

Washington Department of Ecology

Tire Chemicals OECD Toxicity Testing of 6PPD and Related Alternatives

Prepared for: Washington State Department of Ecology

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

Results verified by: _____



Peter Arth, Director

INTRODUCTION

Washington Department of Ecology engaged Enthalpy Analytical (Enthalpy) to generate acute toxicity data on the sensitivity of rainbow trout to 6PPD, 6PPD-quinone, and four alternative compounds. 6PPD-quinone, a chemical found to be present in roadway runoff, was identified as a chemical linked to acute mortality in coho salmon (*Oncorhynchus kisutch*) in stormwater-impacted watersheds throughout the Puget Sound basin. Notably, 6PPD-quinone is a transformation product of 6PPD, an additive in the process of tire manufacturing to protect the rubber polymers from ozone, and it has a published effect to juvenile coho salmon at concentrations below 0.1 µg/L (Tian 2022). The objectives of this project are to compare the relative toxicities to rainbow trout of 6PPD and 6PPD-quinone to potential alternative anti-ozonates (IPPD, 7PPD, TQM, 77PD; alternatives) that might be used in tire production.

Testing was conducted in accordance with the Organization of Economic Cooperation and Development (OECD) method 203, as it would pertain to the United Nations Economic Commission for Europe (UNECE) Globally Harmonized System of Classification and Labeling of Chemicals (GHS; UNECE 2013). Testing was augmented by guidance in OECD method 23 for preparation of difficult to test substances.

The purpose of the GHS is to provide standard criteria for the determination and classification of health, physical, and environmental hazards of chemicals. As part of the current iteration of the GHS hazard characterization system, acute aquatic toxicity tests are conducted to measure the potential of chemicals to cause injury to aquatic organisms subjected to short-term exposure.

Testing was performed to measure acute survival effects to the rainbow trout, *Oncorhynchus mykiss*. All testing was conducted at the Enthalpy Analytical laboratory in San Diego, California. The 6PPD test was conducted between March 9 and 13, 2023; the 6PPD-quinone and four alternative compound tests were conducted between April 13 and 17, 2023.

MATERIALS AND METHODS

The test materials were purchased directly from the supplier and had a listed purity of greater than 95 percent. Upon receipt at the laboratory, the products were stored in cool, dry conditions until used for using.

Compound	CAS Number	Supplier	Product Description
6PPD	793-24-8	Alfa Chemistry	Dark brown solid
IPPD	101-72-4	Alfa Chemistry	Purple liquid
7PPD	3081-01-4	Alfa Chemistry	Light brown liquid
TMQ	26780-96-1	Alfa Chemistry	Yellow liquid
77PD	3081-14-9	Alfa Chemistry	Light purple liquid
6PPD-quinone	unknown	HPC Standards	Orange liquid

Based on the relatively low solubility of the chemical compounds in water, each compound was dissolved in acetone, a solvent vehicle, prior to being introduced to water and exposed to the organisms for testing. Stock solutes containing the compounds and solvents were produced and test dilutions were subsequently created by taking an aliquot of the stock and adding it to water to create the final desired exposure concentrations. A solvent control, consisting of the highest concentration of solvent used in the test series, was added to laboratory dilution water, and tested concurrently to ensure the addition of the solvent itself did not cause detrimental effects to the test organisms.

An initial test was performed with 6PPD to identify the concentration which would show an effect to the rainbow trout and could then be used to inform the test concentrations for the alternative products to understand whether they had increased or decreased effects relative to 6PPD. The 6PPD concentration which produced a 50 percent effect to the test organisms (i.e. EC₅₀) would be used as the target for the middle test concentration in the alternative test series; two concentrations above and below that value were tested to form the dose response curve in the alternative compounds.

Nominal concentrations for the 6PPD exposure were 500, 250, 100, 20, 4, 0.8, and 0.16 micrograms per liter (µg/L). A 1000 milligram per liter (mg/L) acetone control was also tested with this compound.

Nominal concentrations for the IPPD, TMQ, and 77PD tests were 2000, 1000, 500, 250, and 125 µg/L. A 1000 mg/L acetone control was also tested with each of these compounds. Nominal concentrations for the 7PPD test were 2000, 1000, 500, 250, and 125 µg/L. A 2000 mg/L acetone control was also tested with this compound; the increased solvent concentration was required to fully dissolve the compound prior to testing. Nominal concentrations for the 6PPD-quinone test were 1.6, 0.8, 0.4, 0.2, and 0.1 µg/L. A 1000 mg/L acetone control was also tested with this compound. All concentrations were prepared individually.

No subsamples for verification of compound concentrations were collected and analyzed during the testing period. Nominal concentrations were used for all data analysis and reporting.

Toxicity tests were conducted using a listed fish species in accordance with OECD method 203. Concurrent laboratory reference toxicant tests used for quality assurance followed OECD guidelines. Effects were evaluated statistically using the Comprehensive Environmental Toxicity Information System™ (CETIS, version 2.1.2.3) from Tidepool Scientific Software. Organism performance in each test was compared to that observed in the concurrent control exposure. The No Observed Effect Levels (NOEL) and Lowest Observed Effect Levels (LOEL) were calculated using a parametric or nonparametric analysis, as appropriate. The concentrations expected to cause a lethal effect to 25 and 50 percent of test organisms (LC₂₅ or LC₅₀, respectively) were calculated using linear interpolation and Spearman-Kärber.

Larval Fish Toxicity Test Specifications

Test Period:	6PPD: 3/9/23, 16:05 to 3/13/23, 17:05 IPPD: 4/13/23, 16:00 to 4/17/23, 16:00 7PPD: 4/13/23, 15:35 to 4/17/23, 15:35 TMQ: 4/13/23, 15:55 to 4/17/23, 15:55 77PD: 4/13/23, 16:15 to 4/17/23, 16:15 6PPD-quinone: 4/13/23, 16:15 to 4/17/23, 16:15
Test Organism:	<i>Oncorhynchus mykiss</i> (rainbow trout)
Endpoint(s):	96-hour Acute Survival
Test Organism Source, Size:	Thomas Fish Company (Anderson, CA), 3-6 cm
Test Chamber:	4-L glass jars
Volume per Replicate, Number of Replicates:	3 L, 2 Replicates per concentration
Number of Organisms per Replicate:	5
Photoperiod:	16 hours light:8 hours darkness, ambient laboratory levels (50 – 100 ft-c)
Feeding:	None during the test
Control/Dilution Water:	Moderately hard freshwater
Test Concentrations:	6PPD: 500, 250, 100, 20, 4, 0.8, and 0.16 µg/L; lab and solvent controls IPPD, 7PPD, TMQ, 77PD: 2000, 1000, 500, 250, and 125 µg/L; lab and solvent controls 6PPD-quinone: 1.6, 0.8, 0.4, 0.2, and 0.1 µg/L; lab and solvent controls
Protocol Used:	OECD 203 Fish, Acute Toxicity Test (OECD 2019)
Acceptability Criteria:	Mean lab control survival ≥ 90%
Reference Toxicant Test:	A concurrent reference toxicant test using copper chloride was conducted with the April 13 tests.

RESULTS

A statistically significant effect was detected in the 500 µg/L concentration for the 6PPD test, resulting in a NOEC of 250 µg/L. The LC₅₀ was calculated as 375 µg/L, and the LC₂₅ was calculated as 312 µg/L.

Statistically significant effects were detected in the 2000 and 1000 µg/L concentrations for the IPPD test, resulting in a NOEC of 500 µg/L. The LC₅₀ was calculated as 750 µg/L, and the LC₂₅ was calculated as 625 µg/L.

Statistically significant effects were detected in the 2000 and 1000 µg/L concentrations for the 7PPD test, resulting in a NOEC of 500 µg/L. The LC₅₀ was calculated as 643 µg/L, and the LC₂₅ was calculated as 458 µg/L.

No statistically significant effects were detected in any concentration tested for the TMQ test, resulting in a NOEC of 2000 µg/L. The LC₅₀ was calculated as greater than 2000 µg/L, and the LC₂₅ was calculated as 1830 µg/L.

Statistically significant effects were detected in the 2000, 1000, and 500 µg/L concentrations for the 77PD test, resulting in a NOEC of 250 µg/L. The LC₅₀ was calculated as 321 µg/L, and the LC₂₅ was calculated as 229 µg/L.

Out of 6PPD and the 4 alternatives, TMQ exhibited the least mortality to the rainbow trout.

No statistically significant effects were detected in any concentration tested for the 6PPD-quinone test, resulting in a NOEC of 1.6 µg/L. The LC₅₀ and LC₂₅ were both calculated as greater than 1.6 µg/L.

No sublethal abnormalities were observed in any of the chemicals tested.

Summaries of statistical results are provided in Tables 1 to 3. Raw datasheets and complete statistical summaries are provided in Appendix A.

Table 1. Summary of Toxicity Test Results – 6PPD

Test Concentration ($\mu\text{g/L}$)	Mean 96-hr Survival (%)
Lab Control	100
Solvent Control	100
0.16	100
0.8	100
4	100
20	100
100	100
250	100
500	0.0
NOEL (mg/L)	250
LOEL (mg/L)	500
LC ₅₀ (mg/L)	375
LC ₂₅ (mg/L)	312

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

LC₅₀ = the concentration at which 50 percent of the organisms show a lethal effect

LC₂₅ = the concentration at which 25 percent of the organisms show a lethal effect

Table 2. Summary of Toxicity Test Results – IPPD, 7PPD, TMQ, 77PD

Test Concentration (µg/L)	IPPD	7PPD	TMQ	77PD
	Mean 96-hr Survival (%)	Mean 96-hr Survival (%)	Mean 96-hr Survival (%)	Mean 96-hr Survival (%)
Lab Control	100	100	100	100
Solvent Control	100	100	100	100
125	100	100	100	100
250	100	100	100	70.0
500	100	70.0	100	0.0
1000	0.0	0.0	100	0.0
2000	0.0	0.0	70.0	0.0
NOEL (mg/L)	500	500	2000	250
LOEL (mg/L)	1000	1000	>2000	500
LC ₅₀ (mg/L)	750	643	>2000	321
LC ₂₅ (mg/L)	625	458	1830	229

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

LC₅₀ = the concentration at which 50 percent of the organisms show a lethal effect

LC₂₅ = the concentration at which 25 percent of the organisms show a lethal effect

Table 3. Summary of Toxicity Test Results – 6PPD-quinone

Test Concentration (µg/L)	Mean 96-hr Survival (%)
Lab Control	100
Solvent Control	100
0.1	100
0.2	100
0.4	100
0.8	100
1.6	100
NOEL (mg/L)	1.6
LOEL (mg/L)	>1.6
LC ₅₀ (mg/L)	>1.6
LC ₂₅ (mg/L)	>1.6

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

LC₅₀ = the concentration at which 50 percent of the organisms show a lethal effect

LC₂₅ = the concentration at which 25 percent of the organisms show a lethal effect

QUALITY ASSURANCE

The product material was received in good condition. Mean control responses in all tests met minimum test acceptability criteria, and all procedures followed protocol conditions and requirements, unless otherwise noted. The fish were acclimated to the required test temperature and laboratory control water source upon receipt and were held for a period of at least 9 days before test initiation. Fish were fed to satiation in holding (as often as daily); and feeding was discontinued 24 hours before the exposure began.

The 6PPD test exceeded the loading rate of 0.8 g/L, with a loading rate of 1.18 g/L. However, the dissolved oxygen of the test remained above 60 percent saturation and the test organisms showed sensitivity to the chemical. Therefore, it is unlikely the loading rate affected the final outcome of the test.

Minor QA/QC issues that were not likely to have any bearing on the test results are noted on the data sheets, and a list of data qualifier codes is available in Appendix B.

Reference Toxicant Tests

Concurrent reference toxicant test results are summarized in Table 4 and presented in full in Appendix C. The reference toxicant test met minimum test acceptability criteria, and the EC₅₀ was within two standard deviations of the historical mean, indicating the organisms exhibited typical sensitivity to copper as is usually observed in the laboratory.

Table 4. Reference Toxicant Test Results

Species & Endpoint	NOEL (µg/L copper)	LC ₅₀ (µg/L copper)	Historical LC ₅₀ ± 2 SD (µg/L copper)	CV (%)
Fathead Minnow: 96-hour Survival	50	107	82.9 ± 70.9	42.8

NOEL = No Observed Effect Level

LC₅₀ = the concentration at which 50 percent of the organisms show a lethal effect

Historical LC₅₀ ± 2 SD = the mean LC₅₀ from the previous tests performed by Enthelphy, plus or minus two standard deviations

CV= Coefficient of Variation

REFERENCES

- Quality Assurance Project Plan- Tire Chemicals OECD Toxicity Testing of 6PPD and Related Alternatives using the Rainbow Trout, (*Onchorynchus mykiss*)- October 2022
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DOI: 10.1021/acs.estlett.1c00453
- OECD. 2019b. Test No. 203: Fish, Acute Toxicity Test. OECD Guidelines for the Testing of Chemicals, Section 2.
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- Tian Z, Et al. A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon. *Science*. 2021 Jan 8;371(6525):185-189. doi: 10.1126/science.abd6951. Epub 2020 Dec 3. Erratum in: *Science*. 2022 Feb 18;375(6582):eabo5785. PMID: 33273063.
- Tian, Z.; Gonzalez, M.; Rideout, C. A.; Zhao, H. N.; Hu, X.; Wetzel, J.; Mudrock, E.; James, C. A.; McIntyre, J. K.; Kolodziej, E. P. 6PPD-Quinone: Revised Toxicity Assessment and Quantification with a Commercial Standard. *Environmental Science & Technology Letters* 2022, 9 (2), 140– 146,
- Tidepool Scientific Software. 2000-2022. CETIS Comprehensive Environmental Toxicity Information System Software, Version 2.1.2.3.

UNECE. 2013. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Fifth Revised Edition.

