ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

Washington Department of Ecology

Tire Chemicals OECD Toxicity Testing of Ozonated 6PPD and Related Alternatives (7PPD and 77PD)

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: _____
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INTRODUCTION

Washington Department of Ecology engaged Enthalpy Analytical (Enthalpy) to generate acute toxicity data on the sensitivity of rainbow trout (*Oncorhynchus mykiss)* to 6PPD and potential alternative replacement compounds. 6PPD is an additive used in the process of tire manufacturing to protect the rubber polymers from ozone. When exposed to ozone, 6PPD creates the transformation product 6PPD-quinone, which has a published lethal effect to juvenile coho salmon (*Oncorhynchus kisutch*) at concentrations below 0.1 µg/L (Tian 2022). 6PPD-quinone has been found to be present in roadway runoff and stormwater-impacted watersheds throughout the Puget Sound basin (WDOE 2022).

In 2023, Enthalpy generated acute toxicity data on the sensitivity of rainbow trout to 6PPD, 6PPD-quinone, and four alternative parent (i.e. non-ozonated) compounds. This report contains the results of a follow-on study, where the objective is to compare the relative toxicity to rainbow trout of ozonated 6PPD and two ozonated alternatives (7PPD and 77PD). While the initial study in 2023 sought to understand the toxicity of the parent compounds and reagent grade 6PPD-quinone, this study measured the relative toxicity of the ozonated derivatives of the alternative compounds relative to ozonated 6PPD.

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. Testing was conducted between February 21 and 25, 2024.

MATERIALS AND METHODS

The test materials were not produced at Enthalpy, but were generated by Flexsys, using the following procedure.

To determine how much ozone to expose the compounds to: 0.5 grams (g) of solid 6PPD was dissolved in 200 mililiters (mL) of chloroform. Ozone (approx. 7 mg/L at a 4 L/min flow rate) was then bubbled through the solution for 120 minutes and 1 mL aliquots were taken in tared vials at the following times: 0, 20, 40, 60, 80, 100, and 120 minutes. Each aliquot was evaporated for 20 minutes under a stream of nitrogen. Each residue aliquot was dissolved in 10 mL acetonitrile, and evaluated by High Performance Liquid Chromatography (HPLC). HPLC-UV (ultra violet) peak retention times for 6PPD and 6PPD-quinone were each identified using compound standards. 40 minutes of ozonation was identified as the condition to convert the greatest percentage of 6PPD into 6PPD-quinone; therefore, it was selected as the exposure time for creating the ozonated compounds used for toxicity testing.

On November 9, 2023, the compounds were created by dissolving 0.5g of each of the three test products (6PPD, 7PPD, and 77PD) in 200 mL of chloroform. Ozone (approx. 7 mg/L at 4 L/min flow rate) was bubbled through the solution for 40 minutes. The solution was then evaporated under reduced pressure at 40 °C and shipped in the evaporation flask (100 mL round bottom). Throughout this report, the material shipped in the final evaporation flask is referred to as the “final product”. The portion of that final compound estimated to be in the quinone form is referred to as the “transformation product”.

Upon receipt at the Enthalpy Analytical, the products were stored in cool, dry conditions until used for using.

Compound	Product Description
Ozonated 6PPD	Black tar
Ozonated 7PPD	Black tar
Ozonated 77PD	Black tar

Based on the relatively low solubility of the chemical compounds in water, each compound was weighed and then dissolved in acetone, a solvent vehicle, prior to being introduced to water and exposed to the organisms for toxicity testing. Stock solutes containing the compounds and solvents were produced and test dilutions were subsequently created by

taking an aliquot of the solvent and compound 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.

The concentrations used in this study were based on the results of the testing that was performed in 2023 with a reagent grade pure 6PPD-quinone, bracketing the LC_{50} concentration of 1.8 µg/L 6PPD-quinone from that study. Based on the initial trials performed at Flexsys, an approximately 5 percent conversation rate for 6PPD quinone and the two alternatives was assumed to be present in the final product delivered to Enthalpy. Therefore, the nominal concentrations for the all exposures were 240, 110, 50, 22, 10, and 4 micrograms per liter (µg/L) of product; this translates to estimated nominal quinone concentrations of 12, 5.5, 2.5, 1.1, 0.5, and 0.2 µg/L of 6PPD, 7PPD, and 77PD quinone (i.e. transformation product). It should be noted that conversion trials for the two alternatives (7PPD and 77PD) were not performed by Flexsys; therefore, a conversion rate similar to 6PPD was assumed for the purposes of estimating relative toxicity among the alternatives. Once the flasks of the ozonated products were opened, less than one hour elapsed before the solids were dissolved in acetone at Enthalpy. However, there is the potential for further degradation of the ozonated products between the time the flasks were unsealed and when they were introduced to acetone; it is not possible to estimate the extent of the degradation during the time the final products were exposed to air.

A 467 milligram per liter (mg/L) acetone control (the amount added to the highest test concentration) was also tested. 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. The concurrent laboratory reference toxicant test used for quality assurance followed OECD guidelines. Effects were evaluated statistically using the Comprehensive Environmental Toxicity Information System™ (CETIS, version 2.1.4.11) 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 (LC_{25} or LC_{50}, respectively) were calculated using linear interpolation and Spearman-Karber.

Larval Fish Toxicity Test Specifications

Test Period:	2/21/24, 16:00 to 2/25/24, 16:00
Test Organism:	*Oncorhynchus mykiss* (rainbow trout)
Endpoint(s):	96-hour Acute Survival
Test Organism Source, Size:	Thomas Fish Company (Anderson, CA), 3-6 cm
Test Chamber:	3.5-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:	240, 110, 50, 22, 10, and 4 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

RESULTS

All results are presented as the true exposure concentrations of the final products provided by Flexys; note that the actual quinone concentration (transformation product), provided in parentheses after the test concentration, is an estimation at the assumed 5 percent conversion rate.

A statistically significant effect was detected in the 240 µg/L (12 µg/L) concentration for the ozonated 6PPD test, resulting in a NOEL of 110 µg/L (5.5 µg/L). The LC_{50} was calculated as 128 µg/L (6.4 µg/L), and the LC_{25} was calculated as 100 µg/L (5 ug/L).

No statistically significant effects were detected in any concentration tested for the ozonated 7PPD and ozonated 77PD tests, resulting in a NOEL of 240 µg/L (12 µg/L). The LC_{50} and LC_{25} values for both samples were calculated as greater than 240 µg/L (12 µg/L).

It should be noted that the LC_{50} of 6PPD quinone in this round of testing was higher (i.e. less toxic) than the testing performed in 2023. Reasons for this might include 1) the 2023 6PPD quinone testing was performed with a reagent grade product where this study tested ozonated parent compounds to create the transformation product, 2) the nominal test concentrations were derived with the assumption that 5 percent of the final product was present in the transformation product (i.e. quinone) form; if this conversion rate was actually lower than 5 percent, that would reduce the proportion of quinone in the final product tested, 3) other transformation products could be present in the remaining 95 percent of the final product which made the 6PPD quinone less toxic.

Regardless, since all three parent compounds were ozonated using the same method, these data suggest that the quinone forms of both 7PPD and 77PD are relatively less toxic than 6PPD quinone.

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

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

Table 1. Summary of Toxicity Test Results

Test Concentration (µg/L)	Ozonated 6PPD	Ozonated 7PPD	Ozonated 77PD
	Mean 96-hr Survival (%)	Mean 96-hr Survival (%)	Mean 96-hr Survival (%)
Lab Control	100	100	100
Solvent Control	100	100	100
4	100	100	100
10	100	100	100
22	100	100	100
50	100	100	100
110	70.0	100	100
240	0.0	100	100
NOEL (µg/L)	110 (5.5 ug/L)	240 (12 ug/L)	240 (12 ug/L)
LOEL (µg/L)	240 (12 ug/L)	>240 (12 ug/L)	>240 (12 ug/L)
LC_{50} (µg/L)	128 (6.4 ug/L)	>240 (12 ug/L)	>240 (12 ug/L)
LC_{25} (µg/L)	100 (5 ug/L)	>240 (12 ug/L)	>240 (12 ug/L)

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
Values in parentheses are an estimation at the assumed 5 percent conversion rate from the parent compound to quinone.

