

# TOXICITY TESTING RESULTS

## 6PPD ALTERNATIVE TESTING

**Prepared for**

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All testing reported herein was performed consistent with our laboratory's quality assurance program. All results are intended to be considered in their entirety, and EcoAnalysts is not responsible for use of less than the complete report. The test results summarized in this report apply only to the sample(s) evaluated. This document is uncontrolled when printed or accessed from electronic distribution.

**APPROVED BY**



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## APPENDIX

Appendix A:	Laboratory Documents
Appendix B:	Available Certificates of Analysis

## ACRONYMS AND ABBREVIATIONS

ASTM	American Society for Testing and Materials
CoA	Certificate of Analysis
EPA	Environmental Protection Agency
LC <sub>25</sub>	Lethal Concentration to 25% of test population
LC <sub>50</sub>	Lethal Concentration that results in a 50% reduction in survival
LOEL	Lowest Observed Effect Level
mg/L	Milligrams per liter
NOEL	No Observed Effect Level
QM	Quality manual
µg/L	Micrograms per liter
SOP	Standard operation procedure
WDOE	Washington Department of Ecology

## 1. EXECUTIVE SUMMARY

EcoAnalysts conducted toxicity testing on seven compounds as part of an evaluation of the relative toxicity of potential alternatives to N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) in tire manufacturing. The toxicity to three species was evaluated: the invertebrates *Ceriodaphnia dubia* (water flea) and *Hyalella azteca* (amphipod), and the vertebrate *Oncorhynchus mykiss* (rainbow trout).

A summary of the results is provided below (Table 1-1).

**Table 1-1. Toxicity Test Results Summary.**

Test	Compound	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> (µg/L)	LC <sub>50</sub> (µg/L)
Water Flea ( <i>Ceriodaphnia dubia</i> ) 48-hour Survival	6PPD	250	500	353.6	500.0
	6PPD Ozonate	500	1000	548.4	691.0
	Alpha-tocopherol	8403	>8403	>8403	>8403
	Rambutan Peel Extract	10400	>10400	>10400	>10400
	Sinapic Acid	70000	>70000	>70000	>70000
	Durazone 37	1400	>1400	>1400	>1400
	Irganox 1076 <sup>1</sup>	340000	>340000	>340000	>340000
Amphipod ( <i>Hyalella azteca</i> ) 24-hour Survival	6PPD	500	1000	530.7	655.5
	6PPD Ozonate	500	1000	591.0	752.3
	Alpha-tocopherol <sup>2</sup>	3232	8403	4174	>8403
	Rambutan Peel Extract	10400	>10400	>10400	>10400
	Sinapic Acid <sup>2</sup>	70000	140000	94360	>140000
	Durazone 37	1400	>1400	>1400	>1400
	Irganox 1076 <sup>1</sup>	500000	>500000	>500000	>500000
Rainbow Trout ( <i>Oncorhynchus mykiss</i> ) 96-hour Survival	6PPD	70	140	88.24	122.7
	6PPD Ozonate	35	70	58.91	86.49
	Alpha-tocopherol <sup>2</sup>	8403	>8403	>8403	>8403
	Rambutan Peel Extract	10400	>10400	>10400	>10400
	Sinapic Acid	24000	>24000	>24000	>24000
	Durazone 37	136	>136	>136	>136
	Irganox 1076 <sup>1</sup>	3400000	>340000	>340000	>340000

<sup>1</sup> Compound fell out of solution upon contact with water. Actual concentrations tested likely much lower.

<sup>2</sup> Compared against acetone control. See Section 2.1 for more information.

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

LC<sub>25</sub> = Lethal Concentration to 25% of test population

LC<sub>50</sub> = Lethal Concentration to 50% of test population

## 2. METHODS

The samples analyzed for toxicity were tested using criteria outlined in the Environmental Protection Agency’s (EPA) and American Society for Testing and Materials’ (ASTM) most recently promulgated effluent guidance documents outlined in Section 4.

Bioassay testing for this project consisted of three acute bioassays. The tests conducted in support of this project are summarized in Table 2-1. All tests were conducted in glass containers to minimize adsorption of the chemicals to the test chambers. This necessitated a decrease in volume for the rainbow trout test from the recommended 4L to 3.25L. The maximum loading rate of 0.8 g fish/L was not exceeded, however, therefore this deviation is not expected to have affected the results.

**Table 2-1. Biological Testing Performed.**

Test Type	Test Descriptor	Species	Method
Acute	Static 48-hour Survival	<i>Ceriodaphnia dubia</i> Water Flea	EPA-821-R-02-012 Method 2000.0; SOP TOX004.11
	Static 24-Hour Survival	<i>Hyalella azteca</i> Amphipod	ASTM E1706-20; SOP TOX022.06 Modified
	Static Renewal 96-hour Survival	<i>Oncorhynchus mykiss</i> Rainbow Trout	EPA-821-R-02-012 Method 2019.0; SOP TOX016.08

### 2.1 Compounds Tested

Table 2-2 summarizes the compounds that were tested. The Certificates of Analysis (CoA), available for all compounds except the rambutan peel extract and the 6PPD ozonate, can be found in Appendix B. The purity of 6PPD, Durazone 37, sinapic acid and Irganox 1076 were all ≥90%, therefore the dilutions for these compounds, along with 6PPD ozonate, were prepared with no adjustment for purity. Rambutan peel extract, though no CoA was available from the supplier, was reported to have a purity of approximately 48 to 50%. A purity of 49% was assumed, and the amount of material added to the exposures was increased accordingly to reach the target nominal concentrations. The CoA for the lot of alpha-tocopherol used was acquired post testing and indicated a purity of 80.8%. The concentrations tested were adjusted for purity post testing on the datasheets and in the statistics, and the adjusted concentrations are reported here.

All stock solutions and test dilutions were prepared in glass to minimize adsorption of the test material, and the time between test concentration preparation and test initiation was minimized (≤ 1 hr) to avoid substantial compound transformation prior to exposure.

All compounds except the rambutan peel extract were dissolved in acetone, and an acetone control was prepared at the highest concentration of acetone used per round of testing. The rambutan peel extract was received dissolved in 22-25% glycerin therefore was already miscible in water without the use of acetone (and acetone and glycerin are immiscible). A glycerin control was prepared at the highest concentration of glycerin used in each test, assuming a 25% glycerin concentration in the test material.

During the first round of testing performed with alpha-tocopherol and sinapic acid on amphipods and rainbow trout, it was noted that the acetone control, with a concentration of 0.5%, was toxic to the test organisms. The acetone concentration was decreased to a maximum of 0.25% for subsequent testing. For those tests where the acetone was believed to have contributed to the toxicity observed in the test material (alpha-tocopherol for amphipods and rainbow trout, and sinapic acid for amphipods), statistical comparisons were made to the acetone control instead of the dilution control to remove the influence of the acetone toxicity. The target sinapic acid concentration series was decreased by half for the water

flea test, because less material would dissolve in the amount of acetone allowed at a maximum concentration of 0.25%.

All test compound concentrations reported are nominal values.

It was noted that Irganox 1076 fell out of solution upon contact with water at all concentrations in all three exposures. It is unlikely that the actual dissolved concentration was similar to the nominal values reported.

**Table 2-2. Compounds Tested.**

Compound	Manufacturer/ Supplier	Lot #	CAS #	Date Received
6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine)	HB Chemical/Akron Rubber Development	5221031128	793-24-8	8/30/24
6PPD ozonate	HB chemical/Akron Rubber Development	5221031128 (ozonated)	793-24-8 (ozonated)	8/30/24
Alpha-tocopherol	Spectrum Chemical Mfg Corp/VWR	1NG0212	59-02-9	8/2/24
Rambutan Peel Extract	Esentials of Australia	1704001	93165-68-5	8/20/24
Sinapic Acid (3,5-dimethoxy-4-hydroxycinnamic acid)	Indofine Chem Co./VWR	1512719	530-59-6	8/22/24
Durazone 37 (2,4,6 Tris-(N-1, 4-dimethylpentyl- p-phenylenediamino)- 1,3,5-triazine)	Akrochem	CD1J23P008	121246-28-4	8/22/24
Irganox 1076 (Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate)	TCI America/VWR	C2PNC-GN	2082-79-3	8/13/24

## 2.2 Water for Bioassay Testing

Freshwater diluent used in the water flea and amphipod tests was prepared using the EPA method for standard, synthetic moderately hard, reconstituted water, using the reagent grade chemicals, with the addition of selenium for the water flea test (USEPA 2002). The diluent in the rainbow trout test was carbon filtered tap water. Extensive testing on a variety of test species has shown that there is no significant potential for toxicity or bioaccumulation of contaminants from these water supplies. Chemical analysis of the water sources is conducted and reviewed on an annual basis. The hardness and alkalinity of the diluent waters used are listed in Table 2-3.

**Table 2-3. Hardness and Alkalinity of Dilution Water.**

Water Type (Test Organism)	Hardness (mg CaCO <sub>3</sub> /L)	Alkalinity (mg CaCO <sub>3</sub> /L)
Synthetic Moderately-Hard Water (Water Flea)	82-87	55-58
Synthetic Moderately-Hard Water (Amphipod)	87	55
Carbon Filtered Tap Water (Rainbow Trout)	59-69	70

### **2.3 Quality Assurance/Quality Control**

The quality assurance objectives for toxicity testing conducted by the testing laboratory are detailed in the laboratory's quality manual (QM) as well as the method specific guidance documents (USEPA 2002; ASTM 2020). The methods employed in every phase of the toxicity testing program are detailed in the EcoAnalysts Standard Operating Practices (SOP). All EcoAnalysts staff members receive regular, documented training in all SOPs and test methods. Finally, all data collected and produced because of these analyses were recorded on approved data sheets.

### **2.4 Data Management and Analysis**

Endpoint data were calculated for each replicate and the mean value and standard deviation were determined for each test treatment. All hand-entered data were reviewed for data entry errors, which were corrected prior to summary calculations. A minimum of 10% of all calculations and data sorting were reviewed for errors. Review counts were conducted on any apparent outliers.

Statistical comparisons were made according to the EPA guidance. Statistical comparisons were performed using CETIS™ software (CETIS 2022).

### 3. RESULTS

The results of the effluent testing are presented in this section. Statistical comparisons and laboratory documents are provided in Appendix A.

#### 3.1 Water Flea (*Ceriodaphnia dubia*) Acute Test Results

The acute toxicity tests with *C. dubia* on 6PPD, 6PPD ozonate, Durazone 37 and Irganox 1076 were initiated on September 5, 2024. The tests with alpha-tocopherol and sinapic acid were initiated on September 11, 2024, and the test with rambutan peel extract was initiated on September 26, 2024. All tests met the survival acceptability criterion of  $\geq 90\%$  with a mean control survival of 100% in both the dilution water and solvent controls. Mean survival and statistical results are summarized in Table 3-1 through Table 3-7. The test conditions are summarized in Table 3-8. The Irganox 1076 test had an interrupted dose response, where the 85,000  $\mu\text{g/L}$  concentration was significantly different than the control but none of the other concentrations were. The higher NOEL/LOEL pair was selected for reporting. The variable response in the Irganox 1076 test was likely due to the presence of flaky material in the test chambers caused by the material falling out of solution upon contact with water.

Water quality parameters were within the acceptable limits throughout the duration of the 48-hour static test. Six organisms were added to Replicate 4 of the sinapic acid acetone control, Replicate 1 of the 6PPD acetone control, Replicate 1 of the 400  $\mu\text{g/L}$  concentration of alpha-tocopherol, and Replicate 2 of the 170,000  $\mu\text{g/L}$  concentration of Irganox 1076. The start count was adjusted for statistical analysis.

The  $\text{LC}_{50}$  for the copper chloride reference-toxicant test was 7.14  $\mu\text{g Cu/L}$  for survival. These results were within two standard deviations of the laboratory mean for survival and reproduction (Table 3-8). This indicates that the organisms are of a similar sensitivity to those previously tested at the EcoAnalysts laboratory.

**Table 3-1. Endpoint Summary for the *Ceriodaphnia dubia* Acute Test – 6PPD**

Conc. ( $\mu\text{g/L}$ )	6PPD					
	Mean Survival (%)	Standard Deviation	NOEL ( $\mu\text{g/L}$ )	LOEL ( $\mu\text{g/L}$ )	$\text{LC}_{25}$ Value ( $\mu\text{g/L}$ )	$\text{LC}_{50}$ Value ( $\mu\text{g/L}$ )
Control (0)	100	0	250	500	353.6	500.0
Acetone Control (0.25%)	100	0				
125	100	0				
250	100	0				
500	50.0	11.6				
1000	10.0	11.6				
2000	0	0				

**Table 3-2. Endpoint Summary for the *Ceriodaphnia dubia* Acute Test – 6PPD ozonate**

Conc. (µg/L)	6PPD ozonate					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	100	0	500	1000	548.4	691.0
Acetone Control (0.25%)	100	0				
125	100	0				
250	100	0				
500	85.0	10.0				
1000	10.0	11.6				
2000	0	0				

**Table 3-3. Endpoint Summary for the *Ceriodaphnia dubia* Acute Test – Alpha-Tocopherol**

Conc. (µg/L)	Alpha-Tocopherol					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	100	0	8403	>8403	>8403	>8403
Acetone Control (0.25%)	100	0				
16	100	0				
323	100	0				
808	100	0				
3232	100	0				
8403	95.0	10.0				

**Table 3-4. Endpoint Summary for the *Ceriodaphnia dubia* Acute Test – Rambutan Peel Extract**

Conc. (µg/L)	Rambutan Peel Extract					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	100	0	10400	>10400	>10400	>10400
Glycerin Control (0.13%)	100	0				
20	100	0				
40	100	0				
1000	100	0				
4000	100	0				
10400	100	0				

**Table 3-5. Endpoint Summary for the *Ceriodaphnia dubia* Acute Test – Sinapic Acid**

Conc. (µg/L)	Sinapic Acid					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	100	0	70000	>70000	>70000	>70000
Acetone Control (0.25%)	100	0				
4375	100	0				
8750	100	0				
17500	100	0				
35000	95.0	10.0				
70000	95.0	10.0				

**Table 3-6. Endpoint Summary for the *Ceriodaphnia dubia* Acute Test – Durazone 37**

Conc. (µg/L)	Durazone 37					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	100	0	1400	>1400	>1400	>1400
Acetone Control (0.25%)	100	0				
87.5	100	0				
175	100	0				
350	100	0				
700	95.0	10.0				
1400	100	0				

**Table 3-7. Endpoint Summary for the *Ceriodaphnia dubia* Acute Test – Irganox 1076**

Conc. (µg/L) <sup>1</sup>	Irganox 1076					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	100	0	340000	>340000	>340000	>340000
Acetone Control (0.25%)	100	0				
21250	95.0	10.0				
42500	95.0	10.0				
85000 <sup>2</sup>	80.0	0				
170000	82.5	23.6				
340000	100	0				

<sup>1</sup>Compound fell out of solution upon contact with water. Actual dissolved concentrations tested likely much lower. Flakey film on surface of all concentrations. Precipitate in highest concentration.

<sup>2</sup>Interrupted dose response. Only concentration significantly different than control.

**Table 3-8. Test Condition Summary for *Ceriodaphnia dubia* Acute Test**

Test Duration / Type		48-hour / Static	
Species		<i>Ceriodaphnia dubia</i>	
Supplier		Aquatic BioSystems	
Test Dates		9/5/24 – 9/7/24: 6PPD, 6PPD ozonate, Durazone 37, Irganox 1076 9/11/24 – 9/13/24: alpha-tocopherol, sinapic acid 9/26/24 – 9/28/24: rambutan peel extract	
Age at test initiation (Recommended: ≤ 24 hours)		≤ 24 hours	
<b>Test Procedures</b>		EPA-821-R-02-012 Method 2000.0; SOP TOX004.11	
Test location		EcoAnalysts; Port Gamble, WA	
Control water / Diluent		Cerio Reconstituted Freshwater (CRFW)	
Test Lighting		16 hour light / 8 hour dark	
Test Chamber		20-mL Glass Chamber	
Exposure volume		15 mL	
Replicates/treatment		4	
Organisms/replicate		5	
Feeding		None	
Test solution renewal		None	
<b>Test Water Quality</b>		<b>Recommended</b>	<b>Actual</b>
Test Dissolved Oxygen (Recommended:)		≥ 2.0 mg/L	8.4 – 9.7 mg/L
Test Temperature (Recommended:)		20 ± 1°C	19.4 – 20.5 °C
Test Conductivity		Not specified	245 – 360 µS/cm
Test pH (Range not specified)		Not specified Targeted Range: 6 – 9 units	7.0 – 8.1 units
<b>Quality Assurance</b>			
<b>Control performance standards</b> Survival (Recommended): ≥ 90%		100%; meets acceptability criterion	
<b>Reference Toxicant Date</b>		9/11/24	
Survival	Reference Toxicant LC <sub>50</sub>	7.14 µg Cu/L	
	Laboratory Mean LC <sub>50</sub>	14.9 µg Cu/L	
	Range LC <sub>50</sub> (±2 SD)	6.79 – 32.9 µg Cu/L	
<b>Deviations from Test Protocol</b>		Start count of 6 in 4 chambers	

### 3.2 Amphipod (*Hyalella azteca*) Acute Test Results

The acute toxicity tests with *H. azteca* on alpha-tocopherol, rambutan peel extract and sinapic acid were initiated on August 29, 2024. The tests with 6PPD, 6PPD ozonate, Durazone 37 and Irganox 1076 were initiated on September 5, 2024. All tests met the survival acceptability criterion of  $\geq 90\%$  in the dilution water with a mean control survival of 90 to 97.5%. The solvent controls had a mean survival of 77.5% to 100%, with survival less than 90% in the acetone controls for alpha-tocopherol and sinapic acid. The low survival in the acetone controls for these two compounds were likely due to the concentration of acetone (0.5%). The concentration of acetone was decreased to a maximum of 0.25% for subsequent testing. Test concentrations were compared to the acetone control for statistical analysis of alpha-tocopherol and sinapic acid. Mean survival and statistical results are summarized in Table 3-9 to Table 3-15. The test conditions are summarized in Table 3-24.

Water quality parameters were within the acceptable limits throughout the duration of the 24-hour static test. The sinapic acid/alpha-tocopherol acetone control Replicate 3, sinapic acid 35,000  $\mu\text{g/L}$  Replicate 4 and sinapic acid 140,000  $\mu\text{g/L}$  Replicate 3 were initiated with 11 organisms instead of 10, and rambutan peel extract 20  $\mu\text{g/L}$  Replicate 1 was initiated with 12 organisms. The start counts were adjusted for statistical analysis.

The  $\text{LC}_{50}$  for the copper chloride reference-toxicant test was 982  $\mu\text{g Cu/L}$  for survival. These results were within two standard deviations of the laboratory mean for survival and reproduction (Table 3-24). This indicates that the organisms are of a similar sensitivity to those previously tested at the EcoAnalysts laboratory.

**Table 3-9. Endpoint Summary for the *Hyalella azteca* Acute Test – 6PPD**

Conc. ( $\mu\text{g/L}$ )	6PPD					
	Mean Survival (%)	Standard Deviation	NOEL ( $\mu\text{g/L}$ )	LOEL ( $\mu\text{g/L}$ )	$\text{LC}_{25}$ Value ( $\mu\text{g/L}$ )	$\text{LC}_{50}$ Value ( $\mu\text{g/L}$ )
Control (0)	97.5	5.0	500	1000	530.7	655.5
Acetone Control (0.13%)	97.5	5.0				
62.5	95.0	10.0				
125	95.0	5.8				
250	97.5	5.0				
500	80.0	18.3				
1000	0.0	0.0				

**Table 3-10. Endpoint Summary for the *Hyalella azteca* Acute Test – 6PPD ozonate**

Conc. (µg/L)	6PPD ozonate					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	97.5	5.0	500	1000	591.0	752.3
Acetone Control (0.13%)	97.5	5.0				
62.5	95.0	5.8				
125	95.0	5.8				
250	97.5	5.0				
500	90.0	11.6				
1000	20.0	8.2				

**Table 3-11. Endpoint Summary for the *Hyalella azteca* Acute Test – Alpha-Tocopherol**

Conc. (µg/L)	Alpha-Tocopherol <sup>1</sup>					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	90.0	8.2	3232	8403	4174	>8403
Acetone Control (0.5%)	77.5	17.1				
16	95.0	10.0				
323	80.0	14.1				
808	67.5	5.0				
3232	75.0	17.3				
8403	47.5	20.6				

<sup>1</sup>Statistical analysis performed against acetone control

**Table 3-12. Endpoint Summary for the *Hyalella azteca* Acute Test – Rambutan Peel Extract**

Conc. (µg/L)	Rambutan Peel Extract					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	90.0	8.2	10400	>10400	>10400	>10400
Glycerin Control (0.13%)	97.5	5.0				
20	95.0	5.8				
400	87.5	12.6				
1000	87.5	12.6				
4000	87.5	9.6				
10400	87.5	15.0				

**Table 3-13. Endpoint Summary for the *Hyalella azteca* Acute Test – Sinapic Acid**

Conc. (µg/L)	Sinapic Acid <sup>1</sup>					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	90.0	8.2	70000	140000	94360	>140000
Acetone Control (0.5%)	77.5	17.1				
8750	87.5	9.6				
17500	70.0	8.2				
35000	78.0	9.8				
70000	85.0	12.9				
140000	41.6	5.9				

<sup>1</sup>Statistical analysis performed against acetone control

**Table 3-14. Endpoint Summary for the *Hyalella azteca* Acute Test – Durazone 37**

Conc. (µg/L)	Durazone 37					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	97.5	5.0	1400	>1400	>1400	>1400
Acetone Control (0.25%)	100	0				
87.5	97.5	5.0				
175	100	0				
350	100	0				
700	97.5	5.0				
1400	95.0	10.0				

**Table 3-15. Endpoint Summary for the *Hyalella azteca* Acute Test – Irganox 1076**

Conc. (µg/L) <sup>1</sup>	Irganox 1076					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	97.5	5.0	500000	>500000	>500000	>500000
Acetone Control (0.25%)	100	0				
31250	97.5	5.0				
62500	95.0	5.8				
125000	100	0				
250000	92.5	9.6				
500000	95.0	5.8				

<sup>1</sup>Compound fell out of solution upon contact with water. Actual dissolved concentrations tested likely much lower. Flakey film on surface of all concentrations. Precipitate in highest concentration.

Table 3-16. Test Condition Summary for *Hyalella azteca* Acute Test

Test Duration / Type		24-hour / Static	
Species		<i>Hyalella azteca</i>	
Supplier		Aquatic Indicators	
Test Dates		9/5/24 – 9/6/24: 6PPD, 6PPD ozonate, Durazone 37, Irganox 1076 8/29/24 – 8/30/24: alpha-tocopherol, sinapic acid, rambutan peel extract	
Age at test initiation (Recommended: 7 to 14 days)		7 to 14 days	
<b>Test Procedures</b>		ASTM E1706-20; SOP TOX022.06 Modified	
Test location		EcoAnalysts; Port Gamble, WA	
Control water / Diluent		Reconstituted Freshwater (RFW)	
Test Lighting		16 hour light / 8 hour dark	
Test Chamber		500-mL Glass Beaker	
Exposure volume		250 mL	
Replicates/treatment		4	
Organisms/replicate		10	
Feeding		None	
Test solution renewal		None	
<b>Test Water Quality</b>		<b>Recommended</b>	<b>Actual</b>
Test Dissolved Oxygen		≥ 4.0 mg/L	8.1 – 9.1 mg/L
Test Temperature		23 ± 1°C	21.9 – 23.6 °C
Test Conductivity		Not specified	307 – 373 µS/cm
Test pH		Not specified Targeted Range: 6 – 9 units	6.6 – 8.6 units
<b>Quality Assurance</b>			
<b>Control performance standards</b> Survival (Recommended): ≥ 90%		90.0 – 97.5%; meets acceptability criterion	
<b>Reference Toxicant Date</b>		9/03/24	
Survival	Reference Toxicant LC <sub>50</sub>	982 µg Cu/L	
	Laboratory Mean LC <sub>50</sub>	733 µg Cu/L	
	Range LC <sub>50</sub> (±2 SD)	157 – 3240 µg Cu/L	
<b>Deviations from Test Protocol</b>		Start count in 5 chambers	

### 3.3 Rainbow Trout (*Oncorhynchus mykiss*) Acute Test Results

The acute toxicity tests with *O. mykiss* on alpha-tocopherol, rambutan peel extract and sinapic acid were initiated on August 29, 2024. The tests with 6PPD, 6PPD ozonate, Durazone 37 and Irganox 1076 were initiated on September 5, 2024. All tests met the survival acceptability criterion of  $\geq 90\%$  in the dilution water with a mean control survival of 100%. The solvent controls had a mean survival of 55.0% to 97.5%, with survival less than 90% in the acetone control for alpha-tocopherol and sinapic acid. The low survival in the acetone control for these two compounds was likely due to the concentration of acetone (0.5%). The concentration of acetone was decreased to a maximum of 0.25% for subsequent testing. Test concentrations were compared to the acetone control for statistical analysis of alpha-tocopherol. There was no toxicity in any concentration of sinapic acid outside of the acetone control, therefore statistical analysis was performed against the dilution water control for this compound. The maximum acetone concentration in the sinapic acid treatments was 0.09%, below that in the acetone control. Mean survival and statistical results are summarized in Table 3-17 to Table 3-23. The test conditions are summarized in Table 3-24.

Water quality parameters were within the acceptable limits throughout the duration of the 96-hour static-renewal test, with the exception of deviations in temperature and dissolved oxygen. The temperature was recorded above the limit of  $12 \pm 1^\circ\text{C}$  on Day 3 in some of the 6PPD and 6PPD ozonate treatments, at a maximum value of  $16.2^\circ\text{C}$  in the 6PPD treatment and  $14.8^\circ\text{C}$  in the 6PPD ozonate treatment. The bath temperature was decreased, and temperatures were within acceptable range by the end of the day. Dissolved oxygen was measured at a concentration of 5.5 mg/L in the 6PPD ozonate 70  $\mu\text{g/L}$  treatment on Day 4. As the test was terminating, no aeration was initiated. These deviations were within the tolerance range of the test organisms and should not have impacted the significance of the test results.

The  $\text{LC}_{50}$  for the sodium dodecyl sulfate (SDS) reference-toxicant test was 4.68 mg/L SDS. These results were within two standard deviations of the laboratory mean at the time of testing. These results suggest the test organisms used in this study of similar sensitivity to those used previously at the EcoAnalysts laboratory.

