ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

Washington Department of Ecology

OECD Toxicity Testing of 6PPD Quinone using *Oncorhynchus mykiss* (Rainbow Trout)

Prepared for: Washington State Department of Ecology

Prepared by: Enthalpy Analytical
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Date Submitted: November 11, 2023

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

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INTRODUCTION

Washington Department of Ecology engaged Enthalpy Analytical (Enthalpy) to generate acute toxicity data on the sensitivity of rainbow trout to 6PPD-quinone. 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 degrading and cracking, and it has a published effect to juvenile coho salmon at concentrations below 0.1 µg/L (Tian 2022). The objective of this study is to compare the relative toxicity of rainbow trout to 6PPD-quinone.

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 test was conducted between September 6 and 10, 2023.

MATERIALS AND METHODS

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

Compound	CAS Number	Supplier	Product Description
6PPD-quinone	unknown	HPC Standards	Dark orange powder

Based on the relatively low solubility of the chemical compound in water, the compound was dissolved in acetone, a solvent vehicle, prior to being introduced to water and exposed to the organisms for testing. A stock solution containing the compound and solvent was 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 acetone 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.

Nominal concentrations for the 6PPD-quinone exposure were 12, 5.5, 2.5, 1.1, 0.5, and 0.2 micrograms per liter (µg/L). A 24 milligram per liter (mg/L) acetone control was also tested with this compound.

Subsamples for verification of compound concentrations were collected and analyzed at the beginning and end of the testing period for the 0.2, 2.5 and 12 µg/L concentrations. The stock solution used to create the individual test solutions was also analyzed. Analysis was provided by WECK Laboratory and a full report is provided in Appendix D. 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 2.1.4.11) 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:	9/6/23, 15:15 to 9/10/23, 16:15
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:	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:	12, 5.5, 2.5, 1.1, 0.5, and 0.2 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

Statistically significant effects were detected in the 12, 5.5, and 2.5 µg/L concentrations tested, resulting in a NOEC of 1.1 µg/L. The LC_{50} was calculated as 1.8 µg/L, and the LC_{25} was calculated as 1.45 µg/L. No sublethal abnormalities were observed in the fish which survived.

Nominal values were used for the calculation of all statistical endpoints. Subsamples were collected from select test concentrations (0.2, 2.5, 12 g/L) at test initiation and termination (or when complete mortality in the test concentration was observed), which represent the lowest, a middle, and highest test concentrations. A subsample of the stock solution used to create the individual test solutions was also collected and measured.

The target concentration of the stock solution was 1000 µg/L and the measured value was 980 µg/L. This confirms that the measurement of the neat compound was precise and there was minimal product loss when it was dissolved in acetone and then water to create the stock solution.

Measurements taken from the individual test solutions at the time of test initiation indicate that nominal targets were achieved within a +/- 25 percent range. However, it should be noted that compound concentrations decreased from the beginning to the end of the test exposure. Compound concentration decreases tended to be higher (as a relative percentage of the initial measured concentration) in test solutions which had higher survival of the test organisms. Loss in the 0.2 µg/L test concentration, which had no mortality, was greater than 80 percent over the course of the test (Table 1). The decrease in the highest test concentration, 12 µg/L was less than 10 percent where complete mortality occurred within 24 hours of test initiation. And finally, a middle concentration, 2.5 µg/L showed a decrease in concentration of approximately 50 percent by the end of the test; this concentration had a steady increase in mortality observed throughout the 96-hour duration of the bioassay.

Significant care was taken during testing, including using glass material where ever possible, to minimize the loss of 6PPD quinone to adsorption of test equipment. The mechanism for toxicity with regard to aquatic life is still under investigation, however it is reasonable to believe that the reductions in measured concentrations can be attributable to a combination of degradation and absorption by the test organisms (Foldvik 2022). The pattern of reduction observed in this study, with the highest reduction in final 6PPD quinone being observed in the test concentration with the highest survival supports the theory that the organisms are absorbing the quinone throughout the test exposure. This pattern is further supported in the remaining two concentrations which were measured where 6PPD quinone concentration reductions at the end of the bioassay varied in parallel with the rate of mortality observed in the test organisms.

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 – 6PPD-quinone

Test Concentration (µg/L)	Measured Concentration at Initiation (µg/L)	Measured Concentration at Termination (µg/L)[a]	Mean 96-hr Survival (%)
Lab Control	--	--	100
Solvent Control	--	--	100
0.2	0.25	0.042	100
0.5	NM	NM	100
1.1	NM	NM	100
2.5	1.9	0.91	0.00
5.5	NM	NM	0.00
12	9.9	9.3	0.00
NOEL (µg/L)			1.1
LOEL (µg/L)			2.5
LC_{50} (µg/L)			1.8
LC_{25} (µg/L)			1.5

[a] 12 µg/L concentration measured at day 1, when full mortality occurred.
NM = Not Measured
NOEL = No Observed Effect Level
LOEL = Lowest Observed Effect Level
LC_{50} = the concentration at which 50 percent of the organisms show a lethal effect
LC_{25} = 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 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	200	66.0	84.1 ± 71.8	42.7

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

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.

Kyoshiro Hiki, et al. Environmental Science & Technology Letters 2021 8 (9), 779-784 DOI: 10.1021/acs.estlett.1c00453

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

OECD. 2019b. Test No. 203: Fish, Acute Toxicity Test. OECD Guidelines for the Testing of Chemicals, Section 2.