US EPA. 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

Datasheets and Statistical Summaries

CETIS Summary Report

Report Date: 25 Apr-23 13:59 (p 1 of 1)
 Test Code/ID: 2304-S122 / 18-3381-8496

Acute Fish Survival Test

Nautilus Environmental (CA)

Batch ID: 05-0885-0010	Test Type: Survival (96h)	Analyst:
Start Date: 09 Mar-23 16:05 PST	Protocol: OECD 203	Diluent: Laboratory Freshwater
Ending Date: 13 Mar-23 17:05 PDT	Species: Oncorhynchus mykiss	Brine: Not Applicable
Test Length: 4d 4h 96 hrs	Taxon:	Source: Thomas Fish Co. Age: 66d
Sample ID: 16-2368-7699	Code: 2304-S122	Project: 6PPD
Sample Date: 09 Mar-23	Material: Chemical Product	Source: Washington Department of Ecology
Receipt Date: 09 Mar-23	CAS (PC): 793-24-8	Station: 6PPD
Sample Age: 16h	Client: Washington Department of Ecology	

post-hatch

Multiple Comparison Summary							
Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
21-0771-0939	96h Survival Rate	Fisher Exact Test	250	500	353.6	---	1

Point Estimate Summary							
Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
02-0749-9952	96h Survival Rate	Linear Interpolation (ICPIN)	EC25	312	312	312	1
			EC50	375	375	375	

96h Survival Rate Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
0	LC	2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
0.16		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
0.8		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
4		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
20		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
100		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
250		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
500		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

96h Survival Rate Detail				MD5: DCE14F656B257D35085F9BE5F3EE86CF
Conc-µg/L	Code	Rep 1	Rep 2	
0	S	1.000	1.000	
0	LC	1.000	1.000	
0.16		1.000	1.000	
0.8		1.000	1.000	
4		1.000	1.000	
20		1.000	1.000	
100		1.000	1.000	
250		1.000	1.000	
500		0.000	0.000	

Ⓐ Q18 RL 4/25/23

Ⓑ Q15 #CS 4/26/23

S = solvent control

CETIS Analytical Report

Report Date: 25 Apr-23 13:59 (p 1 of 1)
 Test Code/ID: 2304-S122 / 18-3381-8496

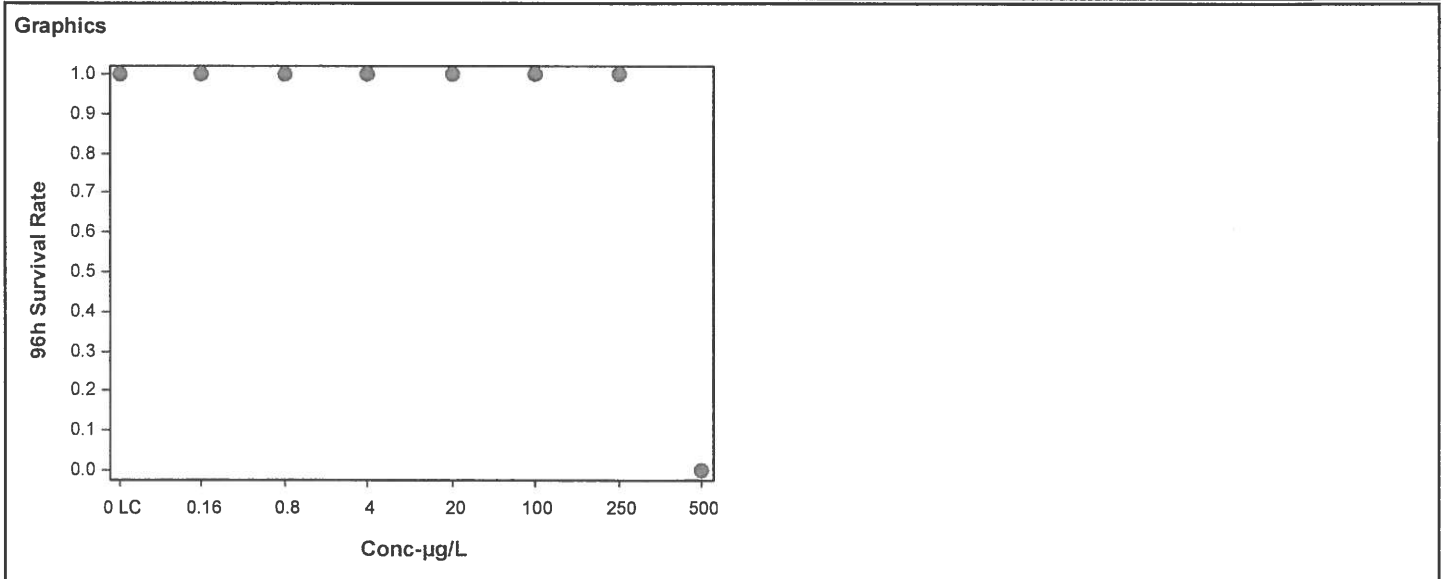
Acute Fish Survival Test			Nautilus Environmental (CA)		
Analysis ID: 21-0771-0939	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.2			
Analyzed: 25 Apr-23 13:58	Analysis: Single 2x2 Contingency Table	Status Level: 1			
Edit Date: 20 Apr-23 13:57	MD5 Hash: 46ECDA54D1369A0F371E3C5D5ED2D6C	Editor ID: 007-803-386-7			

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units
Untransformed	C > T	250	500	353.6	---

Fisher Exact Test						
Control	vs	Conc-µg/L	Test Stat	P-Type	P-Value	Decision(α:5%)
Lab Control		0.16	1.000	Exact	1.0000	Non-Significant Effect
		0.8	1.000	Exact	1.0000	Non-Significant Effect
		4	1.000	Exact	1.0000	Non-Significant Effect
		20	1.000	Exact	1.0000	Non-Significant Effect
		100	1.000	Exact	1.0000	Non-Significant Effect
		250	1.000	Exact	1.0000	Non-Significant Effect

96h Survival Rate Frequencies							
Conc-µg/L	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
0	LC	10	0	10	1.000	0.000	0.00%
0.16		10	0	10	1.000	0.000	0.00%
0.8		10	0	10	1.000	0.000	0.00%
4		10	0	10	1.000	0.000	0.00%
20		10	0	10	1.000	0.000	0.00%
100		10	0	10	1.000	0.000	0.00%
250		10	0	10	1.000	0.000	0.00%
500		0	10	10	0.000	1.000	100.00%

96h Survival Rate Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
0.16		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
0.8		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
4		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
20		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
100		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
250		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
500		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%



CETIS Analytical Report

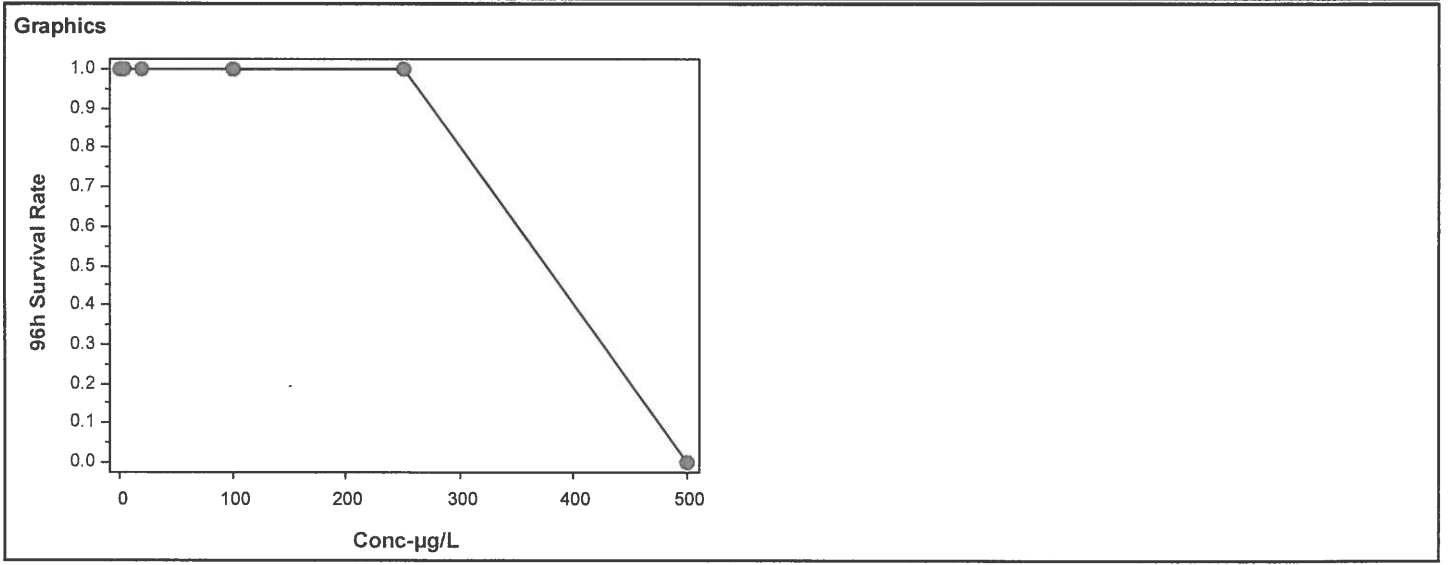
Report Date: 25 Apr-23 13:59 (p 1 of 1)
 Test Code/ID: 2304-S122 / 18-3381-8496

Acute Fish Survival Test			Nautilus Environmental (CA)		
Analysis ID: 02-0749-9952	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.2			
Analyzed: 20 Apr-23 13:59	Analysis: Linear Interpolation (ICPIN)	Status Level: 1			
Edit Date: 20 Apr-23 13:57	MD5 Hash: 46ECDA54D1369A0F371E3C5D5ED2D6C	Editor ID: 007-803-386-7			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1577310	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
EC25	312	312	312
EC50	375	375	375

96h Survival Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
0.16		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
0.8		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
4		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
20		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
100		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
250		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
500		2	0.000	0.000	0.000	0.000	---	100.00%	0/10	0.000	100.00%



Freshwater Acute Bioassay
Static Conditions

DF-018

OECD 203 Product Testing-Definitive

Water Quality Measurements
& Test Organism Survival

Client: Washington Department of Ecology

Sample ID: 6PPD

Test No.: 2304-5122

Test Species: O. mykiss

Start Date/Time: 3/12/23 1605 PST

End Date/Time: 3/13/23 1705 PST

Concentration (ug/L)	Rep	Number of Live Organisms						Conductivity (umhos/cm)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)					Percent Survival
		0	1	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	5	339	333	337	340	341	13.0	10.7	12.8	12.0	11.9	10.6	8.0	6.2	7.7	8.8	7.65	7.12	7.36	7.30	7.24	100
	B	5	5	5	5	5	5	343	337	342	345	344	12.9	10.7	12.6	11.9	11.9	10.4	7.8	6.3	6.4	6.9	7.69	7.25	7.33	7.28	7.20	
Acetone Control	A	5	5	5	5	5	5	342	337	342	345	344	13.0	10.7	12.7	12.0	12.0	10.4	8.3	7.5	8.7	7.9	7.72	7.27	7.32	7.31	7.24	100
	B	5	5	5	5	5	5	342	338	343	346	345	13.0	10.7	12.6	12.0	12.0	10.4	8.4	7.5	8.3	7.8	7.74	7.30	7.37	7.34	7.30	
0.16	A	5	5	5	5	5	5	342	338	342	345	343	12.9	10.8	12.8	12.0	12.1	10.4	8.2	6.3	6.4	6.4	7.73	7.30	7.32	7.25	7.21	100
	B	5	5	5	5	5	5	342	338	343	346	345	13.0	10.9	12.7	12.1	12.1	10.4	8.0	5.9	6.3	6.4	7.77	7.31	7.27	7.22	7.21	
0.8	A	5	5	5	5	5	5	343	338	343	346	344	13.0	10.9	12.7	12.1	12.1	10.3	7.6	6.7	7.8	7.2	7.78	7.30	7.29	7.24	7.22	100
	B	5	5	5	5	5	5	342	338	343	345	344	13.0	10.9	12.7	12.1	12.1	10.4	7.5	6.6	6.2	6.4	7.74	7.31	7.30	7.20	7.23	
4	A	5	5	5	5	5	5	343	338	343	346	345	13.0	10.9	12.8	12.1	12.1	10.3	8.3	7.2	7.7	7.5	7.80	7.35	7.33	7.28	7.26	100
	B	5	5	5	5	5	5	343	338	343	346	344	13.0	11.0	12.9	12.2	12.2	10.3	8.0	6.9	7.1	6.9	7.81	7.34	7.29	7.16	7.20	
20	A	5	5	5	5	5	5	343	338	343	346	345	13.0	11.0	12.9	12.3	12.3	10.3	7.4	6.2	6.2	6.0	7.81	7.31	7.23	7.17	7.20	100
	B	5	5	5	5	5	5	343	338	343	345	344	13.0	11.0	12.9	12.3	12.3	10.3	7.8	6.4	6.8	7.1	7.82	7.32	7.26	7.19	7.26	
100	A	5	5	5	5	5	5	342	338	342	346	344	12.9	10.5	12.3	11.5	11.7	10.4	7.0	5.4	6.4	6.3	7.83	7.26	7.17	7.16	7.21	100
	B	5	5	5	5	5	5	342	337	342	345	344	12.9	10.5	12.3	11.4	11.6	10.4	7.3	5.4	6.1	6.3	7.83	7.28	7.18	7.11	7.17	
250	A	5	5	5	5	5	5	342	339	345	349	348	13.0	10.6	12.3	11.4	11.4	10.4	7.8	5.5	5.2	4.6	7.84	7.31	7.20	7.10	7.13	100
	B	5	5	5	5	5	5	342	338	345	349	347	12.9	10.5	12.2	11.4	11.4	10.4	8.0	6.2	5.9	5.4	7.84	7.33	7.23	7.14	7.19	
500	A	5	5	4	0	-	0	341	338	346	-	-	12.8	10.5	12.4	-	-	10.6	7.9	7.5	-	-	7.85	7.33	7.27	-	-	0
	B	5	5	4	1	1	0	342	339	348	341	349	12.8	10.5	12.4	11.4	11.7	10.3	7.9	7.0	7.0	6.8	7.85	7.33	7.24	7.18	7.23	
Tech Initials	Counts	WF	WF	WF	WF	KR	AKS																					
	WQ	WF	X	WF	WF	FR	KR																					
	QC	AKS																										

Fish Size at test initiation*:

Weights (g): 0.620 0.873 0.552 0.790 0.699 $\mu = 0.707g$

Lengths (cm): 3.8 4.0 3.4 4.0 3.8 $\mu = 3.8$ cm Loading rate = 1.18g/L

Environmental Chamber: F

Sample Description: Dark Brown Solid

Animal Source/Date Received: O. mykiss Thomas Fish Co. 2/28/23 Age at Initiation: 66 days post hatch 52 days post swim up

Comments: *5 random fish are sacrificed at initiation for size determination. Loading must be less than 0.8 g fish/L. Fish must be 3-6 cm in length

AKS KR 3/12/23 AKS KR 3/13/23 AKS 4/26/23

QC Check: RL 4/25/23

Final Review: AKS 4/26/23

CETIS Summary Report

Report Date: 25 Apr-23 14:02 (p 1 of 1)
 Test Code/ID: 2304-S123 / 12-8677-3038

Acute Fish Survival Test

Nautilus Environmental (CA)

Batch ID: 19-5304-1610	Test Type: Survival (96h)	Analyst:
Start Date: 13 Apr-23 16:15	Protocol: OECD 203	Diluent: Laboratory Freshwater
Ending Date: 17 Apr-23 16:15	Species: Oncorhynchus mykiss	Brine: Not Applicable
Test Length: 96h	Taxon:	Source: Thomas Fish Co. Age: 46d
Sample ID: 15-5993-0012	Code: 5CFAA49C	Project: 6PPD-quinone
Sample Date: 13 Apr-23	Material: Chemical Product	Source: Washington Department of Ecology
Receipt Date: 13 Apr-23	CAS (PC):	Station: 6PPD-quinone
Sample Age: 16h	Client: <i>Washington Department of Ecology</i>	

post-hatch

Multiple Comparison Summary							
Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
14-1352-9017	96h Survival Rate	Fisher Exact Test	1.6	>1.6	---	---	1