**Table 3-17. Endpoint Summary for the *Oncorhynchus mykiss* Acute Test – 6PPD**

Conc. ( $\mu\text{g/L}$ )	6PPD					
	Mean Survival (%)	Standard Deviation	NOEL ( $\mu\text{g/L}$ )	LOEL ( $\mu\text{g/L}$ )	$\text{LC}_{25}$ Value ( $\mu\text{g/L}$ )	$\text{LC}_{50}$ Value ( $\mu\text{g/L}$ )
Control (0)	100	0	70	140	88.2	122.7
Acetone Control (0.07%)	97.5	5.0				
35	97.5	5.0				
70	92.5	9.6				
140	40.0	14.1				
280	0	0				
560	0	0				

**Table 3-18. Endpoint Summary for the *Oncorhynchus mykiss* Acute Test – 6PPD ozonate**

Conc. (µg/L)	6PPD ozonate					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	100	0	35	70	58.9	86.5
Acetone Control (0.07%)	97.5	5.0				
35	97.5	5.0				
70	67.5	5.0				
140	10.0	0				
280	0	0				
560	0	0				

**Table 3-19. Endpoint Summary for the *Oncorhynchus mykiss* Acute Test – Alpha-Tocopherol**

Conc. (µg/L)	Alpha-Tocopherol <sup>1</sup>					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	100	0	8403	>8403	>8403	>8403
Acetone Control (0.5%)	55.0	20.8				
16	100	0				
323	100	0				
808	100	0				
3232	100	0				
8403	72.5	9.6				

<sup>1</sup>Statistical analysis performed against acetone control

**Table 3-20. Endpoint Summary for the *Oncorhynchus mykiss* Acute Test – Rambutan Peel Extract**

Conc. (µg/L)	Rambutan Peel Extract					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	100	0	10400	>10400	>10400	>10400
Glycerin Control (0.13%)	97.5	5.0				
20	100	0				
400	100	0				
1000	100	0				
4000	100	0				
10400	100	0				

**Table 3-21. Endpoint Summary for the *Oncorhynchus mykiss* Acute Test – Sinapic Acid**

Conc. (µg/L)	Sinapic Acid					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	100	0	24000	>24000	>24000	>24000
Acetone Control (0.5%)	55.0	20.8				
1500	100	0				
3000	100	0				
6000	100	0				
12000	100	0				
24000	100	0				

**Table 3-22. Endpoint Summary for the *Oncorhynchus mykiss* Acute Test – Durazone 37**

Conc. (µg/L)	Durazone 37					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	100	0	136	>136	>136	>136
Acetone Control (0.02%)	95.0	5.8				
8.5	95.0	5.8				
17	100	0				
34	97.5	5.0				
68	100	0				
136	97.5	5.0				

**Table 3-23. Endpoint Summary for the *Oncorhynchus mykiss* Acute Test – Irganox 1076**

Conc. (µg/L) <sup>1</sup>	Irganox 1076					
	Mean Survival (%)	Standard Deviation	NOEL (µg/L)	LOEL (µg/L)	LC <sub>25</sub> Value (µg/L)	LC <sub>50</sub> Value (µg/L)
Control (0)	100	0	340000	>340000	>340000	>340000
Acetone Control (0.25%)	97.5	5.0				
21250	100	0				
42500	100	0				
85000	100	0				
170000	100	0				
340000	90.0	14.1				

<sup>1</sup>Compound fell out of solution upon contact with water. Actual dissolved concentrations tested likely much lower. Flakey film on surface of all concentrations. Precipitate in highest concentration.

**Table 3-24. Test Condition Summary for *Oncorhynchus mykiss* Acute Test.**

Test Duration / Type		96-Hour; Static-Renewal	
Species		<i>Oncorhynchus mykiss</i>	
Supplier		Thomas Fish Co.	
Test Dates		9/5/24 – 9/9/24: 6PPD, 6PPD ozonate, Durazone 37, Irganox 1076 8/29/24 – 9/2/24: alpha-tocopherol, sinapic acid, rambutan peel extract	
Age at test initiation Recommended: 15 -30 days post swim up		Actual: 19 to 26 days post swim up	
Test Procedures		EPA-821-R-02-012 Method 2019.0; SOP TOX016.08	
Test location		EcoAnalysts; Port Gamble, WA	
Control water / Diluent		Carbon Filtered Tap Water (CFTW)	
Test Lighting		16 hour light / 8 hour dark	
Test Chamber		4-L Glass Chamber	
Exposure volume		3.25 L	
Replicates/treatment		4	
Organisms/replicate		10	
Loading rate Recommended: ≤0.8 g/L		0.6 to 0.7 g/L	
Feeding		None	
Test solution renewal		Day 2	
Test Water Quality		Recommended	Actual
Test Dissolved Oxygen		≥ 6.0 mg/L	5.5 – 11.0 mg/L
Test Temperature		12 ± 1°C	10.5 – 16.2 °C
Test Conductivity		NA	145 – 203 µS/cm
Test pH		6 – 9	6.8 – 9.4
Quality Assurance			
Control performance standard		Recommended: ≥ 90% survival	Actual: 100%, Pass
Reference Toxicant Date		8/28/24	
Survival	Reference Toxicant LC <sub>50</sub>	4.68 mg/L sodium dodecyl sulfate	
	Laboratory Mean LC <sub>50</sub>	4.10 mg/L sodium dodecyl sulfate	
	Acceptable Range LC <sub>50</sub> (± SD)	2.35 – 7.17 mg/L sodium dodecyl sulfate	
Deviations from Test Protocol		Temperature, dissolved oxygen, exposure volume	

## 4. REFERENCES

- ASTM 2020. E1706-20: "Standard Test Method for Measuring the Toxicity of Sediment Associated Contaminants with Freshwater Invertebrates." Annual Book of Standards. Volume 11.06 "Biological Effects and Environmental Fate; Biotechnology." American Society of Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA.
- CETIS. 2022 CETIS™ Comprehensive Environmental Toxicity Information System User's Guide. Tidepool Scientific Software. McKinleyville, CA.
- Lee, D.R. 1980. Reference toxicants in quality control of aquatic bioassays: Aquatic invertebrate bioassays. In Buikema AL Jr, Cairns J Jr, eds, *Proceedings*, 2nd Annual Symposium on Aquatic Toxicology. STP 715. American Society for Testing and Materials, Philadelphia, PA, pp 188–199.
- USEPA. 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. EPA-821-R-02-013.
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## **APPENDIX A**

### **LABORATORY DOCUMENTS**

## **APPENDIX A.1**

### ***CERIODAPHNIA DUBIA* (WATER FLEA) 48-HOUR SURVIVAL TEST**

#### **STATISTICAL COMPARISONS AND LABORATORY DATA SHEETS**

# CETIS Summary Report

Report Date: 03 Oct-24 13:30 (p 1 of 2)  
 Test Code/ID: 6PPD C.du. / 05-4930-9241

## Ceriodaphnia 48-h Acute Survival Test

EcoAnalysts

Batch ID: 07-9720-4568	Test Type: Survival (48h)	Analyst: Mary Ann Rempel-Hester
Start Date: 05 Sep-24 14:40	Protocol: EPA/821/R-02-012 (2002)	Diluent: Cerio Reconstituted Fresh Water
Ending Date: 07 Sep-24 14:30	Species: Ceriodaphnia dubia	Brine: Not Applicable
Test Length: 48h	Taxon: Branchiopoda	Source: Aquatic Biosystems, CO Age: 24H
Sample ID: 12-8955-8383	Code: Lot 5221031128	Project: 6PPD Alternative Testing 2024
Sample Date: 30 Aug-24 12:00	Material: 6PPD	Source: Akron Rubber Development (HB Chem
Receipt Date: 30 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 6d 3h	Client: Washington Department of Ecology	

### Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
04-1821-2276	48h Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	1.0000	Acetone Control passed 48h proportion sur	1

### Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
15-0088-2826	48h Proportion Survived	Steel Many-One Rank Sum Test	250	500	353.6	12.4%	1

### Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
15-3814-3848	48h Proportion Survived	Linear Interpolation (ICPIN)	EC15	307.8	291	334.1	1
			EC20	329.9	306.1	367.8	
			EC25	353.6	321.9	404.8	
			EC40	435.3	373.9	538.8	
			EC50	500	412.8	619	

### Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
04-1821-2276	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
15-0088-2826	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
15-3814-3848	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria

### 48h Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0	AC	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
125		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
250		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
500		4	0.5000	0.3163	0.6837	0.4000	0.6000	0.0577	0.1155	23.09%	50.00%
1000		4	0.1000	-0.0837	0.2837	0.0000	0.2000	0.0577	0.1155	115.47%	90.00%
2000		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

### 48h Proportion Survived Detail

MD5: A2AD39EB8E0533C2E05A39DA6CF55ACD

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
0	AC	1.0000	1.0000	1.0000	1.0000
125		1.0000	1.0000	1.0000	1.0000
250		1.0000	1.0000	1.0000	1.0000
500		0.6000	0.4000	0.4000	0.6000
1000		0.2000	0.0000	0.0000	0.2000
2000		0.0000	0.0000	0.0000	0.0000

# CETIS Summary Report

Report Date: 03 Oct-24 13:30 (p 2 of 2)  
Test Code/ID: 6PPD C.du. / 05-4930-9241

Ceriodaphnia 48-h Acute Survival Test

EcoAnalysts

## 48h Proportion Survived Binomials

Conc- $\mu$ g/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	5/5	5/5	5/5	5/5
0	AC	6/6	5/5	5/5	5/5
125		5/5	5/5	5/5	5/5
250		5/5	5/5	5/5	5/5
500		3/5	2/5	2/5	3/5
1000		1/5	0/5	0/5	1/5
2000		0/5	0/5	0/5	0/5

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 13:30 (p 1 of 1)  
 Test Code/ID: 6PPD C.du. / 05-4930-9241

<b>Ceriodaphnia 48-h Acute Survival Test</b>				<b>EcoAnalysts</b>			
<b>Start Date:</b>	05 Sep-24 14:40	<b>Species:</b>	Ceriodaphnia dubia	<b>Sample Code:</b>	Lot 5221031128		
<b>End Date:</b>	07 Sep-24 14:30	<b>Protocol:</b>	EPA/821/R-02-012 (2002)	<b>Sample Source:</b>	Akron Rubber Development (HB Che		
<b>Sample Date:</b>	30 Aug-24 12:00	<b>Material:</b>	6PPD	<b>Sample Station:</b>			

Conc-µg/L	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Notes
0	AC	1	4	6	5	6	
0	AC	2	24	5	5	5	
0	AC	3	14	5	5	5	
0	AC	4	7	5	5	5	
0	D	1	16	5	5	5	
0	D	2	26	5	5	5	
0	D	3	12	5	5	5	
0	D	4	8	5	5	5	
125		1	6	5	5	5	
125		2	15	5	5	5	
125		3	19	5	5	5	
125		4	1	5	5	5	
250		1	27	5	5	5	
250		2	17	5	5	5	
250		3	18	5	5	5	
250		4	22	5	5	5	
500		1	23	5	5	3	
500		2	5	5	5	2	
500		3	9	5	5	2	
500		4	2	5	5	3	
1000		1	21	5	5	1	
1000		2	28	5	5	0	
1000		3	11	5	3	0	
1000		4	3	5	5	1	
2000		1	25	5	3	0	
2000		2	10	5	3	0	
2000		3	20	5	4	0	
2000		4	13	5	4	0	

Version V.1

GENERAL

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	6PPD
Test Type	48-Hour Acute
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	09/05/24
Test Species	<i>Ceriodaphnia dubia</i>
Organism Batch (Brood Board #)	ABS090524.02
Organism Acquired	In House Culture
Organism Acclimation	NA
Organism Age	<24 hour
Test Protocol	TOX 004
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Test Location	Bath 5
Water Description	cerio reconstituted freshwater
Organisms per Replicate	5
Test Chamber Size	20 mL
Exposure Volume	15 mL
Feeding Information	None
Test Dissolved Oxygen	> 2
Test Temperature	20 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	2	
Temp	18.5	21.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1440 CS
TEST END TIME/INIT:	1430 NL

COMPOUND	LOT #
6PPD	5221031128

Concentrations (µg/L)	
1	Control
2	Acetone Control
3	125
4	250
5	500
6	1000
7	2000

Copy and Past VALUES

Treatment	Rep	Chamber
Control	1	18
Control	2	1
Control	3	26
Control	4	28
Acetone Con	1	11
Acetone Con	2	21
Acetone Con	3	16
Acetone Con	4	5
125	1	12
125	2	10
125	3	13
125	4	27
250	1	7
250	2	17
250	3	25
250	4	14
500	1	24
500	2	6
500	3	8
500	4	22
1000	1	2
1000	2	3
1000	3	15
1000	4	23
2000	1	4
2000	2	20
2000	3	9
2000	4	19

v.1

<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	48-Hour Acute	<b>PROTOCOL</b>	TOX 004
<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	9/5/24	<b>PROJECT NUMBER</b>	PG2032
<b>COMPOUND</b>	6PPD	<b>TEST END DATE</b>	9/7/24	<b>SPECIES</b>	<i>Ceriodaphnia dubia</i>
<b>LOT #</b>	5221031128	<b>MATRIX</b>	Liquid	<b>NO. OF ORGANISMS</b>	5

**48-Hour Acute**

**Stock Preparation**

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
800,000	100	80.0

**Dilution Preparation**

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)
800,000	125	160	0.025
800,000	250	160	0.050
800,000	500	160	0.100
800,000	1000	160	0.200
800,000	2000	160	0.400
			<b>Amt. of Acetone (mL)</b>
	Acetone control	160	0.400

**Test Dilution Prep**

Date	Balance ID	Water Batch ID	Initials	Comments
9/5/2024		7 CRFW082724.02	MARH	

**Water Quality**

		Concentration (µg/L)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH
		> 2	> 2	20 ± 1	(µS/cm)	7.5 ± 1.5
<b>Day 0 (Stock)</b>	Control		8.9	20.4	316	7.8
Date	9/5/2024	Acetone Control	8.9	20.4	315	7.9
Time	1433	125	8.9	20.4	317	7.9
Tech	CS	250	8.9	20.4	317	7.9
Meter #	9	500	8.9	20.4	316	8.0
		1000	8.9	20.4	316	8.0
		2000	8.9	20.5	316	8.0
<b>Day 1</b>	Control		8.9	19.8	311	8.1
Surrogate	Acetone Control		8.8	19.8	310	8.1
Date	9/6/2024	125	8.9	19.8	310	8.1
Time	1212	250	8.9	19.8	312	8.1
Tech	DM	500	8.9	19.9	310	8.1
Meter #	8	1000	8.8	19.8	310	8.1
		2000	8.9	19.8	310	8.1
<b>Day 2</b>	Control		8.9	19.9	328	7.1
Surrogate	Acetone Control		8.9	19.9	312	7.3
Date	9/7/2024	125	9.0	19.9	312	7.4
Time	1000	250	8.9	19.8	314	7.5
Tech	EM	500	9.0	19.9	311	7.5
Meter #	8	1000	8.9	19.9	313	7.6
		2000	9.0	19.8	312	7.6

**Comments**

v.1	<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	48-Hour Acute
	<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	9/5/24
	<b>PROJECT #</b>	PG2032	<b>TEST END DATE</b>	9/7/24
	<b>COMPOUND</b>	6PPD	<b>MATRIX</b>	Liquid
	<b>LOT #</b>	5221031128	<b>SPECIES</b>	<i>Ceriodaphnia dubia</i>
	<b>PROTOCOL</b>	TOX 004	<b>NO. OF ORGANISMS</b>	5

**48-Hour Acute**
**Abbreviation Key:**

NB = No Body  
 FB = Found Body  
 ST = Stranded

Day 1		Day 2	
<b>Date</b>	09/06/24	<b>Date</b>	09/07/24
<b>Time</b>	1213	<b>Time</b>	1430
<b>Tech</b>	DM	<b>Tech</b>	NL

Concentration (µg/L)	REP.	Alive	Dead	Obs	Alive	Dead	Obs	Comments
Control	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
Acetone Control	1	5	0		6	0	1FB	Day 1 likely miscount
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
125	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
250	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
500	1	5	0		3	2		
	2	5	0		2	1	2NB	
	3	5	0		2	3		
	4	5	0		3	2		
1000	1	5	0		1	4		
	2	5	0		0	5		
	3	3	2		0	3		
	4	5	0		1	4		
2000	1	3	2		0	3		
	2	3	2		0	3		
	3	4	1		0	4		
	4	4	1		0	4		

# CETIS Summary Report

Report Date: 03 Oct-24 13:20 (p 1 of 2)  
 Test Code/ID: 6PPDoz C.du. / 04-5344-8471

## Ceriodaphnia 48-h Acute Survival Test

EcoAnalysts

Batch ID: 13-0904-3447	Test Type: Survival (48h)	Analyst: Mary Ann Rempel-Hester
Start Date: 05 Sep-24 14:00	Protocol: EPA/821/R-02-012 (2002)	Diluent: Cerio Reconstituted Fresh Water
Ending Date: 07 Sep-24 14:04	Species: Ceriodaphnia dubia	Brine: Not Applicable
Test Length: 48h	Taxon: Branchiopoda	Source: Aquatic Biosystems, CO Age: 24H
Sample ID: 09-4724-5485	Code: Lot 5221031128	Project: 6PPD Alternative Testing 2024
Sample Date: 30 Aug-24 12:00	Material: 6PPD ozonate	Source: Akron Rubber Development (HB Chem
Receipt Date: 30 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 6d 2h	Client: Washington Department of Ecology	

### Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
17-5143-4115	48h Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	1.0000	Acetone Control passed 48h proportion sur	1

### Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
12-4242-2308	48h Proportion Survived	Steel Many-One Rank Sum Test	500	1000	707.1	12.5%	1

### Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
04-7316-0486	48h Proportion Survived	Linear Interpolation (ICPIN)	EC15	500	372.8	572.4	1
			EC20	523.7	485.8	596.9	
			EC25	548.4	508.8	622.4	
			EC40	630	584.5	705.5	
			EC50	691	641.1	766.9	

### Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits			Overlap	Decision
				Lower	Upper			
04-7316-0486	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
12-4242-2308	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
17-5143-4115	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	

### 48h Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0	AC	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
125		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
250		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
500		4	0.8500	0.6909	1.0090	0.8000	1.0000	0.0500	0.1000	11.76%	15.00%
1000		4	0.1000	-0.0837	0.2837	0.0000	0.2000	0.0577	0.1155	115.47%	90.00%
2000		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

### 48h Proportion Survived Detail

MD5: 0106768800E300E4781AF8AE93CDE886

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
0	AC	1.0000	1.0000	1.0000	1.0000
125		1.0000	1.0000	1.0000	1.0000
250		1.0000	1.0000	1.0000	1.0000
500		0.8000	0.8000	1.0000	0.8000
1000		0.2000	0.0000	0.2000	0.0000
2000		0.0000	0.0000	0.0000	0.0000

# CETIS Summary Report

Report Date: 03 Oct-24 13:20 (p 2 of 2)  
Test Code/ID: 6PPDoz C.du. / 04-5344-8471

## Ceriodaphnia 48-h Acute Survival Test

EcoAnalysts

### 48h Proportion Survived Binomials

Conc- $\mu$ g/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	5/5	5/5	5/5	5/5
0	AC	5/5	5/5	5/5	5/5
125		5/5	5/5	5/5	5/5
250		5/5	5/5	5/5	5/5
500		4/5	4/5	5/5	4/5
1000		1/5	0/5	1/5	0/5
2000		0/5	0/5	0/5	0/5

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 13:20 (p 1 of 1)  
 Test Code/ID: 6PPDoz C.du. / 04-5344-8471

<b>Ceriodaphnia 48-h Acute Survival Test</b>				<b>EcoAnalysts</b>			
<b>Start Date:</b>	05 Sep-24 14:00	<b>Species:</b>	Ceriodaphnia dubia	<b>Sample Code:</b>	Lot 5221031128		
<b>End Date:</b>	07 Sep-24 14:04	<b>Protocol:</b>	EPA/821/R-02-012 (2002)	<b>Sample Source:</b>	Akron Rubber Development (HB Che		
<b>Sample Date:</b>	30 Aug-24 12:00	<b>Material:</b>	6PPD ozonate	<b>Sample Station:</b>			

Conc-µg/L	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Notes
0	AC	1	4	5	5	5	
0	AC	2	24	5	5	5	
0	AC	3	14	5	5	5	
0	AC	4	7	5	5	5	
0	D	1	16	5	5	5	
0	D	2	26	5	5	5	
0	D	3	12	5	5	5	
0	D	4	8	5	5	5	
125		1	6	5	5	5	
125		2	15	5	5	5	
125		3	19	5	5	5	
125		4	1	5	5	5	
250		1	27	5	5	5	
250		2	17	5	5	5	
250		3	18	5	5	5	
250		4	22	5	5	5	
500		1	23	5	5	4	
500		2	5	5	5	4	
500		3	9	5	5	5	
500		4	2	5	5	4	
1000		1	21	5	5	1	
1000		2	28	5	4	0	
1000		3	11	5	5	1	
1000		4	3	5	5	0	
2000		1	25	5	4	0	
2000		2	10	5	5	0	
2000		3	20	5	4	0	
2000		4	13	5	3	0	

Version V.1

GENERAL

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	6PPD ozonate
Test Type	48-Hour Acute
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	09/05/24
Test Species	<i>Ceriodaphnia dubia</i>
Organism Batch (Brood Board #)	ABS090524.02
Organism Acquired	In House Culture
Organism Acclimation	NA
Organism Age	<24 hour
Test Protocol	TOX 004
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Test Location	Bath 5
Water Description	cerio reconstituted freshwater
Organisms per Replicate	5
Test Chamber Size	20 mL
Exposure Volume	15 mL
Feeding Information	None
Test Dissolved Oxygen	> 2
Test Temperature	20 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	2	
Temp	18.5	21.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1400 CS
TEST END TIME/INIT:	1404 NL

COMPOUND	LOT #
6PPD ozonate	5221031128 ozonate

Concentrations (µg/L)	
1	Control
2	Acetone Control
3	125
4	250
5	500
6	1000
7	2000

Copy and Past VALUES

Treatment	Rep	Chamber
Control	1	18
Control	2	1
Control	3	26
Control	4	28
Acetone Con	1	11
Acetone Con	2	21
Acetone Con	3	16
Acetone Con	4	5
125	1	12
125	2	10
125	3	13
125	4	27
250	1	7
250	2	17
250	3	25
250	4	14
500	1	24
500	2	6
500	3	8
500	4	22
1000	1	2
1000	2	3
1000	3	15
1000	4	23
2000	1	4
2000	2	20
2000	3	9
2000	4	19

v.1

<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	48-Hour Acute	<b>PROTOCOL</b>	TOX 004
<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	9/5/24	<b>PROJECT NUMBER</b>	PG2032
<b>COMPOUND</b>	6PPD ozonate	<b>TEST END DATE</b>	9/7/24	<b>SPECIES</b>	<i>Ceriodaphnia dubia</i>
<b>LOT #</b>	5221031128 ozonate	<b>MATRIX</b>	Liquid	<b>NO. OF ORGANISMS</b>	5

**48-Hour Acute**

**Stock Preparation**

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
800,000	100	80.0

**Dilution Preparation**

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)
800,000	125	160	0.025
800,000	250	160	0.050
800,000	500	160	0.100
800,000	1000	160	0.200
800,000	2000	160	0.400
			<b>Amt. of Acetone (mL)</b>
	Acetone control	160	0.400

**Test Dilution Prep**

Date	Balance ID	Water Batch ID	Initials	Comments
9/5/2024		7 CRFW082724.02	MARH	

**Water Quality**

	Concentration (µg/L)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH	
		> 2	20 ± 1		7.5 ± 1.5	
<b>Day 0 (Stock)</b>	Control	8.8	20.1	318	7.4	
Date	9/5/2024	Acetone Control	8.9	20.3	315	7.6
Time	1342	125	9.0	20.3	316	7.7
Tech	CS	250	8.9	20.3	317	7.8
Meter #	9	500	8.9	20.3	317	7.8
		1000	9.0	20.3	317	7.8
		2000	9.0	20.2	317	7.9
<b>Day 1</b>	Control	8.9	19.7	308	8.0	
Surrogate	Acetone Control	9.1	19.6	330	7.9	
Date	9/6/2024	125	9.0	19.6	318	8.0
Time	1116	250	9.0	19.6	322	8.0
Tech	DM	500	9.0	19.6	316	8.0
Meter #	8	1000	9.0	19.7	319	8.0
		2000	9.0	19.7	314	8.1
<b>Day 2</b>	Control	9.0	19.7	310	7.5	
Surrogate	Acetone Control	9.0	19.5	344	7.6	
Date	9/7/2024	125	9.0	19.5	334	7.7
Time	1009	250	9.0	19.4	340	7.8
Tech	EM	500	9.0	19.4	329	7.9
Meter #	8	1000	9.0	19.4	335	7.9
		2000	9.0	19.4	322	7.9

**Comments**

v.1 CLIENT	Washington Department of Ecology	TEST TYPE	48-Hour Acute
PROJECT	6PPD Alternative Testing	TEST START DATE	9/5/24
PROJECT #	PG2032	TEST END DATE	9/7/24
COMPOUND	6PPD ozonate	MATRIX	Liquid
LOT #	5221031128 ozonate	SPECIES	<i>Ceriodaphnia dubia</i>
PROTOCOL	TOX 004	NO. OF ORGANISMS	5

**Abbreviation Key:**

NB = No Body  
 FB = Found Body  
 ST = Stranded

**48-Hour Acute**

Day 1		Day 2	
Date	09/06/24	Date	09/07/24
Time	1124	Time	1404
Tech	DM	Tech	NL

Concentration (µg/L)	Rep.	Alive	Dead	Obs	Alive	Dead	Obs	Comments
Control	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
Acetone Control	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
125	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
250	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
500	1	5	0		4	1		
	2	5	0		4	1		
	3	5	0		5	0		
	4	5	0		4	1		
1000	1	5	0		1	4		
	2	4	1		0	4		
	3	5	0		1	4		
	4	5	0		0	5		
2000	1	4	1		0	4		
	2	5	0		0	5		
	3	4	1		0	4		
	4	3	2		0	3		

# CETIS Summary Report

Report Date: 16 Oct-24 09:11 (p 1 of 2)  
 Test Code/ID: Alpha-toc C.du. / 11-7789-8136

## Ceriodaphnia 48-h Acute Survival Test

EcoAnalysts

Batch ID: 00-8828-8615	Test Type: Survival (48h)	Analyst: Mary Ann Rempel-Hester
Start Date: 11 Sep-24 14:32	Protocol: EPA/821/R-02-012 (2002)	Diluent: Cerio Reconstituted Fresh Water
Ending Date: 13 Sep-24 12:36	Species: Ceriodaphnia dubia	Brine: Not Applicable
Test Length: 46h	Taxon: Branchiopoda	Source: Aquatic Biosystems, CO Age: 24H
Sample ID: 19-9486-4191	Code: Lot 1NG0212	Project: 6PPD Alternative Testing 2024
Sample Date: 02 Aug-24 12:00	Material: Alpha-tocopherol	Source: Spectrum
Receipt Date: 02 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 40d 3h	Client: Washington Department of Ecology	

### Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
01-3736-7022	48h Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	1.0000	Acetone Control passed 48h proportion sur	1

### Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
14-2039-5686	48h Proportion Survived	Steel Many-One Rank Sum Test	8403	>8403	---	9.22%	1

### Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
12-1644-3736	48h Proportion Survived	Linear Interpolation (ICPIN)	EC15	>8403	---	---	1
			EC20	>8403	---	---	
			EC25	>8403	---	---	
			EC40	>8403	---	---	
			EC50	>8403	---	---	

### Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
01-3736-7022	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
12-1644-3736	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
14-2039-5686	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria

### 48h Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0	AC	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
16		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
323		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
808		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
3232		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
8403		4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	5.00%

### 48h Proportion Survived Detail

MD5: 1F0D7C22669BAA865BF49779984E36A6

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
0	AC	1.0000	1.0000	1.0000	1.0000
16		1.0000	1.0000	1.0000	1.0000
323		1.0000	1.0000	1.0000	1.0000
808		1.0000	1.0000	1.0000	1.0000
3232		1.0000	1.0000	1.0000	1.0000
8403		1.0000	1.0000	1.0000	0.8000

# CETIS Summary Report

Report Date: 16 Oct-24 09:11 (p 2 of 2)  
Test Code/ID: Alpha-toc C.du. / 11-7789-8136