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:	12-3487-5269	**Test Type:**	Survival (96h)	**Analyst:**		
Start Date:	06 Sep-23 15:15	**Protocol:**	OECD 203	**Diluent:**	Laboratory Freshwater	
Ending Date:	10 Sep-23 15:15	**Species:**	Oncorhynchus mykiss	**Brine:**	Not Applicable	
Test Length:	96h	**Taxon:**		**Source:**	Thomas Fish Co.	**Age:** 51d post-hatch

Sample ID:	00-5700-2169	**Code:**	365C8B9	**Project:**	6PPD-quinone
Sample Date:	~~06-Sep-23~~ 6-9-23	**Material:**	Chemical Product	**Source:**	Washington Department of Ecology
Receipt Date:	~~06-Sep-23~~ 11/21/23	**CAS (PC):**		**Station:**	6PPD-quinone
Sample Age:	15h	**Client:**	Washington Department of Ecology		

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
18-5393-3670	96h Survival Rate	Fisher Exact Test		1.1	2.5	1.658	---	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	µg/L	95% LCL	95% UCL	S
00-0827-6895	96h Survival Rate	Linear Interpolation (ICPIN)		EC25	1.45	1.45	1.45	1
				EC50	1.8	1.8	1.8	

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%
0.2		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
0.5		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
1.1		2	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
2.5		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
5.5		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
12		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

96h Survival Rate Detail — MD5: 45FC67E29C39E3AD2AE2CCBE7987F509

Conc-µg/L	Code	Rep 1	Rep 2
0	S	1.000	1.000
0	LC	1.000	1.000
0.2		1.000	1.000
0.5		1.000	1.000
1.1		1.000	1.000
2.5		0.000	0.000
5.5		0.000	0.000
12		0.000	0.000

S = solvent control

CETIS Analytical Report

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

Analysis ID:	18-5393-3670	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	30 Oct-23 12:40	**Analysis:**	Single 2x2 Contingency Table	**Status Level:**	1
Edit Date:	30 Oct-23 12:39	**MD5 Hash:**	0DB7DFC4292BA4CD67ECDF2464EBDA8	**Editor ID:**	007-926-968-0

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units
Untransformed	C > T	1.1	2.5	1.658	---

Fisher Exact Test

Control	vs	Conc-µg/L	Test Stat	P-Type	P-Value	Decision(α:5%)
Lab Control		0.2	1.000	Exact	1.0000	Non-Significant Effect
		0.5	1.000	Exact	1.0000	Non-Significant Effect
		1.1	1.000	Exact	1.0000	Non-Significant Effect

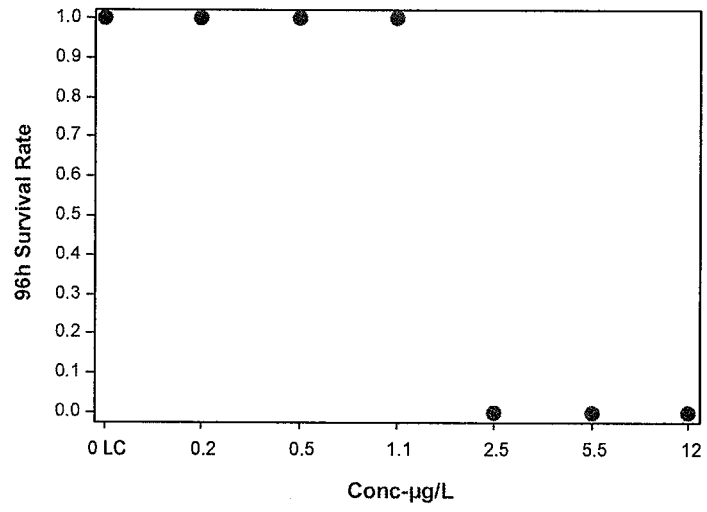
96h Survival Rate Frequencies

Conc-µg/L	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
0	LC	10	0	10	1.000	0.000	0.00%
0.2		10	0	10	1.000	0.000	0.00%
0.5		10	0	10	1.000	0.000	0.00%
1.1		10	0	10	1.000	0.000	0.00%
2.5		0	10	10	0.000	1.000	100.00%
5.5		0	10	10	0.000	1.000	100.00%
12		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%
0.2		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
0.5		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
1.1		2	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
2.5		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
5.5		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
12		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

Graphics



CETIS Analytical Report

Test Code/ID: 2309-S049 / 06-1504-4963

Acute Fish Survival Test — Nautilus Environmental (CA)

Analysis ID:	00-0827-6895	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	30 Oct-23 12:40	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	30 Oct-23 12:39	**MD5 Hash:**	0DB7DFC4292BA4CD67ECDF2464EBDA8	**Editor ID:**	007-926-968-0

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	24428	1000	Yes	Two-Point Interpolation

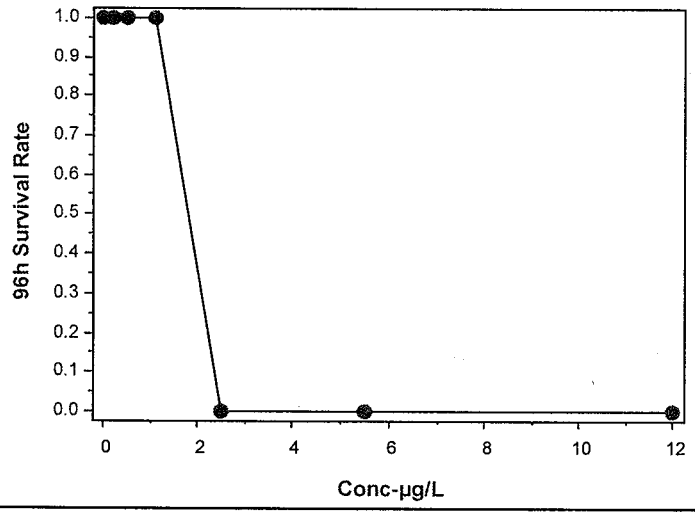
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	1.45	1.45	1.45
EC50	1.8	1.8	1.8