Point Estimate Summary							
Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
20-3653-4282	96h Survival Rate	Linear Interpolation (ICPIN)	EC25	>1.6	---	---	1
			EC50	>1.6	---	---	

96h Survival Rate Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
0	LC	2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
0.1		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
0.2		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
0.4		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
0.8		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
1.6		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%

96h Survival Rate Detail				MD5: 5FE8623225CE764AFBC656420B2041E8							
Conc-µg/L	Code	Rep 1	Rep 2								
0	S	1.000	1.000								
0	LC	1.000	1.000								
0.1		1.000	1.000								
0.2		1.000	1.000								
0.4		1.000	1.000								
0.8		1.000	1.000								
1.6		1.000	1.000								

S = solvent control

CETIS Analytical Report

Report Date: 25 Apr-23 14:02 (p 1 of 1)
 Test Code/ID: 2304-S123 / 12-8677-3038

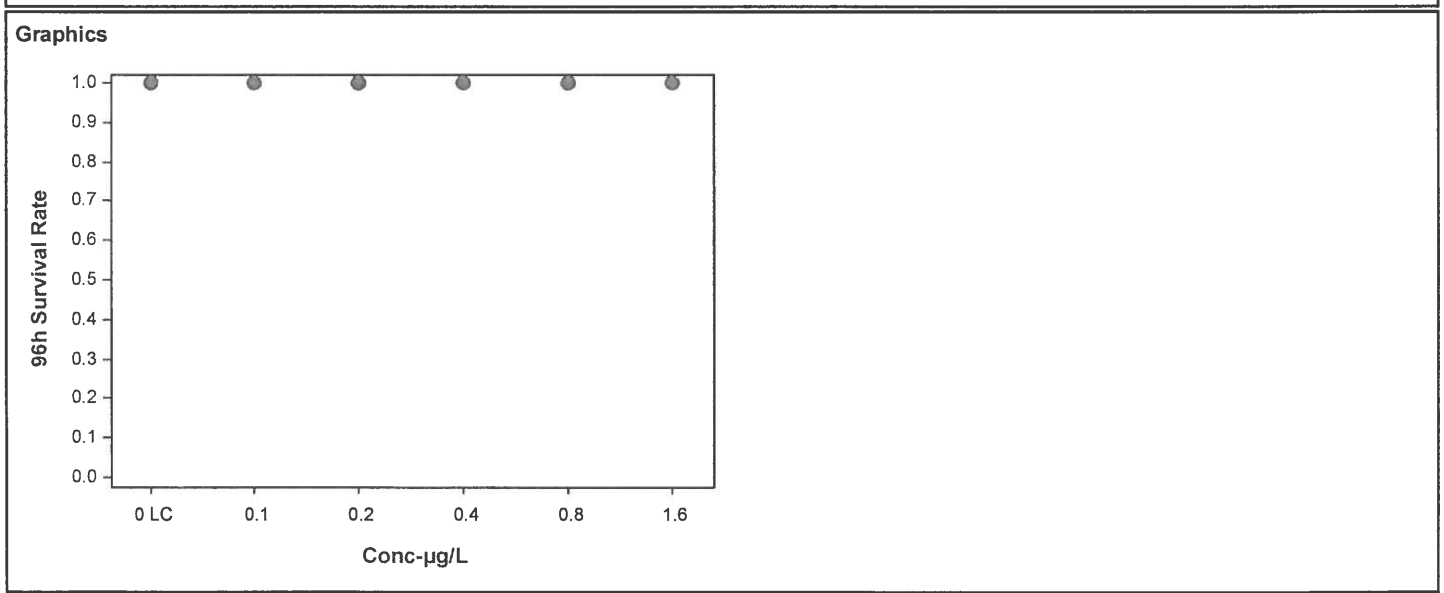
Acute Fish Survival Test			Nautilus Environmental (CA)		
Analysis ID: 14-1352-9017	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.2			
Analyzed: 25 Apr-23 14:01	Analysis: Single 2x2 Contingency Table	Status Level: 1			
Edit Date: 25 Apr-23 14:01	MD5 Hash: BD14F58107757E7FA05135C4E0264C07	Editor ID: 007-803-386-7			

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units
Untransformed	C > T	1.6	>1.6	---	---

Fisher Exact Test						
Control	vs	Conc-µg/L	Test Stat	P-Type	P-Value	Decision(α:5%)
Lab Control		0.1	1.000	Exact	1.0000	Non-Significant Effect
		0.2	1.000	Exact	1.0000	Non-Significant Effect
		0.4	1.000	Exact	1.0000	Non-Significant Effect
		0.8	1.000	Exact	1.0000	Non-Significant Effect
		1.6	1.000	Exact	1.0000	Non-Significant Effect

96h Survival Rate Frequencies							
Conc-µg/L	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
0	LC	10	0	10	1.000	0.000	0.00%
0.1		10	0	10	1.000	0.000	0.00%
0.2		10	0	10	1.000	0.000	0.00%
0.4		10	0	10	1.000	0.000	0.00%
0.8		10	0	10	1.000	0.000	0.00%
1.6		10	0	10	1.000	0.000	0.00%

96h Survival Rate Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
0.1		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
0.2		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
0.4		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
0.8		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
1.6		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%



CETIS Analytical Report

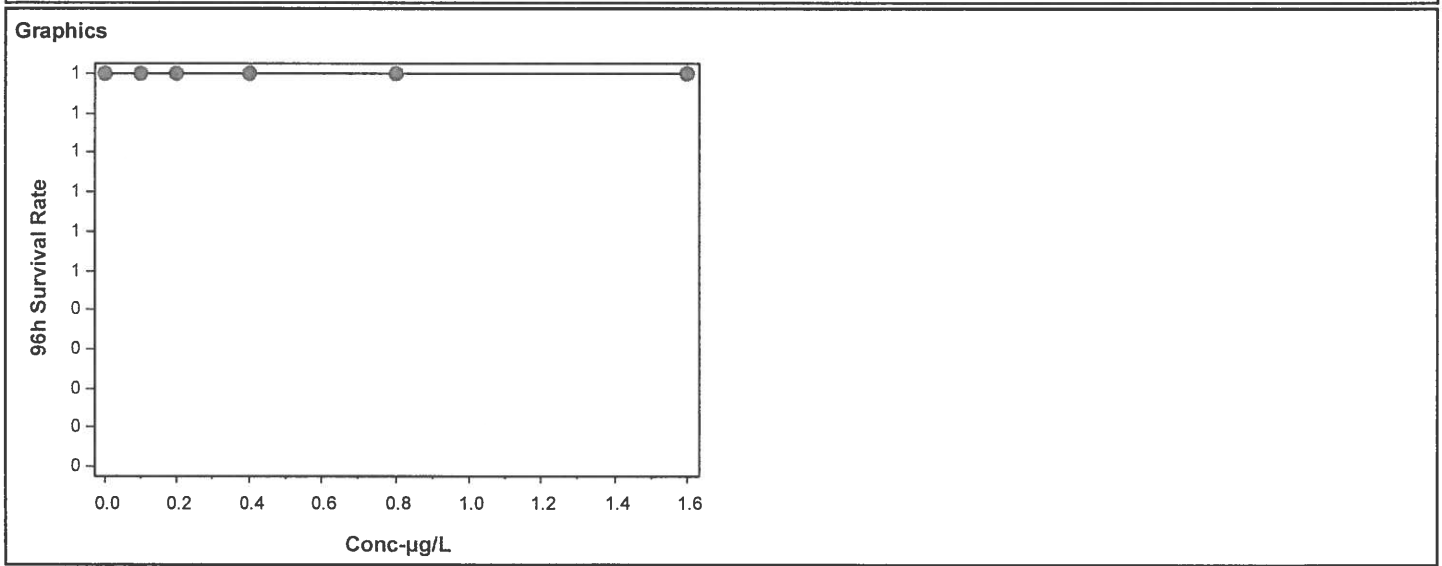
Report Date: 25 Apr-23 14:01 (p 1 of 1)
 Test Code/ID: 2304-S123 / 12-8677-3038

Acute Fish Survival Test			Nautilus Environmental (CA)		
Analysis ID: 20-3653-4282	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.2			
Analyzed: 25 Apr-23 14:01	Analysis: Linear Interpolation (ICPIN)	Status Level: 1			
Edit Date: 25 Apr-23 14:01	MD5 Hash: BD14F58107757E7FA05135C4E0264C07	Editor ID: 007-803-386-7			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	781368	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
EC25	>1.6	---	---
EC50	>1.6	---	---

96h Survival Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
0.1		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
0.2		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
0.4		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
0.8		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
1.6		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%



**Freshwater Acute Bioassay
Static Conditions**

DF-018

OECD 203

**Water Quality Measurements
& Test Organism Survival**

Client: WADOE

Sample ID: 6PPD-quinone

Test No.: 2304-5123

Test Species: O. mykiss

Start Date/Time: 4/13/23 1615

End Date/Time: 4/17/23 1615

Concentration (ug/L)	Rep	Number of Live Organisms						Conductivity (umhos/cm)					Temperature Q1 (°C)					Dissolved Oxygen Q14 (mg/L) Q14 Q14					pH (units)					Percent Survival
		0	1	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	S	S	S	S	S	319	304	302	335	327	11.8	11.1	11.4	11.4	11.4	11.3	9.9	9.1	9.2	8.9	8.03	7.60	7.52	7.42	7.37	100
	B	5	S	S	S	S	S	321	305	303	330	330	11.6	10.9	11.3	11.3	11.3	11.3	9.9	9.2	9.1	9.1	8.04	7.63	7.55	7.49	7.44	
1,000 mg/L	A	5	S	S	S	S	S	320	305	291	337	330	11.5	11.1	11.6	11.6	11.5	11.4	9.8	9.3	9.4	9.4	8.01	7.62	7.54	7.54	7.51	100
Acetone Control	B	5	S	S	S	S	S	320	305	291	335	329	11.4	11.1	11.5	11.5	11.5	11.4	10.1	9.4	9.5	9.6	7.99	7.64	7.57	7.50	7.55	
0.1	A	5	S	S	S	S	S	309	294	292	323	317	12.1	11.0	11.4	11.4	11.3	11.2	9.9	9.0	9.3	9.4	8.02	7.64	7.56	7.55	7.51	100
	B	5	S	S	S	S	S	310	294	292	323	317	12.0	10.9	11.3	11.3	11.2	11.1	9.9	9.1	9.1	9.0	8.01	7.64	7.55	7.55	7.52	
0.2	A	5	S	S	S	S	S	309	294	292	324	318	12.0	11.0	11.4	11.4	11.3	11.3	9.8	8.9	9.1	9.2	8.02	7.64	7.54	7.54	7.54	100
	B	5	S	S	S	S	S	310	294	293	325	319	12.1	10.9	11.2	11.2	11.2	11.2	9.7	9.0	9.0	8.9	8.02	7.63	7.56	7.52	7.52	
0.4	A	5	S	S	S	S	S	309	295	293	324	318	12.1	11.0	11.4	11.4	11.4	11.2	9.5	8.6	8.6	8.3	8.02	7.62	7.52	7.51	7.49	100
	B	5	S	S	S	S	S	310	294	292	323	316	12.1	10.9	11.3	11.3	11.2	11.1	9.6	8.8	8.9	9.0	8.01	7.58	7.54	7.52	7.51	
0.8	A	5	S	S	S	S	S	310	295	293	325	318	12.1	11.0	11.4	11.3	11.3	11.2	10.0	9.1	9.1	9.4	8.03	7.63	7.54	7.54	7.54	100
	B	5	S	S	S	S	S	310	295	293	325	318	12.1	10.9	11.3	11.3	11.2	11.2	10.9	9.0	9.1	9.0	8.02	7.63	7.55	7.54	7.53	
1.6	A	5	S	S	S	S	S	310	295	293	324	317	12.1	11.1	11.4	11.4	11.4	11.1	9.5	8.8	8.8	9.0	8.01	7.61	7.54	7.55	7.50	100
	B	5	S	S	S	S	S	310	295	293	324	318	12.1	10.9	11.3	11.2	11.2	11.1	9.7	8.9	9.2	9.1	8.01	7.60	7.55	7.50	7.51	
Technician Initials		KP	WF	WF	WF	KR	KP																					

Environmental Chamber: F

Weights (mg): 0.346 0.292 0.275 0.341 0.316 0.259 0.340 0.234 0.262 0.306

Lengths (cm): 3.5 3.0 3.3 3.0 3.5 3.0 3.5 2.9 3.0 3.5
3.2 3.1 3.1 3.3 3.2 3.1 3.2 2.9 3.0 3.2

$\mu = \frac{0.304g}{0.296}$
 $\mu = \frac{3.1cm}{3.1cm}$
Loading rate = $\frac{0.51}{0.419/L}$

Sample Description: orange liquid

Animal Source/Date Received: Thomas Fish Co. 4/4/23

Age at Initiation: 46 days post hatch

Comments: ⓐ Q1B WF 4/13/23 ⓑ Q18 RL 4/25/23

QC Check: RL 4/25/23

Final Review: ACS 4/26/23

CETIS Summary Report

Report Date: 25 Apr-23 14:08 (p 1 of 1)
 Test Code/ID: 2304-S127 / 10-0189-6773

Acute Fish Survival Test

Nautilus Environmental (CA)

Batch ID: 03-0476-3407	Test Type: Survival (96h)	Analyst:
Start Date: 13 Apr-23 15:35	Protocol: OECD 203	Diluent: Laboratory Freshwater
Ending Date: 17 Apr-23 15:35	Species: Oncorhynchus mykiss	Brine: Not Applicable
Test Length: 96h	Taxon:	Source: Thomas Fish Co. Age: 46d
Sample ID: 02-3627-4655	Code: E1543DF	Project: 7PPD
Sample Date: 13 Apr-23	Material: Chemical Product	Source: Washington Department of Ecology
Receipt Date: 13 Apr-23	CAS (PC): 3081-01-4	Station: 7PPD
Sample Age: 16h	Client: Washington Department of Ecology	

post-hatch

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
14-6572-9472	96h Survival Rate	Fisher Exact Test	500	1000	707.1	---	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
19-5561-2344	96h Survival Rate	Linear Interpolation (ICPIN)	EC25	458	250	750	1
			EC50	643	405	821	

96h Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
0	LC	2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
125		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
250		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
500		2	0.700	-0.571	1.970	0.600	0.800	0.100	0.141	20.20%	30.00%
1000		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
2000		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

96h Survival Rate Detail

MD5: 0A780B555A60BE610F56473AC38A3CCD

Conc-µg/L	Code	Rep 1	Rep 2
0	S	1.000	1.000
0	LC	1.000	1.000
125		1.000	1.000
250		1.000	1.000
500		0.800	0.600
1000		0.000	0.000
2000		0.000	0.000