## Ceriodaphnia 48-h Acute Survival Test

EcoAnalysts

### 48h Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	5/5	5/5	5/5	5/5
0	AC	5/5	5/5	5/5	5/5
16		5/5	5/5	5/5	5/5
323		6/6	5/5	5/5	5/5
808		5/5	5/5	5/5	5/5
3232		5/5	5/5	5/5	5/5
8403		5/5	5/5	5/5	4/5

**CETIS Test Data Worksheet**

Report Date: 16 Oct-24 09:17 (p 1 of 1)  
 Test Code/ID: Alpha-toc C.du. / 11-7789-8136

<b>Ceriodaphnia 48-h Acute Survival Test</b>				<b>EcoAnalysts</b>			
<b>Start Date:</b>	11 Sep-24 14:32	<b>Species:</b>	Ceriodaphnia dubia	<b>Sample Code:</b>	Lot 1NG0212		
<b>End Date:</b>	13 Sep-24 12:36	<b>Protocol:</b>	EPA/821/R-02-012 (2002)	<b>Sample Source:</b>	Spectrum		
<b>Sample Date:</b>	02 Aug-24 12:00	<b>Material:</b>	Alpha-tocopherol	<b>Sample Station:</b>			

Conc-ug/L	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Notes
0	AC	1	24	5	5	5	
0	AC	2	3	5	5	5	
0	AC	3	27	5	5	5	
0	AC	4	23	5	5	5	
0	D	1	21	5	5	5	
0	D	2	7	5	5	5	
0	D	3	18	5	5	5	
0	D	4	20	5	5	5	
16		1	13	5	5	5	
16		2	5	5	5	5	
16		3	6	5	5	5	
16		4	10	5	5	5	
323		1	28	6	6	6	
323		2	26	5	5	5	
323		3	1	5	5	5	
323		4	15	5	5	5	
808		1	22	5	5	5	
808		2	14	5	5	5	
808		3	4	5	5	5	
808		4	8	5	5	5	
3232		1	2	5	5	5	
3232		2	19	5	5	5	
3232		3	11	5	5	5	
3232		4	12	5	5	5	
8403		1	16	5	5	5	
8403		2	25	5	5	5	
8403		3	9	5	5	5	
8403		4	17	5	4	4	

Version V.1

**GENERAL**

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	alpha-Tocopherol
Test Type	48-Hour Acute
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	09/11/24
Test Species	<i>Ceriodaphnia dubia</i>
Organism Batch (Brood Board #)	ABS091124.05
Organism Acquired	In House Culture
Organism Acclimation	NA
Organism Age	<24 hour
Test Protocol	TOX 004
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Test Location	Bath 5
Water Description	cerio reconstituted freshwater
Organisms per Replicate	5
Test Chamber Size	20 mL
Exposure Volume	15 mL
Feeding Information	None
Test Dissolved Oxygen	> 2
Test Temperature	20 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	2	
Temp	18.5	21.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1432 LG
TEST END TIME/INIT:	1236 MS

COMPOUND	LOT #
alpha-Tocopherol	1NG0212

Concentrations (µg/L)	
1	Control
2	Acetone Control
3	16
4	323
5	808
6	3232
7	8403

**Copy and Past VALUES**

Treatment	Rep	Chamber
Control	1	18
Control	2	1
Control	3	26
Control	4	28
Acetone Co	1	11
Acetone Co	2	21
Acetone Co	3	16
Acetone Co	4	5
16	1	12
16	2	10
16	3	13
16	4	27
323	1	7
323	2	17
323	3	25
323	4	14
808	1	24
808	2	6
808	3	8
808	4	22
3232	1	2
3232	2	3
3232	3	15
3232	4	23
8403	1	4
8403	2	20
8403	3	9
8403	4	19

v.1 CLIENT	Washington Department of Ecology	TEST TYPE	48-Hour Acute	PROTOCOL	TOX 004
PROJECT	6PPD Alternative Testing	TEST START DATE	9/11/24	PROJECT NUMBER	PG2032
COMPOUND	alpha-Tocopherol	TEST END DATE	9/13/24	SPECIES	<i>Ceriodaphnia dubia</i>
LOT #	1NG0212	MATRIX	Liquid	NO. OF ORGANISMS	5

48-Hour Acute

Stock Preparation

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
4,160,000	20	83.2

Dilution Preparation

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)
4,160,000	16	200	0.001
4,160,000	323	200	0.016
4,160,000	808	200	0.039
4,160,000	3232	200	0.155
4,160,000	8403	200	0.404
			<b>Amt. of Acetone (mL)</b>
	Acetone control	200	0.404

Test Dilution Prep

Date	Balance ID	Water Batch ID	Initials	Comments
9/11/2024		7 CRFW091024.01	MARH	

Water Quality

		Concentration (µg/L)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH
		> 2		20 ± 1		7.5 ± 1.5
<b>Day 0 (Stock)</b>	Control		8.6	20.1	282	7.9
Date	9/11/2024	Acetone Control	9.1	19.4	283	7.8
Time	1332	16	8.7	19.7	282	7.9
Tech	MARH	323	8.7	19.8	283	7.9
Meter #	10	808	8.7	19.8	282	8.0
		3232	8.8	19.8	280	8.0
		8403	8.8	19.8	280	8.0
<b>Day 1</b>	Control		8.8	20.1	316	8.0
Surrogate	Acetone Control		8.8	19.6	321	7.9
Date	9/12/2024	16	8.9	19.6	315	8.1
Time	1313	323	8.9	19.5	316	8.1
Tech	CS	808	8.9	19.6	315	8.1
Meter #	7	3232	8.9	19.5	314	8.1
		8403	9.0	19.5	314	8.1
<b>Day 2</b>	Control		8.8	19.9	328	7.1
Surrogate	Acetone Control		8.9	19.6	323	7.2
Date	9/13/2024	16	8.9	19.6	318	7.3
Time	1003	323	9.0	19.5	322	7.6
Tech	EM	808	9.0	19.6	319	7.6
Meter #	8	3232	9.0	19.5	320	7.6
		8403	9.0	19.5	321	7.7

Comments

v.1 CLIENT	Washington Department of Ecology	TEST TYPE	48-Hour Acute
PROJECT	6PPD Alternative Testing	TEST START DATE	9/11/24
PROJECT #	PG2032	TEST END DATE	9/13/24
COMPOUND	alpha-Tocopherol	MATRIX	Liquid
LOT #	1NG0212	SPECIES	<i>Ceriodaphnia dubia</i>
PROTOCOL	TOX 004	NO. OF ORGANISMS	5

**48-Hour Acute**
**Abbreviation Key:**

NB = No Body  
 FB = Found Body  
 ST = Stranded

Day 1		Day 2	
Date	09/12/24	Date	09/13/24
Time	1314	Time	1236
Tech	CS	Tech	MS

Concentration (µg/L)	REP	Alive	Dead	Obs	Alive	Dead	Obs	Comments
Control	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
Acetone Control	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
16	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
323	1	6	0	1 FB	6	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
808	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
3232	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
8403	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	4	1		4	0		

# CETIS Summary Report

Report Date: 03 Oct-24 12:38 (p 1 of 2)  
 Test Code/ID: Rambutan C.du. / 18-1872-8656

## Ceriodaphnia 48-h Acute Survival Test

EcoAnalysts

Batch ID: 12-8634-9138	Test Type: Survival (48h)	Analyst: Mary Ann Rempel-Hester
Start Date: 26 Sep-24 12:30	Protocol: EPA/821/R-02-012 (2002)	Diluent: Cerio Reconstituted Fresh Water
Ending Date: 28 Sep-24 12:35	Species: Ceriodaphnia dubia	Brine: Not Applicable
Test Length: 48h	Taxon: Branchiopoda	Source: Aquatic Biosystems, CO Age: 24H
Sample ID: 20-6536-8638	Code: Lot 1704001	Project: 6PPD Alternative Testing 2024
Sample Date: 20 Aug-24 12:00	Material: Rambutan Peel Extract	Source: Essentials of Australia
Receipt Date: 20 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 37d 1h	Client: Washington Department of Ecology	

### Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
18-6734-5373	48h Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	1.0000	Glycerin Control passed 48h proportion sur	1

### Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
02-7812-8128	48h Proportion Survived	Steel Many-One Rank Sum Test	10400	>10400	---	---	1

### Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
01-0042-7259	48h Proportion Survived	Linear Interpolation (ICPIN)	EC15	>10400	---	---	1
			EC20	>10400	---	---	
			EC25	>10400	---	---	
			EC40	>10400	---	---	
			EC50	>10400	---	---	

### Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits			Overlap	Decision
				Lower	Upper			
01-0042-7259	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
02-7812-8128	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
18-6734-5373	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	

### 48h Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0	GC	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
20		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
400		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
1000		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
4000		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
10400		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%

### 48h Proportion Survived Detail

MD5: D34737E37D9AE04024E8D04676F05515

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
0	GC	1.0000	1.0000	1.0000	1.0000
20		1.0000	1.0000	1.0000	1.0000
400		1.0000	1.0000	1.0000	1.0000
1000		1.0000	1.0000	1.0000	1.0000
4000		1.0000	1.0000	1.0000	1.0000
10400		1.0000	1.0000	1.0000	1.0000

# CETIS Summary Report

Report Date: 03 Oct-24 12:38 (p 2 of 2)  
Test Code/ID: Rambutan C.du. / 18-1872-8656

## Ceriodaphnia 48-h Acute Survival Test

EcoAnalysts

### 48h Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	5/5	5/5	5/5	5/5
0	GC	5/5	5/5	5/5	5/5
20		5/5	5/5	5/5	5/5
400		5/5	5/5	5/5	5/5
1000		5/5	5/5	5/5	5/5
4000		5/5	5/5	5/5	5/5
10400		5/5	5/5	5/5	5/5

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 12:21 (p 1 of 1)  
 Test Code/ID: Rambutan C.du. / 18-1872-8656

<b>Ceriodaphnia 48-h Acute Survival Test</b>				<b>EcoAnalysts</b>			
<b>Start Date:</b>	26 Sep-24 12:30	<b>Species:</b>	Ceriodaphnia dubia	<b>Sample Code:</b>	Lot 1704001		
<b>End Date:</b>	28 Sep-24 12:35	<b>Protocol:</b>	EPA/821/R-02-012 (2002)	<b>Sample Source:</b>	Essentials of Australia		
<b>Sample Date:</b>	03 Oct-24 12:15	<b>Material:</b>	Rambutan Peel Extract	<b>Sample Station:</b>			

Conc-µg/L	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Notes
0	D	1	7	5	5	5	
0	D	2	4	5	5	5	
0	D	3	14	5	5	5	
0	D	4	25	5	5	5	
0	GC	1	16	5	5	5	
0	GC	2	11	5	5	5	
0	GC	3	13	5	5	5	
0	GC	4	8	5	5	5	
20		1	18	5	5	5	
20		2	19	5	5	5	
20		3	27	5	5	5	
20		4	26	5	5	5	
400		1	28	5	5	5	
400		2	23	5	5	5	
400		3	1	5	5	5	
400		4	22	5	5	5	
1000		1	15	5	5	5	
1000		2	20	5	5	5	
1000		3	2	5	5	5	
1000		4	21	5	5	5	
4000		1	17	5	5	5	
4000		2	12	5	5	5	
4000		3	9	5	5	5	
4000		4	5	5	5	5	
10400		1	3	5	5	5	
10400		2	6	5	5	5	
10400		3	10	5	5	5	
10400		4	24	5	5	5	

Version V.1

**GENERAL**

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	Rambutan Peel Extract
Test Type	48-Hour Acute
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	9/26/2024
Test Species	<i>Ceriodaphnia dubia</i>
Organism Batch (Brood Board #)	ABS092624.02
Organism Acquired	9/26/2024
Organism Acclimation	0
Organism Age	<24 hour
Test Protocol	TOX 004
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Test Location	Bath 5
Water Description	cerio reconstituted freshwater
Organisms per Replicate	5
Test Chamber Size	20 mL
Exposure Volume	15 mL
Feeding Information	None
Test Dissolved Oxygen	> 2
Test Temperature	20 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	2	
Temp	18.5	21.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1230 MS
TEST END TIME/INIT:	1235 NL

COMPOUND	LOT #
Rambutan Peel Extract	1704001

Concentrations (µg/L)	
1	Control
2	Glycerin Control
3	20
4	400
5	1000
6	4000
7	10400

**Copy and Past VALUES**

Treatment	Rep	Chamber
Control	1	18
Control	2	1
Control	3	26
Control	4	28
Glycerin Co	1	11
Glycerin Co	2	21
Glycerin Co	3	16
Glycerin Co	4	5
20	1	12
20	2	10
20	3	13
20	4	27
400	1	7
400	2	17
400	3	25
400	4	14
1000	1	24
1000	2	6
1000	3	8
1000	4	22
4000	1	2
4000	2	3
4000	3	15
4000	4	23
10400	1	4
10400	2	20
10400	3	9
10400	4	19

v.1 CLIENT	Washington Department of Ecology	TEST TYPE	48-Hour Acute	PROTOCOL	TOX 004
PROJECT	6PPD Alternative Testing	TEST START DATE	9/26/24	PROJECT NUMBER	PG2032
COMPOUND	Rambutan Peel Extract	TEST END DATE	9/28/24	SPECIES	<i>Ceriodaphnia dubia</i>
LOT #	1704001	MATRIX	Liquid	NO. OF ORGANISMS	5

**48-Hour Acute**

**Stock Preparation**

Target Stock Concentration (µg/L)	Volume of Water (mLs)	Amt. of Toxicant (mg)
2,080,000	20	85.0

**Dilution Preparation**

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)
2,080,000	20	200	0.002
2,080,000	400	200	0.038
2,080,000	1000	200	0.096
2,080,000	4000	200	0.385
2,080,000	10400	200	1.000
			<b>Amt. of Glycerin Stock (mL)</b>
	Glycerin Control	200	1.000

**Test Dilution Prep**

Date	Balance ID	Water Batch ID	Initials	Comments
9/26/2024	7	CRFW092324.01	MARH	

**Water Quality**

	Concentration (µg/L)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH
		> 2	20 ± 1		7.5 ± 1.5
<b>Day 0 (Stock)</b>	Control	8.9	19.9	246	7.8
Date 9/26/2024	Glycerin Control	8.8	20.1	246	7.9
Time 1222	20	8.8	20.1	248	7.9
Tech MARH	400	8.9	20.1	245	8.0
Meter # 10	1000	9.3	20.1	245	8.0
	4000	9.2	20.1	245	8.0
	10400	9.0	20.1	245	8.0
<b>Day 1</b>	Control	9.6	20.2	262	7.8
Surrogate	Glycerin Control	9.7	20.2	247	7.8
Date 9/27/2024	20	9.5	20.1	248	7.9
Time 1526	400	9.1	20.1	247	7.9
Tech MS	1000	9.0	20.0	248	8.0
Meter # 10	4000	9.0	20.0	247	8.0
	10400	9.0	19.9	248	8.0
<b>Day 2</b>	Control	8.6	20.3	360	7.9
Surrogate	Glycerin Control	8.7	20.3	313	8.0
Date 9/28/2024	20	8.7	20.2	316	8.1
Time 1226	400	8.8	20.2	313	8.1
Tech NL	1000	8.8	20.1	316	8.1
Meter # 8	4000	8.9	20.1	313	8.1
	10400	8.8	20.0	316	8.1

**Comments**

v.1 CLIENT	Washington Department of Ecology	TEST TYPE	48-Hour Acute
PROJECT	6PPD Alternative Testing	TEST START DATE	9/26/24
PROJECT #	PG2032	TEST END DATE	9/28/24
COMPOUND	Rambutan Peel Extract	MATRIX	Liquid
LOT #	1704001	SPECIES	<i>Ceriodaphnia dubia</i>
PROTOCOL	TOX 004	NO. OF ORGANISMS	5

**48-Hour Acute**
**Abbreviation Key:**

NB = No Body  
 FB = Found Body  
 ST = Stranded

Day 1		Day 2	
Date	09/27/24	Date	09/28/24
Time	1526	Time	1235
Tech	MS	Tech	NL

Concentration (µg/L)	REP	Alive	Dead	Obs	Alive	Dead	Obs	Comments
Control	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
Glycerin Control	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
20	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
400	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
1000	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
4000	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
10400	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	6	0	1 FB	5	0	1NB	Day 1 likely miscount

# CETIS Summary Report

Report Date: 03 Oct-24 12:37 (p 1 of 2)  
 Test Code/ID: Sinapic C.du. / 12-4388-1959

Ceriodaphnia 48-h Acute Survival Test				EcoAnalysts	
Batch ID: 16-4265-4579	Test Type: Survival (48h)	Analyst: Mary Ann Rempel-Hester			
Start Date: 11 Sep-24 15:01	Protocol: EPA/821/R-02-012 (2002)	Diluent: Cerio Reconstituted Fresh Water			
Ending Date: 13 Sep-24 13:01	Species: Ceriodaphnia dubia	Brine: Not Applicable			
Test Length: 46h	Taxon: Branchiopoda	Source: Aquatic Biosystems, CO	Age: 24H		
Sample ID: 19-4367-4086	Code: Lot 1512719	Project: 6PPD Alternative Testing 2024			
Sample Date: 22 Aug-24 12:00	Material: Sinapic Acid	Source: Indofine Chemical Company			
Receipt Date: 22 Aug-24 12:00	CAS (PC):	Station:			
Sample Age: 20d 3h	Client: Washington Department of Ecology				

Single Comparison Summary					
Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
15-8936-9998	48h Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	1.0000	Acetone Control passed 48h proportion sur	1

Multiple Comparison Summary							
Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
19-2135-1020	48h Proportion Survived	Steel Many-One Rank Sum Test	70000	>70000	---	11.3%	1

Point Estimate Summary							
Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
06-9602-7506	48h Proportion Survived	Linear Interpolation (ICPIN)	EC15	>70000	---	---	1
			EC20	>70000	---	---	
			EC25	>70000	---	---	
			EC40	>70000	---	---	
			EC50	>70000	---	---	

Test Acceptability							
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
06-9602-7506	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
15-8936-9998	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
19-2135-1020	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria

48h Proportion Survived Summary											
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0	AC	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
4375		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
8750		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
17500		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
35000		4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	5.00%
70000		4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	5.00%

48h Proportion Survived Detail						MD5: E8DC4A660421D8050487CCA7AE114314
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	
0	D	1.0000	1.0000	1.0000	1.0000	
0	AC	1.0000	1.0000	1.0000	1.0000	
4375		1.0000	1.0000	1.0000	1.0000	
8750		1.0000	1.0000	1.0000	1.0000	
17500		1.0000	1.0000	1.0000	1.0000	
35000		1.0000	1.0000	1.0000	0.8000	
70000		1.0000	1.0000	1.0000	0.8000	

# CETIS Summary Report

Report Date: 03 Oct-24 12:37 (p 2 of 2)  
Test Code/ID: Sinapic C.du. / 12-4388-1959

## Ceriodaphnia 48-h Acute Survival Test

EcoAnalysts

### 48h Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	5/5	5/5	5/5	5/5
0	AC	5/5	5/5	5/5	6/6
4375		5/5	5/5	5/5	5/5
8750		5/5	5/5	5/5	5/5
17500		5/5	5/5	5/5	5/5
35000		5/5	5/5	5/5	4/5
70000		5/5	5/5	5/5	4/5

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 12:37 (p 1 of 1)  
 Test Code/ID: Sinapic C.du. / 12-4388-1959

<b>Ceriodaphnia 48-h Acute Survival Test</b>				<b>EcoAnalysts</b>			
<b>Start Date:</b>	11 Sep-24 15:01	<b>Species:</b>	Ceriodaphnia dubia	<b>Sample Code:</b>	Lot 1512719		
<b>End Date:</b>	13 Sep-24 13:01	<b>Protocol:</b>	EPA/821/R-02-012 (2002)	<b>Sample Source:</b>	Indofine Chemical Company		
<b>Sample Date:</b>	22 Aug-24 12:00	<b>Material:</b>	Sinapic Acid	<b>Sample Station:</b>			

Conc-µg/L	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Notes
0	AC	1	10	5	5	5	
0	AC	2	14	5	5	5	
0	AC	3	16	5	5	5	
0	AC	4	6	6	6	6	
0	D	1	18	5	5	5	
0	D	2	9	5	5	5	
0	D	3	23	5	5	5	
0	D	4	3	5	5	5	
4375		1	28	5	5	5	
4375		2	11	5	5	5	
4375		3	20	5	5	5	
4375		4	5	5	5	5	
8750		1	15	5	5	5	
8750		2	24	5	5	5	
8750		3	27	5	5	5	
8750		4	17	5	5	5	
17500		1	1	5	5	5	
17500		2	7	5	5	5	
17500		3	13	5	5	5	
17500		4	21	5	5	5	
35000		1	4	5	5	5	
35000		2	12	5	5	5	
35000		3	19	5	5	5	
35000		4	2	5	5	4	
70000		1	8	5	5	5	
70000		2	25	5	5	5	
70000		3	26	5	5	5	
70000		4	22	5	4	4	

Version V.1

**GENERAL**

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	Sinapic Acid
Test Type	48-Hour Acute
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	09/11/24
Test Species	<i>Ceriodaphnia dubia</i>
Organism Batch (Brood Board #)	ABS091124.05
Organism Acquired	In House Culture
Organism Acclimation	NA
Organism Age	<24 hour
Test Protocol	TOX 004
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Test Location	Bath 5
Water Description	cerio reconstituted freshwater
Organisms per Replicate	5
Test Chamber Size	20 mL
Exposure Volume	15 mL
Feeding Information	None
Test Dissolved Oxygen	> 2
Test Temperature	20 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	2	
Temp	18.5	21.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1501 LG
TEST END TIME/INIT:	1301 MS

COMPOUND	LOT #
Sinapic Acid	1512719

Concentrations (µg/L)	
1	Control
2	Acetone Control
3	4375
4	8750
5	17500
6	35000
7	70000

**Copy and Past VALUES**

Treatment	Rep	Chamber
Control	1	18
Control	2	1
Control	3	26
Control	4	28
Acetone Co	1	11
Acetone Co	2	21
Acetone Co	3	16
Acetone Co	4	5
4375	1	12
4375	2	10
4375	3	13
4375	4	27
8750	1	7
8750	2	17
8750	3	25
8750	4	14
17500	1	24
17500	2	6
17500	3	8
17500	4	22
35000	1	2
35000	2	3
35000	3	15
35000	4	23
70000	1	4
70000	2	20
70000	3	9
70000	4	19

v.1

<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	48-Hour Acute	<b>PROTOCOL</b>	TOX 004
<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	9/11/24	<b>PROJECT NUMBER</b>	PG2032
<b>COMPOUND</b>	Sinapic Acid	<b>TEST END DATE</b>	9/13/24	<b>SPECIES</b>	<i>Ceriodaphnia dubia</i>
<b>LOT #</b>	1512719	<b>MATRIX</b>	Liquid	<b>NO. OF ORGANISMS</b>	5

**48-Hour Acute**

**Stock Preparation**

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
28,000,000	20	560

**Dilution Preparation**

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)
28,000,000	4375	200	0.031
28,000,000	8750	200	0.063
28,000,000	17500	200	0.125
28,000,000	35000	200	0.250
28,000,000	70000	200	0.500
			<b>Amt. of Acetone (mL)</b>
	Acetone control	200	0.500

**Test Dilution Prep**

Date	Balance ID	Water Batch ID	Initials	Comments
9/11/2024	7	CRFW091024.01	MARH	Wasn't able to get 56 g/L to dissolve. Made stock at 28 g/L instead and halved concentration series to keep acetone to max of 0.25%.

**Water Quality**

	Concentration (µg/L)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH	
		> 2	20 ± 1		7.5 ± 1.5	
<b>Day 0 (Stock)</b>	Control	8.6	20.1	282	7.9	
Date	9/11/2024	Acetone Control	8.4	19.6	282	7.8
Time	1440	4375	8.7	19.8	282	7.8
Tech	MARH	8750	8.9	19.8	282	7.7
Meter #	10	17500	8.8	19.8	280	7.6
		35000	8.7	19.8	279	7.3
		70000	8.8	19.9	274	7.0
<b>Day 1</b>	Control	8.8	20.1	316	8.0	
Surrogate	Acetone Control	8.9	19.9	314	8.0	
Date	9/12/2024	4375	8.9	19.9	313	8.1
Time	1250	8750	9.0	19.8	314	8.1
Tech	CS	17500	9.0	19.7	312	8.0
Meter #	7	35000	8.9	19.7	310	8.0
		70000	8.9	19.8	305	7.8
<b>Day 2</b>	Control	8.8	19.9	328	7.1	
Surrogate	Acetone Control	8.8	19.6	318	7.7	
Date	9/13/2024	4375	8.9	19.6	317	7.7
Time	1003	8750	8.9	19.5	320	7.8
Tech	EM	17500	8.9	19.6	316	7.8
Meter #	8	35000	8.9	19.5	316	7.8
		70000	8.9	19.6	310	7.8

**Comments**

v.1 CLIENT	Washington Department of Ecology	TEST TYPE	48-Hour Acute
PROJECT	6PPD Alternative Testing	TEST START DATE	9/11/24
PROJECT #	PG2032	TEST END DATE	9/13/24
COMPOUND	Sinapic Acid	MATRIX	Liquid
LOT #	1512719	SPECIES	<i>Ceriodaphnia dubia</i>
PROTOCOL	TOX 004	NO. OF ORGANISMS	5

48-Hour Acute

Abbreviation Key:

NB = No Body  
 FB = Found Body  
 ST = Stranded

		Day 1		Day 2	
Date		09/12/24		Date	09/13/24
Time		1253		Time	1301
Tech		CS		Tech	MS

Concentration (µg/L)	REP	Alive	Dead	Obs	Alive	Dead	Obs	Comments
Control	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
Acetone Control	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		6	0	1 FB	Likely miscount on Day 1
4375	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
8750	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
17500	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
35000	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		4	1		
70000	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	4	1		4	0		

**CETIS Summary Report**

Report Date: 03 Oct-24 13:09 (p 1 of 2)  
 Test Code/ID: Durazone C.du. / 05-4204-9716

**Ceriodaphnia 48-h Acute Survival Test**

EcoAnalysts

Batch ID: 08-9228-9087	Test Type: Survival (48h)	Analyst: Mary Ann Rempel-Hester
Start Date: 05 Sep-24 15:37	Protocol: EPA/821/R-02-012 (2002)	Diluent: Cerio Reconstituted Fresh Water
Ending Date: 07 Sep-24 14:53	Species: Ceriodaphnia dubia	Brine: Not Applicable
Test Length: 47h	Taxon: Branchiopoda	Source: Aquatic Biosystems, CO Age: 24H
Sample ID: 08-0904-2923	Code: Lot CD1J23P008	Project: 6PPD Alternative Testing 2024
Sample Date: 22 Aug-24 12:00	Material: Durazone 37	Source: Akrochem
Receipt Date: 22 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 14d 4h	Client: Washington Department of Ecology	

**Single Comparison Summary**

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
07-2605-7282	48h Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	1.0000	Acetone Control passed 48h proportion sur	1

**Multiple Comparison Summary**

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
02-9190-5391	48h Proportion Survived	Steel Many-One Rank Sum Test	1400	>1400	---	9.2%	1

**Point Estimate Summary**

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
09-0845-3825	48h Proportion Survived	Linear Interpolation (ICPIN)	EC15	>1400	---	---	1
			EC20	>1400	---	---	
			EC25	>1400	---	---	
			EC40	>1400	---	---	
			EC50	>1400	---	---	

**Test Acceptability**

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
02-9190-5391	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
07-2605-7282	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
09-0845-3825	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria

**48h Proportion Survived Summary**

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0	AC	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
87.5		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
175		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
350		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
700		4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	5.00%
1400		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%