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%
0.2		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
0.5		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
1.1		2	1.000	1.000	1.000	1.000	0.00%	0.00%	10/10	1.000	0.00%
2.5		2	0.000	0.000	0.000	0.000	---	100.00%	0/10	0.000	100.00%
5.5		2	0.000	0.000	0.000	0.000	---	100.00%	0/10	0.000	100.00%
12		2	0.000	0.000	0.000	0.000	---	100.00%	0/10	0.000	100.00%

Graphics



Freshwater Acute Bioassay
Static Conditions
DF-018

OECD 203

Water Quality Measurements & Test Organism Survival

Client: WADOE
Sample ID: 6PPD-quinone
Test No.: 2309-5049

Test Species: *O. mykiss*
Start Date/Time: 9/6/23 1515
End Date/Time: 9/10/23 1515

Concentration (ug/L)	Rep	Number of Live Organisms						Conductivity (umhos/cm)					Temperature (°C) Q1				Q2	Dissolved Oxygen Q14 Q14 (mg/L) (A) (B)					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	330	336	353	331	324	12.2	10.6	11.1	11.6	10.9	11.7	10.4	9.2	8.8	8.9	7.55	7.40	7.28	7.33	7.30	100
	B	5	5	5	5	5	5	335	338	355	333	330	12.5	10.5	11.0	11.5	10.9	11.4	10.4	9.2	8.5	8.6	7.56	7.38	7.30	7.26	7.30	
0.2	A	5	5	5	5	5	5	336	337	356	333	332	12.6	11.1	11.5	11.9	11.4	11.3	9.7	8.9	8.6	8.4	7.58	7.31	7.26	7.24	7.29	100
	B	5	5	5	5	5	5	336	337	355	332	331	12.5	11.0	11.5	11.9	11.3	11.3	9.6	9.1	8.7	8.6	7.59	7.34	7.31	7.30	7.29	
0.5	A	5	5	5	5	5	5	336	338	356	334	334	12.6	11.2	11.6	12.0	11.5	11.2	9.5	9.0	9.0	9.0	7.58	7.35	7.26	7.24	7.30	100
	B	5	5	5	5	5	5	336	338	355	333	333	12.7	11.1	11.6	12.0	11.4	11.3	9.1	8.3	7.9	8.0	7.58	7.31	7.25	7.27	7.23	
1.1	A	5	5	5	5	5	5	336	338	356	335	335	12.4	11.1	11.7	12.0	11.4	11.3	9.2	8.6	8.8	8.6	7.58	7.33	7.29	7.26	7.28	100
	B	5	5	5	5	5	5	337	338	356	334	334	12.4	11.1	11.6	12.0	11.5	11.3	9.4	8.9	8.8	8.8	7.58	7.36	7.34	7.35	7.31	
2.5	A	5	5 (B)	~~5~~ 3	2	1	0	337	341	363	343	352	12.4	11.1	11.6	12.0	11.4	11.3	9.5	9.2	6.7	4.0	7.58	7.37	7.36	7.25	7.06	0
	B	5	5 (B)	~~5~~ 3	3	3	0	337	340	360	339	345	12.4	11.1	11.7	12.0	11.5	11.3	9.7	9.5	7.7	5.4	7.58	7.40	7.41	7.31	7.14	
5.5	A	5	5 (B)	~~5~~ 0	—	—	—	337	340	—	—	—	12.5	11.0	—	—	—	11.3	10.5	—	—	—	7.58	7.48	—	—	—	0
	B	5	5 (B)	~~5~~ 0	—	—	—	336	340	—	—	—	12.4	11.1	—	—	—	11.3	11.0	—	—	—	7.57	7.49	—	—	—	
12	A	5	5	0	—	—	—	337	346	—	—	—	12.5	11.0	—	—	—	11.3	10.9	—	—	—	7.58	7.47	—	—	—	0
	B	5	5	0	—	—	—	337	340	—	—	—	12.4	11.0	—	—	—	11.3	11.0	—	—	—	7.58	7.54	—	—	—	
Tech Initials	Counts	KR	BO	GM	GM	GM	KR																					
	WQ	KR	X	GM	GM	GM	KR																					
	QC	BO																										

Fish Size at test initiation*:

Weights (g): (A) ~~0.42~~ ~~0.43~~ 0.44 (A) ~~0.41~~ ~~0.47~~ 0.36 (A) ~~0.45~~ 0.58 — (A) ~~0.43~~ ~~0.440~~ 0.45

Lengths (cm): (A) ~~3.8~~ ~~3.7~~ 3.9 (A) ~~3.9~~ ~~4.0~~ 3.8 (A) ~~3.0~~ 4.3 — (A) ~~3.4~~ 3.9

Environmental Chamber: D

Loading rate = (A) ~~0.714~~ ~~0.77~~ 0.75 g/L

Sample Description: Dark Orange Powder

Animal Source/Date Received: Thomas Fish Co. 8/22/23

Age at Initiation: 51 days post-hatch

Comments: *5 random fish are sacrificed at initiation for size determination.
(A) Q18 KR 9/6/23 (B) Q18 GM 9/7/23

QC Check: AES 10/30/23

Final Review: [illegible] 11/12/23

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: 6PPD-quinone
Test No.: 2309-5049

Test Species: *O. mykiss*
Start Date/Time: 9/6/23 1515
End Date/Time: 9/10/23 1515

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	
Acetone	A	5	5	5	5	5	5	334	337	355	334	331	12.4	11.0	11.5	11.9	11.3	11.3	9.9	9.8	9.1	9.0	7.57	7.36	7.35	7.42	7.34	100
Control	B	5	5	5	5	5	5	334	337	354	333	331	12.5	10.9	11.4	11.8	11.2	11.3	9.6	9.4	8.6	8.7	7.58	7.33	7.33	7.33	7.30	
Tech Initials	Counts	KR	BO	GM	GM	GM	HM 09/20 KR																					
	WQ	KR	X	GM	GM	GM	KR																					
	QC	BO																										