S = solvent control

CETIS Analytical Report

Report Date: 25 Apr-23 14:08 (p 1 of 1)
 Test Code/ID: 2304-S127 / 10-0189-6773

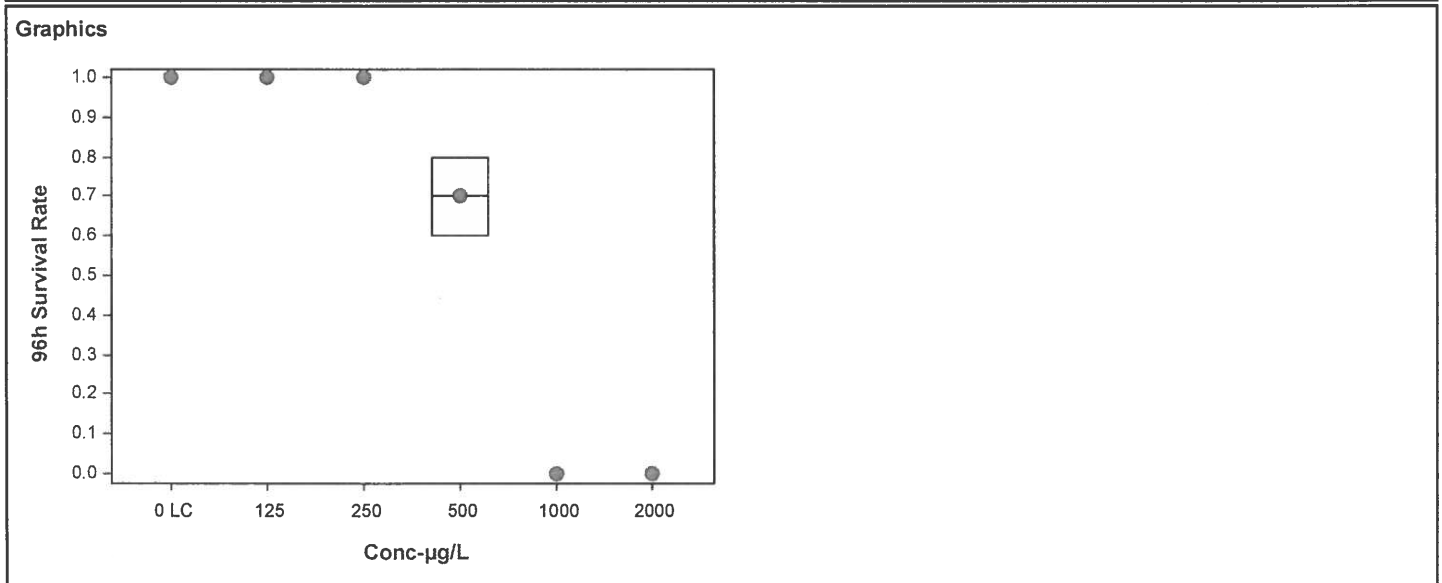
Acute Fish Survival Test			Nautilus Environmental (CA)		
Analysis ID: 14-6572-9472	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.2			
Analyzed: 20 Apr-23 14:06	Analysis: Single 2x2 Contingency Table	Status Level: 1			
Edit Date: 20 Apr-23 14:05	MD5 Hash: 0DAC07DE7DBF7D919A286A1EC55987E	Editor ID: 007-803-386-7			

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units
Untransformed	C > T	500	1000	707.1	---

Fisher Exact Test						
Control	vs	Conc-µg/L	Test Stat	P-Type	P-Value	Decision(α:5%)
Lab Control		125	1.000	Exact	1.0000	Non-Significant Effect
		250	1.000	Exact	1.0000	Non-Significant Effect
		500	0.105	Exact	0.1053	Non-Significant Effect

96h Survival Rate Frequencies							
Conc-µg/L	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
0	LC	10	0	10	1.000	0.000	0.00%
125		10	0	10	1.000	0.000	0.00%
250		10	0	10	1.000	0.000	0.00%
500		7	3	10	0.700	0.300	30.00%
1000		0	10	10	0.000	1.000	100.00%
2000		0	10	10	0.000	1.000	100.00%

96h Survival Rate Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
125		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
250		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
500		2	0.700	0.000	1.000	0.700	0.600	0.800	0.100	20.20%	30.00%
1000		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
2000		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%



CETIS Analytical Report

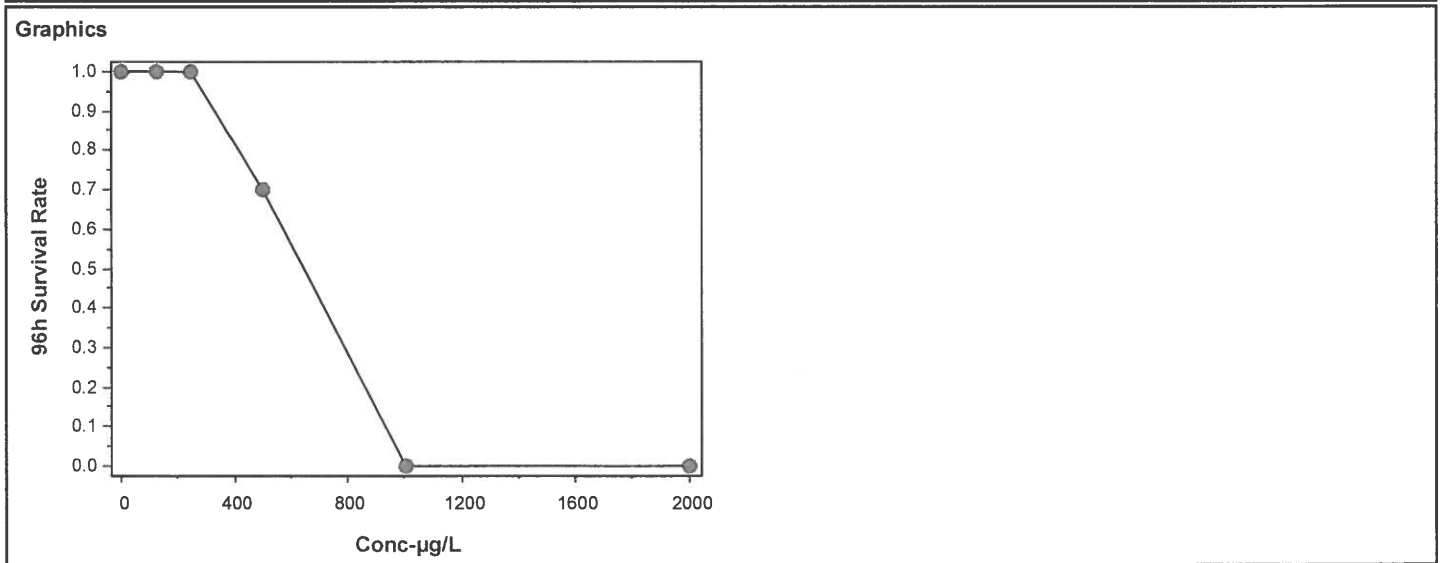
Report Date: 25 Apr-23 14:08 (p 1 of 1)
 Test Code/ID: 2304-S127 / 10-0189-6773

Acute Fish Survival Test			Nautilus Environmental (CA)		
Analysis ID: 19-5561-2344	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.2			
Analyzed: 20 Apr-23 14:07	Analysis: Linear Interpolation (ICPIN)	Status Level: 1			
Edit Date: 20 Apr-23 14:05	MD5 Hash: 0DAC07DE7DBF7D919A286A1EC55987E	Editor ID: 007-803-386-7			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	60714	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
EC25	458	250	750
EC50	643	405	821

96h Survival Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
125		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
250		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
500		2	0.700	0.700	0.600	0.800	20.20%	30.00%	7/10	0.700	30.00%
1000		2	0.000	0.000	0.000	0.000	---	100.00%	0/10	0.000	100.00%
2000		2	0.000	0.000	0.000	0.000	---	100.00%	0/10	0.000	100.00%



Freshwater Acute Bioassay
Static Conditions

DF-018

OECD 203

Water Quality Measurements
& Test Organism Survival

Client: WADOE

Sample ID: 7PPD

Test No.: 2304-527

Test Species: O. mykiss

Start Date/Time: 4/13/23 1535

End Date/Time: 4/17/23 1535

Concentration (ug/L)	Rep	Number of Live Organisms						Conductivity (umhos/cm)					Temperature Q1 (°C)				Dissolved Oxygen Q14 Q14 (mg/L) Q14 Q14					pH (units)					Percent Survival	
		0	1	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	5	314	300	299	337	327	11.4	11.0	11.5	11.4	11.5	11.4	10.0	9.1	9.1	9.3	7.93	7.80	7.41	7.50	7.49	100
	B	5	5	5	5	5	5	320	306	303	319	330	11.4	10.8	11.2	11.4	11.3	11.5	9.8	9.0	9.2	9.1	7.97	7.54	7.44	7.40	7.49	
2,000 mg/L	A	5	5	5	5	5	5	319	304	301	332	328	11.4	11.1	11.5	11.5	11.4	11.5	9.6	8.9	8.9	8.9	7.99	7.55	7.44	7.50	7.49	100
Acetone Control	B	5	5	5	5	5	5	316	303	301	334	329	11.4	10.9	11.4	11.4	11.3	11.4	10.0	9.0	9.2	9.1	7.98	7.58	7.46	7.51	7.50	
125	A	5	5	5	5	5	5	321	306	303	330	329	11.4	11.1	11.5	11.5	11.5	11.4	9.8	8.4	8.5	8.5	7.98	7.59	7.45	7.40	7.48	100
	B	5	5	5	5	5	5	321	306	303	330	329	11.4	11.0	11.4	11.4	11.3	11.4	9.9	8.7	8.7	8.4	8.01	7.60	7.47	7.49	7.47	
250	A	5	5	5	5	5	5	320	305	303	337	329	11.4	11.1	11.5	11.5	11.5	11.4	9.6	8.5	8.3	7.8	7.99	7.60	7.47	7.49	7.43	100
	B	5	5	5	5	5	5	320	305	303	337	330	11.3	11.0	11.4	11.3	11.3	11.4	10.0	8.9	8.3	8.0	7.99	7.60	7.50	7.46	7.42	
500	A	5	5	4	4	4	4	320	305	303	337	330	11.4	11.1	11.5	11.5	11.5	11.4	9.1	7.2	6.1	5.0	7.99	7.57	7.43	7.35	7.26	70
	B	5	5	3	3	3	3	320	306	304	337	330	11.4	11.0	11.4	11.4	11.4	11.4	9.2	7.3	6.7	5.8	7.99	7.56	7.41	7.35	7.28	
1,000	A	5	5	0				320	305				11.4	11.1				11.4	10.5				7.97	7.62				0
	B	5	5	0				320	305				11.4	11.0				11.4	10.3				7.97	7.63				
2,000	A	5	5	0				320	305				11.4	11.2				11.4	10.8				7.96	7.68				0
	B	5	5	0				320	305				11.5	11.1				11.4	10.9				7.95	7.74				
Technician Initials		WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF		

Environmental Chamber: F

Weights (mg): 0.346 0.292 0.275 0.391 0.316 0.254 0.340 0.234 0.282 0.306

Lengths (cm): 3.5 3.0 3.0 3.3 3.1 3.1 3.2 3.1 3.2 3.0 3.2

$\mu = 0.304 \text{ g}$

$\mu = 3.1 \text{ cm}$

Loading rate = 0.19 3/L

Sample Description: Light Brown Liquid

Animal Source/Date Received: Thomas Fish Co. 4/4/23 Age at Initiation: 46 days post hatch

Comments: Ⓐ Q14 OR 04/13/23 Ⓑ Q12 WF 4/13/23 Ⓒ Q14 DR 04/17/23 Ⓓ Q16 RL 4/25/23 Ⓔ Q14 HCS 4/26/23

QC Check: RL 4/25/23

Final Review: ALS 4/26/23

CETIS Summary Report

Report Date: 25 Apr-23 14:07 (p 1 of 1)
 Test Code/ID: 2304-S126 / 07-9618-8936

Acute Fish Survival Test

Nautilus Environmental (CA)

Batch ID: 20-4180-6527	Test Type: Survival (96h)	Analyst:
Start Date: 13 Apr-23 16:15	Protocol: OECD 203	Diluent: Laboratory Freshwater
Ending Date: 17 Apr-23 16:15	Species: Oncorhynchus mykiss	Brine: Not Applicable
Test Length: 96h	Taxon:	Source: Thomas Fish Co. Age: 46d
Sample ID: 10-7906-6284	Code: 40513EAC	Project: 77PD
Sample Date: 13 Apr-23	Material: Chemical Product	Source: Washington Department of Ecology
Receipt Date: 13 Apr-23	CAS (PC): 3081-14-9	Station: 77PD
Sample Age: 16h	Client: <i>Washington Department of Ecology</i>	

post-wash

Multiple Comparison Summary							
Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
15-5632-5297	96h Survival Rate	Fisher Exact/Bonferroni-Holm Test	250	500	353.6	---	1

Point Estimate Summary							
Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
02-2680-4330	96h Survival Rate	Linear Interpolation (ICPIN)	EC25	229	20.8	562	1
			EC50	321	---	536	

96h Survival Rate Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
0	LC	2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
125		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
250		2	0.700	-3.110	4.510	0.400	1.000	0.300	0.424	60.61%	30.00%
500		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
1000		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
2000		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

96h Survival Rate Detail				MD5: 0C387EC8DD093F35EE4A94630F4BDCD2							
Conc-µg/L	Code	Rep 1	Rep 2								
0	S	1.000	1.000								
0	LC	1.000	1.000								
125		1.000	1.000								
250		1.000	0.400								
500		0.000	0.000								
1000		0.000	0.000								
2000		0.000	0.000								