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.

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 2 and presented in full in Appendix C. The reference toxicant test met minimum test acceptability criteria, and the EC_{50} 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 2. 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	25	64.5	83.0 ± 71.8	43.2

NOEL = No Observed Effect Level

LC_{50} = the concentration at which 50 percent of the organisms show a lethal effect

Historical LC_{50} ± 2 SD = the mean LC_{50} from the previous tests performed by Enthalpy, plus or minus two standard deviations

CV= Coefficient of Variation

REFERENCES

Washington Department of Ecology 2022. Quality Assurance Project Plan- Tire Chemicals OECD Toxicity Testing of 6PPD and Related Alternatives using the Rainbow Trout, (*Onchorynchus mykiss*)- October 2022

Brinkmann, M., Et. Al. 2022. Acute Toxicity of the Tire Rubber-Derived Chemical 6PPD-quinone to Four Fishes of Commercial, Cultural, and Ecological Importance. Environmental Science & Technology Letters 2022 9 (4), 333-338.

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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.

OECD. 2019a. Test No. 23: GUIDANCE DOCUMENT ON AQUEOUS-PHASE AQUATIC TOXICITY TESTING OF DIFFICULT TEST CHEMICALS, Second Edition.

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 2.1.4.11.

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

Acute Fish Survival Test — **Nautilus Environmental (CA)**

Batch ID:	04-6430-1924	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	21 Feb-24 16:00	**Protocol:**	OECD 203	**Diluent:**	Laboratory Freshwater
Ending Date:	25 Feb-24 16:00	**Species:**	Oncorhynchus mykiss	**Brine:**	Not Applicable
Test Length:	96h	**Taxon:**		**Source:**	Thomas Fish Co. **Age:** 51d

Sample ID:	01-8368-3120	**Code:**	24-5060	**Project:**	6PPD-~~quinone~~ (B)
Sample Date:	~~26-Mar-24 17:07~~ (A)	**Material:**	Chemical Product	**Source:**	Washington Department of Ecology
Receipt Date:	21 Feb-24 09:30	**CAS (PC):**		**Station:**	Ozonated 6PPD
Sample Age:	---	**Client:**	Washington Department of Ecology		

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
11-0229-6633	96h Survival Rate	Dunnett Multiple Comparison Test		110	240	162.5	47.9%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	µg/L	95% LCL	95% UCL	S
09-4437-4080	96h Survival Rate	Linear Interpolation (ICPIN)		EC25	100	---	270	1
19-3363-1923	96h Survival Rate	Spearman-Kärber		EC50	128	102	161	1

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%
4		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
10		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
22		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
50		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
110		2	0.700	-3.110	4.510	0.400	1.000	0.300	0.424	60.61%	30.00%
240		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

96h Survival Rate Detail — MD5: 3D8D44996C88EF654D1CE358A7AEABEB

Conc-µg/L	Code	Rep 1	Rep 2
0	S	1.000	1.000
0	LC	1.000	1.000
4		1.000	1.000
10		1.000	1.000
22		1.000	1.000
50		1.000	1.000
110		0.400	1.000
240		0.000	0.000

(A) QIBV 3/27/24

(B) QIBACS 4/4/24

Acute Fish Survival Test — Nautilus Environmental (CA)

Analysis ID:	11-0229-6633	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Mar-24 17:10	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	26 Mar-24 17:08	**MD5 Hash:**	999C642D3B390E591AFABD38B0EF138E	**Editor ID:**	007-803-386-7

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	110	240	162.5	---	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		4	2	0	2.83	0.539	CDF	0.8333	Non-Significant Effect
		10	2	0	2.83	0.539	CDF	0.8333	Non-Significant Effect
		22	2	0	2.83	0.539	CDF	0.8333	Non-Significant Effect
		50	2	0	2.83	0.539	CDF	0.8333	Non-Significant Effect
		110	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%
4		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
10		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
22		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
50		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
110		2	0.700	0.000	1.000	0.700	0.400	1.000	0.300	60.61%	30.00%
240		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

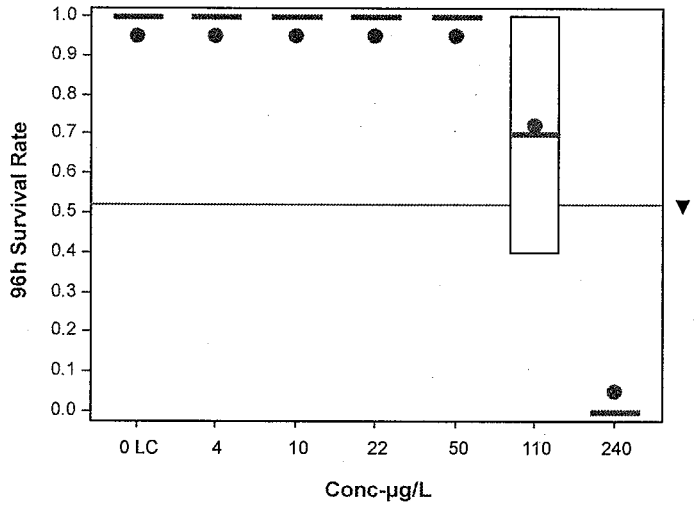
Angular (Corrected) Transformed Summary

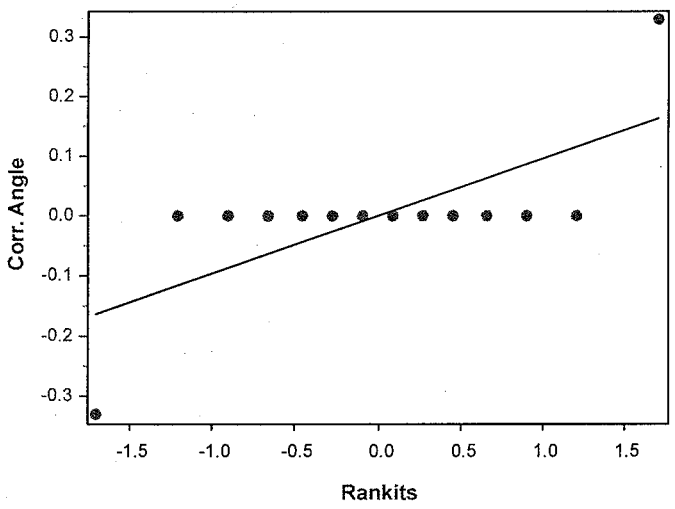
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	2	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
4		2	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
10		2	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
22		2	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
50		2	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
110		2	1.020	-3.180	5.210	1.020	0.685	1.350	0.330	46.02%	24.55%
240		2	0.226	0.225	0.226	0.226	0.226	0.226	0.000	0.00%	83.24%

Acute Fish Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	11-0229-6633	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Mar-24 17:10	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	26 Mar-24 17:08	**MD5 Hash:**	999C642D3B390E591AFABD38B0EF138E	**Editor ID:**	007-803-386-7

Graphics





Acute Fish Survival Test					Nautilus Environmental (CA)
Analysis ID:	19-3363-1923	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Mar-24 17:10	**Analysis:**	Untrimmed Spearman-Kärber	**Status Level:**	1
Edit Date:	26 Mar-24 17:08	**MD5 Hash:**	999C642D3B390E591AFABD38B0EF138E	**Editor ID:**	007-803-386-7