Environmental Chamber: D

Fish Size at test initiation*:

Weights (g): (A) ~~0.42~~ 0.42 0.44 0.41 0.38 0.58 μ = 0.45

Lengths (cm): 3.8 3.9 3.9 3.8 4.3 μ = 3.9

Loading rate = ~~0.74~~ 0.75 g/L

Sample Description: Dark Orange Powder

Animal Source/Date Received: Thomas Fish Co 8/22/23

Age at Initiation: 51 days post-hatch

Comments: *5 random fish are sacrificed at initiation for size determination.
(A) 0.98 KR 9/6/23

QC Check: ACS 10/30/23

Final Review: 11/12/23

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

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

Test Code/ID: 230906omra / 19-8080-9942

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

Batch ID:	07-4282-2881	**Test Type:**	Survival (96h)	**Analyst:**			
Start Date:	06 Sep-23 15:30	**Protocol:**	Washington DOE (2009)	**Diluent:**	Laboratory Freshwater		
Ending Date:	10 Sep-23 15:30	**Species:**	Oncorhynchus mykiss	**Brine:**	Not Applicable		
Test Length:	96h	**Taxon:**		**Source:**	Thomas Fish Co.	**Age:**	37d

post swim-up

Sample ID:	09-6435-6717	**Code:**	~~397AEA6D~~ 230906omra	**Project:**	Internal
Sample Date:	06 Sep-23	**Material:**	Copper chloride	**Source:**	Copper Chloride
Receipt Date:	06 Sep-23	**CAS (PC):**		**Station:**	
Sample Age:	16h	**Client:**			

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
10-2125-9240	96h Survival Rate	Dunnett Multiple Comparison Test		200	400	282.8	48.4%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	µg/L	95% LCL	95% UCL	S
18-9941-8060	96h Survival Rate	Trimmed Spearman-Kärber		EC50	66	39.3	111	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	0.750	-1.160	2.660	0.600	0.900	0.150	0.212	28.28%	25.00%
50		2	0.400	0.400	0.400	0.400	0.400	0.000	0.000	0.00%	60.00%
100		2	0.300	-0.971	1.570	0.200	0.400	0.100	0.141	47.14%	70.00%
200		2	0.550	-2.630	3.730	0.300	0.800	0.250	0.354	64.28%	45.00%
400		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

96h Survival Rate Detail — MD5: C61BBA9DCCE02D9961CDDA1036FAFD2

Conc-µg/L	Code	Rep 1	Rep 2
0	LC	1.000	1.000
25		0.900	0.600
50		0.400	0.400
100		0.400	0.200
200		0.300	0.800
400		0.000	0.000

@98 BO 10/6/23

Acute Fish Survival Test — Nautilus Environmental (CA)

Analysis ID:	10-2125-9240	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	13 Sep-23 11:26	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	13 Sep-23 11:24	**MD5 Hash:**	084E3B7EFB51C4B7EF16E4190613B1D8	**Editor ID:**	009-628-326-6

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	200	400	282.8	---	0.484	48.36%

Dunnett Multiple Comparison Test

Control	vs	Conc-µg/L	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Lab Control		25	2	1.61	2.85	0.61	CDF	0.2100	Non-Significant Effect
		50*	2	3.4	2.85	0.61	CDF	0.0276	Significant Effect
		100*	2	3.91	2.85	0.61	CDF	0.0163	Significant Effect
		200	2	2.65	2.85	0.61	CDF	0.0623	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.889206	0.222302	4	4.84	0.0569	Non-Significant Effect
Error	0.229442	0.0458885	5			
Total	1.11865		9			

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	0.96	0.741	0.7909	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%
25		2	0.750	0.000	1.000	0.750	0.600	0.900	0.150	28.28%	25.00%
50		2	0.400	0.399	0.401	0.400	0.400	0.400	0.000	0.00%	60.00%
100		2	0.300	0.000	1.000	0.300	0.200	0.400	0.100	47.14%	70.00%
200		2	0.550	0.000	1.000	0.550	0.300	0.800	0.250	64.28%	45.00%
400		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