S=solvent control

CETIS Analytical Report

Report Date: 25 Apr-23 14:07 (p 1 of 1)
 Test Code/ID: 2304-S126 / 07-9618-8936

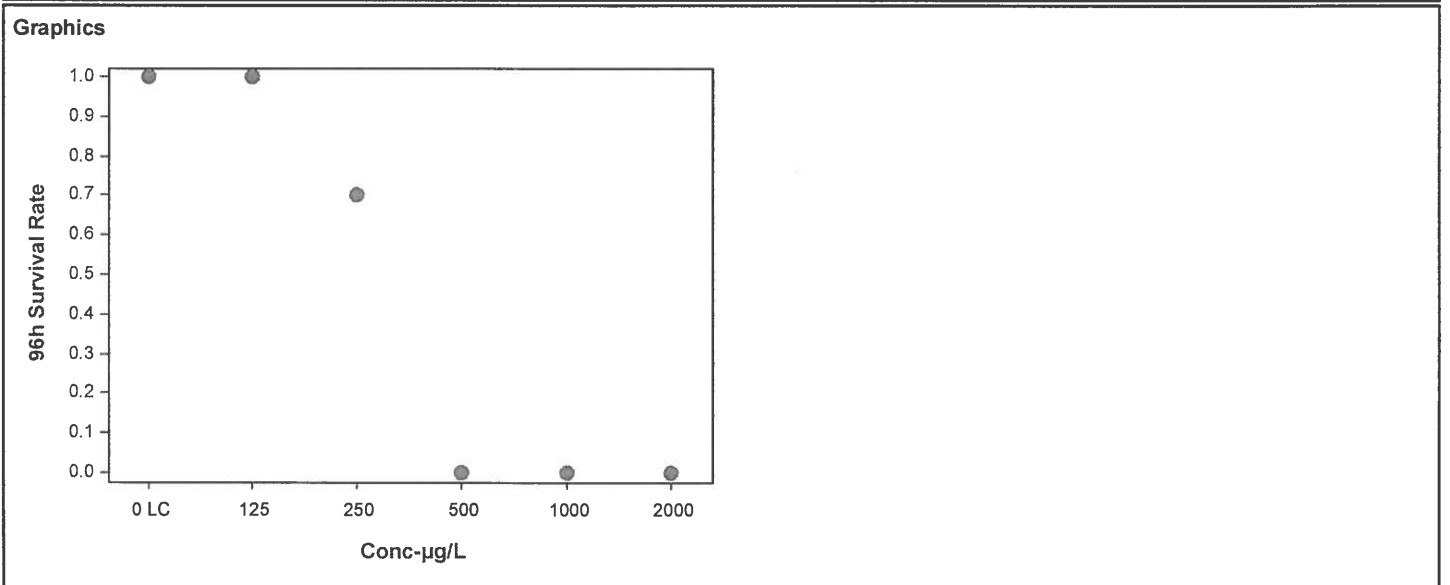
Acute Fish Survival Test			Nautilus Environmental (CA)		
Analysis ID: 15-5632-5297	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.2			
Analyzed: 20 Apr-23 14:09	Analysis: STP 2xK Contingency Tables	Status Level: 1			
Edit Date: 20 Apr-23 14:09	MD5 Hash: 825E0D712267A5EAB1538B2E39E4A7DA	Editor ID: 007-803-386-7			

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units
Untransformed	C > T	250	500	353.6	---

Fisher Exact/Bonferroni-Holm Test						
Control	vs	Conc-µg/L	Test Stat	P-Type	P-Value	Decision(α:5%)
Lab Control		125	1.000	Exact	1.0000	Non-Significant Effect
		250	0.105	Exact	0.2105	Non-Significant Effect
		500*	0.000	Exact	2.7E-05	Significant Effect
		1000*	0.000	Exact	2.7E-05	Significant Effect
		2000*	0.000	Exact	0.0010	Significant Effect

96h Survival Rate Frequencies							
Conc-µg/L	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
0	LC	10	0	10	1.000	0.000	0.00%
125		10	0	10	1.000	0.000	0.00%
250		7	3	10	0.700	0.300	30.00%
500		0	10	10	0.000	1.000	100.00%
1000		0	10	10	0.000	1.000	100.00%
2000		0	5	5	0.000	1.000	100.00%

96h Survival Rate Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
125		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
250		2	0.700	0.000	1.000	0.700	0.400	1.000	0.300	60.61%	30.00%
500		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
1000		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
2000		1	0.000			0.000	0.000	0.000	---	---	100.00%



CETIS Analytical Report

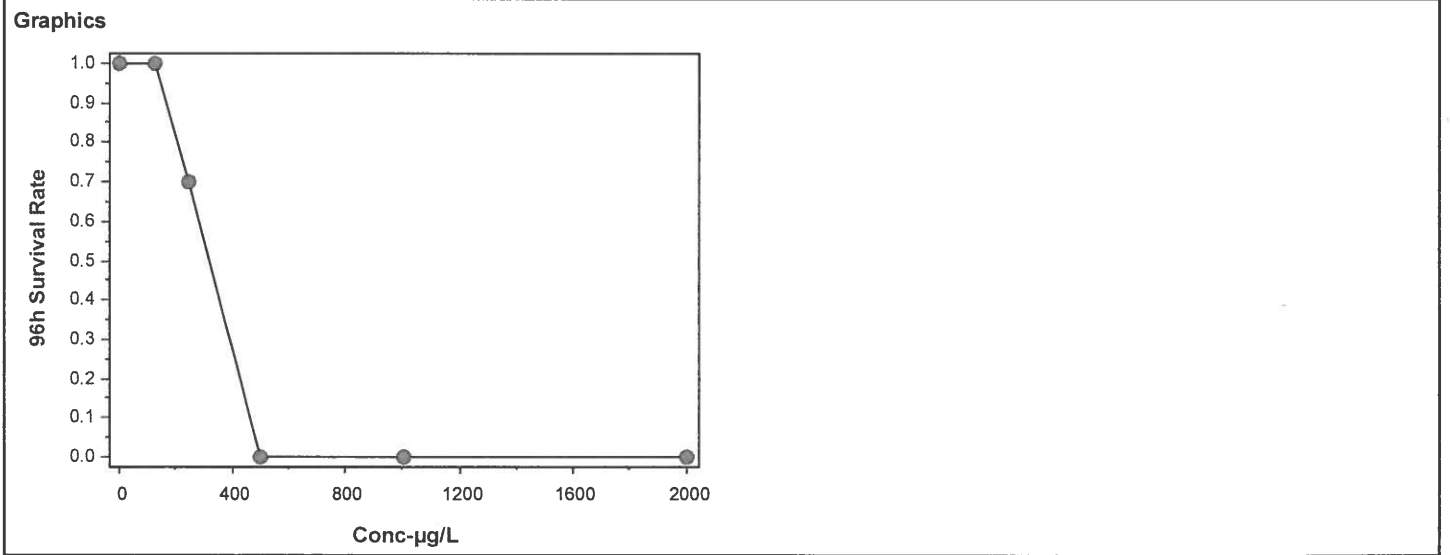
Report Date: 25 Apr-23 14:07 (p 1 of 1)
 Test Code/ID: 2304-S126 / 07-9618-8936

Acute Fish Survival Test			Nautilus Environmental (CA)		
Analysis ID: 02-2680-4330	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.2			
Analyzed: 20 Apr-23 14:09	Analysis: Linear Interpolation (ICPIN)	Status Level: 1			
Edit Date: 20 Apr-23 14:09	MD5 Hash: 825E0D712267A5EAB1538B2E39E4A7DA	Editor ID: 007-803-386-7			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1869126	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
EC25	229	20.8	562
EC50	321	---	536

96h Survival Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
125		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
250		2	0.700	0.700	0.400	1.000	60.61%	30.00%	7/10	0.700	30.00%
500		2	0.000	0.000	0.000	0.000	---	100.00%	0/10	0.000	100.00%
1000		2	0.000	0.000	0.000	0.000	---	100.00%	0/10	0.000	100.00%
2000		1	0.000	0.000	0.000	0.000	---	100.00%	0/5	0.000	100.00%



Freshwater Acute Bioassay
Static Conditions

DF-018

OECD 203

Water Quality Measurements
& Test Organism Survival

Client: WADOE

Sample ID: 77PD

Test No.: 2304-5126

Test Species: O. mykiss

Start Date/Time: 4/13/23 1615

End Date/Time: 4/17/23 1615

Concentration (ug/L)	Rep	Number of Live Organisms						Conductivity (umhos/cm)					Temperature Q1 (°C)					Dissolved Oxygen Q14 (mg/L)					pH (units)					Percent Survival
		0	1	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	5	321	305	301	335	327	11.6	11.0	11.5	11.4	11.4	11.4	9.8	8.9	9.0	9.1	8.04	7.60	7.45	7.49	7.50	100
	B	5	5	5	5	5	5	320	304	301	335	328	11.5	10.9	11.3	11.3	11.3	11.3	9.7	8.9	8.6	8.6	8.05	7.61	7.49	7.46	7.47	
1,000 mg/L	A	5	5	5	5	5	5	308	293	291	320	315	11.9	11.0	11.5	11.5	11.5	11.3	9.3	8.5	8.7	8.5	8.02	7.60	7.48	7.47	7.44	100
Acetone Control	B	5	5	5	5	5	5	308	293	291	320	316	11.8	10.9	11.3	11.3	11.3	11.3	9.5	8.6	8.5	8.4	7.99	7.58	7.48	7.45	7.44	
125	A	5	5	5	5	5	5	310	299	297	328	321	11.9	11.5	11.5	11.5	11.5	11.3	9.9	8.8	8.7	8.8	8.01	7.61	7.52	7.50	7.46	100
	B	5	5	5	5	5	5	310	295	293	323	317	11.8	10.9	11.4	11.3	11.3	11.2	10.0	8.9	9.0	9.2	8.00	7.62	7.54	7.53	7.52	
250	A	5	5	5	5	5	5	309	296	295	320	319	12.0	11.1	11.5	11.5	11.5	11.3	9.3	7.3	7.0	7.1	8.00	7.62	7.47	7.41	7.40	70
	B	5	5	5	5	2	2	309	296	296	330	328	11.9	11.0	11.3	11.3	11.3	11.2	9.3	8.8	7.8	4.5	8.01	7.61	7.47	7.40	7.24	
500	A	5	5	0	/	/	/	310	298	/	/	/	12.0	11.0	/	/	/	11.2	11.2	/	/	/	7.99	7.90	/	/	/	0
	B	5	5	0	/	/	/	309	296	/	/	/	11.9	10.9	/	/	/	11.3	10.1	/	/	/	8.01	7.65	/	/	/	
1,000	A	5	5	0	/	/	/	309	295	/	/	/	11.9	11.0	/	/	/	11.2	10.9	/	/	/	8.01	7.75	/	/	/	0
	B	5	5	0	/	/	/	309	295	/	/	/	11.9	10.9	/	/	/	11.2	10.7	/	/	/	8.02	7.75	/	/	/	
2,000	A	5	5	0	/	/	/	308	297	/	/	/	12.0	11.1	/	/	/	11.2	10.8	/	/	/	8.02	7.83	/	/	/	0
	B	5	5	0	/	/	/	308	299	/	/	/	12.0	11.0	/	/	/	11.2	11.1	/	/	/	8.02	7.85	/	/	/	
Technician Initials		WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	WF	

Environmental Chamber: F

Weights (mg): \textcircled{a} 0.346 0.242 0.275 0.391 0.316 0.259 0.340 0.234 0.282 0.306

Lengths (cm): \textcircled{a} 3.5 3.0 3.0 3.0 3.1 3.0 3.1 3.2 3.0 3.2

\textcircled{a} 0.304 g
 $\mu = 0.296$
 \textcircled{c}
 $\mu = 3.10$ 3.1 cm
 \textcircled{a} 0.51
Loading rate = 0.49 g/L

Sample Description: Light Purple Liquid Animal Source/Date Received: Thomas Fish Co. 4/4/23 Age at Initiation: 46 days post hatch

Comments: \textcircled{a} Q18 WF 4/13/23 \textcircled{b} Q18 WF 4/14/23 \textcircled{c} Q18 RL 4/25/23

QC Check: RL 4/25/23

Final Review: AKS 4/26/23

CETIS Summary Report

Report Date: 25 Apr-23 14:04 (p 1 of 1)

Test Code/ID: 2304-S124 / 14-4803-4024

Acute Fish Survival Test

Nautilus Environmental (CA)

Batch ID: 11-8467-6680	Test Type: Survival (96h)	Analyst:
Start Date: 13 Apr-23 16:00	Protocol: OECD 203	Diluent: Laboratory Freshwater
Ending Date: 17 Apr-23 16:00	Species: Oncorhynchus mykiss	Brine: Not Applicable
Test Length: 96h	Taxon:	Source: Thomas Fish Co. Age: 46d

Sample ID: 20-8574-7031	Code: 7C51F957	Project: IPPD
Sample Date: 13 Apr-23	Material: Chemical Product	Source: Washington Department of Ecology
Receipt Date: 13 Apr-23	CAS (PC): 101-72-4	Station: IPPD
Sample Age: 16h	Client: Washington Department of Ecology	

post-match

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
16-7607-3162	96h Survival Rate	Fisher Exact Test	500	1000	707.1	---	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
10-0781-1029	96h Survival Rate	Linear Interpolation (ICPIN)	EC25	625	625	625	1
			EC50	750	750	750	

96h Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
0	LC	2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
125		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
250		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
500		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
1000		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
2000		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

96h Survival Rate Detail

MD5: 806D11DB5F6C2AFBBEEF9B96A07C7E76

Conc-µg/L	Code	Rep 1	Rep 2
0	S	1.000	1.000
0	LC	1.000	1.000
125		1.000	1.000
250		1.000	1.000
500		1.000	1.000
1000		0.000	0.000
2000		0.000	0.000

S = solvent control

CETIS Analytical Report

Report Date: 25 Apr-23 14:04 (p 1 of 1)
 Test Code/ID: 2304-S124 / 14-4803-4024

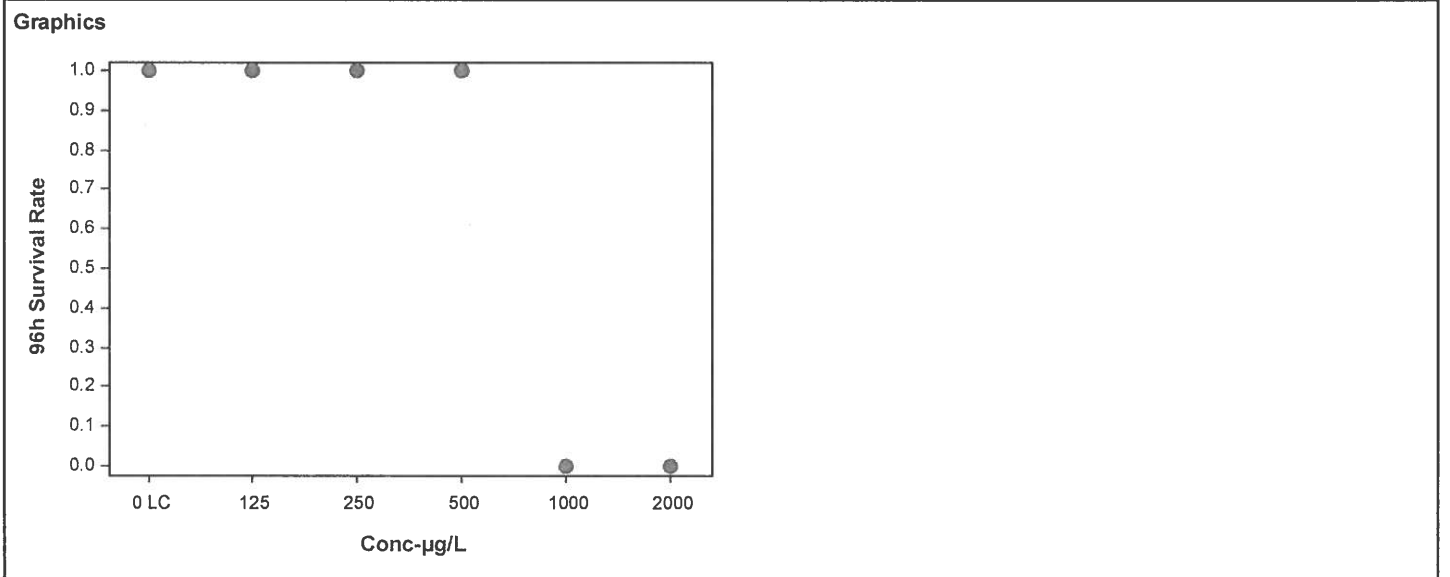
Acute Fish Survival Test			Nautilus Environmental (CA)		
Analysis ID: 16-7607-3162	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.2			
Analyzed: 25 Apr-23 14:04	Analysis: Single 2x2 Contingency Table	Status Level: 1			
Edit Date: 25 Apr-23 14:03	MD5 Hash: EB032BE06328145FED55DED7E39D7E6A	Editor ID: 007-803-386-7			