**48h Proportion Survived Detail**

MD5: 305A302D1CD5DF61D259FE6F1F17D83C

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
0	AC	1.0000	1.0000	1.0000	1.0000
87.5		1.0000	1.0000	1.0000	1.0000
175		1.0000	1.0000	1.0000	1.0000
350		1.0000	1.0000	1.0000	1.0000
700		1.0000	1.0000	0.8000	1.0000
1400		1.0000	1.0000	1.0000	1.0000

# CETIS Summary Report

Report Date: 03 Oct-24 13:09 (p 2 of 2)  
Test Code/ID: Durazone C.du. / 05-4204-9716

## Ceriodaphnia 48-h Acute Survival Test

EcoAnalysts

### 48h Proportion Survived Binomials

Conc- $\mu$ g/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	5/5	5/5	5/5	5/5
0	AC	5/5	5/5	5/5	5/5
87.5		5/5	5/5	5/5	5/5
175		5/5	5/5	5/5	5/5
350		5/5	5/5	5/5	5/5
700		5/5	5/5	4/5	5/5
1400		5/5	5/5	5/5	5/5

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 13:09 (p 1 of 1)  
 Test Code/ID: Durazone C.du. / 05-4204-9716

<b>Ceriodaphnia 48-h Acute Survival Test</b>				<b>EcoAnalysts</b>			
<b>Start Date:</b>	05 Sep-24 15:37	<b>Species:</b>	Ceriodaphnia dubia	<b>Sample Code:</b>	Lot CD1J23P008		
<b>End Date:</b>	07 Sep-24 14:53	<b>Protocol:</b>	EPA/821/R-02-012 (2002)	<b>Sample Source:</b>	Akrochem		
<b>Sample Date:</b>	22 Aug-24 12:00	<b>Material:</b>	Durazone 37	<b>Sample Station:</b>			

Conc-µg/L	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Notes
0	AC	1	7	5	5	5	
0	AC	2	3	5	5	5	
0	AC	3	23	5	5	5	
0	AC	4	19	5	5	5	
0	D	1	5	5	5	5	
0	D	2	4	5	5	5	
0	D	3	22	5	5	5	
0	D	4	12	5	5	5	
87.5		1	14	5	5	5	
87.5		2	2	5	5	5	
87.5		3	24	5	5	5	
87.5		4	9	5	5	5	
175		1	1	5	5	5	
175		2	11	5	5	5	
175		3	16	5	5	5	
175		4	13	5	5	5	
350		1	20	5	5	5	
350		2	6	5	5	5	
350		3	27	5	5	5	
350		4	18	5	5	5	
700		1	10	5	5	5	
700		2	25	5	5	5	
700		3	21	5	4	4	
700		4	15	5	5	5	
1400		1	28	5	5	5	
1400		2	26	5	5	5	
1400		3	8	5	5	5	
1400		4	17	5	5	5	

Version V.1

GENERAL

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	Durazone 37
Test Type	48-Hour Acute
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	09/05/24
Test Species	<i>Ceriodaphnia dubia</i>
Organism Batch (Brood Board #)	ABS090524.02
Organism Acquired	In House Culture
Organism Acclimation	NA
Organism Age	<24 hour
Test Protocol	TOX 004
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Test Location	Bath 5
Water Description	cerio reconstituted freshwater
Organisms per Replicate	5
Test Chamber Size	20 mL
Exposure Volume	15 mL
Feeding Information	None
Test Dissolved Oxygen	> 2
Test Temperature	20 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	2	
Temp	18.5	21.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1537 CS
TEST END TIME/INIT:	1453 NL

COMPOUND	LOT #
Durazone 37	CD1J23P008

Concentrations (µg/L)	
1	Control
2	Acetone Control
3	87.5
4	175
5	350
6	700
7	1400

Copy and Past VALUES

Treatment	Rep	Chamber
Control	1	18
Control	2	1
Control	3	26
Control	4	28
Acetone Co	1	11
Acetone Co	2	21
Acetone Co	3	16
Acetone Co	4	5
88	1	12
88	2	10
88	3	13
88	4	27
175	1	7
175	2	17
175	3	25
175	4	14
350	1	24
350	2	6
350	3	8
350	4	22
700	1	2
700	2	3
700	3	15
700	4	23
1400	1	4
1400	2	20
1400	3	9
1400	4	19

v.1

<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	48-Hour Acute	<b>PROTOCOL</b>	TOX 004
<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	9/5/24	<b>PROJECT NUMBER</b>	PG2032
<b>COMPOUND</b>	Durazone 37	<b>TEST END DATE</b>	9/7/24	<b>SPECIES</b>	<i>Ceriodaphnia dubia</i>
<b>LOT #</b>	CD1J23P008	<b>MATRIX</b>	Liquid	<b>NO. OF ORGANISMS</b>	5

**48-Hour Acute**

**Stock Preparation**

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
560,000	50	28.0

**Dilution Preparation**

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)
560,000	87.5	160	0.025
560,000	175	160	0.050
560,000	350	160	0.100
560,000	700	160	0.200
560,000	1400	160	0.400
			<b>Amt. of Acetone (mL)</b>
	Acetone control	160	0.400

**Test Dilution Prep**

Date	Balance ID	Water Batch ID	Initials	Comments
9/5/2024		7 CRFW082724.02	MARH	

**Water Quality**

		Concentration (µg/L)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH
		> 2	> 2	20 ± 1	(µS/cm)	7.5 ± 1.5
<b>Day 0 (Stock)</b>	Control		8.9	20.5	317	7.7
Date	9/5/2024	Acetone Control	8.9	20.5	315	7.7
Time	1520	87.5	8.9	20.4	317	7.8
Tech	CS	175	8.9	20.4	317	7.8
Meter #	9	350	8.9	20.4	317	7.8
		700	8.9	20.4	317	7.9
		1400	8.9	20.5	316	7.9
<b>Day 1</b>	Control		8.9	20.1	311	7.6
Surrogate	Acetone Control		8.9	19.9	309	7.7
Date	9/6/2024	87.5	9.0	19.8	310	7.8
Time	1007	175	8.9	19.8	311	7.9
Tech	DM	350	8.9	19.9	309	7.9
Meter #	8	700	9.0	19.8	311	7.9
		1400	8.8	19.8	308	8.0
<b>Day 2</b>	Control		9.0	19.8	312	7.9
Surrogate	Acetone Control		9.0	19.8	314	7.9
Date	9/7/2024	87.5	9.0	19.7	315	7.9
Time	1015	175	9.0	19.6	316	7.9
Tech	EM	350	9.0	19.8	313	7.9
Meter #	8	700	9.0	19.7	314	8.0
		1400	9.0	19.7	312	8.0

**Comments**

v.1 CLIENT	Washington Department of Ecology	TEST TYPE	48-Hour Acute
PROJECT	6PPD Alternative Testing	TEST START DATE	9/5/24
PROJECT #	PG2032	TEST END DATE	9/7/24
COMPOUND	Durazone 37	MATRIX	Liquid
LOT #	CD1J23P008	SPECIES	<i>Ceriodaphnia dubia</i>
PROTOCOL	TOX 004	NO. OF ORGANISMS	5

**48-Hour Acute**
**Abbreviation Key:**

NB = No Body  
 FB = Found Body  
 ST = Stranded

Day 1		Day 2	
Date	09/06/24	Date	09/07/24
Time	1045	Time	1453
Tech	DM	Tech	NL

Concentration (µg/L)	Rep	Alive	Dead	Obs	Alive	Dead	Obs	Comments
Control	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
Acetone Control	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
87.5	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
175	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
350	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
700	1	5	0		5	0		
	2	5	0		5	0		
	3	4	1		4	0		
	4	5	0		5	0		
1400	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		

**CETIS Summary Report**

Report Date: 03 Oct-24 13:02 (p 1 of 2)  
 Test Code/ID: Irganox C.du. / 03-2314-9580

**Ceriodaphnia 48-h Acute Survival Test**

EcoAnalysts

Batch ID: 14-1484-6672	Test Type: Survival (48h)	Analyst: Mary Ann Rempel-Hester
Start Date: 05 Sep-24 16:37	Protocol: EPA/821/R-02-012 (2002)	Diluent: Cerio Reconstituted Fresh Water
Ending Date: 07 Sep-24 15:02	Species: Ceriodaphnia dubia	Brine: Not Applicable
Test Length: 46h	Taxon: Branchiopoda	Source: Aquatic Biosystems, CO Age: 24H
Sample ID: 09-5445-9952	Code: Lot C2PNC-GN	Project: 6PPD Alternative Testing 2024
Sample Date: 13 Aug-24 12:00	Material: Irganox 1076	Source: TCI America
Receipt Date: 13 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 23d 5h	Client: Washington Department of Ecology	

**Single Comparison Summary**

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
17-0637-9988	48h Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	1.0000	Acetone Control passed 48h proportion sur	1

**Multiple Comparison Summary**

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
17-5144-0480	48h Proportion Survived	Steel Many-One Rank Sum Test	340000	>340000	---	18.4%	1

**Point Estimate Summary**

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
20-7731-0372	48h Proportion Survived	Linear Interpolation (ICPIN)	EC15	>340000	---	---	1
			EC20	>340000	---	---	
			EC25	>340000	---	---	
			EC40	>340000	---	---	
			EC50	>340000	---	---	

**Test Acceptability**

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
17-0637-9988	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
17-5144-0480	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
20-7731-0372	48h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria

**48h Proportion Survived Summary**

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0	AC	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
21250		4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	5.00%
42500	4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	5.00%	
85000	4	0.8000	0.8000	0.8000	0.8000	0.8000	0.0000	0.0000	0.00%	20.00%	
170000	4	0.8250	0.4490	1.2010	0.5000	1.0000	0.1181	0.2363	28.64%	17.50%	
340000	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%	

**48h Proportion Survived Detail**

MD5: 7956CC6843B025004D2431AF07312FDE

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
0	AC	1.0000	1.0000	1.0000	1.0000
21250		1.0000	0.8000	1.0000	1.0000
42500		0.8000	1.0000	1.0000	1.0000
85000		0.8000	0.8000	0.8000	0.8000
170000		0.8000	0.5000	1.0000	1.0000
340000		1.0000	1.0000	1.0000	1.0000

# CETIS Summary Report

Report Date: 03 Oct-24 13:02 (p 2 of 2)  
Test Code/ID: Irganox C.du. / 03-2314-9580

## Ceriodaphnia 48-h Acute Survival Test

EcoAnalysts

### 48h Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	5/5	5/5	5/5	5/5
0	AC	5/5	5/5	5/5	5/5
21250		5/5	4/5	5/5	5/5
42500		4/5	5/5	5/5	5/5
85000		4/5	4/5	4/5	4/5
170000		4/5	3/6	5/5	5/5
340000		5/5	5/5	5/5	5/5

CETIS Test Data Worksheet

Report Date: 03 Oct-24 13:02 (p 1 of 1)  
 Test Code/ID: Irganox C.du. / 03-2314-9580

<b>Ceriodaphnia 48-h Acute Survival Test</b>				<b>EcoAnalysts</b>			
<b>Start Date:</b>	05 Sep-24 16:37	<b>Species:</b>	Ceriodaphnia dubia	<b>Sample Code:</b>	Lot C2PNC-GN		
<b>End Date:</b>	07 Sep-24 15:02	<b>Protocol:</b>	EPA/821/R-02-012 (2002)	<b>Sample Source:</b>	TCI America		
<b>Sample Date:</b>	13 Aug-24 12:00	<b>Material:</b>	Irganox 1076	<b>Sample Station:</b>			

Conc-µg/L	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Notes
0	AC	1	3	5	5	5	
0	AC	2	7	5	5	5	
0	AC	3	12	5	5	5	
0	AC	4	19	5	5	5	
0	D	1	1	5	5	5	
0	D	2	6	5	5	5	
0	D	3	15	5	5	5	
0	D	4	16	5	5	5	
21250		1	17	5	5	5	
21250		2	11	5	5	4	
21250		3	27	5	5	5	
21250		4	23	5	5	5	
42500		1	22	5	5	4	
42500		2	2	5	5	5	
42500		3	13	5	5	5	
42500		4	8	5	5	5	
85000		1	21	5	5	4	
85000		2	26	5	5	4	
85000		3	25	5	5	4	
85000		4	14	5	5	4	
170000		1	24	5	5	4	
170000		2	9	6	6	3	
170000		3	18	5	5	5	
170000		4	10	5	5	5	
340000		1	28	5	5	5	
340000		2	5	5	5	5	
340000		3	4	5	5	5	
340000		4	20	5	5	5	

Version V.1

GENERAL

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	Irganox 1076
Test Type	48-Hour Acute
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	09/05/24
Test Species	<i>Ceriodaphnia dubia</i>
Organism Batch (Brood Board #)	ABS090524.02
Organism Acquired	In House Culture
Organism Acclimation	NA
Organism Age	<24 hour
Test Protocol	TOX 004
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Test Location	Bath 5
Water Description	cerio reconstituted freshwater
Organisms per Replicate	5
Test Chamber Size	20 mL
Exposure Volume	15 mL
Feeding Information	None
Test Dissolved Oxygen	> 2
Test Temperature	20 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	2	
Temp	18.5	21.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1637 CS
TEST END TIME/INIT:	1502 NL

COMPOUND	LOT #
Irganox 1076	C2PNC-GN

Concentrations (µg/L)

1	Control
2	Acetone Control
3	21250
4	42500
5	85000
6	170000
7	340000

Copy and Past VALUES from

Treatment	Rep	Chamber
Control	1	18
Control	2	1
Control	3	26
Control	4	28
Acetone Co	1	11
Acetone Co	2	21
Acetone Co	3	16
Acetone Co	4	5
21250	1	12
21250	2	10
21250	3	13
21250	4	27
42500	1	7
42500	2	17
42500	3	25
42500	4	14
85000	1	24
85000	2	6
85000	3	8
85000	4	22
170000	1	2
170000	2	3
170000	3	15
170000	4	23
340000	1	4
340000	2	20
340000	3	9
340000	4	19

v.1

<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	48-Hour Acute	<b>PROTOCOL</b>	TOX 004
<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	9/5/24	<b>PROJECT NUMBER</b>	PG2032
<b>COMPOUND</b>	Irganox 1076	<b>TEST END DATE</b>	9/7/24	<b>SPECIES</b>	<i>Ceriodaphnia dubia</i>
<b>LOT #</b>	C2PNC-GN	<b>MATRIX</b>	Liquid	<b>NO. OF ORGANISMS</b>	5

**48-Hour Acute**

**Stock Preparation**

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
136,000,000	250	34000

**Dilution Preparation**

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)
136,000,000	21250	160	0.025
136,000,000	42500	160	0.050
136,000,000	85000	160	0.100
136,000,000	170000	160	0.200
136,000,000	340000	160	0.400
			<b>Amt. of Acetone (mL)</b>
	Acetone control	160	0.400

**Test Dilution Prep**

Date	Balance ID	Water Batch ID	Initials	Comments
9/5/2024		7 CRFW082724.02	MARH	

**Water Quality**

	Concentration (µg/L)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH	
		> 2	20 ± 1		7.5 ± 1.5	
<b>Day 0 (Stock)</b>	Control	8.8	20.4	318	7.9	
Date	9/5/2024	Acetone Control	8.9	20.4	316	7.9
Time	1605	21250	8.9	20.2	319	7.9
Tech	CS	42500	8.8	20.1	317	8.0
Meter #	9	85000	8.9	20.3	319	8.0
		170000	8.9	20.3	318	8.0
		340000	8.9	20.4	317	8.0
<b>Day 1</b>	Control	9.0	19.9	311	7.6	
Surrogate	Acetone Control	9.0	19.8	310	7.5	
Date	9/6/2024	21250	8.9	19.6	320	7.7
Time	929	42500	9.0	19.5	315	7.7
Tech	DM	85000	9.1	19.6	317	7.7
Meter #	8	170000	9.1	19.6	315	7.8
		340000	9.0	19.7	310	7.8
<b>Day 2</b>	Control	8.9	19.8	312	8.0	
Surrogate	Acetone Control	8.9	19.7	314	8.0	
Date	9/7/2024	21250	8.9	19.5	323	7.9
Time	1021	42500	8.9	19.4	324	7.9
Tech	EM	85000	9.0	19.6	322	8.0
Meter #	8	170000	9.0	19.5	321	8.0
		340000	9.0	19.5	314	8.0

**Comments**

v.1 CLIENT	Washington Department of Ecology	TEST TYPE	48-Hour Acute
PROJECT	6PPD Alternative Testing	TEST START DATE	9/5/24
PROJECT #	PG2032	TEST END DATE	9/7/24
COMPOUND	Irganox 1076	MATRIX	Liquid
LOT #	C2PNC-GN	SPECIES	<i>Ceriodaphnia dubia</i>
PROTOCOL	TOX 004	NO. OF ORGANISMS	5

**Abbreviation Key:**

NB = No Body  
 FB = Found Body  
 ST = Stranded

**48-Hour Acute**

Day 1		Day 2	
Date	09/06/24	Date	09/07/24
Time	1014	Time	1502
Tech	DM	Tech	NL

Concentration (µg/L)	REP.	Alive	Dead	Obs	Alive	Dead	Obs	Comments
Control	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
Acetone Control	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
21250	1	5	0		5	0		
	2	5	0		4	1		
	3	5	0		5	0		
	4	5	0		5	0		
42500	1	5	0		4	1		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		
85000	1	5	0		4	1		
	2	5	0		4	1		
	3	5	0		4	1		
	4	5	0		4	1		
170000	1	5	0		4	1		
	2	6	0		3	1	2NB	
	3	5	0		5	0		
	4	5	0		5	0		
340000	1	5	0		5	0		
	2	5	0		5	0		
	3	5	0		5	0		
	4	5	0		5	0		

## **APPENDIX A.2**

### ***HYALELLA AZTECA* 24-HOUR SURVIVAL TEST**

#### **STATISTICAL COMPARISONS AND LABORATORY DATA SHEETS**

# CETIS Summary Report

Report Date: 03 Oct-24 14:30 (p 1 of 1)  
 Test Code/ID: 6PPD H.a. / 08-4431-0226

## Hyalella 24-hour Acute Survival Test

EcoAnalysts

Batch ID: 05-9956-1665	Test Type: Survival	Analyst: Mary Ann Rempel-Hester
Start Date: 05 Sep-24 14:41	Protocol: ASTM E1706-20 (2020)	Diluent: Mod-Hard Synthetic Water
Ending Date: 06 Sep-24 13:55	Species: Hyalella azteca	Brine: Not Applicable
Test Length: 23h	Taxon: Malacostraca	Source: Aquatic Indicators, FL      Age: 14D
Sample ID: 00-0518-2499	Code: Lot 5221031128	Project: 6PPD Alternative Testing 2024
Sample Date: 30 Aug-24 12:00	Material: 6PPD	Source: Akron Rubber Development (HB Chem
Receipt Date: 30 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 6d 3h	Client: Washington Department of Ecology	

### Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
20-9899-2646	Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	0.7857	Acetone Control passed proportion survive	1

### Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
03-4204-4675	Proportion Survived	Dunnett Multiple Comparison Test	500	1000	707.1	15.9%	1

### Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
08-7234-3088	Proportion Survived	Linear Interpolation (ICPIN)	EC15	440.9	250.6	606	1
			EC20	508.7	267.3	597	
			EC25	530.7	319.5	616.8	
			EC40	602.4	474.9	679.8	
			EC50	655.5	538.5	725.2	

### Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
0	AC	4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
62.5		4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	2.56%
125		4	0.9500	0.8581	1.0420	0.9000	1.0000	0.0289	0.0577	6.08%	2.56%
250		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
500		4	0.8000	0.5095	1.0910	0.6000	1.0000	0.0913	0.1826	22.82%	17.95%
1000		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

### Proportion Survived Detail

MD5: 9027F33C78C3AD73445D158322863952

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9000	1.0000	1.0000	1.0000
0	AC	1.0000	0.9000	1.0000	1.0000
62.5		0.8000	1.0000	1.0000	1.0000
125		1.0000	1.0000	0.9000	0.9000
250		1.0000	1.0000	0.9000	1.0000
500		1.0000	0.6000	0.9000	0.7000
1000		0.0000	0.0000	0.0000	0.0000

### Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	9/10	10/10	10/10	10/10
0	AC	10/10	9/10	10/10	10/10
62.5		8/10	10/10	10/10	10/10
125		10/10	10/10	9/10	9/10
250		10/10	10/10	9/10	10/10
500		10/10	6/10	9/10	7/10
1000		0/10	0/10	0/10	0/10

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 14:30 (p 1 of 1)  
 Test Code/ID: 6PPD H.a. / 08-4431-0226

<b>Hyalella 24-hour Acute Survival Test</b>				<b>EcoAnalysts</b>	
<b>Start Date:</b> 05 Sep-24 14:41	<b>Species:</b> Hyalella azteca	<b>Sample Code:</b> Lot 5221031128			
<b>End Date:</b> 06 Sep-24 13:55	<b>Protocol:</b> ASTM E1706-20 (2020)	<b>Sample Source:</b> Akron Rubber Development (HB Che			
<b>Sample Date:</b> 30 Aug-24 12:00	<b>Material:</b> 6PPD	<b>Sample Station:</b>			

Conc-µg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	AC	1	15	10	10	
0	AC	2	13	10	9	
0	AC	3	21	10	10	
0	AC	4	18	10	10	
0	D	1	12	10	9	
0	D	2	1	10	10	
0	D	3	28	10	10	
0	D	4	5	10	10	
62.5		1	8	10	8	
62.5		2	2	10	10	
62.5		3	16	10	10	
62.5		4	6	10	10	
125		1	7	10	10	
125		2	14	10	10	
125		3	4	10	9	
125		4	27	10	9	
250		1	25	10	10	
250		2	10	10	10	
250		3	17	10	9	
250		4	23	10	10	
500		1	19	10	10	
500		2	20	10	6	
500		3	3	10	9	
500		4	22	10	7	
1000		1	9	10	0	
1000		2	24	10	0	
1000		3	26	10	0	
1000		4	11	10	0	

Version V.1

**GENERAL**

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	6PPD
Test Type	24-Hour Acute
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	09/05/24
Test Species	<i>Hyalella azteca</i>
Organism Batch	AI090527.01
Organism Acquired	9/5/2024
Organism Acclimation	0
Organism Age	14 Days
Test Protocol	TOX 022 Modified
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Test Location	Bath 4
Water Description	reconstituted freshwater
Organisms per Replicate	10
Test Chamber Size	500 mL
Exposure Volume	250 mL
Feeding Information	None
Test Dissolved Oxygen	> 4
Test Temperature	23 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	4	
Temp	21.5	24.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1441 TVL/MARH
TEST END TIME/INIT:	1355 RE

COMPOUND	LOT #
6PPD	5221031128

Concentrations (µg/L)	
1	Control
2	Acetone Control
3	62.5
4	125
5	250
6	500
7	1000

**Copy and Past VALUES**

Treatment	Rep	Chamber
Control	1	15
Control	2	
Control	3	
Control	4	
Acetone Co	1	22
Acetone Co	2	23
Acetone Co	3	
Acetone Co	4	
63	1	5
63	2	9
63	3	2
63	4	18
125	1	17
125	2	21
125	3	19
125	4	13
250	1	10
250	2	20
250	3	11
250	4	7
500	1	12
500	2	3
500	3	16
500	4	4
1000	1	14
1000	2	6
1000	3	8
1000	4	1

v.1

<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	24-Hour Acute	<b>PROTOCOL</b>	TOX 022 Modified
<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	9/5/24	<b>PROJECT NUMBER</b>	PG2032
<b>COMPOUND</b>	6PPD	<b>TEST END DATE</b>	9/6/24	<b>SPECIES</b>	<i>Hyaella azteca</i>
<b>LOT #</b>	5221031128	<b>MATRIX</b>	Liquid	<b>NO. OF ORGANISMS</b>	10

**24-Hour Acute**

**Stock Preparation**

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
800,000	100	80.0

**Dilution Preparation**

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)
800,000	62.5	1000	0.078
800,000	125	1000	0.156
800,000	250	1000	0.313
800,000	500	1000	0.625
800,000	1000	1000	1.250
			<b>Amt. of Acetone (mL)</b>
	Acetone control	1000	1.250

**Test Dilution Prep**

Date	Balance ID	Water Batch ID	Initials	Comments
9/5/2024		7 RFW082724.01	MARH	

**Water Quality**

		Concentration (µg/L)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH
		> 4		23 ± 1		7.5 ± 1.5
<b>Day 0 (Stock)</b>	Control		8.5	22.1	318	7.5
Date	9/5/2024	Acetone Control	8.5	22.3	316	7.6
Time	1409	62.5	8.8	22.2	322	7.6
Tech	MARH	125	8.3	22.3	318	7.6
Meter #	8	250	8.3	22.3	318	7.7
		500	8.3	22.4	318	7.8
		1000	8.3	22.4	317	7.9
<b>Day 1</b>	Control		8.1	22.4	336	7.5
Rep 1	Acetone Control		8.2	22.4	326	7.6
Date	9/6/2024	62.5	8.3	22.4	330	7.6
Time	1355	125	8.3	22.3	342	7.7
Tech	RE	250	8.2	22.1	324	7.8
Meter #	8	500	8.3	21.9	324	7.7
		1000	8.2	21.9	326	7.8

**Comments**

v.1	<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	24-Hour Acute
	<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	9/5/24
	<b>PROJECT #</b>	PG2032	<b>TEST END DATE</b>	9/6/24
	<b>COMPOUND</b>	6PPD	<b>MATRIX</b>	Liquid
	<b>LOT #</b>	5221031128	<b>SPECIES</b>	<i>Hyalella azteca</i>
	<b>PROTOCOL</b>	TOX 022 Modified	<b>NO. OF ORGANISMS</b>	10

**24-Hour Acute**
**Abbreviation Key:**

NB = No Body  
 FB = Found Body  
 ST = Stranded

		Day 1			
		Date	09/06/24		
		Time	13:55		
		Tech	RE		
Concentration (µg/L)	REP	Alive	Dead	Obs	Comments
Control	1	9	1		
	2	10	0		
	3	10	0		
	4	10	0		
Acetone Control	1	10	0		
	2	9	1		
	3	10	0		
	4	10	0		
62.5	1	8	2		
	2	10	0		
	3	10	0		
	4	10	0		
125	1	10	0		
	2	10	0		
	3	9	1		
	4	9	1		
250	1	10	0		
	2	10	0		
	3	9	1		
	4	10	0		
500	1	10	0		
	2	6	4		
	3	9	1		
	4	7	3		
1000	1	0	10		
	2	0	10		
	3	0	10		
	4	0	10		

# CETIS Summary Report

Report Date: 03 Oct-24 14:25 (p 1 of 1)  
 Test Code/ID: 6PPDoz H.a. / 01-3764-1514

## Hyalella 24-hour Acute Survival Test

EcoAnalysts

Batch ID: 12-3859-1648	Test Type: Survival	Analyst: Mary Ann Rempel-Hester
Start Date: 05 Sep-24 13:58	Protocol: ASTM E1706-20 (2020)	Diluent: Mod-Hard Synthetic Water
Ending Date: 06 Sep-24 13:10	Species: Hyalella azteca	Brine: Not Applicable
Test Length: 23h	Taxon: Malacostraca	Source: Aquatic Indicators, FL Age: 14D
Sample ID: 15-7992-7641	Code: Lot 5221031128	Project: 6PPD Alternative Testing 2024
Sample Date: 30 Aug-24 12:00	Material: 6PPD ozonate	Source: Akron Rubber Development (HB Chem
Receipt Date: 30 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 6d 2h	Client: Washington Department of Ecology	

### Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
15-3167-6954	Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	0.7857	Acetone Control passed proportion survive	1

### Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
11-2855-9668	Proportion Survived	Dunnett Multiple Comparison Test	500	1000	707.1	12.1%	1

### Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
08-6515-5574	Proportion Survived	Linear Interpolation (ICPIN)	EC15	536.6	324.5	590.4	1
			EC20	563.1	462.1	618.3	
			EC25	591	500	648.2	
			EC40	683.1	608.3	740.7	
			EC50	752.3	680.1	813.4	

### Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
0	AC	4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
62.5		4	0.9500	0.8581	1.0420	0.9000	1.0000	0.0289	0.0577	6.08%	2.56%
125		4	0.9500	0.8581	1.0420	0.9000	1.0000	0.0289	0.0577	6.08%	2.56%
250		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
500		4	0.9000	0.7163	1.0840	0.8000	1.0000	0.0577	0.1155	12.83%	7.69%
1000		4	0.2000	0.0701	0.3299	0.1000	0.3000	0.0408	0.0817	40.82%	79.49%

### Proportion Survived Detail

MD5: 65E5448A9BE5DA70B60E23C381DB7408

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9000	1.0000	1.0000	1.0000
0	AC	1.0000	0.9000	1.0000	1.0000
62.5		1.0000	0.9000	1.0000	0.9000
125		1.0000	0.9000	0.9000	1.0000
250		1.0000	1.0000	0.9000	1.0000
500		0.8000	1.0000	1.0000	0.8000
1000		0.2000	0.2000	0.3000	0.1000

### Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	9/10	10/10	10/10	10/10
0	AC	10/10	9/10	10/10	10/10
62.5		10/10	9/10	10/10	9/10
125		10/10	9/10	9/10	10/10
250		10/10	10/10	9/10	10/10
500		8/10	10/10	10/10	8/10
1000		2/10	2/10	3/10	1/10

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 14:25 (p 1 of 1)  
 Test Code/ID: 6PPDoz H.a. / 01-3764-1514

<b>Hyalella 24-hour Acute Survival Test</b>				<b>EcoAnalysts</b>	
<b>Start Date:</b> 05 Sep-24 13:58	<b>Species:</b> Hyalella azteca	<b>Sample Code:</b> Lot 5221031128			
<b>End Date:</b> 06 Sep-24 13:10	<b>Protocol:</b> ASTM E1706-20 (2020)	<b>Sample Source:</b> Akron Rubber Development (HB Che			
<b>Sample Date:</b> 30 Aug-24 12:00	<b>Material:</b> 6PPD ozonate	<b>Sample Station:</b>			

Conc-µg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	AC	1	15	10	10	
0	AC	2	13	10	9	
0	AC	3	21	10	10	
0	AC	4	18	10	10	
0	D	1	12	10	9	
0	D	2	1	10	10	
0	D	3	28	10	10	
0	D	4	5	10	10	
62.5		1	8	10	10	
62.5		2	2	10	9	
62.5		3	16	10	10	
62.5		4	6	10	9	
125		1	7	10	10	
125		2	14	10	9	
125		3	4	10	9	
125		4	27	10	10	
250		1	25	10	10	
250		2	10	10	10	
250		3	17	10	9	
250		4	23	10	10	
500		1	19	10	8	
500		2	20	10	10	
500		3	3	10	10	
500		4	22	10	8	
1000		1	9	10	2	
1000		2	24	10	2	
1000		3	26	10	3	
1000		4	11	10	1	

Version V.1

GENERAL

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	6PPD ozonate
Test Type	24-Hour Acute
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	09/05/24
Test Species	<i>Hyalella azteca</i>
Organism Batch	AI090524.01
Organism Acquired	9/5/2024
Organism Acclimation	0
Organism Age	14 days
Test Protocol	TOX 022 Modified
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Test Location	Bath 4
Water Description	reconstituted freshwater
Organisms per Replicate	10
Test Chamber Size	500 mL
Exposure Volume	250 mL
Feeding Information	None
Test Dissolved Oxygen	> 4
Test Temperature	23 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	4	
Temp	21.5	24.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1358 TVL/MARH
TEST END TIME/INIT:	1310 MS

COMPOUND	LOT #
6PPD ozonate	5221031128 ozonate

Concentrations (µg/L)

1	Control
2	Acetone Control
3	62.5
4	125
5	250
6	500
7	1000

Copy and Past VALUES

Treatment	Rep	Chamber
Control	1	
Control	2	15
Control	3	
Control	4	
Acetone Con	1	
Acetone Con	2	
Acetone Con	3	22
Acetone Con	4	23
63	1	5
63	2	9
63	3	2
63	4	18
125	1	17
125	2	21
125	3	19
125	4	13
250	1	10
250	2	20
250	3	11
250	4	7
500	1	12
500	2	3
500	3	16
500	4	4
1000	1	14
1000	2	6
1000	3	8
1000	4	1

v.1

<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	24-Hour Acute	<b>PROTOCOL</b>	TOX 022 Modified
<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	9/5/24	<b>PROJECT NUMBER</b>	PG2032
<b>COMPOUND</b>	6PPD ozonate	<b>TEST END DATE</b>	9/6/24	<b>SPECIES</b>	<i>Hyaella azteca</i>
<b>LOT #</b>	5221031128 ozonate	<b>MATRIX</b>	Liquid	<b>NO. OF ORGANISMS</b>	10

**24-Hour Acute**
**Stock Preparation**

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
800,000	100	80.0

**Dilution Preparation**

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)
800,000	62.5	1000	0.078
800,000	125	1000	0.156
800,000	250	1000	0.313
800,000	500	1000	0.625
800,000	1000	1000	1.250
			<b>Amt. of Acetone (mL)</b>
	Acetone control	1000	1.250

**Test Dilution Prep**

Date	Balance ID	Water Batch ID	Initials	Comments
9/5/2024	7	RFW082724.01	MARH	

**Water Quality**

	Concentration (µg/L)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH	
		> 4	23 ± 1		7.5 ± 1.5	
<b>Day 0 (Stock)</b>	Control	8.5	22.1	318	7.5	
Date	9/5/2024	Acetone Control	8.5	22.3	316	7.6
Time	1308	62.5	8.5	22.4	317	7.8
Tech	MARH	125	8.3	22.4	318	7.8
Meter #	8	250	8.3	22.4	317	7.9
		500	8.3	22.3	317	8.0
		1000	8.3	22.4	316	8.0
<b>Day 1</b>	Control	8.1	22.4	336	7.5	
Rep 1	Acetone Control	8.2	22.4	326	7.6	
Date	9/6/2024	62.5	8.4	22.4	329	7.6
Time	13:10	125	8.4	22.4	334	7.6
Tech	MS	250	8.2	22.4	322	7.7
Meter #	8	500	8.2	22.5	324	7.7
		1000	8.1	22.5	323	7.8

**Comments**

v.1 CLIENT	Washington Department of Ecology	TEST TYPE	24-Hour Acute
PROJECT	6PPD Alternative Testing	TEST START DATE	9/5/24
PROJECT #	PG2032	TEST END DATE	9/6/24
COMPOUND	6PPD ozonate	MATRIX	Liquid
LOT #	5221031128 ozonate	SPECIES	<i>Hyalella azteca</i>
PROTOCOL	TOX 022 Modified	NO. OF ORGANISMS	10

24-Hour Acute

Abbreviation Key:

NB = No Body  
 FB = Found Body  
 ST = Stranded

		Day 1			
		Date	09/06/24		
		Time	13:10		
		Tech	MS		
Concentration (µg/L)	REP	Alive	Dead	Obs	Comments
Control	1	9	1		
	2	10	0		
	3	10	0		
	4	10	0		
Acetone Control	1	10	0		
	2	9	1		
	3	10	0		
	4	10	0		
62.5	1	10	0		
	2	9	1		
	3	10	0		
	4	9	1		
125	1	10	0		
	2	9	1		
	3	9	1		
	4	10	0		
250	1	10	0		
	2	10	0		
	3	9	0	1 NB	
	4	10	0		
500	1	8	2		
	2	10	0		
	3	10	0		
	4	8	2		
1000	1	2	8		
	2	2	8		
	3	3	7		
	4	1	9		

# CETIS Summary Report

Report Date: 16 Oct-24 09:25 (p 1 of 2)  
 Test Code/ID: Alpha-toc H.a. / 04-6280-7570

## Hyalella 24-hour Acute Survival Test

EcoAnalysts

Batch ID: 05-3366-7981	Test Type: Survival	Analyst: Mary Ann Rempel-Hester
Start Date: 29 Aug-24 14:50	Protocol: ASTM E1706-20 (2020)	Diluent: Mod-Hard Synthetic Water
Ending Date: 30 Aug-24 13:01	Species: Hyalella azteca	Brine: Not Applicable
Test Length: 22h	Taxon: Malacostraca	Source: Aquatic Indicators, FL Age: 7D
Sample ID: 20-0070-1778	Code: Lot 1NG0212	Project: 6PPD Alternative Testing 2024
Sample Date: 02 Aug-24 12:00	Material: Alpha-tocopherol	Source: Spectrum
Receipt Date: 02 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 27d 3h	Client: Washington Department of Ecology	

### Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
04-8555-2463	Proportion Survived	Equal Variance t Two-Sample Test	0.1433	Acetone Control passed proportion survive	1

### Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
03-5684-5445	Proportion Survived	Dunnett Multiple Comparison Test	✓ 3232	8403	5211	23.9% <i>DI 0</i>	1
07-7198-3004	Proportion Survived	Dunnett Multiple Comparison Test	✓ 3232	8403	5211	34.9% <i>Acetone 0</i>	1

### Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
02-2196-4177	Proportion Survived	Linear Interpolation (ICPIN)	✓ EC15	373.1	---	5112	1
			✓ EC20	605.8	---	6081	
			✓ EC25	3485	---	6002	
			✓ EC40	6091	3018	---	
			✓ EC50	>8403	---	---	
08-8801-8161	Proportion Survived	Linear Interpolation (ICPIN)	EC15	635.3	---	6617	1
			EC20	3507	---	7674	
			EC25	4174	---	10940	
			EC40	7036	3077	---	
			✓ EC50	>8403	---	---	

### Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9000	0.7701	1.0300	0.8000	1.0000	0.0408	0.0817	9.07%	0.00%
0	AC	4	0.7750	0.5032	1.0470	0.6000	1.0000	0.0854	0.1708	22.04%	13.89%
16		4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	-5.56%
323		4	0.8000	0.5750	1.0250	0.6000	0.9000	0.0707	0.1414	17.68%	11.11%
808		4	0.6750	0.5954	0.7546	0.6000	0.7000	0.0250	0.0500	7.41%	25.00%
3232		4	0.7500	0.4744	1.0260	0.6000	0.9000	0.0866	0.1732	23.09%	16.67%
8403		4	0.4750	0.1470	0.8030	0.2000	0.7000	0.1031	0.2062	43.40%	47.22%

### Proportion Survived Detail

MD5: F4C6E83624C3685545FB539695CD5664

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9000	0.8000	1.0000	0.9000
0	AC	0.8000	0.7000	1.0000	0.6000
16		1.0000	1.0000	1.0000	0.8000
323		0.9000	0.9000	0.8000	0.6000
808		0.6000	0.7000	0.7000	0.7000
3232		0.6000	0.6000	0.9000	0.9000
8403		0.5000	0.5000	0.2000	0.7000

# CETIS Summary Report

Report Date: 16 Oct-24 09:25 (p 2 of 2)  
Test Code/ID: Alpha-toc H.a. / 04-6280-7570

## Hyalella 24-hour Acute Survival Test

EcoAnalysts

### Proportion Survived Binomials

Conc- $\mu$ g/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	9/10	8/10	10/10	9/10
0	AC	8/10	7/10	11/11	6/10
16		10/10	10/10	10/10	8/10
323		9/10	9/10	8/10	6/10
808		6/10	7/10	7/10	7/10
3232		6/10	6/10	9/10	9/10
8403		5/10	5/10	2/10	7/10

**CETIS Test Data Worksheet**

Report Date: 16 Oct-24 09:26 (p 1 of 1)  
 Test Code/ID: Alpha-toc H.a. / 04-6280-7570

<b>Hyalella 24-hour Acute Survival Test</b>				<b>EcoAnalysts</b>	
<b>Start Date:</b>	29 Aug-24 14:50	<b>Species:</b>	Hyalella azteca	<b>Sample Code:</b>	Lot 1NG0212
<b>End Date:</b>	30 Aug-24 13:01	<b>Protocol:</b>	ASTM E1706-20 (2020)	<b>Sample Source:</b>	Spectrum
<b>Sample Date:</b>	02 Aug-24 12:00	<b>Material:</b>	Alpha-tocopherol	<b>Sample Station:</b>	

Conc-µg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	AC	1	9	10	8	
0	AC	2	28	10	7	
0	AC	3	3	11	11	
0	AC	4	6	10	6	
0	D	1	5	10	9	
0	D	2	15	10	8	
0	D	3	24	10	10	
0	D	4	23	10	9	
16		1	12	10	10	
16		2	13	10	10	
16		3	2	10	10	
16		4	16	10	8	
323		1	14	10	9	
323		2	25	10	9	
323		3	10	10	8	
323		4	4	10	6	
808		1	26	10	6	
808		2	11	10	7	
808		3	19	10	7	
808		4	27	10	7	
3232		1	18	10	6	
3232		2	1	10	6	
3232		3	17	10	9	
3232		4	20	10	9	
8403		1	21	10	5	
8403		2	22	10	5	
8403		3	8	10	2	
8403		4	7	10	7	

Version V.1

**GENERAL**

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	alpha-Tocopherol
Test Type	24-Hour Acute
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	08/29/24
Test Species	<i>Hyalalella azteca</i>
Organism Batch	A1082824.01
Organism Acquired	8/28/2024
Organism Acclimation	1
Organism Age	7 Days
Test Protocol	TOX 022 Modified
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Test Location	Bath 4
Water Description	reconstituted freshwater
Organisms per Replicate	10
Test Chamber Size	500 mL
Exposure Volume	250 mL
Feeding Information	None
Test Dissolved Oxygen	> 4
Test Temperature	23 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	4	
Temp	21.5	24.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1450 EM/TVL/CS
TEST END TIME/INIT:	1301 MS

COMPOUND	LOT #
alpha-Tocopherol	1NG0212

Concentrations (µg/L)	
1	Control
2	Acetone Control
3	16
4	323
5	808
6	3232
7	8403

**Copy and Past VALUES**

Treatment	Rep	Chamber
Control	1	22
Control	2	
Control	3	
Control	4	
Acetone Co	1	10
Acetone Co	2	
Acetone Co	3	
Acetone Co	4	
16	1	9
16	2	12
16	3	8
16	4	18
323	1	15
323	2	11
323	3	14
323	4	13
808	1	2
808	2	3
808	3	19
808	4	20
3232	1	21
3232	2	16
3232	3	1
3232	4	7
8403	1	5
8403	2	6
8403	3	17
8403	4	4

v.1

<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	24-Hour Acute	<b>PROTOCOL</b>	TOX 022 Modified
<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	8/29/24	<b>PROJECT NUMBER</b>	PG2032
<b>COMPOUND</b>	alpha-Tocopherol	<b>TEST END DATE</b>	8/30/24	<b>SPECIES</b>	<i>Hyalella azteca</i>
<b>LOT #</b>	1NG0212	<b>MATRIX</b>	Liquid	<b>NO. OF ORGANISMS</b>	10

**24-Hour Acute**
**Stock Preparation**

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
2,080,000	300	624.0

**Dilution Preparation**

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)
2,080,000	16	1000	0.008
2,080,000	323	1000	0.155
2,080,000	808	1000	0.388
2,080,000	3232	1000	1.554
2,080,000	8403	1000	4.040
			<b>Amt. of Acetone (mL)</b>
	Acetone control	1000	4.040

**Test Dilution Prep**

Date	Balance ID	Water Batch ID	Initials	Comments
8/29/2024	7	RFW082724.01	MARH	

**Water Quality**

		DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH	
<b>Concentration (µg/L)</b>		> 4	23 ± 1		7.5 ± 1.5	
<b>Day 0 (Stock)</b>	Control	8.3	22.4	324	7.6	
Date	8/29/2024	Acetone Control	8.4	23.5	319	7.7
Time	1353	16	8.4	22.3	324	7.3
Tech	LG	323	8.3	22.9	323	7.5
Meter #	8	808	8.2	23.1	323	7.7
		3232	8.2	23.1	321	7.7
		8403	8.3	23.1	319	7.8
<b>Day 1</b>	Control	8.6	22.4	319	8.1	
Rep 1	Acetone Control	8.8	22.5	343	8.1	
Date	8/30/2024	16	8.8	22.7	336	8.1
Time	1310	323	8.5	22.7	320	8.1
Tech	EM	808	8.5	22.6	320	8.1
Meter #	7	3232	8.5	22.5	318	8.1
		8403	8.5	22.5	317	8.1

**Comments**

v.1 CLIENT	Washington Department of Ecology	TEST TYPE	24-Hour Acute
PROJECT	6PPD Alternative Testing	TEST START DATE	8/29/24
PROJECT #	PG2032	TEST END DATE	8/30/24
COMPOUND	alpha-Tocopherol	MATRIX	Liquid
LOT #	1NG0212	SPECIES	<i>Hyalella azteca</i>
PROTOCOL	TOX 022 Modified	NO. OF ORGANISMS	10

**Abbreviation Key:**

NB = No Body  
 FB = Found Body  
 ST = Stranded

**24-Hour Acute**

		Day 1			
		Date	8/30.24		
		Time	1433		
		Tech	MS/RE/CS		
Concentration (µg/L)	REP	Alive	Dead	Obs	Comments
Control	1	9	1		
	2	8	2		
	3	10	0		
	4	9	0	1 NB	
Acetone Control	1	8	2		
	2	7	3		
	3	11	0		
	4	6	4		
16	1	10	0		
	2	10	0		
	3	10	0		
	4	8	2		
323	1	9	1		
	2	9	1		
	3	8	2		
	4	6	4		
808	1	6	4		
	2	7	3		
	3	7	3		
	4	7	3		
3232	1	6	4		
	2	6	4		
	3	9	1		
	4	9	1		
8403	1	5	4	1 NB	
	2	5	5		
	3	2	8		
	4	7	2	1 NB	

# CETIS Summary Report

Report Date: 03 Oct-24 13:48 (p 1 of 1)  
 Test Code/ID: Rambutan H.a. / 04-0020-4332

## Hyalella 24-hour Acute Survival Test

EcoAnalysts

Batch ID: 18-2370-8842	Test Type: Survival	Analyst: Mary Ann Rempel-Hester
Start Date: 29 Aug-24 12:02	Protocol: ASTM E1706-20 (2020)	Diluent: Mod-Hard Synthetic Water
Ending Date: 30 Aug-24 13:01	Species: Hyalella azteca	Brine: Not Applicable
Test Length: 25h	Taxon: Malacostraca	Source: Aquatic Indicators, FL Age: 7D
Sample ID: 10-7597-4693	Code: Lot 1704001	Project: 6PPD Alternative Testing 2024
Sample Date: 20 Aug-24 12:00	Material: Rambutan Peel Extract	Source: Essentials of Australia
Receipt Date: 20 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 9d 0h	Client: Washington Department of Ecology	

### Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
07-1270-0886	Proportion Survived	Equal Variance t Two-Sample Test	0.9164	Glycerin Control passed proportion survive	1

### Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
15-2511-9062	Proportion Survived	Dunnett Multiple Comparison Test	10400	>10400	---	23.1%	1

### Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
15-1240-2646	Proportion Survived	Linear Interpolation (ICPIN)	EC15	>10400	---	---	1
			EC20	>10400	---	---	
			EC25	>10400	---	---	
			EC40	>10400	---	---	
			EC50	>10400	---	---	

### Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9000	0.7701	1.0300	0.8000	1.0000	0.0408	0.0817	9.07%	0.00%
0	GC	4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	-8.33%
20		4	0.9500	0.8581	1.0420	0.9000	1.0000	0.0289	0.0577	6.08%	-5.56%
400		4	0.8750	0.6748	1.0750	0.7000	1.0000	0.0629	0.1258	14.38%	2.78%
1000		4	0.8750	0.6748	1.0750	0.7000	1.0000	0.0629	0.1258	14.38%	2.78%
4000		4	0.8750	0.7227	1.0270	0.8000	1.0000	0.0479	0.0957	10.94%	2.78%
10400		4	0.8750	0.6363	1.1140	0.7000	1.0000	0.0750	0.1500	17.14%	2.78%

### Proportion Survived Detail

MD5: 6927EA94B2A96C7DA4B30DE91E0C72AA

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9000	0.8000	1.0000	0.9000
0	GC	1.0000	1.0000	1.0000	0.9000
20		1.0000	0.9000	0.9000	1.0000
400		0.7000	0.9000	0.9000	1.0000
1000		0.9000	0.7000	0.9000	1.0000
4000		0.9000	0.8000	0.8000	1.0000
10400		1.0000	1.0000	0.7000	0.8000

### Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	9/10	8/10	10/10	9/10
0	GC	10/10	10/10	10/10	9/10
20		12/12	9/10	9/10	10/10
400		7/10	9/10	9/10	10/10
1000		9/10	7/10	9/10	10/10
4000		9/10	8/10	8/10	10/10
10400		10/10	10/10	7/10	8/10

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 13:49 (p 1 of 1)  
 Test Code/ID: Rambutan H.a. / 04-0020-4332

<b>Hyalella 24-hour Acute Survival Test</b>				<b>EcoAnalysts</b>
<b>Start Date:</b>	29 Aug-24 12:02	<b>Species:</b>	Hyalella azteca	<b>Sample Code:</b> Lot 1704001
<b>End Date:</b>	30 Aug-24 13:01	<b>Protocol:</b>	ASTM E1706-20 (2020)	<b>Sample Source:</b> Escentials of Australia
<b>Sample Date:</b>	20 Aug-24 12:00	<b>Material:</b>	Rambutan Peel Extract	<b>Sample Station:</b>

Conc-µg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	D	1	6	10	9	
0	D	2	1	10	8	
0	D	3	22	10	10	
0	D	4	7	10	9	
0	GC	1	28	10	10	
0	GC	2	9	10	10	
0	GC	3	16	10	10	
0	GC	4	10	10	9	
20		1	13	12	12	
20		2	21	10	9	
20		3	11	10	9	
20		4	3	10	10	
400		1	23	10	7	
400		2	5	10	9	
400		3	20	10	9	
400		4	24	10	10	
1000		1	8	10	9	
1000		2	26	10	7	
1000		3	27	10	9	
1000		4	4	10	10	
4000		1	19	10	9	
4000		2	15	10	8	
4000		3	17	10	8	
4000		4	18	10	10	
10400		1	14	10	10	
10400		2	2	10	10	
10400		3	25	10	7	
10400		4	12	10	8	

Version V.1

GENERAL

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	Rambutan Peel Extract
Test Type	24-Hour Acute
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	08/29/24
Test Species	<i>Hyalalella azteca</i>
Organism Batch	A1082824.01
Organism Acquired	8/28/2024
Organism Acclimation	1
Organism Age	7 Days
Test Protocol	TOX 022 Modified
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Test Location	Bath 4
Water Description	reconstituted freshwater
Organisms per Replicate	10
Test Chamber Size	500 mL
Exposure Volume	250 mL
Feeding Information	None
Test Dissolved Oxygen	> 4
Test Temperature	23 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	4	
Temp	21.5	24.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1202 CS MARH
TEST END TIME/INIT:	1301 MS

COMPOUND	LOT #
Rambutan Peel Extract	1704001

Concentrations (µg/L)	
1	Control
2	Glycerin Control
3	20
4	400
5	1000
6	4000
7	10400

Copy and Past VALUES

Treatment	Rep	Chamber
Control	1	
Control	2	22
Control	3	
Control	4	
Glycerin Co	1	0
Glycerin Co	2	10
Glycerin Co	3	23
Glycerin Co	4	24
20	1	9
20	2	12
20	3	8
20	4	18
400	1	15
400	2	11
400	3	14
400	4	13
1000	1	2
1000	2	3
1000	3	19
1000	4	20
4000	1	21
4000	2	16
4000	3	1
4000	4	7
10400	1	5
10400	2	6
10400	3	17
10400	4	4

v.1 CLIENT	Washington Department of Ecology	TEST TYPE	24-Hour Acute	PROTOCOL	TOX 022 Modified
PROJECT	GPPD Alternative Testing	TEST START DATE	8/29/24	PROJECT NUMBER	PG2032
COMPOUND	Rambutan Peel Extract	TEST END DATE	8/30/24	SPECIES	<i>Hyalella azteca</i>
LOT #	1704001	MATRIX	Liquid	NO. OF ORGANISMS	10

**24-Hour Acute**

**Stock Preparation**

Target Stock Concentration (µg/L)	Volume of Water (mLs)	Amt. of Toxicant (mg)
2,080,000	300	1273.5

**Dilution Preparation**

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)
2,080,000	20	1000	0.010
2,080,000	400	1000	0.192
2,080,000	1000	1000	0.481
2,080,000	4000	1000	1.923
2,080,000	10400	1000	5.000
			<b>Amt. of Glycerin Stock (mL)</b>
	Glycerin Control	1000	5.000

**Test Dilution Prep**

Date	Balance ID	Water Batch ID	Initials	Comments
8/29/2024	7	RFW082724.01	MARH	Glycerine immiscible with acetone. Stock made with DI. Prepared a glycerine control with 156 mg in 300 mL DI to mimic amount of glycerin in rambutan extract.