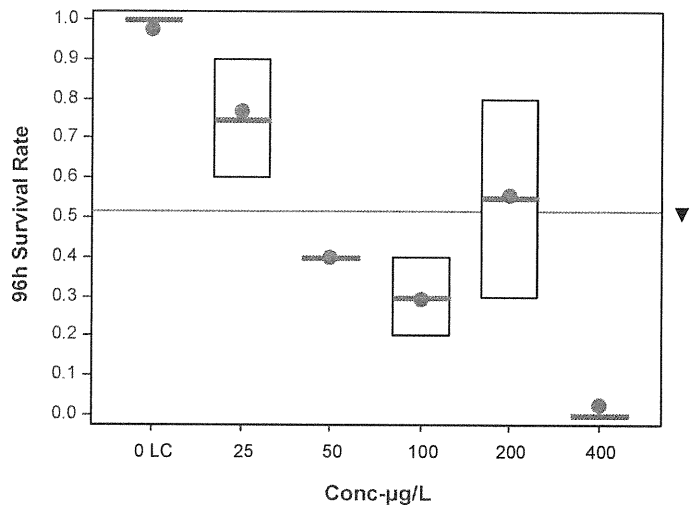
Angular (Corrected) Transformed Summary

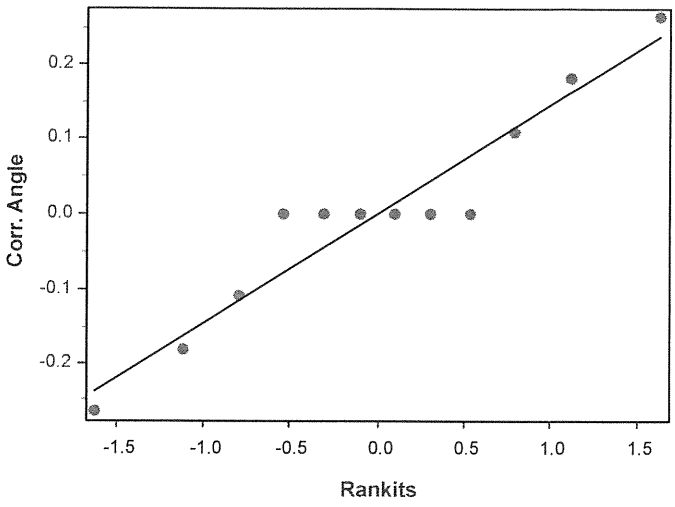
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	2	1.410	1.410	1.420	1.410	1.410	1.410	0.000	0.00%	0.00%
25		2	1.070	-1.240	3.370	1.070	0.886	1.250	0.181	24.04%	24.39%
50		2	0.685	0.684	0.685	0.685	0.685	0.685	0.000	0.00%	51.51%
100		2	0.574	-0.830	1.980	0.574	0.464	0.685	0.111	27.22%	59.34%
200		2	0.843	-2.510	4.190	0.843	0.580	1.110	0.264	44.23%	40.27%
400		2	0.159	0.159	0.159	0.159	0.159	0.159	0.000	0.00%	88.76%

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

Analysis ID:	10-2125-9240	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	13 Sep-23 11:26	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	13 Sep-23 11:24	**MD5 Hash:**	084E3B7EFB51C4B7EF16E4190613B1D8	**Editor ID:**	009-628-326-6

Graphics





CETIS Analytical Report

Test Code/ID: 230906omra / 19-8080-9942

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

Analysis ID:	18-9941-8060	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	13 Sep-23 11:26	**Analysis:**	Trimmed Spearman-Kärber	**Status Level:**	1
Edit Date:	13 Sep-23 11:24	**MD5 Hash:**	084E3B7EFB51C4B7EF16E4190613B1D8	**Editor ID:**	009-628-326-6

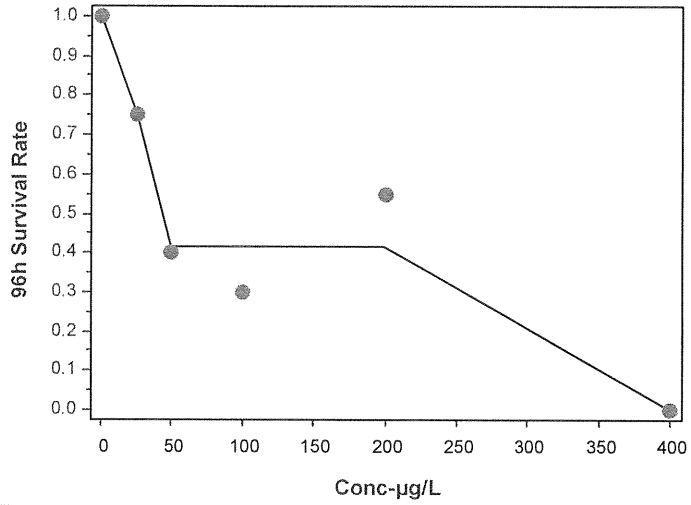
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	25.00%	1.82	0.112	66	39.3	111

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	0.750	0.750	0.600	0.900	28.28%	25.00%	15/20	0.750	25.00%
50		2	0.400	0.400	0.400	0.400	0.00%	60.00%	8/20	0.417	58.30%
100		2	0.300	0.300	0.200	0.400	47.14%	70.00%	6/20	0.417	58.30%
200		2	0.550	0.550	0.300	0.800	64.28%	45.00%	11/20	0.417	58.30%
400		2	0.000	0.000	0.000	0.000	---	100.00%	0/20	0.000	100.00%