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units
Untransformed	C > T	500	1000	707.1	---

Fisher Exact Test						
Control	vs	Conc-µg/L	Test Stat	P-Type	P-Value	Decision(α:5%)
Lab Control		125	1.000	Exact	1.0000	Non-Significant Effect
		250	1.000	Exact	1.0000	Non-Significant Effect
		500	1.000	Exact	1.0000	Non-Significant Effect

96h Survival Rate Frequencies							
Conc-µg/L	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
0	LC	10	0	10	1.000	0.000	0.00%
125		10	0	10	1.000	0.000	0.00%
250		10	0	10	1.000	0.000	0.00%
500		10	0	10	1.000	0.000	0.00%
1000		0	10	10	0.000	1.000	100.00%
2000		0	10	10	0.000	1.000	100.00%

96h Survival Rate Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
125		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
250		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
500		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
1000		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
2000		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%



CETIS Analytical Report

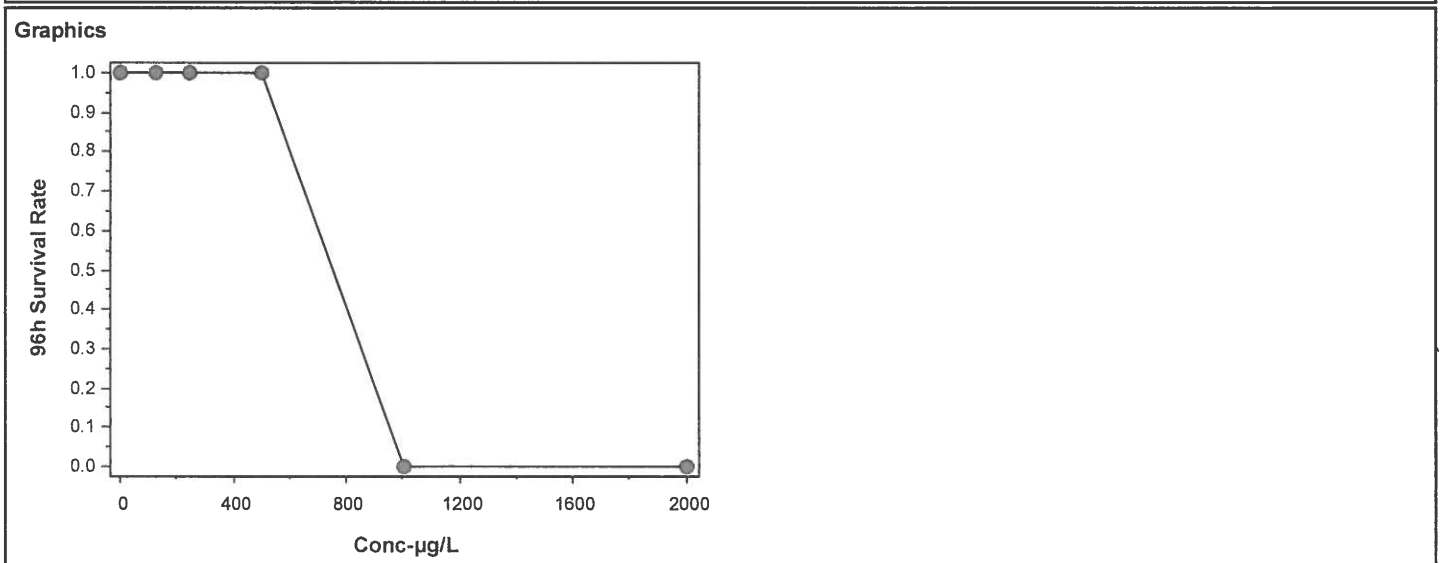
Report Date: 25 Apr-23 14:04 (p 1 of 1)
 Test Code/ID: 2304-S124 / 14-4803-4024

Acute Fish Survival Test			Nautilus Environmental (CA)		
Analysis ID: 10-0781-1029	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.2			
Analyzed: 25 Apr-23 14:04	Analysis: Linear Interpolation (ICPIN)	Status Level: 1			
Edit Date: 25 Apr-23 14:03	MD5 Hash: EB032BE06328145FED55DED7E39D7E6A	Editor ID: 007-803-386-7			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1429582	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
EC25	625	625	625
EC50	750	750	750

96h Survival Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
125		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
250		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
500		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
1000		2	0.000	0.000	0.000	0.000	---	100.00%	0/10	0.000	100.00%
2000		2	0.000	0.000	0.000	0.000	---	100.00%	0/10	0.000	100.00%



Freshwater Acute Bioassay
Static Conditions
DF-018

OECD 203

Water Quality Measurements
& Test Organism Survival

Client: WADOE

Test Species: O. mykiss

Sample ID: IPPD

Start Date/Time: 4/13/23 1600

Test No.: 2304-5124

End Date/Time: 4/17/23 1600

Concentration (ug/L)	Rep	Number of Live Organisms						Conductivity (umhos/cm)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)					Percent Survival
		0	1	24	48	72	96	0	24	48	72	96	0	24	48	72	96	Q14	Q14	Q14	Q14	Q14	0	24	48	72	96	
Lab Control	A	5	5	5	5	5	5	320	305	302	335	328	11.6	11.1	11.5	11.5	11.5	11.4	9.4	8.1	8.0	8.6	8.05	7.62	7.43	7.45	7.40	100
	B	5	5	5	5	5	5	320	305	302	335	328	11.4	11.0	11.4	11.4	11.3	11.4	9.7	8.9	8.7	8.9	8.06	7.62	7.49	7.47	7.45	
1,000 mg/L	A	5	5	5	5	5	5	308	293	291	323	317	11.7	11.1	11.5	11.5	11.5	11.3	9.5	9.0	9.0	9.0	8.05	7.60	7.51	7.52	7.47	100
Acetone Control	B	5	5	5	5	5	5	308	293	291	323	316	11.7	11.0	11.4	11.4	11.4	11.3	9.6	8.7	9.0	8.9	8.03	7.60	7.52	7.53	7.48	100
125	A	5	5	5	5	5	5	309	294	292	324	314	11.8	11.1	11.5	11.5	11.5	11.2	9.5	8.3	8.5	8.4	8.03	7.60	7.50	7.49	7.46	100
	B	5	5	5	5	5	5	309	294	292	325	319	11.7	11.1	11.5	11.4	11.4	11.2	9.6	8.9	8.8	8.6	8.04	7.60	7.51	7.52	7.48	
250	A	5	5	5	5	5	5	309	297	294	327	320	11.7	11.2	11.6	11.5	11.5	11.3	9.2	8.3	8.2	8.3	8.04	7.59	7.49	7.48	7.46	100
	B	5	5	5	5	5	5	309	294	292	325	318	11.7	11.1	11.5	11.5	11.5	11.3	9.6	8.6	8.4	8.8	8.04	7.59	7.49	7.50	7.49	
500	A	5	5	5	5	5	5	309	300	293	325	319	11.8	11.2	11.6	11.6	11.6	11.3	9.5	8.6	8.4	7.9	8.03	7.59	7.52	7.48	7.44	100
	B	5	5	5	5	5	5	309	295	293	325	319	11.7	11.1	11.5	11.5	11.5	11.3	9.7	8.7	8.1	7.7	8.02	7.61	7.53	7.47	7.43	
1,000	A	5	5	0				309	295				11.8	11.2				11.3	9.7				8.04	7.63				0
	B	5	5	0				309	295				11.7	11.1				11.2	9.8				8.02	7.63				
2,000	A	5	5	0				309	295				11.8	11.2				11.3	9.8				8.02	7.66				0
	B	5	5	0				308	295				11.8	11.2				11.3	9.7				8.03	7.65				

Technician Initials: KR WF RL WF KR WF KR KR

Environmental Chamber: F

Weights (mg): 0.316 0.292 0.275 0.311 0.316 0.254 0.340 0.234 0.282 0.306

$\mu = \frac{0.304}{6} = 0.296$

Lengths (cm): 31.5 31.0 30.5 33.0 31.5 30.5 31.5 29.0 30.0 31.5
3.2 3.1 3.1 3.3 3.2 3.1 3.2 2.9 3.0 3.2

$\mu = \frac{31.0}{10} = 3.1$ cm

Loading rate = $\frac{0.51}{6} = 0.49$ g/L

Sample Description: Purple Liquid Animal Source/Date Received: Thomas Fish Co. 4/14/23 Age at Initiation: 46 days post hatch

Comments: Ⓐ Q18 WF 4/13/23 Ⓑ Q18 WF 4/14/23 Ⓒ Q18 RL 4/25/23

QC Check: RL 4/25/23

Final Review: ACS 4/26/23

CETIS Summary Report

Report Date: 25 Apr-23 14:06 (p 1 of 1)
 Test Code/ID: 2304-S125 / 05-9352-1959

Acute Fish Survival Test

Nautilus Environmental (CA)

Batch ID: 03-0476-3407	Test Type: Survival (96h)	Analyst:
Start Date: 13 Apr-23 15:55	Protocol: OECD 203	Diluent: Laboratory Freshwater
Ending Date: 17 Apr-23 15:55	Species: Oncorhynchus mykiss	Brine: Not Applicable
Test Length: 96h	Taxon:	Source: Thomas Fish Co. Age: 46d
Sample ID: 00-6153-2429	Code: 3AAE90D	Project: TMQ
Sample Date: 13 Apr-23	Material: Chemical Product	Source: Washington Department of Ecology
Receipt Date: 13 Apr-23	CAS (PC): 26780-96-1	Station: TMQ
Sample Age: 16h	Client: <i>Washington Department of Ecology</i>	

post-natch

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
00-6861-0642	96h Survival Rate	Dunnett Multiple Comparison Test	2000	>2000	---	47.9%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
03-2312-3728	96h Survival Rate	Linear Interpolation (ICPIN)	EC25	1830	167	---	1
			EC50	>2000	---	---	

96h Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
0	LC	2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
125		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
250		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
500		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
1000		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
2000		2	0.700	-3.110	4.510	0.400	1.000	0.300	0.424	60.61%	30.00%

96h Survival Rate Detail

MD5: 685637667C5CD16C7CE40BEB1E16F2FC

Conc-µg/L	Code	Rep 1	Rep 2
0	S	1.000	1.000
0	LC	1.000	1.000
125		1.000	1.000
250		1.000	1.000
500		1.000	1.000
1000		1.000	1.000
2000		1.000	0.400

S = solvent control

CETIS Analytical Report

Report Date: 25 Apr-23 14:06 (p 1 of 2)
 Test Code/ID: 2304-S125 / 05-9352-1959

Acute Fish Survival Test			Nautilus Environmental (CA)		
Analysis ID: 00-6861-0642	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.2			
Analyzed: 20 Apr-23 14:03	Analysis: Parametric-Control vs Treatments	Status Level: 1			
Edit Date: 20 Apr-23 14:01	MD5 Hash: D20AD7B0EAD5DCE4585D2BAFEA6575C	Editor ID: 007-803-386-7			

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	2000	>2000	---	---	0.479	47.92%

Dunnett Multiple Comparison Test									
Control	vs	Conc-µg/L	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Lab Control		125	2	0	2.83	0.539	CDF	0.8333	Non-Significant Effect
		250	2	0	2.83	0.539	CDF	0.8333	Non-Significant Effect
		500	2	0	2.83	0.539	CDF	0.8333	Non-Significant Effect
		1000	2	0	2.83	0.539	CDF	0.8333	Non-Significant Effect
		2000	2	1.73	2.83	0.539	CDF	0.1959	Non-Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.18181	0.036362	5	1	0.4894	Non-Significant Effect
Error	0.218172	0.036362	6			
Total	0.399982		11			

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	0.599	0.802	0.0001	Non-Normal Distribution	

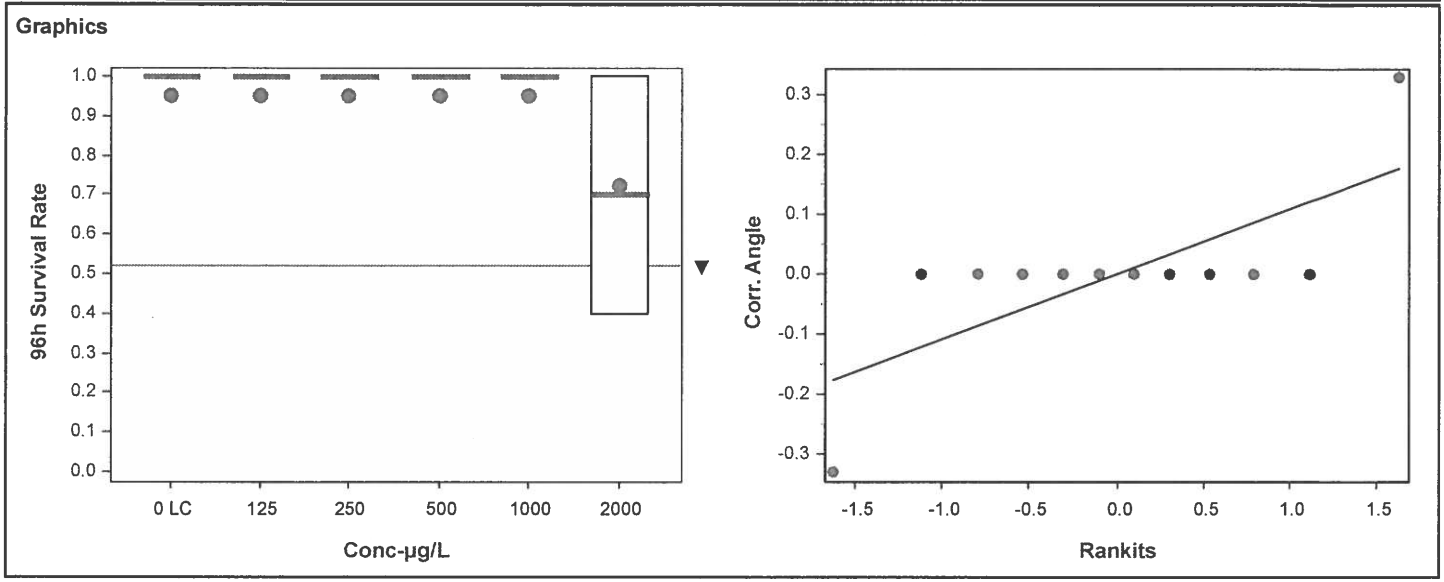
96h Survival Rate Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
125		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
250		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
500		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
1000		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
2000		2	0.700	0.000	1.000	0.700	0.400	1.000	0.300	60.61%	30.00%