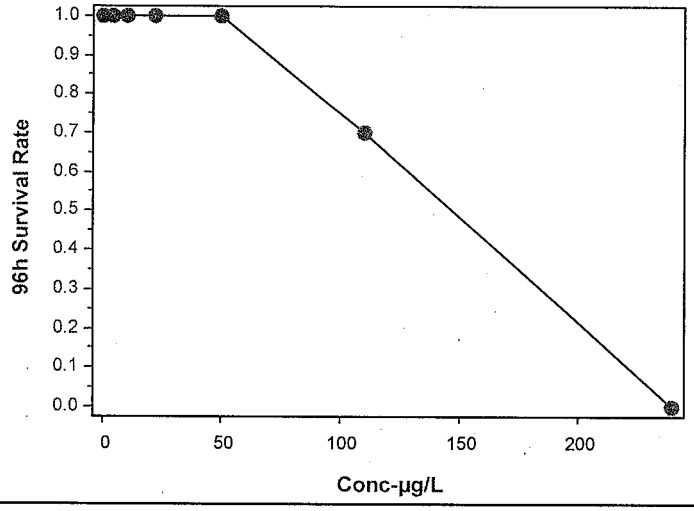
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	2.11	0.0494	128	102	161

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%
4		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
10		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
22		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
50		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
110		2	0.700	0.700	0.400	1.000	60.61%	30.00%	7/10	0.700	30.00%
240		2	0.000	0.000	0.000	0.000	---	100.00%	0/10	0.000	100.00%

Graphics



CETIS Analytical Report

Acute Fish Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	09-4437-4080	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Mar-24 17:10	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	26 Mar-24 17:08	**MD5 Hash:**	999C642D3B390E591AFABD38B0EF138E	**Editor ID:**	007-803-386-7

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	884398	1000	Yes	Two-Point Interpolation

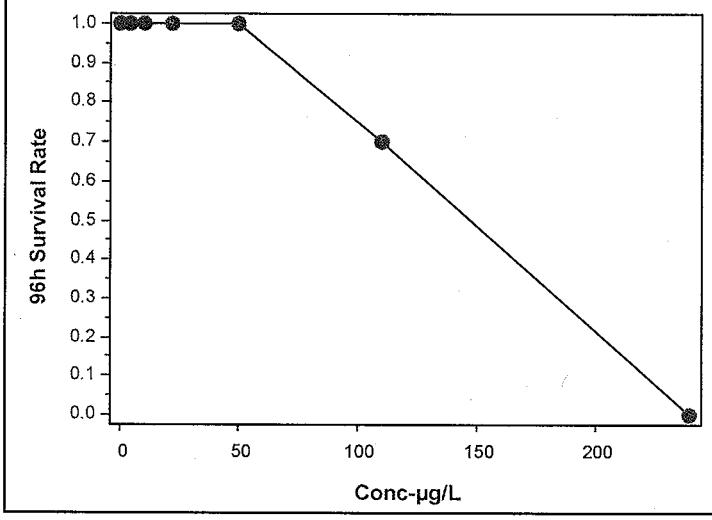
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	100	---	270

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%
4		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
10		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
22		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
50		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
110		2	0.700	0.700	0.400	1.000	60.61%	30.00%	7/10	0.700	30.00%
240		2	0.000	0.000	0.000	0.000	---	100.00%	0/10	0.000	100.00%

Graphics



CETIS Summary Report

Acute Fish Survival Test — **Nautilus Environmental (CA)**

Batch ID:	07-0442-8221	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	21 Feb-24 16:00	**Protocol:**	OECD 203	**Diluent:**	Laboratory Freshwater
Ending Date:	25 Feb-24 16:00	**Species:**	Oncorhynchus mykiss	**Brine:**	Not Applicable
Test Length:	96h	**Taxon:**		**Source:**	Thomas Fish Co. **Age:** 51d

Sample ID:	03-3228-0861	**Code:**	24-5061	**Project:**	6PPD-~~quinone~~ (B)
Sample Date:	~~26-Mar-24 17:14~~ (A)	**Material:**	Chemical Product	**Source:**	Washington Department of Ecology
Receipt Date:	21 Feb-24 09:30	**CAS (PC):**		**Station:**	Ozonated 7PPD
Sample Age:	---	**Client:**	Washington Department of Ecology		

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
12-8584-6715	96h Survival Rate	Fisher Exact Test		240	>240	---	---	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	µg/L	95% LCL	95% UCL	S
14-4112-7177	96h Survival Rate	Linear Interpolation (ICPIN)		EC25	>240	---	---	1
				EC50	>240	---	---	

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%
4		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
10		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
22		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
50		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
110		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
240		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%

96h Survival Rate Detail — MD5: 77C407B2F718B6D24E8A534DE8412863

Conc-µg/L	Code	Rep 1	Rep 2
0	S	1.000	1.000
0	LC	1.000	1.000
4		1.000	1.000
10		1.000	1.000
22		1.000	1.000
50		1.000	1.000
110		1.000	1.000
240		1.000	1.000

(A) QA OK 3/27/24

(B) QA ACS 4/4/24

Acute Fish Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	12-8584-6715	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Mar-24 17:23	**Analysis:**	Single 2x2 Contingency Table	**Status Level:**	1
Edit Date:	26 Mar-24 17:15	**MD5 Hash:**	5014DA71A339DB760C490E96A6AC1B18	**Editor ID:**	007-803-386-7

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units
Untransformed	C > T	240	>240	---	---

Fisher Exact Test

Control	vs	Conc-µg/L	Test Stat	P-Type	P-Value	Decision(α:5%)
Lab Control		4	1.000	Exact	1.0000	Non-Significant Effect
		10	1.000	Exact	1.0000	Non-Significant Effect
		22	1.000	Exact	1.0000	Non-Significant Effect
		50	1.000	Exact	1.0000	Non-Significant Effect
		110	1.000	Exact	1.0000	Non-Significant Effect
		240	1.000	Exact	1.0000	Non-Significant Effect

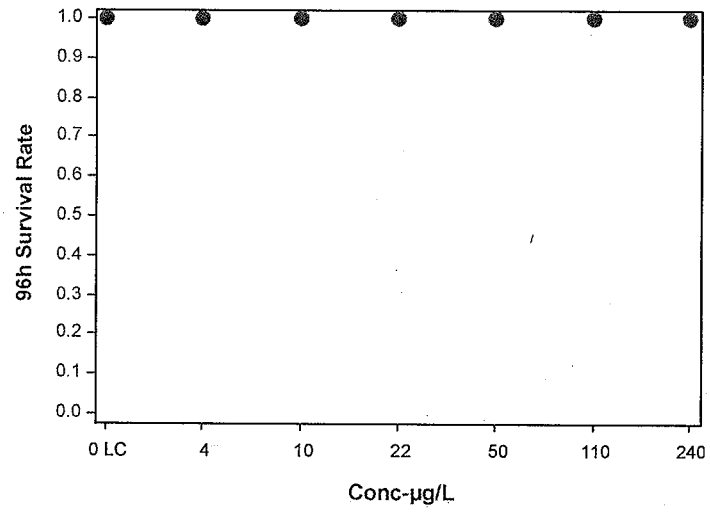
96h Survival Rate Frequencies

Conc-µg/L	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
0	LC	10	0	10	1.000	0.000	0.00%
4		10	0	10	1.000	0.000	0.00%
10		10	0	10	1.000	0.000	0.00%
22		10	0	10	1.000	0.000	0.00%
50		10	0	10	1.000	0.000	0.00%
110		10	0	10	1.000	0.000	0.00%
240		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%
4		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
10		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
22		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
50		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
110		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
240		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%