Graphics

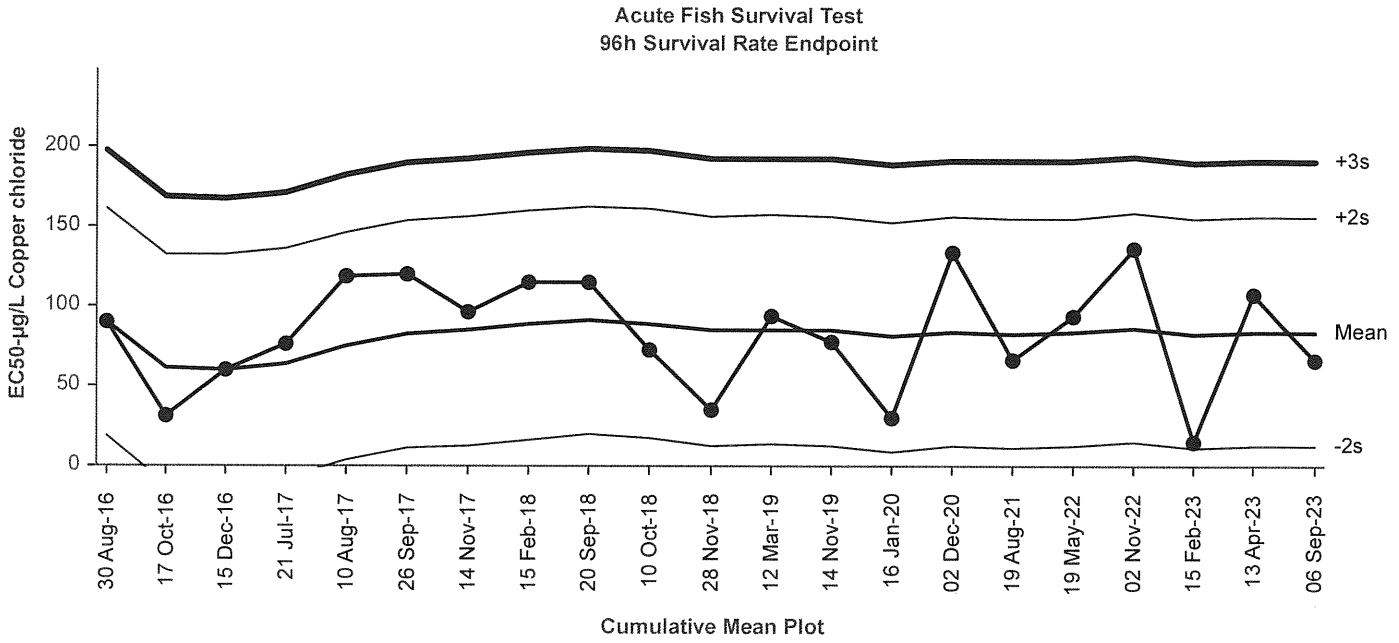


CETIS QC Plot

Report Date: 13 Sep-23 11:27 (1 of 1)

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



Mean:	84.1	**Count:**	20	**-2s Warning Limit:**	12.3	**-3s Action Limit:**	-23.5
Sigma:	35.88	**CV:**	42.70%	**+2s Warning Limit:**	156	**+3s Action Limit:**	192

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2016	Aug	30	13:05	90.13	6.025	0.1679			06-6522-4245	11-7821-6703
2		Oct	17	15:45	31.5	-52.6	-1.466			11-9706-2514	03-3027-4661
3		Dec	15	13:00	59.46	-24.64	-0.6867			07-2059-1930	21-0698-8947
4	2017	Jul	21	11:45	75.79	-8.314	-0.2317			12-6230-4373	10-5665-4943
5		Aug	10	13:35	119.1	34.96	0.9745			09-7390-4688	11-1885-9400
6		Sep	26	15:10	120.1	35.97	1.003			12-0767-7259	14-1478-0761
7		Nov	14	11:25	96.22	12.12	0.3379			21-0521-5529	14-2305-2435
8	2018	Feb	15	15:00	114.5	30.44	0.8483			08-5122-1964	18-9847-1069
9		Sep	20	14:05	114.9	30.77	0.8576			14-1527-8451	21-3828-6142
10		Oct	10	16:40	72.55	-11.55	-0.3219			06-8408-1163	05-7761-5868
11		Nov	28	12:00	35.36	-48.74	-1.359			21-0374-7072	19-9377-5872
12	2019	Mar	12	12:10	93.3	9.203	0.2565			11-1972-1376	05-1051-7815
13		Nov	14	11:55	77.34	-6.764	-0.1885			08-3948-6775	01-9304-4998
14	2020	Jan	16	12:50	30.63	-53.47	-1.49			15-5355-8442	09-8383-1081
15		Dec	2	13:30	133.3	49.23	1.372			07-0223-4669	10-8492-8883
16	2021	Aug	19	14:25	65.98	-18.12	-0.5051			11-4973-5943	08-1400-5422
17	2022	May	19	12:45	93.3	9.203	0.2565			03-2996-8953	07-9626-2312
18		Nov	2	10:05	136.6	52.5	1.463			14-6511-1746	10-0042-0505
19	2023	Feb	15	11:30	14.71	-69.39	-1.934			17-0773-8757	02-8314-6307
20		Apr	13	15:15	107.2	23.08	0.6432			04-5815-1771	04-7188-7445
21		Sep	6	15:30	65.98	-18.12	-0.5051			19-8080-9942	18-9941-8060

Freshwater Acute Bioassay
Static Conditions
DF-019

Dangerous Waste Characterization

Water Quality Measurements & Test Organism Survival

Client: Internal
Sample ID: $CuCl_2$
Test No.: 230906OMRA

Test Species: *O. mykiss*
Start Date/Time: 9/6/23 1530
End Date/Time: 9/10/23 1530

Concentration (µg/L)	RAND #	Number of Live Organisms					Conductivity (umhos/cm)					Temperature (°C) Q1 Q2					Dissolved Oxygen (mg/L) Q14 Q14 Q14					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	329	329	339	323	323	12.8	10.7	11.4	11.9	11.0	11.3	11.6	11.5	9.6	9.8	7.35	7.28	7.43	7.34	7.34	100
	8	10	10	10	10	10	332	334	351	329	330	11.9	10.7	11.5	11.7	11.1	11.1	10.6	10.4	9.7	9.10	7.35	7.32	7.40	7.34	7.33	100
25	9	10	10	9	9	9	334	335	351	329	331	11.7	10.6	11.4	11.7	11.0	10.9	10.1	9.1	7.8	8.0	7.35	7.25	7.30	7.24	7.16	90
	5	10	10	10	7	6	335	336	352	330	330	11.6	10.2	11.0	11.2	10.6	11.0	10.2	9.2	8.3	8.8	7.36	7.28	7.30	7.24	7.27	60
50	7	10	10	8	8	4	335	335	354	331	332	11.7	10.7	11.6	11.8	11.1	10.9	9.9	8.8	8.2	8.8	7.36	7.27	7.27	7.28	7.30	40
	4	10	10	10	7	4	336	335	352	330	331	11.6	10.3	11.0	11.2	10.7	11.0	9.9	8.5	7.1	7.7	7.35	7.26	7.23	7.17	7.19	40
100	12	10	10	9	5	4	336	336	353	331	331	11.6	10.4	11.1	11.5	10.8	11.0	9.8	8.8	8.3	8.9	7.36	7.25	7.24	7.23	7.26	40
	3	10	10	9	7	2	336	335	353	331	332	11.6	10.3	11.0	11.3	10.7	10.9	9.8	8.6	8.7	9.1	7.36	7.27	7.26	7.29	7.30	20
200	1	10	10	10	8	3	336	336	354	332	334	11.7	10.5	11.3	11.5	10.9	10.9	9.6	8.3	8.3	9.3	7.33	7.24	7.22	7.23	7.32	30
	11	10	10	10	9	8	337	337	355	333	335	11.6	10.5	11.3	11.5	10.9	10.9	9.7	8.7	8.6	8.9	7.34	7.23	7.28	7.22	7.15	80
400	2	10	10	1	0	-	336	336	355	331	-	11.6	10.4	11.2	11.4	-	10.9	9.9	9.5	10.0	-	7.29	7.22	7.38	7.38	-	0
	6	10	10	1	0	-	335	336	354	332	-	11.5	10.2	11.0	11.2	-	10.9	9.9	9.5	10.2	-	7.31	7.27	7.35	7.41	-	0
Tech Initials:	Counts	KR	GM	GM	GM	KR													Q14	Q14		Recorded in Log Pass/Fail:					
	Readings	GM	GM	GM	GM	KR																					
	QC	BO																									