Angular (Corrected) Transformed Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	2	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
125		2	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
250		2	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
500		2	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
1000		2	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
2000		2	1.020	-3.180	5.210	1.020	0.685	1.350	0.330	46.02%	24.55%

CETIS Analytical Report

Report Date: 25 Apr-23 14:06 (p 2 of 2)
 Test Code/ID: 2304-S125 / 05-9352-1959

Acute Fish Survival Test		Nautilus Environmental (CA)	
Analysis ID: 00-6861-0642	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.2	
Analyzed: 20 Apr-23 14:03	Analysis: Parametric-Control vs Treatments	Status Level: 1	
Edit Date: 20 Apr-23 14:01	MD5 Hash: D20AD7B0EAD5DCE4585D2BAFEA6575C	Editor ID: 007-803-386-7	



CETIS Analytical Report

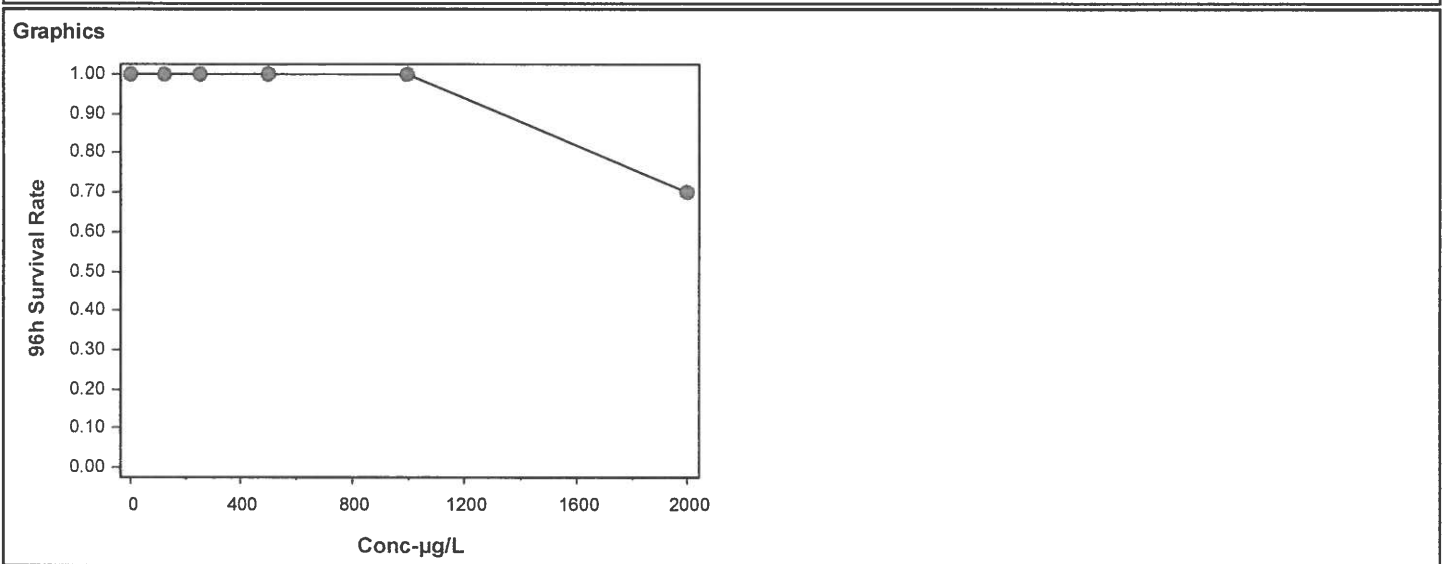
Report Date: 25 Apr-23 14:06 (p 1 of 1)
 Test Code/ID: 2304-S125 / 05-9352-1959

Acute Fish Survival Test			Nautilus Environmental (CA)		
Analysis ID: 03-2312-3728	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.2			
Analyzed: 20 Apr-23 14:03	Analysis: Linear Interpolation (ICPIN)	Status Level: 1			
Edit Date: 20 Apr-23 14:01	MD5 Hash: D20AD7B0EAD5DCE4585D2BAFEA6575C	Editor ID: 007-803-386-7			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1774031	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
EC25	1830	167	---
EC50	>2000	---	---

96h Survival Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
125		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
250		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
500		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
1000		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
2000		2	0.700	0.700	0.400	1.000	60.61%	30.00%	7/10	0.700	30.00%



Freshwater Acute Bioassay
Static Conditions

DF-018

OECD 203

Water Quality Measurements
& Test Organism Survival

Client: WADOE

Sample ID: TMQ

Test No.: 2304-5125

Test Species: O. mykiss

Start Date/Time: 4/13/23 1555

End Date/Time: 4/17/23 1555

Concentration (ug/L)	Rep	Number of Live Organisms						Conductivity (umhos/cm)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)					Percent Survival
		0	1	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	5	320	305	303	336	329	11.4	11.1	11.5	11.4	11.6	11.4	9.8	9.0	8.9	8.8	7.62	7.62	7.47	7.51	7.42	100
	B	5	5	5	5	5	5	321	305	303	337	330	11.3	11.0	11.5	11.4	11.4	11.4	9.8	9.0	8.9	8.7	8.03	7.62	7.51	7.52	7.47	
1,000 mg/L	A	5	5	5	5	5	5	320	305	303	337	330	11.5	11.1	11.6	11.4	11.5	11.4	9.8	9.3	9.4	9.4	8.01	7.62	7.54	7.54	7.51	100
Acetone Control	B	5	5	5	5	5	5	320	305	302	335	329	11.4	11.1	11.5	11.5	11.5	11.4	10.1	9.4	9.5	9.6	7.99	7.64	7.57	7.56	7.55	
125	A	5	5	5	5	5	5	321	306	302	335	330	11.4	11.1	11.6	11.5	11.4	11.4	9.8	8.9	8.9	9.0	7.99	7.65	7.50	7.53	7.52	100
	B	5	5	5	5	5	5	321	305	303	334	330	11.4	11.1	11.6	11.5	11.3	11.3	9.8	9.2	9.2	9.2	7.98	7.63	7.54	7.55	7.54	
250	A	5	5	5	5	5	5	322	306	304	337	331	11.4	10.9	11.6	11.4	11.4	11.4	9.5	8.5	8.5	8.9	7.97	7.61	7.51	7.51	7.52	100
	B	5	5	5	5	5	5	321	306	303	334	330	11.4	11.1	11.6	11.4	11.3	11.4	9.9	9.2	9.2	9.0	7.98	7.62	7.56	7.55	7.53	
500	A	5	5	5	5	5	5	321	306	303	336	330	11.4	11.2	11.6	11.4	11.4	11.4	9.7	8.8	8.8	8.7	7.96	7.62	7.54	7.54	7.52	100
	B	5	5	5	5	5	5	321	306	304	336	331	11.3	11.1	11.6	11.4	11.3	11.4	9.7	8.5	8.3	8.6	7.94	7.61	7.52	7.46	7.51	
1,000	A	5	5	5	5	5	5	321	306	304	336	332	11.3	11.1	11.6	11.4	11.4	11.4	9.8	8.5	8.5	8.3	7.95	7.61	7.52	7.50	7.50	100
	B	5	5	5	5	5	5	321	306	304	337	331	11.3	11.1	11.6	11.4	11.3	11.4	9.5	8.5	8.3	7.9	7.94	7.59	7.52	7.46	7.46	
2,000	A	5	5	5	5	5	5	309	295	293	324	320	11.7	11.1	11.7	11.7	11.5	11.39	8.98	7.9	6.6	6.7	8.02	7.60	7.49	7.41	7.38	70
	B	5	5	5	5	2	2	309	294	293	325	319	11.6	11.1	11.6	11.4	11.4	11.3	9.4	8.1	6.9	7.4	8.04	7.59	7.50	7.30	7.39	
Technician Initials		WF WF WF KR																										

Environmental Chamber: F

Weights (mg): \textcircled{B} 0.346 0.292 0.275 0.391 0.316 0.259 0.340 0.234 0.282 0.306
 Lengths (cm): \textcircled{B} 3.15 3.10 3.05 3.30 3.15 3.05 3.15 2.90 3.00 3.15
 3.1 3.1 3.1 3.3 3.2 3.1 3.2 2.9 3.0 3.2

\textcircled{A} 0.304 g
 $\mu = 0.296$
 \textcircled{B}
 $\mu = 3.10 \pm 0.1 \text{ cm}$
 Loading rate = \textcircled{A} 0.51
 \textcircled{B} 0.44 g/L

Sample Description: Yellow Liquid Animal Source/Date Received: Thomas Fish Co. 4/14/23 Age at Initiation: 46 days post-hatch

Comments: \textcircled{A} Q18 WF 4/13/23 \textcircled{B} Q18 RL 4/25/23

QC Check: RL 4/25/23

Final Review: ACS 4/26/23

Appendix B

Data Qualifier Codes

Glossary of Qualifier Codes

- Q1 - Temperature out of recommended range; corrective action taken and recorded in Test Temperature Correction Log
- Q2 - Temperature out of recommended range; no action taken, test terminated same day
- Q3 - Sample pH adjusted to within range of 6-9 with reagent grade NaOH or HCl, as needed
- Q4 - Test aerated; D.O. levels dropped below 4.0 mg/L
- Q5 - Test initiated with continuous 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, partial 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. Test results were reviewed and reported in accordance with guidance found in EPA-833-R-00-003, 2000 unless otherwise specified.
- 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. Test results were reviewed and reported in accordance with EPA-833-R-00-003, 2000 guidance unless otherwise specified.
- Q18 - Incorrect or illegible Entry
- Q19 - Miscalculation
- Q20 - PMSD criteria do not apply to the test of significant toxicity (TST) analysis
- Q21 - Other (provide reason in comments section)
- Q22 - Greater than 10% batch mortality observed upon receipt and/or in holding prior to test initiation. Organisms acclimated to test conditions at Enthalpy and ultimately deemed fit to use for testing.
- Q23 - Test organisms experienced a temperature shift greater than 3°C within 1 day or were received at a temperature greater than 3°C outside the recommended test temperature range and had minimal time to acclimate prior to test initiation. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate test(s). Organisms were ultimately deemed fit to use for testing.
- Q24 - Test organisms experienced a salinity shift greater than 3 ppt within 1 day or were received at a salinity greater than 3 ppt outside the recommended test salinity range and had minimal time to acclimate prior to test initiation. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate test(s). Organisms were ultimately deemed fit to use for testing.

Appendix C

Reference Toxicant Test Data

CETIS Summary Report

Report Date: 26 Apr-23 11:47 (p 1 of 1)
 Test Code/ID: 230413omra / 04-5815-1771

Acute Fish Survival Test

Nautilus Environmental (CA)

Batch ID: 12-0462-1472	Test Type: Survival (96h)	Analyst:
Start Date: 13 Apr-23 15:15	Protocol: Washington DOE (2009)	Diluent: Laboratory Freshwater
Ending Date: 17 Apr-23 15:15	Species: Oncorhynchus mykiss	Brine: Not Applicable
Test Length: 96h	Taxon:	Source: Thomas Fish Co. Age: 32d
Sample ID: 16-1695-6281	Code: 230413omra	Project: Internal
Sample Date: 13 Apr-23	Material: Copper chloride	Source: Copper Chloride
Receipt Date: 13 Apr-23	CAS (PC):	Station:
Sample Age: 15h	Client:	

post
summary

Multiple Comparison Summary							
Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
16-6843-3165	96h Survival Rate	Fisher Exact Test	50	100	70.71	---	1

Point Estimate Summary							
Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
04-7188-7445	96h Survival Rate	Spearman-Kärber	EC50	107	91.1	126	1

96h Survival Rate Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
25		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
50		2	0.950	0.315	1.590	0.900	1.000	0.050	0.071	7.44%	5.00%
100		2	0.650	0.015	1.290	0.600	0.700	0.050	0.071	10.88%	35.00%
200		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
400		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

96h Survival Rate Detail				MD5: 1D770E2714660A0D9BBA0485AD912D6F							
Conc-µg/L	Code	Rep 1	Rep 2								
0	LC	1.000	1.000								
25		1.000	1.000								
50		0.900	1.000								
100		0.700	0.600								
200		0.000	0.000								
400		0.000	0.000								

CETIS Analytical Report

Report Date: 26 Apr-23 11:47 (p 1 of 1)
 Test Code/ID: 230413omra / 04-5815-1771

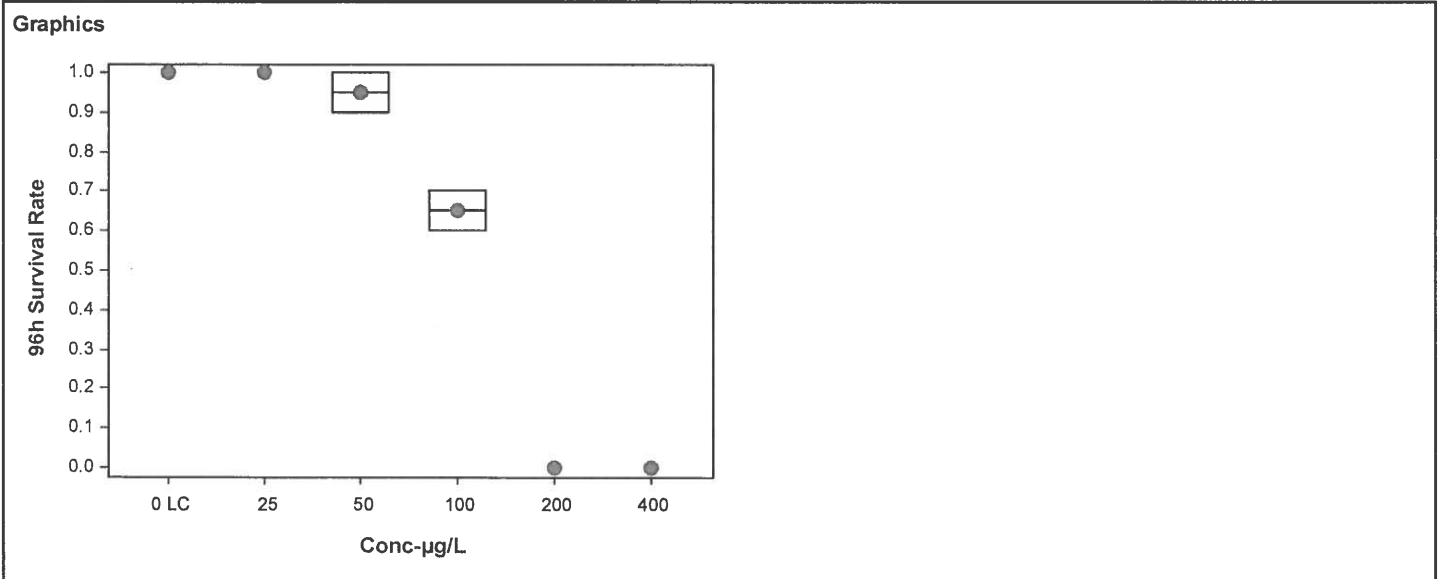
Acute Fish Survival Test			Nautilus Environmental (CA)		
Analysis ID: 16-6843-3165	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.2			
Analyzed: 26 Apr-23 11:46	Analysis: Single 2x2 Contingency Table	Status Level: 1			
Edit Date: 26 Apr-23 11:45	MD5 Hash: 1D770E2714660A0D9BBA0485AD912D6F	Editor ID: 007-803-386-7			