**Water Quality**

		DO (mg/L)	TEMP (°C)	CONDUCTIVITY	pH	
Concentration (µg/L)		> 4	23 ± 1	(µS/cm)	7.5 ± 1.5	
<b>Day 0 (Stock)</b>	Control	8.4	23.3	324	7.5	
Date	8/29/2024	Glycerin Control	8.4	22.5	323	7.6
Time	1023	20	8.4	23.0	323	7.8
Tech	MARH	400	8.3	22.9	323	7.8
Meter #	8	1000	8.3	23.0	323	7.9
	4000	8.3	23.0	322	8.0	
	10400	8.3	23.2	321	8.1	
<b>Day 1</b>	Control	8.8	22.5	324	8.0	
Rep 1	Glycerin Control	8.5	22.4	321	8.1	
Date	8/30/2024	20	8.6	22.2	322	8.1
Time	1248	400	8.8	22.3	322	8.1
Tech	EM	1000	9.1	22.3	321	8.1
Meter #	7	4000	8.9	22.4	321	8.1
	10400	8.6	22.4	320	8.0	

**Comments**

v.1 CLIENT	Washington Department of Ecology	TEST TYPE	24-Hour Acute
PROJECT	6PPD Alternative Testing	TEST START DATE	8/29/24
PROJECT #	PG2032	TEST END DATE	8/30/24
COMPOUND	Rambutan Peel Extract	MATRIX	Liquid
LOT #	1704001	SPECIES	<i>Hyalella azteca</i>
PROTOCOL	TOX 022 Modified	NO. OF ORGANISMS	10

24-Hour Acute

Abbreviation Key:

NB = No Body  
 FB = Found Body  
 ST = Stranded

Day 1	
Date	08/30/24
Time	1301
Tech	MS

Concentration (µg/L)	REP	Alive	Dead	Obs	Comments
Control	1	9	1		
	2	8	2		
	3	10	0		
	4	9	0	1 NB	
Glycerin Control	1	10	0		
	2	10	0		
	3	10	0		
	4	9	0	1 NB	
20	1	12	0		
	2	9	1		
	3	9	1		
	4	10	0		
400	1	7	3		
	2	9	1		
	3	9	1		
	4	10	0		
1000	1	9	1		
	2	7	2	1 NB	
	3	9	1		
	4	10	0		
4000	1	9	1		
	2	8	2		
	3	8	2		
	4	10	0		
10400	1	10	0		
	2	10	0		
	3	7	3		
	4	8	2		

**CETIS Summary Report**

Report Date: 03 Oct-24 14:08 (p 1 of 2)  
 Test Code/ID: Sinapic H.a. / 02-4359-6019

**Hyalella 24-hour Acute Survival Test**

EcoAnalysts

Batch ID: 13-4484-4087	Test Type: Survival	Analyst: Mary Ann Rempel-Hester
Start Date: 29 Aug-24 13:48	Protocol: ASTM E1706-20 (2020)	Diluent: Mod-Hard Synthetic Water
Ending Date: 30 Aug-24 13:29	Species: Hyalella azteca	Brine: Not Applicable
Test Length: 24h	Taxon: Malacostraca	Source: Aquatic Indicators, FL Age: 7D
Sample ID: 05-8170-9350	Code: Lot 1512719	Project: 6PPD Alternative Testing 2024
Sample Date: 22 Aug-24 12:00	Material: Sinapic Acid	Source: Indofine Chemical Company
Receipt Date: 22 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 7d 2h	Client: Washington Department of Ecology	

**Single Comparison Summary**

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
17-7407-5195	Proportion Survived	Equal Variance t Two-Sample Test	0.1433	Acetone Control passed proportion survive	1

**Multiple Comparison Summary**

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
00-8222-2518	Proportion Survived	Dunnett Multiple Comparison Test	✓ 70000	140000	98990	27.7% Acetone $\emptyset$	1
18-4896-1419	Proportion Survived	Dunnett Multiple Comparison Test	✓ 70000	140000	98990	17.5% Dil $\emptyset$	1

**Point Estimate Summary**

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
00-4497-5449	Proportion Survived	Linear Interpolation (ICPIN)	EC15	80530	---	93810	1
			EC20	87170	60350	102300	
			EC25	94360	66390	111000	
			EC40	119700	91160	142900	
			EC50	>140000	---	---	
18-1469-6987	Proportion Survived	Linear Interpolation (ICPIN)	✓ EC15	71600	---	85260	1
			✓ EC20	78040	---	91040	
			✓ EC25	85060	68110	97960	
			✓ EC40	110100	92960	125000	
			✓ EC50	130800	110000	---	

**Proportion Survived Summary**

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9000	0.7701	1.0300	0.8000	1.0000	0.0408	0.0817	9.07%	0.00%
0	AC	4	0.7750	0.5032	1.0470	0.6000	1.0000	0.0854	0.1708	22.04%	13.89%
8750		4	0.8750	0.7227	1.0270	0.8000	1.0000	0.0479	0.0957	10.94%	2.78%
17500		4	0.7000	0.5701	0.8299	0.6000	0.8000	0.0408	0.0817	11.66%	22.22%
35000		4	0.7795	0.6240	0.9351	0.7000	0.9000	0.0489	0.0977	12.54%	13.38%
70000		4	0.8500	0.6446	1.0550	0.7000	1.0000	0.0646	0.1291	15.19%	5.56%
140000		4	0.4159	0.3226	0.5092	0.3636	0.5000	0.0293	0.0586	14.10%	53.79%

**Proportion Survived Detail**

MD5: 93C108A477CA8A57D43D62605B2714BA

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9000	0.8000	1.0000	0.9000
0	AC	0.8000	0.7000	1.0000	0.6000
8750		1.0000	0.8000	0.9000	0.8000
17500		0.8000	0.7000	0.6000	0.7000
35000		0.7000	0.7000	0.9000	0.8182
70000		0.8000	0.9000	1.0000	0.7000
140000		0.4000	0.5000	0.3636	0.4000

# CETIS Summary Report

Report Date: 03 Oct-24 14:08 (p 2 of 2)  
Test Code/ID: Sinapic H.a. / 02-4359-6019

## Hyalella 24-hour Acute Survival Test

EcoAnalysts

### Proportion Survived Binomials

Conc- $\mu$ g/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	9/10	8/10	10/10	9/10
0	AC	8/10	7/10	11/11	6/10
8750		10/10	8/10	9/10	8/10
17500		8/10	7/10	6/10	7/10
35000		7/10	7/10	9/10	9/11
70000		8/10	9/10	10/10	7/10
140000		4/10	5/10	4/11	4/10

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 13:56 (p 1 of 1)  
 Test Code/ID: Sinapic H.a. / 02-4359-6019

<b>Hyalella 24-hour Acute Survival Test</b>				<b>EcoAnalysts</b>
<b>Start Date:</b> 29 Aug-24 13:48	<b>Species:</b> Hyalella azteca	<b>Sample Code:</b> Lot 1512719		
<b>End Date:</b> 30 Aug-24 13:29	<b>Protocol:</b> ASTM E1706-20 (2020)	<b>Sample Source:</b> Indofine Chemical Company		
<b>Sample Date:</b> 22 Aug-24 12:00	<b>Material:</b> Sinapic Acid	<b>Sample Station:</b>		

Conc-µg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	AC	1	26	10	8	
0	AC	2	5	10	7	
0	AC	3	6	11	11	
0	AC	4	11	10	6	
0	D	1	23	10	9	
0	D	2	16	10	8	
0	D	3	1	10	10	
0	D	4	14	10	9	
8750		1	20	10	10	
8750		2	28	10	8	
8750		3	9	10	9	
8750		4	25	10	8	
17500		1	8	10	8	
17500		2	7	10	7	
17500		3	24	10	6	
17500		4	13	10	7	
35000		1	12	10	7	
35000		2	2	10	7	
35000		3	3	10	9	
35000		4	17	11	9	
70000		1	19	10	8	
70000		2	15	10	9	
70000		3	4	10	10	
70000		4	27	10	7	
140000		1	10	10	4	
140000		2	22	10	5	
140000		3	21	11	4	
140000		4	18	10	4	

Version V.1

**GENERAL**

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	Sinapic Acid
Test Type	24-Hour Acute
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	08/29/24
Test Species	<i>Hyaella azteca</i>
Organism Batch	A1082824.01
Organism Acquired	8/28/2024
Organism Acclimation	1
Organism Age	7 Days
Test Protocol	TOX 022 Modified
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Test Location	Bath 4
Water Description	reconstituted freshwater
Organisms per Replicate	10
Test Chamber Size	500 mL
Exposure Volume	250 mL
Feeding Information	None
Test Dissolved Oxygen	> 4
Test Temperature	23 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	4	
Temp	21.5	24.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1348 EM/TVL/CS
TEST END TIME/INIT:	1329 CS

COMPOUND	LOT #
Sinapic Acid	1512719

Concentrations (µg/L)	
1	Control
2	Acetone Control
3	8750
4	17500
5	35000
6	70000
7	140000

**Copy and Past VALUES**

Treatment	Rep	Chamber
Control	1	
Control	2	
Control	3	8
Control	4	12
Acetone Co	1	
Acetone Co	2	
Acetone Co	3	14
Acetone Co	4	16
8750	1	9
8750	2	25
8750	3	22
8750	4	27
17500	1	6
17500	2	20
17500	3	17
17500	4	23
35000	1	4
35000	2	11
35000	3	18
35000	4	10
70000	1	24
70000	2	15
70000	3	26
70000	4	28
140000	1	13
140000	2	21
140000	3	5
140000	4	2

v.1

<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	24-Hour Acute	<b>PROTOCOL</b>	TOX 022 Modified
<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	8/29/24	<b>PROJECT NUMBER</b>	PG2032
<b>COMPOUND</b>	Sinapic Acid	<b>TEST END DATE</b>	8/30/24	<b>SPECIES</b>	<i>Hyaella azteca</i>
<b>LOT #</b>	1512719	<b>MATRIX</b>	Liquid	<b>NO. OF ORGANISMS</b>	10

**24-Hour Acute**

**Stock Preparation**

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
28,000,000	100	2800.0

**Dilution Preparation**

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)
28,000,000	8750	1000	0.313
28,000,000	17500	1000	0.625
28,000,000	35000	1000	1.250
28,000,000	70000	1000	2.500
28,000,000	140000	1000	5.000
			<b>Amt. of Acetone (mL)</b>
	Acetone control	1000	5.000

**Test Dilution Prep**

Date	Balance ID	Water Batch ID	Initials	Comments
8/29/2024		7 RFW082724.01	MARH	

**Water Quality**

		Concentration (µg/L)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH
		> 4	> 4	23 ± 1		7.5 ± 1.5
<b>Day 0 (Stock)</b>	Control		8.3	22.4	324	7.6
Date	8/29/2024	Acetone Control	8.4	23.5	319	7.7
Time	1303	8750	8.2	23.0	322	7.6
Tech	LG	17500	8.3	23.1	321	7.5
Meter #	8	35000	8.2	23.1	319	7.3
		70000	8.2	23.1	315	7.1
		140000	8.3	23.2	307	6.6
<b>Day 1</b>	Control		9.0	21.9	324	8.1
Rep 1	Acetone Control		8.8	21.9	321	8.1
Date	8/30/2024	8750	8.8	22.1	321	8.1
Time	1259	17500	8.7	22.2	320	8.1
Tech	EM	35000	8.9	22.0	320	8.0
Meter #	7	70000	8.7	22.1	316	8.6
		140000	8.7	22.3	320	8.0

**Comments**

v.1 CLIENT	Washington Department of Ecology	TEST TYPE	24-Hour Acute
PROJECT	6PPD Alternative Testing	TEST START DATE	8/29/24
PROJECT #	PG2032	TEST END DATE	8/30/24
COMPOUND	Sinapic Acid	MATRIX	Liquid
LOT #	1512719	SPECIES	<i>Hyalella azteca</i>
PROTOCOL	TOX 022 Modified	NO. OF ORGANISMS	10

24-Hour Acute

Abbreviation Key:

NB = No Body  
 FB = Found Body  
 ST = Stranded

Day 1	
Date	08/30/24
Time	1301
Tech	CS/MS

Concentration (µg/L)	REP	Alive	Dead	Obs	Comments
Control	1	9	1		
	2	8	2		
	3	10	0		
	4	9	0	1 NB	
Acetone Control	1	8	2		
	2	7	3		
	3	11	0	1 FB	
	4	6	4		
8750	1	10	0		
	2	8	2		
	3	9	0	1 NB	
	4	8	1	1 NB	
17500	1	8	2		
	2	7	3		
	3	6	4		
	4	7	3		
35000	1	7	3		
	2	7	3		
	3	9	1		
	4	9	2		11 total organisms in chamber
70000	1	8	2		
	2	9	1		
	3	10	0		
	4	7	3		
140000	1	4	6		
	2	5	5		
	3	4	7		11 total organisms in chamber
	4	4	6		

# CETIS Summary Report

Report Date: 03 Oct-24 14:20 (p 1 of 1)  
 Test Code/ID: Durazone H.a. / 02-9319-1959

## Hyalella 24-hour Acute Survival Test

EcoAnalysts

Batch ID: 02-2620-6149	Test Type: Survival	Analyst: Mary Ann Rempel-Hester
Start Date: 05 Sep-24 15:30	Protocol: ASTM E1706-20 (2020)	Diluent: Mod-Hard Synthetic Water
Ending Date: 06 Sep-24 14:05	Species: Hyalella azteca	Brine: Not Applicable
Test Length: 23h	Taxon: Malacostraca	Source: Aquatic Indicators, FL Age: 14D
Sample ID: 20-6510-2258	Code: Lot CD1J23P008	Project: 6PPD Alternative Testing 2024
Sample Date: 22 Aug-24 12:00	Material: Durazone 37	Source: Akrochem
Receipt Date: 22 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 14d 4h	Client: Washington Department of Ecology	

### Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
03-2429-4529	Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	1.0000	Acetone Control passed proportion survive	1

### Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
12-9570-6147	Proportion Survived	Steel Many-One Rank Sum Test	1400	>1400	---	9.09%	1

### Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
03-9578-9500	Proportion Survived	Linear Interpolation (ICPIN)	EC15	>1400	---	---	1
			EC20	>1400	---	---	
			EC25	>1400	---	---	
			EC40	>1400	---	---	
			EC50	>1400	---	---	

### Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
0	AC	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-2.56%
87.5		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
175		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-2.56%
350		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-2.56%
700		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
1400		4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	2.56%

### Proportion Survived Detail

MD5: 492FFB6BB3AEEF27DC78EAA0E08DDAE

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9000	1.0000	1.0000	1.0000
0	AC	1.0000	1.0000	1.0000	1.0000
87.5		1.0000	1.0000	0.9000	1.0000
175		1.0000	1.0000	1.0000	1.0000
350		1.0000	1.0000	1.0000	1.0000
700		1.0000	0.9000	1.0000	1.0000
1400		1.0000	1.0000	1.0000	0.8000

### Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	9/10	10/10	10/10	10/10
0	AC	10/10	10/10	10/10	10/10
87.5		10/10	10/10	9/10	10/10
175		10/10	10/10	10/10	10/10
350		10/10	10/10	10/10	10/10
700		10/10	9/10	10/10	10/10
1400		10/10	10/10	10/10	8/10

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 14:20 (p 1 of 1)  
 Test Code/ID: Durazone H.a. / 02-9319-1959

<b>Hyalella 24-hour Acute Survival Test</b>				<b>EcoAnalysts</b>
<b>Start Date:</b> 05 Sep-24 15:30	<b>Species:</b> Hyalella azteca	<b>Sample Code:</b> Lot CD1J23P008		
<b>End Date:</b> 06 Sep-24 14:05	<b>Protocol:</b> ASTM E1706-20 (2020)	<b>Sample Source:</b> Akrochem		
<b>Sample Date:</b> 22 Aug-24 12:00	<b>Material:</b> Durazone 37	<b>Sample Station:</b>		

Conc-µg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	AC	1	25	10	10	
0	AC	2	10	10	10	
0	AC	3	5	10	10	
0	AC	4	1	10	10	
0	D	1	8	10	9	
0	D	2	16	10	10	
0	D	3	26	10	10	
0	D	4	22	10	10	
87.5		1	11	10	10	
87.5		2	12	10	10	
87.5		3	27	10	9	
87.5		4	14	10	10	
175		1	23	10	10	
175		2	24	10	10	
175		3	21	10	10	
175		4	9	10	10	
350		1	7	10	10	
350		2	28	10	10	
350		3	2	10	10	
350		4	15	10	10	
700		1	3	10	10	
700		2	17	10	9	
700		3	6	10	10	
700		4	19	10	10	
1400		1	20	10	10	
1400		2	13	10	10	
1400		3	18	10	10	
1400		4	4	10	8	

Version V.1

**GENERAL**

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	Durazone 37
Test Type	24-Hour Acute
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	09/05/24
Test Species	<i>Hyaella azteca</i>
Organism Batch	AI090524.01
Organism Acquired	9/5/2024
Organism Acclimation	0
Organism Age	14 days
Test Protocol	TOX 022 Modified
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Test Location	Bath 4
Water Description	reconstituted freshwater
Organisms per Replicate	10
Test Chamber Size	500 mL
Exposure Volume	250 mL
Feeding Information	None
Test Dissolved Oxygen	> 4
Test Temperature	23 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	4	
Temp	21.5	24.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1530 TVL/MARH
TEST END TIME/INIT:	1405 DM

COMPOUND	LOT #
Durazone 37	CD1J23P008

Concentrations (µg/L)	
1	Control
2	Acetone Control
3	87.5
4	175
5	350
6	700
7	1400

**Copy and Past VALUES**

Treatment	Rep	Chamber
Control	1	
Control	2	
Control	3	
Control	4	15
Acetone Co	1	22
Acetone Co	2	23
Acetone Co	3	
Acetone Co	4	
88	1	5
88	2	9
88	3	2
88	4	18
175	1	17
175	2	21
175	3	19
175	4	13
350	1	10
350	2	20
350	3	11
350	4	7
700	1	12
700	2	3
700	3	16
700	4	4
1400	1	14
1400	2	6
1400	3	8
1400	4	1

v.1

<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	24-Hour Acute	<b>PROTOCOL</b>	TOX 022 Modified
<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	9/5/24	<b>PROJECT NUMBER</b>	PG2032
<b>COMPOUND</b>	Durazone 37	<b>TEST END DATE</b>	9/6/24	<b>SPECIES</b>	<i>Hyaella azteca</i>
<b>LOT #</b>	CD1J23P008	<b>MATRIX</b>	Liquid	<b>NO. OF ORGANISMS</b>	10

**24-Hour Acute**

**Stock Preparation**

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
560,000	50	28.0

**Dilution Preparation**

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)
560,000	88	1000	0.156
560,000	175	1000	0.313
560,000	350	1000	0.625
560,000	700	1000	1.250
560,000	1400	1000	2.500
			<b>Amt. of Acetone (mL)</b>
	Acetone control	1000	2.500

**Test Dilution Prep**

Date	Balance ID	Water Batch ID	Initials	Comments
9/5/2024		7 RFW082724.01	MARH	

**Water Quality**

	Concentration (µg/L)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH	
		> 4	23 ± 1		7.5 ± 1.5	
<b>Day 0 (Stock)</b>	Control	8.5	22.1	318	7.5	
Date	9/5/2024	Acetone Control	8.4	22.3	328	7.6
Time	1452	87.5	8.3	22.3	318	7.7
Tech	MARH	175	8.3	22.4	318	7.8
Meter #	8	350	8.3	22.4	317	7.9
		700	8.3	22.4	317	7.9
		1400	8.3	22.4	316	7.9
<b>Day 1</b>	Control	8.1	22.4	336	7.5	
Rep 1	Acetone Control	8.1	22.1	335	8.0	
Date	9/6/2024	87.5	8.3	22.3	373	7.8
Time	1405	175	8.2	22.2	330	7.9
Tech	DM	350	8.2	22.1	327	7.9
Meter #	8	700	8.2	22.0	325	8.0
		1400	8.1	22.1	329	8.0

**Comments**

v.1 CLIENT	Washington Department of Ecology	TEST TYPE	24-Hour Acute
PROJECT	6PPD Alternative Testing	TEST START DATE	9/5/24
PROJECT #	PG2032	TEST END DATE	9/6/24
COMPOUND	Durazone 37	MATRIX	Liquid
LOT #	CD1J23P008	SPECIES	<i>Hyalella azteca</i>
PROTOCOL	TOX 022 Modified	NO. OF ORGANISMS	10

24-Hour Acute

Abbreviation Key:

NB = No Body  
 FB = Found Body  
 ST = Stranded

Day 1	
Date	09/06/24
Time	1405
Tech	DM

Concentration (µg/L)	REP	Alive	Dead	Obs	Comments
Control	1	9	1		
	2	10	0		
	3	10	0		
	4	10	0		
Acetone Control	1	10	0		
	2	10	0		
	3	10	0		
	4	10	0		
87.5	1	10	0		
	2	10	0		
	3	9	1		
	4	10	0		
175	1	10	0		
	2	10	0		
	3	10	0		
	4	10	0		
350	1	10	0		
	2	10	0		
	3	10	0		
	4	10	0		
700	1	10	0		
	2	9	0	1NB	
	3	10	0		
	4	10	0		
1400	1	10	0		
	2	10	0		
	3	10	0		
	4	8	2		

**CETIS Summary Report**

Report Date: 03 Oct-24 14:15 (p 1 of 1)  
 Test Code/ID: Irganox H.a. / 10-8234-4694

**Hyalella 24-hour Acute Survival Test**

EcoAnalysts

Batch ID: 14-8275-4056	Test Type: Survival	Analyst: Mary Ann Rempel-Hester
Start Date: 05 Sep-24 16:29	Protocol: ASTM E1706-20 (2020)	Diluent: Mod-Hard Synthetic Water
Ending Date: 06 Sep-24 14:34	Species: Hyalella azteca	Brine: Not Applicable
Test Length: 22h	Taxon: Malacostraca	Source: Aquatic Indicators, FL Age: 14D
Sample ID: 20-8616-7840	Code: Lot C2PNC-GN	Project: 6PPD Alternative Testing 2024
Sample Date: 13 Aug-24 12:00	Material: Irganox 1076	Source: TCI America
Receipt Date: 13 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 23d 4h	Client: Washington Department of Ecology	

**Single Comparison Summary**

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
08-1669-6778	Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	1.0000	Acetone Control passed proportion survive	1

**Multiple Comparison Summary**

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
05-0203-3441	Proportion Survived	Steel Many-One Rank Sum Test	500000	>500000	---	10.1%	1

**Point Estimate Summary**

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
02-0718-9167	Proportion Survived	Linear Interpolation (ICPIN)	EC15	>500000	---	---	1
			EC20	>500000	---	---	
			EC25	>500000	---	---	
			EC40	>500000	---	---	
			EC50	>500000	---	---	

**Proportion Survived Summary**

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
0	AC	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-2.56%
31250		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
62500		4	0.9500	0.8581	1.0420	0.9000	1.0000	0.0289	0.0577	6.08%	2.56%
125000		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-2.56%
250000		4	0.9250	0.7727	1.0770	0.8000	1.0000	0.0479	0.0957	10.35%	5.13%
500000		4	0.9500	0.8581	1.0420	0.9000	1.0000	0.0289	0.0577	6.08%	2.56%

**Proportion Survived Detail**

MD5: A7A8ABA0C062E1CCDC6889A82A7CCCCF

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9000	1.0000	1.0000	1.0000
0	AC	1.0000	1.0000	1.0000	1.0000
31250		1.0000	1.0000	1.0000	0.9000
62500		0.9000	0.9000	1.0000	1.0000
125000		1.0000	1.0000	1.0000	1.0000
250000		0.8000	0.9000	1.0000	1.0000
500000		0.9000	1.0000	0.9000	1.0000

**Proportion Survived Binomials**

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	9/10	10/10	10/10	10/10
0	AC	10/10	10/10	10/10	10/10
31250		10/10	10/10	10/10	9/10
62500		9/10	9/10	10/10	10/10
125000		10/10	10/10	10/10	10/10
250000		8/10	9/10	10/10	10/10
500000		9/10	10/10	9/10	10/10

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 14:15 (p 1 of 1)  
 Test Code/ID: Irganox H.a. / 10-8234-4694

<b>Hyalella 24-hour Acute Survival Test</b>				<b>EcoAnalysts</b>
<b>Start Date:</b> 05 Sep-24 16:29	<b>Species:</b> Hyalella azteca	<b>Sample Code:</b> Lot C2PNC-GN		
<b>End Date:</b> 06 Sep-24 14:34	<b>Protocol:</b> ASTM E1706-20 (2020)	<b>Sample Source:</b> TCI America		
<b>Sample Date:</b> 13 Aug-24 12:00	<b>Material:</b> Irganox 1076	<b>Sample Station:</b>		

Conc-µg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	AC	1	25	10	10	
0	AC	2	16	10	10	
0	AC	3	8	10	10	
0	AC	4	14	10	10	
0	D	1	7	10	9	
0	D	2	6	10	10	
0	D	3	13	10	10	
0	D	4	12	10	10	
31250		1	9	10	10	
31250		2	10	10	10	
31250		3	3	10	10	
31250		4	28	10	9	
62500		1	24	10	9	
62500		2	19	10	9	
62500		3	1	10	10	
62500		4	23	10	10	
125000		1	15	10	10	
125000		2	27	10	10	
125000		3	5	10	10	
125000		4	17	10	10	
250000		1	20	10	8	
250000		2	21	10	9	
250000		3	26	10	10	
250000		4	11	10	10	
500000		1	22	10	9	
500000		2	2	10	10	
500000		3	4	10	9	
500000		4	18	10	10	

Version V.1

**GENERAL**

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	Irganox 1076
Test Type	24-Hour Acute
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	09/05/24
Test Species	<i>Hyalella azteca</i>
Organism Batch	AI090524.01
Organism Acquired	9/5/2024
Organism Acclimation	0
Organism Age	14 days
Test Protocol	TOX 022 Modified
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Test Location	Bath 4
Water Description	reconstituted freshwater
Organisms per Replicate	10
Test Chamber Size	500 mL
Exposure Volume	250 mL
Feeding Information	None
Test Dissolved Oxygen	> 4
Test Temperature	23 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	4	
Temp	21.5	24.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1629 MARH/TVL
TEST END TIME/INIT:	1434 MS

COMPOUND	LOT #
Irganox 1076	C2PNC-GN

Concentrations (µg/L)	
1	Control
2	Acetone Control
3	31250
4	62500
5	125000
6	250000
7	500000

**Copy and Past VALUES**

Treatment	Rep	Chamber
Control	1	
Control	2	
Control	3	15
Control	4	
Acetone Co	1	
Acetone Co	2	
Acetone Co	3	22
Acetone Co	4	23
31250	1	5
31250	2	9
31250	3	2
31250	4	18
62500	1	17
62500	2	21
62500	3	19
62500	4	13
125000	1	10
125000	2	20
125000	3	11
125000	4	7
250000	1	12
250000	2	3
250000	3	16
250000	4	4
500000	1	14
500000	2	6
500000	3	8
500000	4	1

v.1

<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	24-Hour Acute	<b>PROTOCOL</b>	TOX 022 Modified
<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	9/5/24	<b>PROJECT NUMBER</b>	PG2032
<b>COMPOUND</b>	Irganox 1076	<b>TEST END DATE</b>	9/6/24	<b>SPECIES</b>	<i>Hyalella azteca</i>
<b>LOT #</b>	C2PNC-GN	<b>MATRIX</b>	Liquid	<b>NO. OF ORGANISMS</b>	10

**24-Hour Acute**

**Stock Preparation**

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
200,000,000	175	35000.0

**Dilution Preparation**

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)
200,000,000	31250	1000	0.156
200,000,000	62500	1000	0.313
200,000,000	125000	1000	0.625
200,000,000	250000	1000	1.250
200,000,000	500000	1000	2.500
			<b>Amt. of Acetone (mL)</b>
	Acetone control	1000	2.500

**Test Dilution Prep**

Date	Balance ID	Water Batch ID	Initials	Comments
9/5/2024	7	RFW082724.01	MARH	Film formed on surface of water

**Water Quality**

		Concentration (µg/L)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH
		> 4	> 4	23 ± 1	(µS/cm)	7.5 ± 1.5
<b>Day 0 (Stock)</b>	Control		8.5	22.1	318	7.5
Date	9/5/2024	Acetone Control	8.4	22.3	328	7.6
Time	1545	31250	8.4	22.2	344	7.6
Tech	MARH	62500	8.3	22.3	318	7.7
Meter #	8	125000	8.3	22.4	318	7.8
		250000	8.3	22.4	317	7.9
		500000	8.4	22.4	317	7.9
<b>Day 1</b>	Control		8.1	22.4	336	7.5
Rep 1	Acetone Control		8.1	22.1	335	8.0
Date	9/6/2024	31250	8.2	23.3	348	8.0
Time	1457	62500	8.2	23.3	327	8.0
Tech	DM	125000	8.1	23.4	326	8.0
Meter #	8	250000	8.1	23.4	327	8.0
		500000	8.1	23.6	328	8.0

**Comments**

v.1	<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	24-Hour Acute
	<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	9/5/24
	<b>PROJECT #</b>	PG2032	<b>TEST END DATE</b>	9/6/24
	<b>COMPOUND</b>	Irganox 1076	<b>MATRIX</b>	Liquid
	<b>LOT #</b>	C2PNC-GN	<b>SPECIES</b>	<i>Hyalella azteca</i>
	<b>PROTOCOL</b>	TOX 022 Modified	<b>NO. OF ORGANISMS</b>	10