Graphics



CETIS Analytical Report

Acute Fish Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	14-4112-7177	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Mar-24 17:24	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	26 Mar-24 17:15	**MD5 Hash:**	5014DA71A339DB760C490E96A6AC1B18	**Editor ID:**	007-803-386-7

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1170682	1000	Yes	Two-Point Interpolation

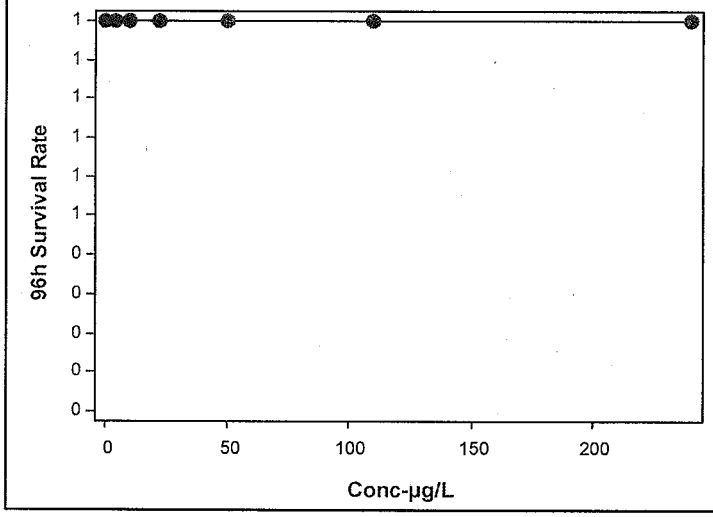
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	>240	---	---
EC50	>240	---	---

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%
4		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
10		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
22		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
50		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
110		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
240		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%

Graphics



CETIS Summary Report

Acute Fish Survival Test — **Nautilus Environmental (CA)**

Batch ID:	07-2807-0381	**Test Type:**	Survival (96h)	**Analyst:**			
Start Date:	21 Feb-24 16:00	**Protocol:**	OECD 203	**Diluent:**	Laboratory Freshwater		
Ending Date:	25 Feb-24 16:00	**Species:**	Oncorhynchus mykiss	**Brine:**	Not Applicable		
Test Length:	96h	**Taxon:**		**Source:**	Thomas Fish Co.	**Age:**	51d

Sample ID:	13-6777-6296	**Code:**	24-5062	**Project:**	6PPD-~~quinone~~ (B)
Sample Date:	~~26-Mar-24 17:18~~ (A)	**Material:**	Chemical Product	**Source:**	Washington Department of Ecology
Receipt Date:	21 Feb-24 09:30	**CAS (PC):**		**Station:**	Ozonated 77PD
Sample Age:	---	**Client:**	Washington Department of Ecology		

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
20-0593-3733	96h Survival Rate	Fisher Exact Test		240	>240	---	---	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	µg/L	95% LCL	95% UCL	S
10-6092-1087	96h Survival Rate	Linear Interpolation (ICPIN)		EC25	>240	---	---	1
				EC50	>240	---	---	

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%
4		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
10		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
22		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
50		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
110		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
240		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%

96h Survival Rate Detail — MD5: 77C407B2F718B6D24E8A534DE8412863

Conc-µg/L	Code	Rep 1	Rep 2
0	S	1.000	1.000
0	LC	1.000	1.000
4		1.000	1.000
10		1.000	1.000
22		1.000	1.000
50		1.000	1.000
110		1.000	1.000
240		1.000	1.000

(A) QA OK 3/27/24

(B) QA OK 4/4/24

Acute Fish Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	20-0593-3733	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Mar-24 17:19	**Analysis:**	Single 2x2 Contingency Table	**Status Level:**	1
Edit Date:	26 Mar-24 17:18	**MD5 Hash:**	5014DA71A339DB760C490E96A6AC1B18	**Editor ID:**	007-803-386-7

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units
Untransformed	C > T	240	>240	---	---

Fisher Exact Test

Control	vs	Conc-µg/L	Test Stat	P-Type	P-Value	Decision(α:5%)
Lab Control		4	1.000	Exact	1.0000	Non-Significant Effect
		10	1.000	Exact	1.0000	Non-Significant Effect
		22	1.000	Exact	1.0000	Non-Significant Effect
		50	1.000	Exact	1.0000	Non-Significant Effect
		110	1.000	Exact	1.0000	Non-Significant Effect
		240	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%
4		10	0	10	1.000	0.000	0.00%
10		10	0	10	1.000	0.000	0.00%
22		10	0	10	1.000	0.000	0.00%
50		10	0	10	1.000	0.000	0.00%
110		10	0	10	1.000	0.000	0.00%
240		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%
4		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
10		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
22		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
50		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
110		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
240		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%

CETIS Analytical Report

Acute Fish Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	10-6092-1087	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Mar-24 17:19	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	26 Mar-24 17:18	**MD5 Hash:**	5014DA71A339DB760C490E96A6AC1B18	**Editor ID:**	007-803-386-7

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	222430	1000	Yes	Two-Point Interpolation

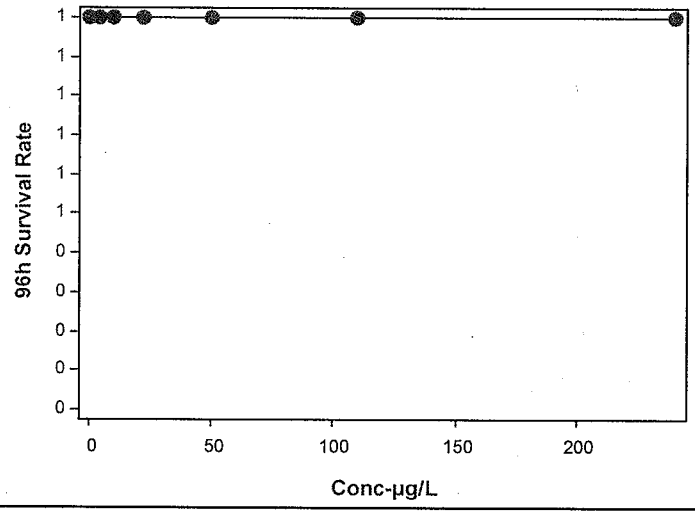
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	>240	---	---
EC50	>240	---	---

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%
4		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
10		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
22		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
50		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
110		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
240		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%

Graphics



Freshwater Acute Bioassay
Static Conditions
DF-018

OECD 203

Water Quality Measurements & Test Organism Survival

Client: WADOE
Sample ID: Acetone control
Test No.: 2402 - 5163 to 5165

Test Species: *O. mykiss*
Start Date/Time: 2/21/24 1600
End Date/Time: 2/25/24 1600

Concentration	Rep	Number of Live Organisms						pH (units)					Dissolved Oxygen (mg/L)					Conductivity (umhos/cm)					Temperature (°C)					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	
Acetone	A	5	5	5	5	5	5	7.82	7.27	7.26	7.29	7.34	11.9	8.5 ~~7.4~~	8.8	9.3	8.9	299	300	297	298	295	12.5	11.9	12.0	12.1	12.1	100
Control	B	5	5	5	5	5	5	7.86	7.32	7.32	7.35	7.38	11.8	8.6 ~~7.8~~	8.6	9.3	8.9	299	300	296	298	295	12.5	11.8	11.9	11.9	12.0	

Tech Initials								
	Counts	WF	WF	WF	WF	WF	WF	Gm
	WQ	WF	X	WF	WF	WF	WF	Gm
	QC	MM						

Fish Size at test initiation*:

Weights (g): 0.341 0.422 0.364 0.397 0.367 μ = 0.378

Lengths (cm): 3.20 3.35 3.35 3.30 3.25 μ = 3.29

Environmental Chamber: F

Loading rate = 0.63 g/L

Sample Description: Clear Liquid

Animal Source/Date Received: Thomas Fish Co. 2/9/24

Age at Initiation: 51 days

Comments: *5 random fish are sacrificed at initiation for size determination. AQ18 WF 2/22/24

QC Check: KL 3/26/24

Final Review: AES 4/4/24

Enthalpy Analytical. 4340 Vandever Avenue. San Diego, CA 92120.