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units
Untransformed	C > T	50	100	70.71	---

Fisher Exact Test						
Control	vs	Conc-µg/L	Test Stat	P-Type	P-Value	Decision(α:5%)
Lab Control		25	1.000	Exact	1.0000	Non-Significant Effect
		50	0.500	Exact	0.5000	Non-Significant Effect
		100*	0.004	Exact	0.0042	Significant Effect

96h Survival Rate Frequencies							
Conc-µg/L	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
0	LC	20	0	20	1.000	0.000	0.00%
25		20	0	20	1.000	0.000	0.00%
50		19	1	20	0.950	0.050	5.00%
100		13	7	20	0.650	0.350	35.00%
200		0	20	20	0.000	1.000	100.00%
400		0	20	20	0.000	1.000	100.00%

96h Survival Rate Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
25		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
50		2	0.950	0.315	1.000	0.950	0.900	1.000	0.050	7.44%	5.00%
100		2	0.650	0.015	1.000	0.650	0.600	0.700	0.050	10.88%	35.00%
200		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
400		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%



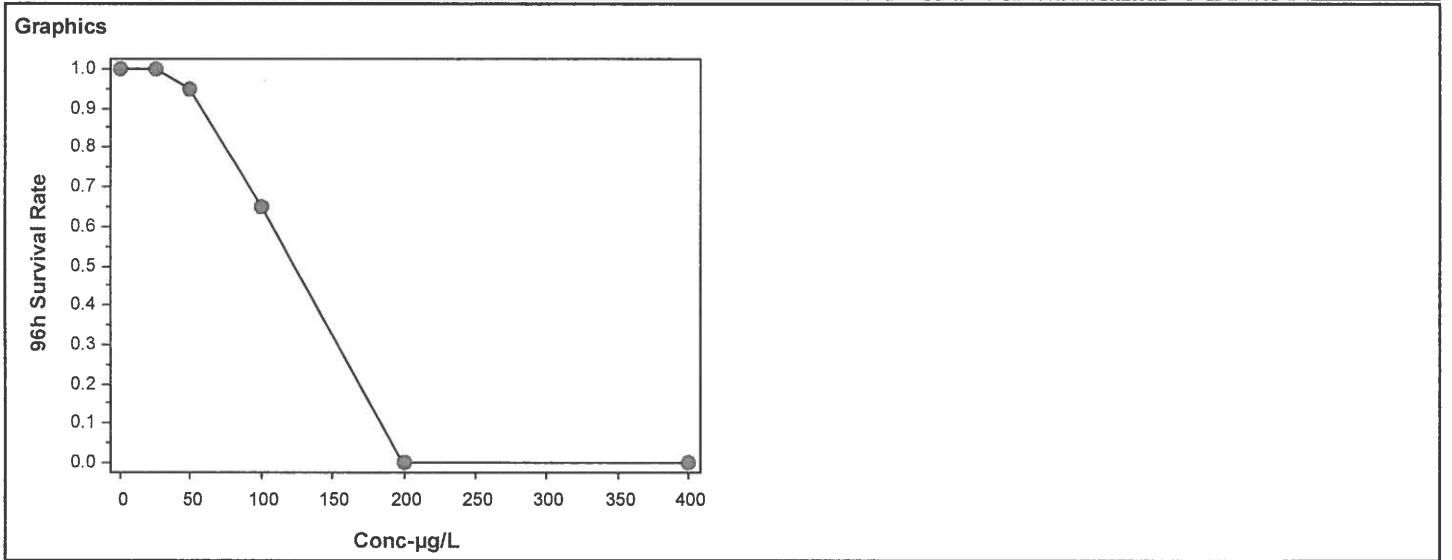
CETIS Analytical Report

Report Date: 26 Apr-23 11:47 (p 1 of 1)
 Test Code/ID: 230413omra / 04-5815-1771

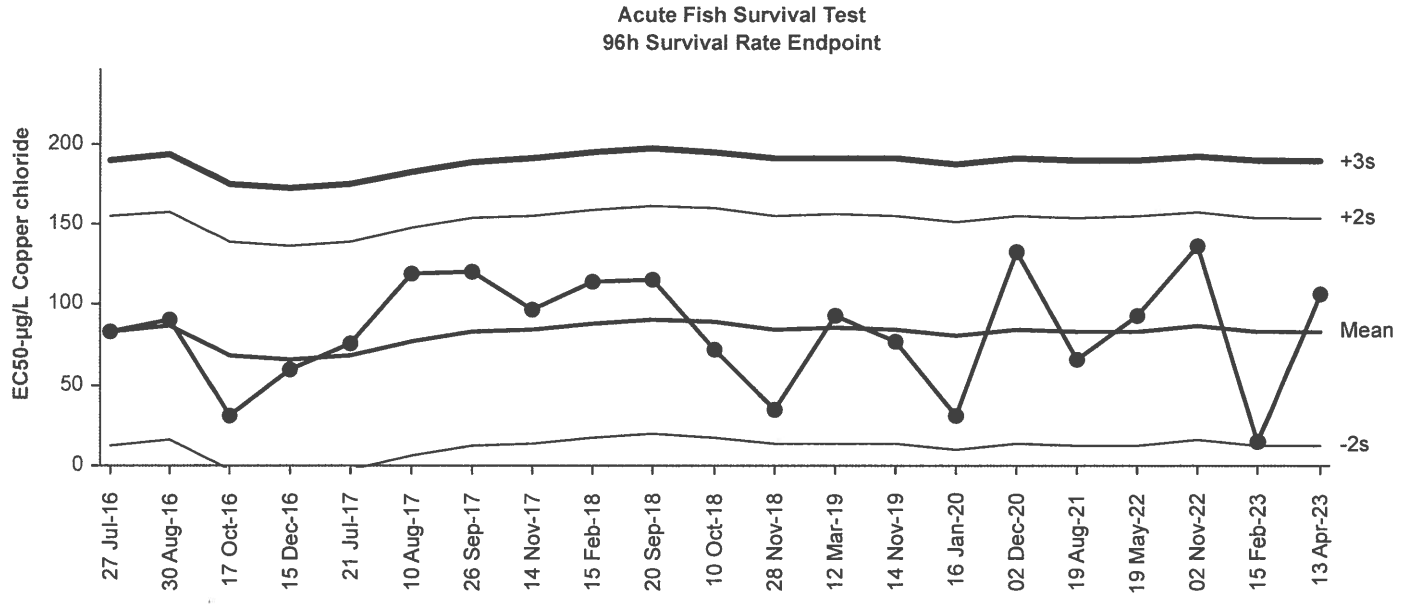
Acute Fish Survival Test			Nautilus Environmental (CA)		
Analysis ID: 04-7188-7445	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.2			
Analyzed: 26 Apr-23 11:46	Analysis: Untrimmed Spearman-Kärber	Status Level: 1			
Edit Date: 26 Apr-23 11:45	MD5 Hash: 1D770E2714660A0D9BBA0485AD912D6F	Editor ID: 007-803-386-7			

Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	2.03	0.0353	107	91.1	126

96h Survival Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	2	1.000	1.000	1.000	1.000	0.00%	0.00%	20/20	1.000	0.00%
25		2	1.000	1.000	1.000	1.000	0.00%	0.00%	20/20	1.000	0.00%
50		2	0.950	0.950	0.900	1.000	7.44%	5.00%	19/20	0.950	5.00%
100		2	0.650	0.650	0.600	0.700	10.88%	35.00%	13/20	0.650	35.00%
200		2	0.000	0.000	0.000	0.000	---	100.00%	0/20	0.000	100.00%
400		2	0.000	0.000	0.000	0.000	---	100.00%	0/20	0.000	100.00%



Acute Fish Survival Test		Nautilus Environmental (CA)	
Test Type: Survival (96h)	Organism: Oncorhynchus mykiss	Material: Copper chloride	
Protocol: Washington DOE (2009)	Endpoint: 96h Survival Rate	Source: Copper Chloride-CU	



Cumulative Mean Plot

Mean: 82.91	Count: 20	-2s Warning Limit: 12	-3s Action Limit: -23.5
Sigma: 35.46	CV: 42.80%	+2s Warning Limit: 154	+3s Action Limit: 189

Quality Control Data											
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2016	Jul	27	14:00	83.55	0.6417	0.0181			03-8490-8254	08-7411-8094
2		Aug	30	13:05	90.13	7.215	0.2035			06-6522-4245	11-7821-6703
3		Oct	17	15:45	31.5	-51.41	-1.45			11-9706-2514	03-3027-4661
4		Dec	15	13:00	59.46	-23.45	-0.6613			07-2059-1930	21-0698-8947
5	2017	Jul	21	11:45	75.79	-7.124	-0.2009			12-6230-4373	10-5665-4943
6		Aug	10	13:35	119.1	36.15	1.02			09-7390-4688	11-1885-9400
7		Sep	26	15:10	120.1	37.16	1.048			12-0767-7259	14-1478-0761
8		Nov	14	11:25	96.22	13.31	0.3754			21-0521-5529	14-2305-2435
9	2018	Feb	15	15:00	114.5	31.63	0.892			08-5122-1964	18-9847-1069
10		Sep	20	14:05	114.9	31.96	0.9013			14-1527-8451	21-3828-6142
11		Oct	10	16:40	72.55	-10.36	-0.2922			06-8408-1163	05-7761-5868
12		Nov	28	12:00	35.36	-47.55	-1.341			21-0374-7072	19-9377-5872
13	2019	Mar	12	12:10	93.3	10.39	0.2931			11-1972-1376	05-1051-7815
14		Nov	14	11:55	77.34	-5.574	-0.1572			08-3948-6775	01-9304-4998
15	2020	Jan	16	12:50	30.63	-52.28	-1.474			15-5355-8442	09-8383-1081
16		Dec	2	13:30	133.3	50.42	1.422			07-0223-4669	10-8492-8883
17	2021	Aug	19	14:25	65.98	-16.93	-0.4776			11-4973-5943	08-1400-5422
18	2022	May	19	12:45	93.3	10.39	0.2931			03-2996-8953	07-9626-2312
19		Nov	2	10:05	136.6	53.69	1.514			14-6511-1746	10-0042-0505
20	2023	Feb	15	11:30	14.71	-68.2	-1.923			17-0773-8757	02-8314-6307
21		Apr	13	15:15	107.2	24.27	0.6844			04-5815-1771	04-7188-7445

Freshwater Acute Bioassay
Static Conditions

DF-019

Dangerous Waste Characterization

Water Quality Measurements
& Test Organism Survival

Client: Internal

Sample ID: CuCl₂

Test No.: 230413 onra

Test Species: *O. mykiss*

Start Date/Time: 4/13/23 1515

End Date/Time: 4/17/23 1515

Concentration (µg/L)	RAND #	Number of Live Organisms					Conductivity (umhos/cm)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)					Percent Survival
		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	10	10	10	10	10	10	320	302	301	334	328	11.2	11.0	11.4	11.0	11.0	11.5	10.6	9.7	9.9	9.9	8.04	7.65	7.52	7.49	7.46	100
	8	10	10	10	10	10	321	305	304	336	330	11.2	11.0	11.3	11.1	11.1	11.4	10.3	9.6	9.8	9.8	8.04	7.67	7.53	7.45	7.47	100
25	9	10	10	10	10	10	322	306	304	337	331	11.2	11.0	11.4	11.0	11.0	11.4	10.4	9.6	9.9	9.9	8.06	7.67	7.54	7.48	7.48	100
	5	10	10	10	10	10	322	306	304	336	330	11.3	11.1	11.4	11.2	11.3	11.4	10.4	9.4	9.6	9.6	8.06	7.67	7.52	7.47	7.45	100
50	7	10	10	9	9	9	322	306	305	336	330	11.4	11.1	11.4	11.1	11.1	11.5	10.2	9.2	9.8	9.8	8.06	7.67	7.50	7.44	7.43	90
	4	10	10	10	10	10	322	308	307	336	331	11.6	12.1	12.4	12.0	12.1	11.3	9.8	9.0	9.3	9.3	8.02	7.66	7.50	7.49	7.44	100
100	12	10	10	7	7	7	322	306	304	336	331	11.0	11.0	11.2	11.0	11.0	11.5	10.5	9.5	10.1	10.4	8.04	7.68	7.50	7.53	7.53	70
	3	10	8	6	6	6	322	307	309	340	332	11.6	12.1	12.4	12.1	12.1	11.3	9.9	9.5	9.9	10.0	8.02	7.66	7.51	7.51	7.50	60
200	1	10	5	0	/	/	322	308	305	/	/	11.7	11.9	12.3	/	/	11.3	10.0	10.0	/	/	8.01	7.64	7.55	/	/	0
	11	10	3	0	/	/	322	307	304	/	/	11.1	11.0	11.3	/	/	11.5	10.6	10.6	/	/	8.02	7.64	7.61	/	/	0
400	2	10	2	0	/	/	321	308	305	/	/	11.7	12.0	12.4	/	/	11.4	10.2	10.3	/	/	7.93	7.62	7.62	/	/	0
	6	10	0	0	/	/	321	306	-	/	/	11.3	11.1	-	/	/	11.4	10.9	-	/	/	7.91	7.63	-	/	/	0
Tech Initials:	Counts	WF	WF	WF	KR	KR											Recorded in Log Pass/Fail:										
	Readings	DR	WF	WF	KR	DR																					
	QC	WF/KR	DR																								

Dilution Calcs (final volume 8L) made by: RT

Conc. µg/L	25	50	100	200	400
Vol. Cu stock added (mL)	2.0	4.1	8.2	16.3	32.7
Cu Stock Conc. (µg/L)	18000	48000	98000	98000	98000

Environmental Chamber: F

Weights (g): 0.346 0.292 0.275 0.341 0.316 0.259 0.340 0.234 0.282 0.306

Lengths (mm): 31.5 31.0 30.5 33.0 31.5 30.5 31.5 29.0 30.0 31.5

Loading: 0.44 g/L 0.38 g/L

$\mu = 0.3049$
 $\mu = 0.296$
 $\mu = 31.0$ cm

Animal Source/Date Received: Thomas Fish Co/ 4/4/23

Hatch Date: 2/26/23
Swim-up Date: 3/12/23
Days post Swim-up: 32

Length max/min = 29.0/33.0
Ratio of longest to shortest = 1.138

Comments: ^b10 random fish are sacrificed at initiation for size determination. The standard length of the longest fish should be no more than 2X the shortest fish.
Q15 DR 04/13/23 Q18 RA 4/13/23

QC Check: RL 4/26/23

Final Review: ACS 4/26/23