Environmental Chamber: D

Dilution Calcs (final volume 8L) made by: KR/GM

Conc. µg/L	25	50	100	200	400
Vol. Cu stock added (mL)	2.1	4.2	8.3	16.7	33.3
Cu Stock Conc. (µg/L)	10,000 →				

Weights (g): 0.43 0.44 0.47 0.36 0.45 0.50 0.41 0.52 0.58 0.58 µ = 0.48

[b] Lengths (mm): 3.7 3.9 4.0 3.8 4.0 4.1 3.9 3.9 4.3 4.2 µ = 4.0 (4) ~~3.98~~

Loading: 0.69/L

Length max/min = 4.3/3.7

Ratio of longest to shortest = ~~1.10~~ 1.2 (4)

Animal Source/Date Received: (A) ~~ABS~~ Thomas Fish Co. 8/22/23

Hatch Date: 7/17/23

Swim-up Date: 7/31/23

Days post Swim-up: 37 days

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) Q1 KR 9/10/23 (B) Q1 BO 9/8/23 (d) Q18 CM 9/10/23

QC Check: CM 9/13/23

Final Review: BO 10/6/23

Appendix D

Chemical Analysis

WECK LABORATORIES, INC.

Certificate of Analysis

FINAL REPORT

Work Orders:	3I12041	**Report Date:**	10/25/2023
		Received Date:	9/12/2023
Project:	6PPD	**Turnaround Time:**	Normal
		Phones:	(858) 587-7333
		Fax:	(858) 587-3961
Attn:	Barbara Orelo	**P.O. #:**	
Client:	Enthalpy Analytical - San Diego 4340 Vandever Avenue San Diego, CA 92120	**Billing Code:**	

ELAP-CA #1132 • EPA-UCMR #CA00211 • LACSD #10143

This is a complete final report. The information in this report applies to the samples analyzed in accordance with the chain-of-custody document. Weck Laboratories certifies that the test results meet all requirements of TNI unless noted by qualifiers or written in the Case Narrative. This analytical report must be reproduced in its entirety.

Dear Barbara Orelo,

Enclosed are the results of analyses for samples received 9/12/23 with the Chain-of-Custody document. The samples were received in good condition, at 4.3 °C and on ice. All analyses met the method criteria except as noted in the case narrative or in the report with data qualifiers.

Reviewed by:

Ryan J. Gasio
Project Manager

14859 Clark Avenue,City of Industry CA, 91745 | Phone: (626) 336-2139 | Fax: (626) 336-2634

www.wecklabs.com

WECK LABORATORIES, INC.

Certificate of Analysis

FINAL REPORT

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

Project Number: 6PPD

Project Manager: Barbara Orelo

Reported:
10/25/2023 17:25

Sample Summary

Sample Name	Sampled By	Lab ID	Matrix	Sampled	Qualifiers
6PPD quinone - 1,000 ug/L stock	BO	3I12041-01	Water	09/06/23 14:00	
6PPD quinone - 0.2 ug/L - Day 0	BO	3I12041-02	Water	09/06/23 14:00	
6PPD quinone - 2.5 ug/L - Day 0	BO	3I12041-03	Water	09/06/23 14:00	
6PPD quinone - 12 ug/L - Day 0	BO	3I12041-04	Water	09/06/23 14:00	
6PPD quinone - 0.2 ug/L - Day 4	BO	3I12041-05	Water	09/10/23 10:30	
6PPD quinone - 2.5 ug/L - Day 4	BO	3I12041-06	Water	09/10/23 10:35	
6PPD quinone - 12 ug/L - Day 1	BO	3I12041-07	Water	09/07/23 13:45	

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

Project Number: 6PPD

Project Manager: Barbara Orelo

Reported: 10/25/2023 17:25

Sample Results

Sample: 6PPD quinone - 1,000 ug/L stock — Sampled: 09/06/23 14:00 by BO

3I12041-01RE1 (Water)

Analyte	Result	MRL	Units	Dil	Analyzed	Qualifier
Organic Compounds - Low Level by Tandem LC/MS/MS						
Method: EPA 1694M		**Instr:** LCMS03				
Batch ID: W3I1112 **Preparation:** _NONE (LC)		**Prepared:** 09/14/23 11:14				**Analyst:** jna
6PPD-quinone	**980000**	20000	ng/l	10000	09/15/23	M-06

Sample Results

Sample: 6PPD quinone - 0.2 ug/L - Day 0 — Sampled: 09/06/23 14:00 by BO

3I12041-02RE1 (Water)