Freshwater Acute Bioassay
Static Conditions
DF-018

OECD 203

Water Quality Measurements & Test Organism Survival

Client: WADOE
Sample ID: Ozonated 6PPD
Test No.: 2402-S163

Test Species: *O. mykiss*
Start Date/Time: 2/21/24 1600
End Date/Time: 2/25/24 1600

Concentration (ug/L)	Rep	Number of Live Organisms						pH (units)					Dissolved Oxygen Q14 Q14 (mg/L) Q14					Conductivity (umhos/cm)					Temperature (°C)					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	7.81	7.28	7.19	7.32	7.27	12.0	9.5	8.6	9.2	9.6	300	299	295	297	293	12.5	12.0	12.0	12.0	12.1	100
	B	5	5	5	5	5	5	7.85	7.32	7.24	7.32	7.27	11.9	9.2	8.4	8.5	8.6	300	300	296	298	294	12.5	11.9	12.0	12.0	12.1	
4	A	5	5	5	5	5	5	7.87	7.31	7.27	7.31	7.35	11.8	8.4	8.6	8.8	8.7	301	300	297	299	296	12.8	12.0	12.1	12.0	12.1	100
	B	5	5	5	5	5	5	7.89	7.33	7.31	7.36	7.38	11.8	8.4	9.0	8.8	8.6	301	299	296	298	294	12.8	11.9	12.0	12.0	12.1 (3)	
10	A	5	5	5	5	5	5	7.87	7.33	7.31	7.36	7.39	11.8	8.2	8.5	8.7	8.5	301	300	297	299	295	12.9	12.0	12.1	12.0	12.1	100
	B	5	5	5	5	5	5	7.86	7.32	7.31	7.36	7.38	11.8	8.2	8.5	9.0	8.6	301	299	297	298	295	12.8	12.0	12.1	12.0	12.1	
22	A	5	5	5	5	5	5	7.84	7.33	7.30	7.38	7.37	11.8	8.2	8.1	8.8	8.1	302	300	295	297	294	13.0	12.2	12.1	12.0	12.1 (2)	100
	B	5	5	5	5	5	5	7.86	7.29	7.29	7.33	7.35	11.7	8.0	8.3	8.9	8.0	302	300	296	298	294	13.0	12.1	12.1	12.1	12.2	
50	A	5	5	5	5	5	5	7.87	7.24	7.23	7.35	7.39	11.9	8.5	8.7	8.9	8.8	301	299	295	297	292	12.8	11.8	11.8	11.7	11.8	100
	B	5	5	5	5	5	5	7.87	7.30	7.27	7.35	7.35	11.8	8.6	8.6	8.6	8.6	301	300	297	299	294	12.7	11.7	11.7	11.7	11.7	
110	A	5	5	2	2	2	2	7.86	7.33	7.32	7.35	7.36	11.8	8.7	9.0	8.7	8.8	301	301	296	297	292	12.8	11.8	11.9	11.8	11.9	70
	B	5	5	5	5	5	5	7.85	7.32	7.36	7.35	7.34	11.8	8.5	8.4	8.4	8.5	301	299	295	297	293	12.8	11.6	11.7	11.7	11.7	
240	A	5	5	1	0	\		7.83	7.38	7.38	\		11.8	9.0	9.5	\		301	303	300	\		12.9	11.8	11.8	\		0
	B	5	5	0	—		\	7.87	7.42	—		\	11.7	9.4	—		\	301	300	—		\	12.9	11.7	—		\	
Tech Initials	Counts	WF	WF	WF	WF	WF	Gm																					
	WQ	WF	X	WF	WF	WF	Gm																					
	QC	MW																										

Environmental Chamber: F

Fish Size at test initiation*:

Weights (g): 0.341 0.422 0.364 0.397 0.367 μ = 0.378

Lengths (cm): 3.20 3.35 3.35 3.30 3.25 μ = 3.29

Loading rate = 0.63 g/L

Sample Description: Black Solid

Animal Source/Date Received: Thomas Fish Co. 2/1/24

Age at Initiation: 51 days

Comments: *5 random fish are sacrificed at initiation for size determination. (3) Q18 Gm 2/25/24

QC Check: KL 3/26/24

Final Review: AMS 4/9/24

Enthalpy Analytical. 4340 Vandever Avenue. San Diego, CA 92120.

Freshwater Acute Bioassay
Static Conditions
DF-018

OECD 203

Water Quality Measurements & Test Organism Survival

Client: WADOE
Sample ID: Ozonated 7PPD
Test No.: 2402-5164

Test Species: *O. mykiss*
Start Date/Time: 2/21/24 1600
End Date/Time: 2/25/24 1600

Concentration (ug/L)	Rep	Number of Live Organisms						pH (units)					Dissolved Oxygen (mg/L)					Conductivity (umhos/cm)					Temperature (°C)					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	7.84	7.33	7.29	7.35	7.35	12.0	9.1	9.3	9.5	9.2	301	300	296	298	294	12.2	11.8	11.9	11.8	11.9	100
	B	5	5	5	5	5	5	7.84	7.34	7.34	7.37	7.34	12.2	9.2	9.0	9.7	9.0	301	300	295	297	294	12.2	11.7	11.8	11.7	11.8	
4	A	5	5	5	5	5	5	7.86	7.36	7.34	7.38	7.39	12.1	9.1	9.0	9.4	9.1	301	300	296	298	295	12.3	11.9	11.9	11.9	11.9	100
	B	5	5	5	5	5	5	7.86	7.36	7.36	7.40	7.38	11.9	8.7	9.0	9.4	8.8	301	300	297	300	297	12.2	11.7	11.8	11.8	11.9	
10	A	5	5	5	5	5	5	7.86	7.34	7.35	7.39	7.34	11.8	8.2	8.1	9.0	8.5	301	300	297	299	296	12.4	11.8	11.9	11.9	11.9	100
	B	5	5	5	5	5	5	7.85	7.34	7.30	7.35	7.35	11.8	8.5	8.2	9.0	8.8	301	300	296	298	294	12.3	11.8	11.9	11.9	11.9	
22	A	5	5	5	5	5	5	7.87	7.33	7.30	7.35	7.34	11.7	8.3	8.3	8.8	8.5	301	300	296	298	295	12.5	11.9	11.9	11.9	11.9	100
	B	5	5	5	5	5	5	7.86	7.33	7.30	7.35	7.34	11.6	8.6	8.2	8.8	8.7	301	300	296	298	294	12.7	11.8	11.9	11.9	12.0	
50	A	5	5	5	5	5	5	7.84	7.30	7.31	7.36	7.36	11.8	8.5	8.5	8.7	8.9	301	300	296	298	295	12.4	11.9	12.0	12.0	12.0	100
	B	5	5	5	5	5	5	7.84	7.32	7.32	7.33	7.32	11.8	8.2	8.6	8.4	8.6	301	300	296	298	294	12.7	11.9	12.0	12.0	12.1	
110	A	5	5	5	5	5	5	7.85	7.34	7.30	7.32	7.34	11.8	8.9	8.5	8.6	8.7	301	300	297	298	295	12.8	11.9	12.1	12.0	12.1	100
	B	5	5	5	5	5	5	7.86	7.34	7.32	7.36	7.35	11.7	8.6	8.6	8.6	8.5	302	299	295	297	293	13.0	11.9	12.1	12.1	12.1	
240	A	5	5	5	5	5	5	7.85	7.33	7.33	7.28	7.28	11.7	8.4	8.0	8.4	7.5	302	299	295	297	293	13.0	12.0	12.1	12.1	12.1	100
	B	5	5	5	5	5	5	7.84	7.35	7.29	7.27	7.24	11.7	8.5	7.9	8.4	7.5	302	299	295	297	294	13.0	12.0	12.2	12.1	12.1	
Tech Initials	Counts	WF	WF	WF	WF	WF	LM																					
	WQ	WF	X	WF	WF	WF	LM																					
	QC	MM																										

Fish Size at test initiation*:

Environmental Chamber: F

Weights (g): 0.341 0.422 0.364 0.397 0.367 μ = 0.378

Lengths (cm): 3.20 3.35 3.35 3.30 3.25 μ = 3.29

Loading rate = 0.63g/L

Sample Description: Black Solid

Animal Source/Date Received: Thomas Fish Co. 2/14/24

Age at Initiation: 51 days

Comments: *5 random fish are sacrificed at initiation for size determination.

QC Check: KL 3/26/24

Final Review: ACS 4/4/24

Enthalpy Analytical. 4340 Vandever Avenue. San Diego, CA 92120.

Freshwater Acute Bioassay
Static Conditions
DF-018

OECD 203

Water Quality Measurements & Test Organism Survival

Client: WADOE
Sample ID: Ozonated 77PD
Test No.: 2402-S165

Test Species: *O. mykiss*
Start Date/Time: 2/21/24 1600
End Date/Time: 2/25/24 1600

Concentration (ug/L)	Rep	Number of Live Organisms						pH (units)					Dissolved Oxygen Q14 Q14 (mg/L) Q14 Q14					Conductivity (umhos/cm)					Temperature (°C)					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	7.86	7.37	7.21	7.32	7.31	12.2	10.8	9.0	9.2	9.2	295	299	296	296	294	11.8	11.8	11.9	11.8	11.9	100
	B	5	5	5	5	5	5	7.89	7.39	7.28	7.35	7.34	12.2	8.9	8.6	9.2	9.1	298	298	295	295	292	11.6	11.7	11.8	11.7	11.8	
4	A	5	5	5	5	5	5	7.89	7.37	7.32	7.36	7.34	12.0	8.2	8.3	8.6	8.6	300	299	296	296	293	11.9	11.9	11.9	11.8	11.9	100
	B	5	5	5	5	5	5	7.90	7.36	7.32	7.35	7.38	12.0	8.5	8.6	9.0	8.8	300	300	297	298	295	11.9	11.7	11.7	11.7	11.8	
10	A	5	5	5	5	5	5	7.88	7.37	7.33	7.38	7.37	12.1	8.2	8.3	8.7	8.6	300	300	297	299	296	12.1	11.8	11.9	11.8	11.9	100
	B	5	5	5	5	5	5	7.88	7.37	7.37	7.38	7.37	11.9	8.4	8.5	8.6	8.6	300	299	295	296	293	12.0	11.7	11.8	11.7	11.8	
22	A	5	5	5	5	5	5	7.88	7.36	7.33	7.39	7.35	12.4	8.3	8.5	8.4	8.7	300	299	297	298	295	12.4	11.9	11.9	11.9	11.9	100
	B	5	5	5	5	5	5	7.88	7.38	7.34	7.36	7.36	11.9	7.7	8.7	8.6	8.4	300	299	296	297	294	12.2	11.7	11.8	11.8	11.8	
50	A	5	5	5	5	5	5	7.86	7.40	7.33	7.32	7.38	11.9	8.6	8.3	8.8	8.8	301	300	296	294	294	12.3	11.9	11.9	11.9	12.0	100
	B	5	5	5	5	5	5	7.87	7.36	7.33	7.35	7.37	12.0	8.4	9.3	8.5	8.6	300	300	297	298	295	12.1	11.7	11.8	11.8	11.8	
110	A	5	5	5	5	5	5	7.88	7.38	7.34	7.36	7.37	11.9	8.4	8.8	8.4	8.6	300	300	297	298	295	12.2	11.9	11.9	11.9	12.0	100
	B	5	5	5	5	5	5	7.87	7.38	7.34	7.38	7.33	11.8	8.4	8.7	8.8	8.0	300	300	297	299	296	12.1	11.7	11.8	11.8	11.8	
240	A	5	5	5	5	5	5	7.87	7.36	7.33	7.33	7.33	12.0	8.0	8.4	8.0	8.1	300	299	296	298	296	12.1	11.9	12.0	12.0	12.0	100
	B	5	5	5	5	5	5	7.89	7.32	7.29	7.33	7.37	11.9	8.5	9.0	8.8	9.0	300	299	296	298	294	12.1	11.8	11.9	11.9	11.9	
Tech Initials	Counts	WF	WF	WF	WF	WF	KM																					
	WQ	WF	X	WF	WF	WF	KM																					
	QC	MK																										

Environmental Chamber: F

Fish Size at test initiation*:

Weights (g): 0.341 0.422 0.364 0.397 0.367 μ = 0.378

Lengths (cm): ~~3.20~~ ~~32.0~~ 3.35 ~~33.5~~ 3.35 ~~33.5~~ 3.30 ~~33.0~~ 3.25 ~~32.5~~ (A) (A) (A) (A) (A) μ = (A) 3.29 ~~32.9~~

Loading rate = 0.63 g/L

Sample Description: Blue Solid

Animal Source/Date Received: (A) ~~ABS~~ Thomas Fish Co. 2/9/24

Age at Initiation: 51 days

Comments: *5 random fish are sacrificed at initiation for size determination. (A) Q18 WF 2/21/24 (B) Q18 WF 2/22/24

QC Check: KL 3/26/24

Final Review: AES 4/4/24

Enthalpy Analytical
4340 Vandever Avenue
San Diego, CA 92120

Chemical Product
Sample Check-in Information
DC-009

Client: WADOE

Test No.: 2402-S163 to S165

Test Type: OECD O. mykiss acute

Enthalpy Log-in 24-xxxx	Sample ID	Collection Date & Time	Receipt Date & Time	No. Containers	Container Type	Approx. amount of sample received	Sample Description	Receipt Condition	Tech Initials
5060	Ozonated 6PPD	N/A	2/21/24 0930	1	boiling flask	500mg	black tar	Good	BO
5061	Ozonated 7PPD	N/A	2/21/24 0930	1	boiling flask	500mg	black tar	Good	↓
5062	Ozonated 77PD	N/A	2/21/24 0930	1	boiling flask	500mg	black tar	Good	↓

Samples Shipped Via: Fedex

COC Present? Y (N)

Sub-samples for additional chemistry: No

Comments: N/A = not applicable

QC Check: VL 3/26/24

Final Review: AFS 4/4/24

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 ≤ 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 Mar-24 16:57 (p 1 of 1)
Test Code/ID: 240221omra / 14-8789-5260

Acute Fish Survival Test — **Nautilus Environmental (CA)**

Batch ID:	11-9729-1219	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	21 Feb-24 16:00	**Protocol:**	Washington DOE (2009)	**Diluent:**	Laboratory Freshwater
Ending Date:	25 Feb-24 ~~17:00~~ (A) 16:00	**Species:**	Oncorhynchus mykiss	**Brine:**	Not Applicable
Test Length:	4d ~~16~~ (A)	**Taxon:**		**Source:**	Aquatic Biosystems, CO **Age:** 37 d post swim up

Sample ID:	11-9598-7582	**Code:**	240221omra	**Project:**	Internal
Sample Date:	21 Feb-24	**Material:**	Copper chloride	**Source:**	Copper Chloride
Receipt Date:	21 Feb-24	**CAS (PC):**		**Station:**	
Sample Age:	16h	**Client:**			