Analyte	Result	MRL	Units	Dil	Analyzed	Qualifier
Organic Compounds - Low Level by Tandem LC/MS/MS						
Method: EPA 1694M		**Instr:** LCMS03				
Batch ID: W3I1112 **Preparation:** _NONE (LC)		**Prepared:** 09/14/23 11:14				**Analyst:** jna
6PPD-quinone	**250**	4.0	ng/l	2	09/15/23	M-06

Sample Results

Sample: 6PPD quinone - 2.5 ug/L - Day 0 — Sampled: 09/06/23 14:00 by BO

3I12041-03RE1 (Water)

Analyte	Result	MRL	Units	Dil	Analyzed	Qualifier
Organic Compounds - Low Level by Tandem LC/MS/MS						
Method: EPA 1694M		**Instr:** LCMS03				
Batch ID: W3I1112 **Preparation:** _NONE (LC)		**Prepared:** 09/14/23 11:14				**Analyst:** jna
6PPD-quinone	**1900**	20	ng/l	10	09/15/23	M-06

Sample Results

Sample: 6PPD quinone - 12 ug/L - Day 0 — Sampled: 09/06/23 14:00 by BO

3I12041-04RE1 (Water)

Analyte	Result	MRL	Units	Dil	Analyzed	Qualifier
Organic Compounds - Low Level by Tandem LC/MS/MS						
Method: EPA 1694M		**Instr:** LCMS03				
Batch ID: W3I1112 **Preparation:** _NONE (LC)		**Prepared:** 09/14/23 11:14				**Analyst:** jna
6PPD-quinone	**9900**	100	ng/l	50	09/15/23	M-06

Sample Results

Sample: 6PPD quinone - 0.2 ug/L - Day 4 — Sampled: 09/10/23 10:30 by BO

3I12041-05RE1 (Water)

Analyte	Result	MRL	Units	Dil	Analyzed	Qualifier
Organic Compounds - Low Level by Tandem LC/MS/MS						
Method: EPA 1694M		**Instr:** LCMS03				
Batch ID: W3I1112 **Preparation:** _NONE (LC)		**Prepared:** 09/14/23 11:14				**Analyst:** jna
6PPD-quinone	**42**	2.0	ng/l	1	09/15/23	

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10/25/2023 17:25

Sample Results

(Continued)

Sample: 6PPD quinone - 2.5 ug/L - Day 4

Sampled: 09/10/23 10:35 by BO

3I12041-06RE1 (Water)

Analyte	Result	MRL	Units	Dil	Analyzed	Qualifier
Organic Compounds - Low Level by Tandem LC/MS/MS						
Method: EPA 1694M		**Instr:** LCMS03				
Batch ID: W3I1112 **Preparation:** _NONE (LC)		**Prepared:** 09/14/23 11:14				**Analyst:** jna
6PPD-quinone	**910**	20	ng/l	10	09/15/23	M-06

Sample Results

(Continued)

Sample: 6PPD quinone - 12 ug/L - Day 1

Sampled: 09/07/23 13:45 by BO

3I12041-07RE1 (Water)

Analyte	Result	MRL	Units	Dil	Analyzed	Qualifier
Organic Compounds - Low Level by Tandem LC/MS/MS						
Method: EPA 1694M		**Instr:** LCMS03				
Batch ID: W3I1112 **Preparation:** _NONE (LC)		**Prepared:** 09/14/23 11:14				**Analyst:** jna
6PPD-quinone	**9300**	100	ng/l	50	09/15/23	M-06

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Reported:
10/25/2023 17:25

Quality Control Results

Organic Compounds - Low Level by Tandem LC/MS/MS

Analyte	Result	MRL	Units	Spike Level	Source Result	%REC	%REC Limits	RPD	RPD Limit	Qualifier
Batch: W3I1112 - EPA 1694M										
Blank (W3I1112-BLK1)				**Prepared: 09/14/23**	**Analyzed: 09/15/23**					
6PPD-quinone	ND	2.0	ng/l							
IPPD-quinone	ND	2.0	ng/l							
LCS (W3I1112-BS1)				**Prepared: 09/14/23**	**Analyzed: 09/15/23**					
6PPD-quinone	23.7	2.0	ng/l	25.0		95	50-150			
IPPD-quinone	24.7	2.0	ng/l	25.0		99	50-150			
Matrix Spike (W3I1112-MS1)	**Source: 3H30053-01**			**Prepared: 09/14/23**	**Analyzed: 09/15/23**					
6PPD-quinone	25.0	2.0	ng/l	25.0	ND	100	50-150			
IPPD-quinone	26.1	2.0	ng/l	25.0	ND	104	50-150			
Matrix Spike Dup (W3I1112-MSD1)	**Source: 3H30053-01**			**Prepared: 09/14/23**	**Analyzed: 09/15/23**					
6PPD-quinone	27.1	2.0	ng/l	25.0	ND	108	50-150	8	200	
IPPD-quinone	26.8	2.0	ng/l	25.0	ND	107	50-150	3	200	

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10/25/2023 17:25

Notes and Definitions

Item	Definition
M-06	Due to the high concentration of analyte inherent in the sample, sample was diluted prior to preparation and/or analysis. The MDL and MRL were raised due to this dilution.
%REC	Percent Recovery
Dil	Dilution
MRL	The minimum levels, concentrations, or quantities of a target variable (e.g., target analyte) that can be reported with a specified degree of confidence. The MRL is also known as Limit of Quantitation (LOQ)
ND	NOT DETECTED at or above the Method Reporting Limit (MRL). If Method Detection Limit (MDL) is reported, then ND means not detected at or above the MDL.
RPD	Relative Percent Difference
Source	Sample that was matrix spiked or duplicated.

Any remaining sample(s) will be disposed of one month from the final report date unless other arrangements are made in advance.

All results are expressed on wet weight basis unless otherwise specified.

All samples collected by Weck Laboratories have been sampled in accordance to laboratory SOP Number MIS002.

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