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
05-8113-2774	96h Survival Rate	Fisher Exact Test		25	50	35.36	---	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	µg/L	95% LCL	95% UCL	S
12-7192-5702	96h Survival Rate	Trimmed Spearman-Kärber		EC50	64.5	52.3	79.6	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	0.950	0.315	1.590	0.900	1.000	0.050	0.071	7.44%	5.00%
50		2	0.700	-0.571	1.970	0.600	0.800	0.100	0.141	20.20%	30.00%
100		2	0.200	0.200	0.200	0.200	0.200	0.000	0.000	0.00%	80.00%
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: B4A820F1FDADE62B6DAC5F53C22F44F3

Conc-µg/L	Code	Rep 1	Rep 2
0	LC	1.000	1.000
25		0.900	1.000
50		0.800	0.600
100		0.200	0.200
200		0.000	0.000
400		0.000	0.000

(A) EIS/ATS 4/4/24

CETIS Analytical Report

Test Code/ID: 240221omra / 14-8789-5260

Acute Fish Survival Test — Nautilus Environmental (CA)

Analysis ID:	05-8113-2774	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Mar-24 16:56	**Analysis:**	Single 2x2 Contingency Table	**Status Level:**	1
Edit Date:	26 Mar-24 16:54	**MD5 Hash:**	B4A820F1FDADE62B6DAC5F53C22F44F3	**Editor ID:**	007-803-386-7

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units
Untransformed	C > T	25	50	35.36	---

Fisher Exact Test

Control	vs	Conc-µg/L	Test Stat	P-Type	P-Value	Decision(α:5%)
Lab Control		25	0.500	Exact	0.5000	Non-Significant Effect
		50*	0.010	Exact	0.0101	Significant Effect
		100*	0.000	Exact	<1.0E-05	Significant Effect

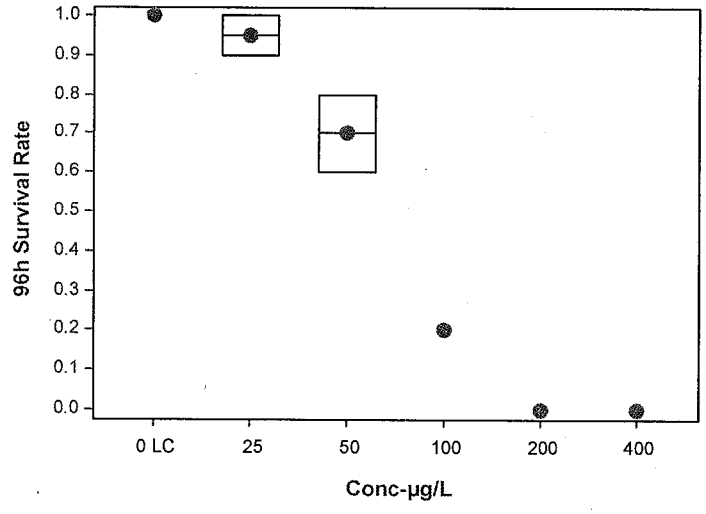
96h Survival Rate Frequencies

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		19	1	20	0.950	0.050	5.00%
50		14	6	20	0.700	0.300	30.00%
100		4	16	20	0.200	0.800	80.00%
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	0.950	0.315	1.000	0.950	0.900	1.000	0.050	7.44%	5.00%
50		2	0.700	0.000	1.000	0.700	0.600	0.800	0.100	20.20%	30.00%
100		2	0.200	0.199	0.201	0.200	0.200	0.200	0.000	0.00%	80.00%
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%

Graphics



CETIS Analytical Report

Acute Fish Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	12-7192-5702	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Mar-24 16:57	**Analysis:**	Trimmed Spearman-Kärber	**Status Level:**	1
Edit Date:	26 Mar-24 16:54	**MD5 Hash:**	B4A820F1FDADE62B6DAC5F53C22F44F3	**Editor ID:**	007-803-386-7

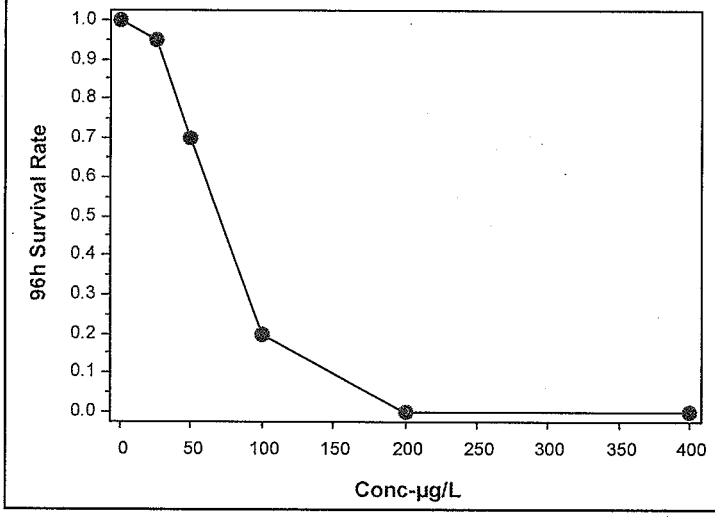
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	5.00%	1.81	0.0456	64.5	52.3	79.6

96h Survival Rate Summary

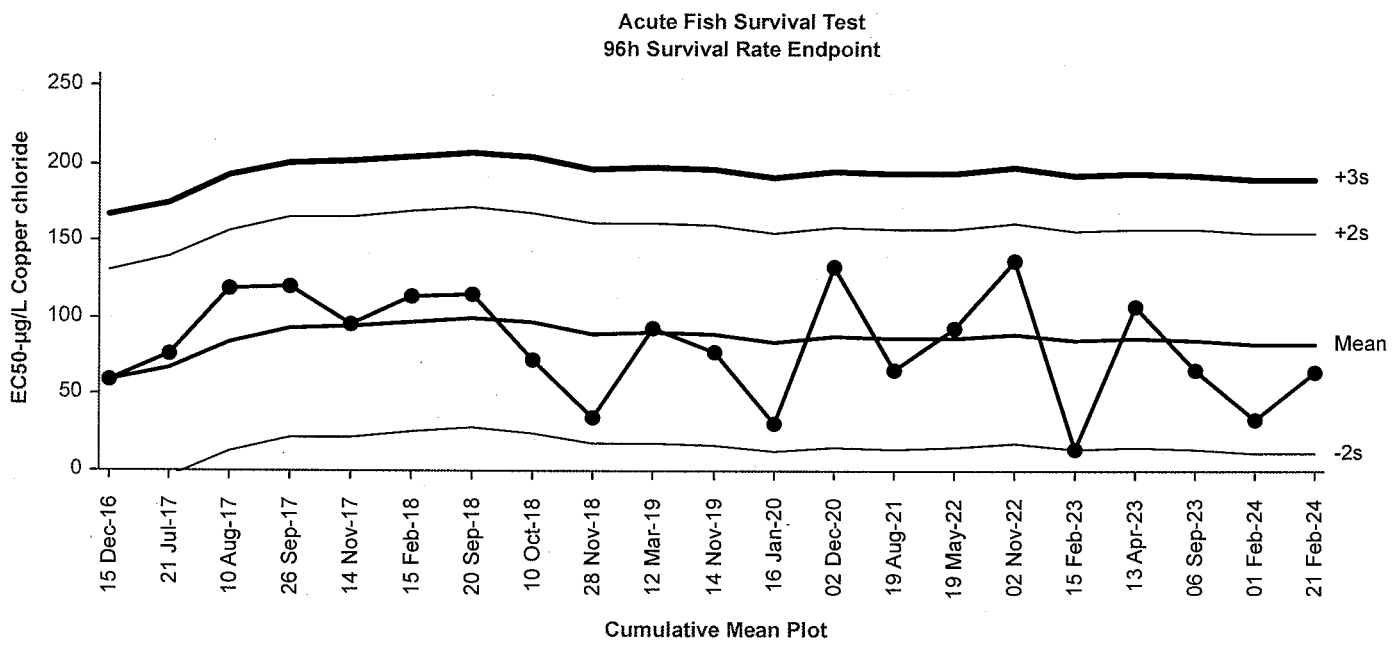
			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	2	1.000	1.000	1.000	1.000	0.00%	0.00%	20/20	1.000	0.00%
25		2	0.950	0.950	0.900	1.000	7.44%	5.00%	19/20	0.950	5.00%
50		2	0.700	0.700	0.600	0.800	20.20%	30.00%	14/20	0.700	30.00%
100		2	0.200	0.200	0.200	0.200	0.00%	80.00%	4/20	0.200	80.00%
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%

Graphics



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:	83.01	**Count:**	20	**-2s Warning Limit:**	11.3	**-3s Action Limit:**	-24.6
Sigma:	35.88	**CV:**	43.20%	**+2s Warning Limit:**	155	**+3s Action Limit:**	191

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2016	Dec	15	13:00	59.46	-23.55	-0.6563			07-2059-1930	21-0698-8947
2	2017	Jul	21	11:45	75.79	-7.224	-0.2013			12-6230-4373	10-5665-4943
3		Aug	10	13:35	119.1	36.05	1.005			09-7390-4688	11-1885-9400
4		Sep	26	15:10	120.1	37.06	1.033			12-0767-7259	14-1478-0761
5		Nov	14	11:25	96.22	13.21	0.3682			21-0521-5529	14-2305-2435
6	2018	Feb	15	15:00	114.5	31.53	0.8787			08-5122-1964	18-9847-1069
7		Sep	20	14:05	114.9	31.86	0.888			14-1527-8451	21-3828-6142
8		Oct	10	16:40	72.55	-10.46	-0.2915			06-8408-1163	05-7761-5868
9		Nov	28	12:00	35.36	-47.65	-1.328			21-0374-7072	19-9377-5872
10	2019	Mar	12	12:10	93.3	10.29	0.2869			11-1972-1376	05-1051-7815
11		Nov	14	11:55	77.34	-5.674	-0.1581			08-3948-6775	01-9304-4998
12	2020	Jan	16	12:50	30.63	-52.38	-1.46			15-5355-8442	09-8383-1081
13		Dec	2	13:30	133.3	50.32	1.403			07-0223-4669	10-8492-8883
14	2021	Aug	19	14:25	65.98	-17.03	-0.4748			11-4973-5943	08-1400-5422
15	2022	May	19	12:45	93.3	10.29	0.2869			03-2996-8953	07-9626-2312
16		Nov	2	10:05	136.6	53.59	1.494			14-6511-1746	10-0042-0505
17	2023	Feb	15	11:30	14.71	-68.3	-1.904			17-0773-8757	02-8314-6307
18		Apr	13	15:15	107.2	24.17	0.6736			04-5815-1771	04-7188-7445
19		Sep	6	15:30	65.98	-17.03	-0.4748			19-8080-9942	18-9941-8060
20	2024	Feb	1	12:35	34.02	-48.99	-1.365			00-8109-2501	16-6938-0720
21			21	16:00	64.53	-18.48	-0.515			14-8789-5260	12-7192-5702

Freshwater Acute Bioassay
Static Conditions
DF-019

Dangerous Waste Characterization

Water Quality Measurements & Test Organism Survival

Client: Internal
Sample ID: $CuCl_2$
Test No.: 240221omra

Test Species: *O. mykiss*
Start Date/Time: 2/21/24 1600
End Date/Time: 2/25/24 1600

Concentration (µg/L)	RAND #	Number of Live Organisms					pH (units)					Dissolved Oxygen (mg/L)					Conductivity (umhos/cm)					Temperature (°C)					Percent Survival
		0	24	48	72	96	0	24	48	72	96	Q14 0	Q14 24	48	Q14 72	Q14 96	0	24	48	72	96	0	24	48	72	96	
Lab Control	10	10	10	10	10	10	7.73	7.17	7.23	7.16	7.46	12.2	9.4	8.2	8.3	9.2	296	290	287	289	281	12.0	12.1	12.1	12.1	12.2	100
	8	10	10	10	10	10	7.78	7.32	7.26	7.21	7.29	11.8	8.8	8.6	8.4	8.8	299	296	289	291	285	12.5	11.8	12.0	12.0	12.2	100
25	9	10	10	10	9	9	7.79	7.33	7.29	7.24	7.36	11.8	8.6	9.0	8.7	9.2	299	297	291	292	288	12.5	12.0	~~21.1~~	12.0	12.1	90
	5	10	10	10	10	10	7.81	7.34	7.31	7.27	7.32	11.8	9.9	8.6	8.4	8.8	299	296	291	292	288	12.5	11.8	12.0	12.0	12.0	100
50	7	10	10	10	8	8	7.78	7.32	7.31	7.26	7.26	11.8	9.0	8.9	8.5	8.9	299	296	292	293	289	12.5	11.8	11.9	12.0	12.0	80
	4	10	10	9	6	6	7.78	7.32	7.28	7.28	7.32	11.8	9.0	9.0	9.0	9.3	298	297	292	292	288	12.5	11.9	12.0	~~12.9~~ 11.9	12.0	60
100	12	10	5	4	2	2	7.74	7.30	7.28	7.35	7.39	11.8	~~12.2~~ 6.8	8.6	9.0	9.4	299	298	293	292	287	12.6	12.2	12.2	12.1	12.1	20
	3	10	8	4	4	2	7.77	7.29	7.30	7.36	7.37	11.9	8.9	8.8	9.2	9.3	298	297	293	292	287	12.4	11.9	11.9	11.8	11.9	20
200	1	10	2	0	\		7.72	7.27	7.30	\		11.6	8.5	9.2	\		302	300	294	\		12.9	11.8	11.9	\		0
	11	10	1	0		\	7.72	7.26	7.31		\	11.8	8.7	9.4		\	299	299	293		\	12.5	12.1	12.1		\	0
400	2	10	0	\			7.76	7.29	\			11.8	9.2	\			299	299	\			12.7	11.9	\			0
	6	10	0			\	7.63	7.33			\	11.8	9.2			\	298	299			\	12.5	11.9			\	0
Tech Initials:	Counts	WF	WF	WF	WF	GM																					
	Readings	WF	WF	WF	WF	GM																					
	QC	MW																									

Recorded in Log Pass/Fail: N/A

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

Conc. µg/L	25	50	100	200	400
Vol. Cu stock added (mL)	2.2	4.4	8.9	17.8	35.6
Cu Stock Conc. (µg/L)	10,000	10,000	20,000	20,000	20,000

Environmental Chamber: F

Weights (g): 0.341 0.422 0.364 0.397 0.367 0.365 0.344 0.354 0.364 0.304

µ = 0.3672 g

Lengths (mm): 32.0 33.5 33.5 33.0 32.5 31.5 32.0 32.0 32.5 30.5

µ = 32.3 mm

Loading: 0.453 g/L

Length max/min = 33.5/30.5

Ratio of longest to shortest = 1.10

Animal Source/Date Received: Thomas Fish Co. 2/9/24

Hatch Date: 1/1/24

Swim-up Date: 1/15/24

Days post Swim-up: ~~36~~ 37

Comments: [b]10 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.

A Q18 WF 2/21/24 B Q18 WF 2/22/24 C Q18 WF 2/23/24 D Q18 WF 2/24/24 N/A = not applicable

E QA/HS 4/4/24

QC Check: KL 3/26/24

Final Review: AJS 4/4/24

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