**24-Hour Acute**
**Abbreviation Key:**

NB = No Body  
 FB = Found Body  
 ST = Stranded

Day 1	
<b>Date</b>	09/06/24
<b>Time</b>	14:34
<b>Tech</b>	MS

Concentration (µg/L)	REP	Alive	Dead	Obs	Comments
Control	1	9	1		
	2	10	0		
	3	10	0		
	4	10	0		
Acetone Control	1	10	0		
	2	10	0		
	3	10	0		
	4	10	0		
31250	1	10	0		
	2	10	0		
	3	10	0		
	4	9	1		
62500	1	9	0	1 NB	
	2	9	0	1 NB	
	3	10	0		
	4	10	0		
125000	1	10	0		
	2	10	0		
	3	10	0		
	4	10	0		
250000	1	8	2		
	2	9	1		
	3	10	0		
	4	10	0		
500000	1	9	1		
	2	10	0		
	3	9	1		
	4	10	0		

## **APPENDIX A.3**

### ***ONCORHYNCHUS MYKISS* (RAINBOW TROUT) 96-HOUR SURVIVAL TEST**

#### **STATISTICAL COMPARISONS AND LABORATORY DATA SHEETS**

**CETIS Summary Report**

Report Date: 03 Oct-24 15:39 (p 1 of 2)  
 Test Code/ID: 6PPD O.m. / 17-6195-8889

**Fish 96-h Acute Survival Test**

EcoAnalysts

Batch ID: 08-0039-8561	Test Type: Survival (96h)	Analyst: Mary Ann Rempel-Hester
Start Date: 05 Sep-24 11:15	Protocol: EPA/821/R-02-012 (2002)	Diluent: Carbon Filtered Municipal Water
Ending Date: 09 Sep-24 10:42	Species: Oncorhynchus mykiss	Brine: Not Applicable
Test Length: 95h	Taxon: Actinopterygii	Source: Thomas Fish Co. Age: 26p
Sample ID: 19-3444-6399	Code: Lot 5221031128	Project: 6PPD Alternative Testing 2024
Sample Date: 30 Aug-24 12:00	Material: 6PPD	Source: Akron Rubber Development (HB Chem
Receipt Date: 30 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 5d 23h	Client: Washington Department of Ecology	

**Single Comparison Summary**

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
18-7449-0701	96h Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	0.5000	Acetone Control passed 96h proportion sur	1

**Multiple Comparison Summary**

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
11-6394-4399	96h Proportion Survived	Steel Many-One Rank Sum Test	70	140	98.99	11.0%	1

**Point Estimate Summary**

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
17-4661-0873	96h Proportion Survived	Linear Interpolation (ICPIN)	EC15	77.31	65.61	87.53	1
			EC20	82.6	70.11	95.77	
			EC25	88.24	74.91	105.5	
			EC40	107.6	93.95	135.1	
			EC50	122.7	105.9	157.9	

**Test Acceptability**

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits			Overlap	Decision
				Lower	Upper			
11-6394-4399	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
17-4661-0873	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
18-7449-0701	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
	96h Proportion Survived	Control Resp	0.975	0.9	<<	Yes	Passes Criteria	

**96h Proportion Survived Summary**

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0	AC	4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	2.50%
35		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	2.50%
70	4	0.9250	0.7727	1.0770	0.8000	1.0000	0.0479	0.0957	10.35%	7.50%	
140	4	0.4000	0.1750	0.6250	0.3000	0.6000	0.0707	0.1414	35.36%	60.00%	
280	4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%	
560	4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%	

**96h Proportion Survived Detail**

MD5: 42A2AD03E57E5087E29E7A1C8F591F0B

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
0	AC	1.0000	0.9000	1.0000	1.0000
35		1.0000	1.0000	1.0000	0.9000
70	1.0000	0.8000	1.0000	0.9000	
140	0.3000	0.6000	0.3000	0.4000	
280	0.0000	0.0000	0.0000	0.0000	
560	0.0000	0.0000	0.0000	0.0000	

# CETIS Summary Report

Report Date: 03 Oct-24 15:39 (p 2 of 2)  
Test Code/ID: 6PPD O.m. / 17-6195-8889

## Fish 96-h Acute Survival Test

EcoAnalysts

### 96h Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	10/10	10/10
0	AC	10/10	9/10	10/10	10/10
35		10/10	10/10	10/10	9/10
70		10/10	8/10	10/10	9/10
140		3/10	6/10	3/10	4/10
280		0/10	0/10	0/10	0/10
560		0/10	0/10	0/10	0/10

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 15:39 (p 1 of 1)  
 Test Code/ID: 6PPD O.m. / 17-6195-8889

<b>Fish 96-h Acute Survival Test</b>				<b>EcoAnalysts</b>
<b>Start Date:</b> 05 Sep-24 11:15	<b>Species:</b> Oncorhynchus mykiss	<b>Sample Code:</b> Lot 5221031128		
<b>End Date:</b> 09 Sep-24 10:42	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Sample Source:</b> Akron Rubber Development (HB Che		
<b>Sample Date:</b> 30 Aug-24 12:00	<b>Material:</b> 6PPD	<b>Sample Station:</b>		

Conc-µg/L	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Survival 72h	Survival 96h	Notes
0	AC	1	26	10	10	10	10	10	
0	AC	2	9	10	10	10	9	9	
0	AC	3	15	10	10	10	10	10	
0	AC	4	2	10	10	10	10	10	
0	D	1	10	10	10	10	10	10	
0	D	2	23	10	10	10	10	10	
0	D	3	5	10	10	10	10	10	
0	D	4	19	10	10	10	10	10	
35		1	1	10	10	10	10	10	
35		2	13	10	10	10	10	10	
35		3	25	10	10	10	10	10	
35		4	21	10	10	9	9	9	
70		1	24	10	10	10	10	10	
70		2	20	10	10	10	10	8	
70		3	4	10	10	10	10	10	
70		4	11	10	10	10	10	9	
140		1	12	10	10	10	6	3	
140		2	3	10	10	9	9	6	
140		3	6	10	10	10	8	3	
140		4	17	10	10	9	8	4	
280		1	27	10	10	6	0	0	
280		2	7	10	10	5	1	0	
280		3	16	10	10	9	1	0	
280		4	28	10	10	6	2	0	
560		1	14	10	0	0	0	0	
560		2	22	10	0	0	0	0	
560		3	18	10	0	0	0	0	
560		4	8	10	0	0	0	0	

**GENERAL**

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	6PPD
Test Type	96-Hour Acute Static Renewal
Matrix	Liquid
Test Acceptability	≥ 90% average survival of control
Test Start Date	09/05/24
Test Species	<i>Oncorhynchus mykiss</i>
Organism Batch	TF082224.01
Organism Acquired	8/22/2024
Organism Acclimation	14
Organism Age	26 days from swimup
Test Protocol	TOX 017
Regional Protocol	WDOE WQ-R-95-80
Test Location	Bath 5
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	carbon filtered tap water
Organisms per Replicate	10
Test Chamber Size	5 L
Exposure Volume	4 L
Feeding Information	None
Test Dissolved Oxygen	> 6
Test Temperature	12 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	6	
Temp	10.5	13.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1115 CS/EM
TEST END TIME/INIT:	1042 TW

REFERENCE TOXICANT TEST ID	LOT #
6PPD	5221031128

Concentrations (µg/L)	
1	Control
2	Acetone Control
3	35
4	70
5	140
6	280
7	560

**Copy and Past VALUES**

Treatment	Rep	Chamber
Control	1	22
Control	2	
Control	3	
Control	4	
Acetone Co	1	10
Acetone Co	2	23
Acetone Co	3	
Acetone Co	4	
35	1	9
35	2	12
35	3	8
35	4	18
70	1	15
70	2	11
70	3	14
70	4	13
140	1	2
140	2	3
140	3	19
140	4	20
280	1	21
280	2	16
280	3	1
280	4	7
560	1	5
560	2	6
560	3	17
560	4	4

CLIENT	Washington Department of Ecology	TEST TYPE	Acute Static Renewal	PROTOCOL	TOX 017
PROJECT	6PPD Alternative Testing	TEST START DATE	9/5/24	PROJECT NUMBER	PG2032
COMPOUND	6PPD	TEST END DATE	9/9/24	SPECIES	<i>Oncorhynchus mykiss</i>
LOT #	5221031128	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Static Renewal

Stock Preparation

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
800,000	100	80.0

Day 0 Dilution Preparation

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)	Total Volume (mLs per rep)
800,000	35	3249.86	0.142	3250
800,000	70	3249.72	0.284	
800,000	140	3249.43	0.569	
800,000	280	3248.86	1.138	
800,000	560	3247.73	2.275	
Acetone control			3247.73	Amt. of Acetone (mL) 2.275

Day 2 Dilution Preparation

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)	Total Volume (mLs renewal stock)
800,000	35	12999.43	0.569	13000
800,000	70	12998.86	1.138	
800,000	140	12997.73	2.275	
800,000	280	12995.45	4.550	
800,000	560	12990.90	9.100	
Acetone control			12990.90	Amt. of Acetone (mL) 9.100

Test Dilution Prep

Date	Balance ID	Water Batch ID	Initials	Comments
9/5/2024	7	CFTW082724.01	EM	Diluent prepared on 9/4/24, spiked with toxicant on 9/5/24 - EM
9/7/2024	volumetric	CFTW090624.01	MCK	

Water Quality

	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH	
	> 6	12 ± 1		7.5 ± 1.5	
	Concentration (µg/L)				
<b>Day 0 (Stock)</b>	Control	9.0	13.0	177	8.0
Date	Acetone Control	9.4	12.9	177	8.1
Time	35	9.1	12.8	177	8.2
Tech	70	9.3	12.9	177	8.2
Meter #	140	9.3	12.7	177	8.3
	280	9.3	12.9	177	8.3
	560	9.4	12.8	177	9.4
<b>Day 1</b>	Control	6.5	11.2	172	7.6
Rep 1	Acetone Control	8.0	10.8	175	7.8
Date	35	8.2	10.6	176	7.8
Time	70	6.8	11.0	175	7.7
Tech	140	8.0	11.6	178	7.7
Meter #	280	8.0	11.5	174	7.8
	560	7.9	11.6	145	7.9
<b>Day 2</b>	Control	7.8	11.9	178	6.8
Rep 2 (old)	Acetone Control	7.9	12.2	174	6.9
Date	35	8.0	10.6	175	6.9
Time	70	7.6	10.7	177	7.0
Tech	140	7.5	13.2	176	7.2
Meter #	280	6.3	11.9	177	7.2
	560				
<b>Day 2</b>	Control	10.9	10.9	156	8.1
Renewal Stock	Acetone Control	11.0	11.4	182	8.1
Date	35	11.0	11.5	157	8.0
Time	70	10.8	11.6	163	7.9
Tech	140	11.0	11.4	181	8.1
Meter #	280	10.9	11.8	156	8.0
	560				
<b>Day 3</b>	Control	7.9	13.0	166	7.3
Rep 3	Acetone Control	8.1	13.3	166	7.4
Date	35	7.3	13.2	164	7.4
Time	70	7.1	12.3	170	7.4
Tech	140	7.6	15.0	164	7.5
Meter #	280	8.9	16.2	163	7.7
	560				
<b>Day 4</b>	Control	7.9	12.7	176	7.5
Rep 4	Acetone Control	8.0	11.7	172	7.6
Date	35	7.6	11.5	169	7.6
Time	70	6.2	11.9	178	7.6
Tech	140	7.0	11.7	173	7.6
Meter #	280	8.1	11.9	170	7.7
	560				

Comments

v.1

<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	96-Hour Acute Static Renewal	<b>PROTOCOL</b>	TOX 017
<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	9/5/24	<b>PROJECT NUMBER</b>	PG2032
<b>COMPOUND</b>	6PPD	<b>TEST END DATE</b>	9/9/24	<b>SPECIES</b>	<i>Oncorhynchus mykiss</i>
<b>LOT #</b>	5.221E+09	<b>MATRIX</b>	Liquid	<b>NO. OF ORGANISMS</b>	10

**Abbreviation Key:**  
 NB = No Body  
 FB = Found Body  
 ST = Stranded

**96-Hour Acute Static Renewal**

Concentration (µg/L)	Rep	Day 1			Day 2			Day 3			Day 4			Comments
		Date	09/06/24		Date	09/07/24		Date	09/08/24		Date	09/09/24		
		Time	1411		Time	905		Time	1213		Time	1042		
		Tech	CS		Tech	MCK		Tech	LG		Tech	TW		
Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs			
Control	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
Acetone Control	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		9	1		9	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
35	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		9	1		9	0		9	0		
70	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		8	2		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		9	1		
140	1	10	0		10	0		6	4		3	3	LOE	
	2	10	0		9	1		9	0		6	3	LOE	
	3	10	0		10	0		8	2		3	5	LOE	
	4	10	0		9	1		8	1		4	4	LOE	
280	1	10	0		6	4		0	6					
	2	10	0		5	5		1	4		0	1		
	3	10	0		9	1		1	8		0	1		
	4	10	0		6	4		2	4		0	2		
560	1	0	10											
	2	0	10											
	3	0	10											
	4	0	10											

**CETIS Summary Report**

Report Date: 03 Oct-24 15:33 (p 1 of 2)  
 Test Code/ID: 6PPDoz O.m. / 21-3442-4038

**Fish 96-h Acute Survival Test**

EcoAnalysts

Batch ID: 02-4783-0168	Test Type: Survival (96h)	Analyst: Mary Ann Rempel-Hester
Start Date: 05 Sep-24 09:56	Protocol: EPA/821/R-02-012 (2002)	Diluent: Carbon Filtered Municipal Water
Ending Date: 09 Sep-24 10:06	Species: Oncorhynchus mykiss	Brine: Not Applicable
Test Length: 4d 0h	Taxon: Actinopterygii	Source: Thomas Fish Co. Age: 26p
Sample ID: 06-9409-5348	Code: Lot 5221031128	Project: 6PPD Alternative Testing 2024
Sample Date: 30 Aug-24 12:00	Material: 6PPD ozonate	Source: Akron Rubber Development (HB Chem
Receipt Date: 30 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 5d 22h	Client: Washington Department of Ecology	

**Single Comparison Summary**

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
20-9048-6846	96h Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	0.5000	Acetone Control passed 96h proportion sur	1

**Multiple Comparison Summary**

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
19-3573-2577	96h Proportion Survived	Steel Many-One Rank Sum Test	35	70	49.5	5.52%	1

**Point Estimate Summary**

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
11-0199-6146	96h Proportion Survived	Linear Interpolation (ICPIN)	EC15	46.78	40.95	51.23	1
			EC20	52.5	47.79	57.49	
			EC25	58.91	53.64	64.49	
			EC40	76.65	69.78	79.77	
			EC50	86.49	80.27	89.3	

**Test Acceptability**

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits			Overlap	Decision
				Lower	Upper			
11-0199-6146	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
19-3573-2577	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
20-9048-6846	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
	96h Proportion Survived	Control Resp	0.975	0.9	<<	Yes	Passes Criteria	

**96h Proportion Survived Summary**

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0	AC	4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	2.50%
35		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	2.50%
70	4	0.6750	0.5954	0.7546	0.6000	0.7000	0.0250	0.0500	7.41%	32.50%	
140	4	0.1000	0.1000	0.1000	0.1000	0.1000	0.0000	0.0000	0.00%	90.00%	
280	4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%	
560	4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%	

**96h Proportion Survived Detail**

MD5: BE19DEEB1A51509A15608C023E2FCC37

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
0	AC	1.0000	0.9000	1.0000	1.0000
35		1.0000	1.0000	0.9000	1.0000
70		0.7000	0.7000	0.6000	0.7000
140		0.1000	0.1000	0.1000	0.1000
280		0.0000	0.0000	0.0000	0.0000
560		0.0000	0.0000	0.0000	0.0000

# CETIS Summary Report

Report Date: 03 Oct-24 15:33 (p 2 of 2)  
Test Code/ID: 6PPDoz O.m. / 21-3442-4038

Fish 96-h Acute Survival Test

EcoAnalysts

## 96h Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	10/10	10/10
0	AC	10/10	9/10	10/10	10/10
35		10/10	10/10	9/10	10/10
70		7/10	7/10	6/10	7/10
140		1/10	1/10	1/10	1/10
280		0/10	0/10	0/10	0/10
560		0/10	0/10	0/10	0/10

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 15:33 (p 1 of 1)  
 Test Code/ID: 6PPDoz O.m. / 21-3442-4038

<b>Fish 96-h Acute Survival Test</b>				<b>EcoAnalysts</b>					
<b>Start Date:</b>	05 Sep-24 09:56	<b>Species:</b>	Oncorhynchus mykiss	<b>Sample Code:</b>	Lot 5221031128				
<b>End Date:</b>	09 Sep-24 10:06	<b>Protocol:</b>	EPA/821/R-02-012 (2002)	<b>Sample Source:</b>	Akron Rubber Development (HB Che				
<b>Sample Date:</b>	30 Aug-24 12:00	<b>Material:</b>	6PPD ozonate	<b>Sample Station:</b>					

Conc-µg/L	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Survival 72h	Survival 96h	Notes
0	AC	1	15	10	10	10	10	10	
0	AC	2	28	10	10	10	9	9	
0	AC	3	4	10	10	10	10	10	
0	AC	4	20	10	10	10	10	10	
0	D	1	8	10	10	10	10	10	
0	D	2	9	10	10	10	10	10	
0	D	3	13	10	10	10	10	10	
0	D	4	17	10	10	10	10	10	
35		1	22	10	10	10	10	10	
35		2	21	10	10	10	10	10	
35		3	25	10	10	10	10	9	
35		4	6	10	10	10	10	10	
70		1	14	10	10	8	8	7	
70		2	5	10	10	9	9	7	
70		3	12	10	10	9	8	6	
70		4	2	10	10	10	9	7	
140		1	19	10	10	3	2	1	
140		2	11	10	10	6	4	1	
140		3	3	10	10	7	5	1	
140		4	18	10	10	6	3	1	
280		1	10	10	7	1	0	0	
280		2	16	10	3	0	0	0	
280		3	26	10	4	0	0	0	
280		4	1	10	7	2	0	0	
560		1	24	10	0	0	0	0	
560		2	7	10	0	0	0	0	
560		3	27	10	0	0	0	0	
560		4	23	10	0	0	0	0	

**GENERAL**

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	6PPD ozonate
Test Type	96-Hour Acute Static Renewal
Matrix	Liquid
Test Acceptability	≥ 90% average survival of control
Test Start Date	09/05/24
Test Species	<i>Oncorhynchus mykiss</i>
Organism Batch	TF082224.01
Organism Acquired	8/22/2024
Organism Acclimation	14
Organism Age	26 days from swimup
Test Protocol	TOX 017
Regional Protocol	WDOE WQ-R-95-80
Test Location	Bath 5
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	carbon filtered tap water
Organisms per Replicate	10
Test Chamber Size	5 L
Exposure Volume	4 L
Feeding Information	None
Test Dissolved Oxygen	> 6
Test Temperature	12 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	6	
Temp	10.5	13.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	0956 CS/TVL
TEST END TIME/INIT:	1006 TW

REFERENCE TOXICANT TEST ID	LOT #
6PPD ozonate	5221031128 ozonate

Concentrations (µg/L)	
1	Control
2	Acetone Control
3	35
4	70
5	140
6	280
7	560

**Copy and Past VALUES**

Treatment	Rep	Chamber
Control	1	
Control	2	22
Control	3	
Control	4	
Acetone Co	1	
Acetone Co	2	
Acetone Co	3	10
Acetone Co	4	23
35	1	9
35	2	12
35	3	8
35	4	18
70	1	15
70	2	11
70	3	14
70	4	13
140	1	2
140	2	3
140	3	19
140	4	20
280	1	21
280	2	16
280	3	1
280	4	7
560	1	5
560	2	6
560	3	17
560	4	4

CLIENT	Washington Department of Ecology	TEST TYPE	Acute Static Renewal	PROTOCOL	TOX 017
PROJECT	6PPD Alternative Testing	TEST START DATE	9/5/24	PROJECT NUMBER	PG2032
COMPOUND	6PPD ozonate	TEST END DATE	9/9/24	SPECIES	<i>Oncorhynchus mykiss</i>
LOT #	5221031128 ozonate	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Static Renewal

Stock Preparation

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
800,000	100	80.0

Day 0 Dilution Preparation

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)	Total Volume (mLs per rep)
800,000	35	3249.86	0.142	3250
800,000	70	3249.72	0.284	
800,000	140	3249.43	0.569	
800,000	280	3248.86	1.138	
800,000	560	3247.73	2.275	
			Amt. of Acetone (mL)	
	Acetone control	3247.73	2.275	

Day 2 Dilution Preparation

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)	Total Volume (mLs renewal stock)
800,000	35	12999.43	0.569	13000
800,000	70	12998.86	1.138	
800,000	140	12997.73	2.275	
800,000	280	12995.45	4.550	
800,000	560	12990.90	9.100	
			Amt. of Acetone (mL)	
	Acetone control	12990.90	9.100	

Test Dilution Prep

Date	Balance ID	Water Batch ID	Initials	Comments
9/5/2024		7 CFTW082724.01	EM	Diluent prepared on 9/4/24, spiked with toxicant on 9/5/24 - EM
9/7/2024	volumetric	CFTW090624.01	MCK	

Water Quality

		Concentration (µg/L)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH
			> 6	12 ± 1		7.5 ± 1.5
<b>Day 0 (Stock)</b>		Control	9.3	13.0	178	8.1
Date	9/5/2024	Acetone Control	9.3	12.9	177	8.2
Time	934	35	9.3	12.8	178	8.2
Tech	CS	70	9.3	12.8	178	8.3
Meter #	9	140	9.3	12.7	177	8.3
		280	9.1	12.7	178	8.3
		560	9.3	12.6	177	8.3
<b>Day 1</b>		Control	7.6	10.6	178	7.6
Rep 1		Acetone Control	8.4	10.5	177	7.6
Date	9/6/2024	35	8.0	11.5	178	7.6
Time	1445	70	7.9	11.9	177	7.7
Tech	CS	140	8.3	12.6	178	7.7
Meter #	7	280	8.1	12.8	174	7.7
		560	7.9	11.9	176	7.9
<b>Day 2</b>		Control	8.0	11.1	181	7.2
Rep 2 (old)		Acetone Control	8.2	10.9	178	7.2
Date	9/7/2024	35	8.3	12.0	178	7.4
Time	839	70	7.5	11.7	178	7.3
Tech	EM	140	7.2	12.3	177	7.3
Meter #	8	280	7.5	12.0	177	7.3
		560				
<b>Day 2</b>		Control	10.9	10.9	156	8.1
Renewal Stock		Acetone Control	11.0	11.4	182	8.1
Date	9/7/2024	35	10.6	12.1	168	8.0
Time	1129	70	10.9	12.1	161	7.9
Tech	MCK	140	10.7	12.5	158	8.0
Meter #	7	280	10.7	12.5	159	7.9
		560				
<b>Day 3</b>		Control	7.9	13.0	166	7.3
Rep 3		Acetone Control	8.1	13.3	166	7.4
Date	9/8/2024	35	7.7	13.8	166	7.3
Time	1313	70	7.5	13.8	166	7.5
Tech	LG	140	8.9	14.5	162	7.7
Meter #	9	280	9.4	14.8	164	7.9
		560				
<b>Day 4</b>		Control	7.9	12.7	176	7.5
Rep 4		Acetone Control	8.0	11.7	172	7.6
Date	9/9/2024	35	7.1	11.8	172	7.6
Time	816	70	5.5	12.9	174	7.5
Tech	TW	140	7.8	12.8	169	7.7
Meter #	9	280				
		560				

Comments

CLIENT	Washington Department of Ecology	TEST TYPE	96-Hour Acute Static Renewal	PROTOCOL	TOX 017
PROJECT	GPPD Alternative Testing	TEST START DATE	9/5/24	PROJECT NUMBER	PG2032
COMPOUND	GPPD ozonate	TEST END DATE	9/9/24	SPECIES	<i>Oncorhynchus mykiss</i>
LOT #	5221031128 ozonate	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Static Renewal

Abbreviation Key:

NB = No Body

FB = Found Body

ST = Stranded

Concentration (µg/L)	Dose	Day 1			Day 2			Day 3			Day 4			Comments
		Date	09/06/24		Date	09/07/24		Date	09/08/24		Date	09/09/24		
		Time	1502		Time	1129		Time	1318		Time	1006		
		Tech	CS		Tech	MCK		Tech	LG		Tech	TW		
Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs			
Control	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
Acetone Control	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		9	1		9	1		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
35	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		9	1		
	4	10	0		10	0		10	0		10	0		
70	1	10	0		8	2		8	0		7	1		2 fish observed not swimming with strained gill action on final day -TW 9/9/24
	2	10	0		9	1		9	0		7	2		1 fish observed not swimming with strained gill action on final day -TW 9/9/24
	3	10	0		9	1		8	1		6	2		
	4	10	0		10	0		9	1		7	2		
140	1	10	0		3	7		2	1		1	0	1NB	
	2	10	0		6	4		4	2		1	3		
	3	10	0		7	3		5	2		1	4		
	4	10	0		6	4		3	3		1	2		
280	1	7	3		1	6		0	1					
	2	3	7		0	3								
	3	4	6		0	4								
	4	7	3		2	5		0	2					
560	1	0	10											
	2	0	10											
	3	0	10											
	4	0	10											

# CETIS Summary Report

Report Date: 16 Oct-24 09:30 (p 1 of 2)  
 Test Code/ID: Alpha-toc O.m. / 08-9740-2968

## Fish 96-h Acute Survival Test

EcoAnalysts

Batch ID: 11-9883-1451	Test Type: Survival (96h)	Analyst: Mary Ann Rempel-Hester
Start Date: 29 Aug-24 14:00	Protocol: EPA/821/R-02-012 (2002)	Diluent: Carbon Filtered Municipal Water
Ending Date: 02 Sep-24 14:04	Species: Oncorhynchus mykiss	Brine: Not Applicable
Test Length: 4d 0h	Taxon: Actinopterygii	Source: Thomas Fish Co. Age: 19p
Sample ID: 15-8735-8062	Code: Lot 1NG0212	Project: 6PPD Alternative Testing 2024
Sample Date: 02 Aug-24 12:00	Material: Alpha-tocopherol	Source: Spectrum
Receipt Date: 02 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 27d 2h	Client: Washington Department of Ecology	

## Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
20-6806-0867	96h Proportion Survived	Unequal Variance t Two-Sample Test	0.0068	Acetone Control failed 96h proportion survi	1

## Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
04-2536-4783	96h Proportion Survived	Steel Many-One Rank Sum Test	8403	>8403	---	29.8% Acetone $\emptyset$	1
14-1894-5482	96h Proportion Survived	Steel Many-One Rank Sum Test	3232	8403	5211	5.32% Dil $\emptyset$	1

## Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S	
05-0137-6716	96h Proportion Survived	Linear Interpolation (ICPIN)	✓ EC15	5443	4523	7322	1	
			✓ EC20	6475	5042	9560		
			✓ EC25	7704	5611	---		Dilution $\emptyset$
			✓ EC40	>8403	---	---		
			✓ EC50	>8403	---	---		
10-0799-8259	96h Proportion Survived	Linear Interpolation (ICPIN)	EC15	6541	4450	---	1	
			EC20	8274	4870	---		
			EC25	>8403	---	---		Acetone $\emptyset$
			✓ EC40	>8403	---	---		
			✓ EC50	>8403	---	---		

## Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits			Overlap	Decision
				Lower	Upper			
04-2536-4783	96h Proportion Survived	Control Resp	0.55	0.9	<<	Yes	Below Criteria	
05-0137-6716	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
10-0799-8259	96h Proportion Survived	Control Resp	0.55	0.9	<<	Yes	Below Criteria	
14-1894-5482	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
20-6806-0867	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
	96h Proportion Survived	Control Resp	0.55	0.9	<<	Yes	Below Criteria	

## 96h Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0	AC	4	0.5500	0.2188	0.8812	0.3000	0.8000	0.1041	0.2082	37.85%	45.00%
16		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
323		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
808		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
3232		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
8403		4	0.7250	0.5727	0.8773	0.6000	0.8000	0.0479	0.0957	13.21%	27.50%

# CETIS Summary Report

Report Date: 16 Oct-24 09:30 (p 2 of 2)  
 Test Code/ID: Alpha-toc O.m. / 08-9740-2968

## Fish 96-h Acute Survival Test

EcoAnalysts

### 96h Proportion Survived Detail

MD5: 971D011F28DF866564EBA7088AB07AB2

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
0	AC	0.3000	0.6000	0.8000	0.5000
16		1.0000	1.0000	1.0000	1.0000
323		1.0000	1.0000	1.0000	1.0000
808		1.0000	1.0000	1.0000	1.0000
3232		1.0000	1.0000	1.0000	1.0000
8403		0.8000	0.8000	0.7000	0.6000

### 96h Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	10/10	10/10
0	AC	3/10	6/10	8/10	5/10
16		10/10	10/10	10/10	10/10
323		10/10	10/10	10/10	10/10
808		10/10	10/10	10/10	10/10
3232		10/10	10/10	10/10	10/10
8403		8/10	8/10	7/10	6/10

**CETIS Test Data Worksheet**

Report Date: 16 Oct-24 09:30 (p 1 of 1)  
 Test Code/ID: Alpha-toc O.m. / 08-9740-2968

<b>Fish 96-h Acute Survival Test</b>				<b>EcoAnalysts</b>					
<b>Start Date:</b>	29 Aug-24 14:00	<b>Species:</b>	Oncorhynchus mykiss	<b>Sample Code:</b>	Lot 1NG0212				
<b>End Date:</b>	02 Sep-24 14:04	<b>Protocol:</b>	EPA/821/R-02-012 (2002)	<b>Sample Source:</b>	Spectrum				
<b>Sample Date:</b>	02 Aug-24 12:00	<b>Material:</b>	Alpha-tocopherol	<b>Sample Station:</b>					

Conc-µg/L	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Survival 72h	Survival 96h	Notes
0	AC	1	21	10	7	7	6	3	
0	AC	2	1	10	9	8	7	6	
0	AC	3	20	10	9	9	9	8	
0	AC	4	28	10	8	6	5	5	
0	D	1	7	10	10	10	10	10	
0	D	2	27	10	10	10	10	10	
0	D	3	22	10	10	10	10	10	
0	D	4	3	10	10	10	10	10	
16		1	15	10	10	10	10	10	
16		2	2	10	10	10	10	10	
16		3	19	10	10	10	10	10	
16		4	8	10	10	10	10	10	
323		1	17	10	10	10	10	10	
323		2	12	10	10	10	10	10	
323		3	14	10	10	10	10	10	
323		4	25	10	10	10	10	10	
808		1	13	10	10	10	10	10	
808		2	5	10	10	10	10	10	
808		3	18	10	10	10	10	10	
808		4	24	10	10	10	10	10	
3232		1	4	10	10	10	10	10	
3232		2	16	10	10	10	10	10	
3232		3	23	10	10	10	10	10	
3232		4	11	10	10	10	10	10	
8403		1	26	10	10	10	10	8	
8403		2	6	10	10	10	10	8	
8403		3	9	10	10	10	9	7	
8403		4	10	10	8	8	7	6	

GENERAL

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	alpha-Tocopherol
Test Type	96-Hour Acute Static Renewal
Matrix	Liquid
Test Acceptability	≥ 90% average survival of control
Test Start Date	08/29/24
Test Species	<i>Oncorhynchus mykiss</i>
Organism Batch	TF082224.01
Organism Acquired	8/22/2024
Organism Acclimation	7
Organism Age	19 days from swimup
Test Protocol	TOX 017
Regional Protocol	WDOE WQ-R-95-80
Test Location	Bath 5
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	carbon filtered tap water
Organisms per Replicate	10
Test Chamber Size	1 gal
Exposure Volume	3.25 L
Feeding Information	None
Test Dissolved Oxygen	> 6
Test Temperature	12 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	6	
Temp	10.5	13.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1400 AR CS
TEST END TIME/INIT:	1404 MARH

REFERENCE TOXICANT TEST ID	LOT #
alpha-Tocopherol	1NG0212

Concentrations (µg/L)	
1	Control
2	Acetone Control
3	16
4	323
5	808
6	3232
7	8403

Copy and Past VALUES

Treatment	Rep	Chamber
Control	1	22
Control	2	
Control	3	
Control	4	
Acetone Co	1	10
Acetone Co	2	
Acetone Co	3	
Acetone Co	4	
16	1	9
16	2	12
16	3	8
16	4	18
323	1	15
323	2	11
323	3	14
323	4	13
808	1	2
808	2	3
808	3	19
808	4	20
3232	1	21
3232	2	16
3232	3	1
3232	4	7
8403	1	5
8403	2	6
8403	3	17
8403	4	4

CLIENT	Washington Department of Ecology	TEST TYPE	Acute Static Renewal	PROTOCOL	TOX 017
PROJECT	6PPD Alternative Testing	TEST START DATE	8/29/24	PROJECT NUMBER	PG2032
COMPOUND	alpha-Tocopherol	TEST END DATE	9/2/24	SPECIES	<i>Oncorhynchus mykiss</i>
LOT #	1NG0212	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Static Renewal

Stock Preparation

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
2,080,000	300	624.0

Day 0 Dilution Preparation

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)	Total Volume (mLs per rep)
2,080,000	16	3249.98	0.025	3250
2,080,000	323	3249.50	0.505	
2,080,000	808	3248.74	1.263	
2,080,000	3232	3244.95	5.050	
2,080,000	8403	3236.87	13.130	
			Amt. of Acetone (mL)	
	Acetone control	3236.87	13.130	

Day 2 Dilution Preparation

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)	Total Volume (mLs renewal stock)
2,080,000	16	12999.90	0.100	13000
2,080,000	323	12997.98	2.019	
2,080,000	808	12994.95	5.050	
2,080,000	3232	12979.80	20.200	
2,080,000	8403	12947.48	52.519	
			Amt. of Acetone (mL)	
	Acetone control	12947.48	52.519	

Test Dilution Prep

Date	Balance ID	Water Batch ID	Initials	Comments
8/29/2024	7	CFTW082724.01	Spikes AR	AR 08/28/24 Diluent Prep
8/31/2024	Volumetric	CFTW082724.01 - 083024.01	MARH	

Water Quality

	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH	
	> 6	12 ± 1		7.5 ± 1.5	
<b>Day 0 (Stock)</b>	Control	9.5	13.4	176	8.3
Date	Acetone Control	9.9	13.3	173	8.3
Time	16	9.6	13.4	175	8.4
Tech	323	9.6	13.2	175	8.4
Meter #	808	9.6	13.4	175	8.4
	3232	9.6	13.4	174	8.4
	8403	9.8	13.4	172	8.4
<b>Day 1</b>	Control	8.2	12.2	185	6.8
Rep 1	Acetone Control	8.6	12.7	181	6.9
Date	16	8.7	11.6	183	7.1
Time	323	8.4	11.5	183	7.2
Tech	808	8.8	11.8	183	7.2
Meter #	3232	9.4	11.9	183	7.4
	8403	8.4	12.1	181	7.2
<b>Day 2</b>	Control	8.2	11.6	186	7.6
Rep 2 (old)	Acetone Control	8.3	12.8	183	7.0
Date	16	8.6	11.8	186	7.3
Time	323	8.5	11.7	186	7.4
Tech	808	8.6	11.9	186	7.5
Meter #	3232	8.5	12.3	185	7.5
	8403	8.8	12.4	183	7.5
<b>Day 2</b>	Control	9.5	13.0	195	7.7
Renewal Stock	Acetone Control	9.7	12.3	184	7.3
Date	16	9.6	13.4	196	7.8
Time	323	10.0	13.4	186	7.5
Tech	808	9.7	13.4	193	7.3
Meter #	3232	9.7	13.4	183	7.4
	8403	9.8	12.5	182	7.3
<b>Day 3</b>	Control	8.0	12.3	191	7.6
Rep 3	Acetone Control	8.1	12.3	182	7.7
Date	16	8.3	12.0	190	7.7
Time	323	8.5	11.9	185	7.7
Tech	808	8.7	12.5	189	7.8
Meter #	3232	8.2	11.8	183	7.7
	8403	8.5	12.0	183	7.7
<b>Day 4</b>	Control	8.2	13.0	190	7.6
Rep 4	Acetone Control	9.1	13.3	179	7.7
Date	16	8.2	12.1	191	7.7
Time	323	8.6	12.4	185	7.7
Tech	808	8.5	12.3	188	7.7
Meter #	3232	7.9	12.5	180	7.7
	8403	8.2	13.4	182	7.7

Comments

CLIENT	Washington Department of Ecology	TEST TYPE	96-Hour Acute Static Renewal	PROTOCOL	TOX 017
PROJECT	6PPD Alternative Testing	TEST START DATE	8/29/24	PROJECT NUMBER	PG2032
COMPOUND	alpha-Tocopherol	TEST END DATE	9/2/24	SPECIES	<i>Oncorhynchus mykiss</i>
LOT #	1NG0212	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Static Renewal

Abbreviation Key:

NB = No Body  
 FB = Found Body  
 ST = Stranded

Concentration (µg/L)	R.D. R.C.	Day 1			Day 2			Day 3			Day 4			Comments
		Date	08/30/24		Date	08/31/24		Date	09/01/24		Date	09/02/24		
		Time	944		Time	1416		Time	1056		Time	1404		
		Tech	MCK		Tech	MARH		Tech	LG		Tech	MARH		
		Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	
Control	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
Acetone Control	1	7	3		7	0		6	1		3	3		
	2	9	1		8	1	1 dark	7	1		6	1		
	3	9	1		9	0		9	0		8	1		
	4	8	2		6	2		5	1		5	0		
16	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
323	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
808	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
3232	1	10	0		10	0	water cloudy	10	0		10	0	water cloudy	
	2	10	0		10	0	water cloudy	10	0		10	0	water cloudy	
	3	10	0		10	0	water cloudy	10	0		10	0	water cloudy	
	4	10	0		10	0	water cloudy	10	0		10	0	water cloudy	
8403	1	10	0		10	0	water cloudy	10	0		8	2	water cloudy	
	2	10	0		10	0	water cloudy	10	0		8	2	water cloudy	
	3	10	0		10	0	water cloudy	9	1		7	2	water cloudy	
	4	8	2		8	0	water cloudy	7	1		6	1	water cloudy	

# CETIS Summary Report

Report Date: 03 Oct-24 14:41 (p 1 of 2)  
 Test Code/ID: Rambutan O.m. / 10-6260-5809

## Fish 96-h Acute Survival Test

EcoAnalysts

Batch ID: 11-8834-9469	Test Type: Survival (96h)	Analyst: Mary Ann Rempel-Hester
Start Date: 29 Aug-24 10:22	Protocol: EPA/821/R-02-012 (2002)	Diluent: Carbon Filtered Municipal Water
Ending Date: 02 Sep-24 10:07	Species: Oncorhynchus mykiss	Brine: Not Applicable
Test Length: 96h	Taxon: Actinopterygii	Source: Thomas Fish Co. Age: 19pSU
Sample ID: 19-6037-9792	Code: Lot 1704001	Project: 6PPD Alternative Testing 2024
Sample Date: 20 Aug-24 12:00	Material: Rambutan Peel Extract	Source: Essentials of Australia
Receipt Date: 20 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 8d 22h	Client: Washington Department of Ecology	

## Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
08-8041-7359	96h Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	0.5000	Glycerin Control passed 96h proportion sur	1

## Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
02-2557-8860	96h Proportion Survived	Steel Many-One Rank Sum Test	10400	>10400	---	---	1

## Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
11-6111-8081	96h Proportion Survived	Linear Interpolation (ICPIN)	EC15	>10400	---	---	1
			EC20	>10400	---	---	
			EC25	>10400	---	---	
			EC40	>10400	---	---	
			EC50	>10400	---	---	

## Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
02-2557-8860	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
08-8041-7359	96h Proportion Survived	Control Resp	0.975	0.9	<<	Yes	Passes Criteria
	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
11-6111-8081	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria

## 96h Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0	GC	4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	2.50%
20		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
400		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
1000		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
4000		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
10400		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%

## 96h Proportion Survived Detail

MD5: B2A0D05DE9CE67D1A8D6B76192784CE3

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
0	GC	0.9000	1.0000	1.0000	1.0000
20		1.0000	1.0000	1.0000	1.0000
400		1.0000	1.0000	1.0000	1.0000
1000		1.0000	1.0000	1.0000	1.0000
4000		1.0000	1.0000	1.0000	1.0000
10400		1.0000	1.0000	1.0000	1.0000

# CETIS Summary Report

Report Date: 03 Oct-24 14:41 (p 2 of 2)  
Test Code/ID: Rambutan O.m. / 10-6260-5809

Fish 96-h Acute Survival Test

EcoAnalysts

## 96h Proportion Survived Binomials

Conc- $\mu$ g/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	10/10	10/10
0	GC	9/10	10/10	10/10	10/10
20		10/10	10/10	10/10	10/10
400		10/10	10/10	10/10	10/10
1000		10/10	10/10	10/10	10/10
4000		10/10	10/10	10/10	10/10
10400		10/10	10/10	10/10	10/10

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 14:41 (p 1 of 1)  
 Test Code/ID: Rambutan O.m. / 10-6260-5809

<b>Fish 96-h Acute Survival Test</b>				<b>EcoAnalysts</b>					
<b>Start Date:</b>	29 Aug-24 10:22	<b>Species:</b>	Oncorhynchus mykiss	<b>Sample Code:</b>	Lot 1704001				
<b>End Date:</b>	02 Sep-24 10:07	<b>Protocol:</b>	EPA/821/R-02-012 (2002)	<b>Sample Source:</b>	Essentials of Australia				
<b>Sample Date:</b>	20 Aug-24 12:00	<b>Material:</b>	Rambutan Peel Extract	<b>Sample Station:</b>					

Conc-µg/L	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Survival 72h	Survival 96h	Notes
0	D	1	14	10	10	10	10	10	
0	D	2	2	10	10	10	10	10	
0	D	3	10	10	10	10	10	10	
0	D	4	3	10	10	10	10	10	
0	GC	1	4	10	10	10	9	9	
0	GC	2	21	10	10	10	10	10	
0	GC	3	15	10	10	10	10	10	
0	GC	4	19	10	10	10	10	10	
20		1	6	10	10	10	10	10	
20		2	13	10	10	10	10	10	
20		3	9	10	10	10	10	10	
20		4	20	10	10	10	10	10	
400		1	16	10	10	10	10	10	
400		2	26	10	10	10	10	10	
400		3	7	10	10	10	10	10	
400		4	17	10	10	10	10	10	
1000		1	11	10	10	10	10	10	
1000		2	12	10	10	10	10	10	
1000		3	8	10	10	10	10	10	
1000		4	5	10	10	10	10	10	
4000		1	22	10	10	10	10	10	
4000		2	27	10	10	10	10	10	
4000		3	25	10	10	10	10	10	
4000		4	1	10	10	10	10	10	
10400		1	28	10	10	10	10	10	
10400		2	24	10	10	10	10	10	
10400		3	23	10	10	10	10	10	
10400		4	18	10	10	10	10	10	

**GENERAL**

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	Rambutan Peel Extract
Test Type	96-Hour Acute Static Renewal
Matrix	Liquid
Test Acceptability	≥ 90% average survival of control
Test Start Date	08/29/24
Test Species	<i>Oncorhynchus mykiss</i>
Organism Batch	TF082224.01
Organism Acquired	8/22/2024
Organism Acclimation	7
Organism Age	19 days from swimup
Test Protocol	TOX 017
Regional Protocol	WDOE WQ-R-95-80
Test Location	Bath 5
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	carbon filtered tap water
Organisms per Replicate	10
Test Chamber Size	1 Gal
Exposure Volume	3.25 L
Feeding Information	None
Test Dissolved Oxygen	> 6
Test Temperature	12 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	6	
Temp	10.5	13.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1022 CS/AR
TEST END TIME/INIT:	1007 MARH

REFERENCE TOXICANT TEST ID	LOT #
Rambutan Peel Extract	1704001

Concentrations (µg/L)	
1	Control
2	Glycerine Control
3	20
4	400
5	1000
6	4000
7	10400

**Copy and Past VALUES**

Treatment	Rep	Chamber
Control	1	
Control	2	22
Control	3	
Control	4	
Glycerine C	1	0
Glycerine C	2	10
Glycerine C	3	23
Glycerine C	4	24
20	1	9
20	2	12
20	3	8
20	4	18
400	1	15
400	2	11
400	3	14
400	4	13
1000	1	2
1000	2	3
1000	3	19
1000	4	20
4000	1	21
4000	2	16
4000	3	1
4000	4	7
10400	1	5
10400	2	6
10400	3	17
10400	4	4

CLIENT	Washington Department of Ecology	TEST TYPE	Acute Static Renewal	PROTOCOL	TOX 017
PROJECT	6PPD Alternative Testing	TEST START DATE	8/29/24	PROJECT NUMBER	PG2032
COMPOUND	Rambutan Peel Extract	TEST END DATE	9/2/24	SPECIES	<i>Oncorhynchus mykiss</i>
LOT #	1704001	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Static Renewal

Stock Preparation

Target Stock Concentration (µg/L)	Volume of Water (mLs)	Amt. of Toxicant (mg)
2,080,000	300	624.0

Day 0 Dilution Preparation

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)	Total Volume (mLs per rep)
2,080,000	20	3249.97	0.031	3250
2,080,000	400	3249.38	0.625	
2,080,000	1000	3248.44	1.563	
2,080,000	4000	3243.75	6.250	
2,080,000	10400	3233.75	16.250	
			<b>Amt. of Glycerine Stock (mL)</b>	
	Acetone control	3233.75	16.250	

Day 2 Dilution Preparation

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)	Total Volume (mLs renewal stock)
2,080,000	20	12999.88	0.125	13000
2,080,000	400	12997.50	2.500	
2,080,000	1000	12993.75	6.250	
2,080,000	4000	12975.00	25.000	
2,080,000	10400	12935.00	65.000	
			<b>Amt. of Glycerine Stock (mL)</b>	
	Acetone control	12935.00	65.000	

Test Dilution Prep

Date	Balance ID	Water Batch ID	Initials	Comments
8/28/2029		7 cftw082724.01	Water spiked 8/29 AR	AR 08/28/24 Diluent Prep.stock made 8/29 in water (glycerine immiscible in acetone). Prepared a glycerine control instead of acetone control
8/31/2024	Volumetric	CFTW082924.01	MARH	

Water Quality

		DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH	
Concentration (µg/L)		> 6	12 ± 1		7.5 ± 1.5	
<b>Day 0 (Stock)</b>	Control	9.5	12.4	175	8.3	
Date	8/29/2024	Glycerine Control	9.2	13.4	174	8.3
Time	952	20	9.5	12.3	174	8.3
Tech	AR	400	9.5	12.2	175	8.4
Meter #	7	1000	9.6	12.3	175	8.4
		4000	9.6	12.4	174	8.4
		10400	9.7	12.1	175	8.4
<b>Day 1</b>	Control	8.2	12.2	185	6.8	
Rep 1	Glycerine Control	8.4	12.3	182	6.8	
Date	8/30/2024	20	8.8	11.6	184	6.8
Time	1041	400	9.2	11.3	183	6.9
Tech	MCK	1000	8.3	11.4	183	7.0
Meter #	8	4000	8.5	11.5	183	7.1
		10400	8.7	11.6	184	7.1
<b>Day 2</b>	Control	8.2	11.6	186	7.6	
Rep 2 (old)	Glycerine Control	8.7	11.7	185	7.6	
Date	8/31/2024	20	8.6	11.5	186	7.6
Time	920	400	8.4	11.4	187	7.6
Tech	AR	1000	8.7	11.6	187	7.6
Meter #	9	4000	8.6	11.7	187	7.7
		10400	8.3	11.6	186	7.7
<b>Day 2</b>	Control	9.5	12.6	196	8.0	
Renewal Stock	Glycerine Control	9.7	12.6	196	7.6	
Date	8/31/2024	20	9.5	13.2	195	7.6
Time	1001	400	9.6	12.9	203	7.5
Tech	MARH	1000	9.5	13.1	196	7.5
Meter #	8	4000	9.2	12.9	195	7.3
		10400	9.5	13.3	195	7.4
<b>Day 3</b>	Control	8.0	12.3	191	7.6	
Rep 3	Glycerine Control	8.2	12.5	190	7.6	
Date	9/1/2024	20	8.5	12.2	192	7.7
Time	1150	400	8.5	11.8	197	7.7
Tech	LG	1000	8.5	11.8	192	7.7
Meter #	7	4000	8.7	12.0	193	7.7
		10400	8.5	12.1	192	7.7
<b>Day 4</b>	Control	7.0	12.4	188	7.5	
Rep 4	Glycerine Control	7.6	12.4	191	7.7	
Date	9/2/2024	20	8.2	12.0	192	7.7
Time	928	400	8.4	11.9	198	7.6
Tech	EM	1000	8.2	12.3	189	7.7
Meter #	7	4000	7.9	12.1	190	7.6
		10400	7.8	12.4	190	7.6

Comments

v.1

<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	96-Hour Acute Static Renewal	<b>PROTOCOL</b>	TOX 017
<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	8/29/24	<b>PROJECT NUMBER</b>	PG2032
<b>COMPOUND</b>	Rambutan Peel Extract	<b>TEST END DATE</b>	9/2/24	<b>SPECIES</b>	<i>Oncorhynchus mykiss</i>
<b>LOT #</b>	1704001	<b>MATRIX</b>	Liquid	<b>NO. OF ORGANISMS</b>	10

**96-Hour Acute Static Renewal**

**Abbreviation Key:**  
 NB = No Body  
 FB = Found Body  
 ST = Stranded

Day 1			Day 2			Day 3			Day 4		
Date	08/30/24		Date	08/31/24		Date	09/01/24		Date	09/02/24	
Time	1057		Time	1005		Time	1210		Time	1007	
Tech	MCK		Tech	MARH		Tech	LG		Tech	MARH	

Concentration (µg/L)	Rep	Day 1			Day 2			Day 3			Day 4			Comments
		Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	
Control	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
Glycerine Control	1	10	0		10	0	1LOE	9	1		9	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
20	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
400	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
1000	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
4000	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
10400	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		

# CETIS Summary Report

Report Date: 03 Oct-24 15:01 (p 1 of 2)  
 Test Code/ID: Sinapic O.m. / 09-6386-2368

## Fish 96-h Acute Survival Test

EcoAnalysts

Batch ID: 09-6492-8574	Test Type: Survival (96h)	Analyst: Mary Ann Rempel-Hester
Start Date: 29 Aug-24 13:00	Protocol: EPA/821/R-02-012 (2002)	Diluent: Carbon Filtered Municipal Water
Ending Date: 02 Sep-24 12:55	Species: Oncorhynchus mykiss	Brine: Not Applicable
Test Length: 96h	Taxon: Actinopterygii	Source: Thomas Fish Co. Age: 19p\$U
Sample ID: 14-2192-1874	Code: Lot 1512719	Project: 6PPD Alternative Testing 2024
Sample Date: 22 Aug-24 12:00	Material: Sinapic Acid	Source: Indofine Chemical Company
Receipt Date: 22 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 7d 1h	Client: Washington Department of Ecology	

## Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
03-9457-7434	96h Proportion Survived	Unequal Variance t Two-Sample Test	0.0068	Acetone Control failed 96h proportion survi	1

## Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
04-1611-7651	96h Proportion Survived	Steel Many-One Rank Sum Test	24000	>24000	---	---	1

## Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
08-4408-1904	96h Proportion Survived	Linear Interpolation (ICPIN)	EC15	>24000	---	---	1
			EC20	>24000	---	---	
			EC25	>24000	---	---	
			EC40	>24000	---	---	
			EC50	>24000	---	---	

## Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits			Overlap	Decision
				Lower	Upper			
03-9457-7434	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
	96h Proportion Survived	Control Resp	0.55	0.9	<<	Yes	Below Criteria	
04-1611-7651	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
08-4408-1904	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	

## 96h Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0	AC	4	0.5500	0.2188	0.8812	0.3000	0.8000	0.1041	0.2082	37.85%	45.00%
1500		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
3000		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
6000		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
12000		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
24000		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%

## 96h Proportion Survived Detail

MD5: 0BDA157550ADD961E99B1B964894FFDA

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
0	AC	0.3000	0.6000	0.8000	0.5000
1500		1.0000	1.0000	1.0000	1.0000
3000		1.0000	1.0000	1.0000	1.0000
6000		1.0000	1.0000	1.0000	1.0000
12000		1.0000	1.0000	1.0000	1.0000
24000		1.0000	1.0000	1.0000	1.0000

# CETIS Summary Report

Report Date: 03 Oct-24 15:01 (p 2 of 2)  
Test Code/ID: Sinapic O.m. / 09-6386-2368

## Fish 96-h Acute Survival Test

EcoAnalysts

### 96h Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	10/10	10/10
0	AC	3/10	6/10	8/10	5/10
1500		10/10	10/10	10/10	10/10
3000		10/10	10/10	10/10	10/10
6000		10/10	10/10	10/10	10/10
12000		10/10	10/10	10/10	10/10
24000		10/10	10/10	10/10	10/10

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 14:56 (p 1 of 1)  
 Test Code/ID: Sinapic O.m. / 09-6386-2368

<b>Fish 96-h Acute Survival Test</b>				<b>EcoAnalysts</b>					
<b>Start Date:</b>	29 Aug-24 13:00	<b>Species:</b>	Oncorhynchus mykiss	<b>Sample Code:</b>	Lot 1512719				
<b>End Date:</b>	02 Sep-24 12:55	<b>Protocol:</b>	EPA/821/R-02-012 (2002)	<b>Sample Source:</b>	Indofine Chemical Company				
<b>Sample Date:</b>	22 Aug-24 12:00	<b>Material:</b>	Sinapic Acid	<b>Sample Station:</b>					

Conc-µg/L	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Survival 72h	Survival 96h	Notes
0	AC	1	18	10	7	7	6	3	
0	AC	2	4	10	9	8	7	6	
0	AC	3	25	10	9	9	9	8	
0	AC	4	9	10	8	6	5	5	
0	D	1	1	10	10	10	10	10	
0	D	2	24	10	10	10	10	10	
0	D	3	26	10	10	10	10	10	
0	D	4	28	10	10	10	10	10	
1500		1	22	10	10	10	10	10	
1500		2	12	10	10	10	10	10	
1500		3	14	10	10	10	10	10	
1500		4	2	10	10	10	10	10	
3000		1	3	10	10	10	10	10	
3000		2	20	10	10	10	10	10	
3000		3	16	10	10	10	10	10	
3000		4	19	10	10	10	10	10	
6000		1	7	10	10	10	10	10	
6000		2	15	10	10	10	10	10	
6000		3	10	10	10	10	10	10	
6000		4	8	10	10	10	10	10	
12000		1	21	10	10	10	10	10	
12000		2	6	10	10	10	10	10	
12000		3	23	10	10	10	10	10	
12000		4	11	10	10	10	10	10	
24000		1	17	10	10	10	10	10	
24000		2	13	10	10	10	10	10	
24000		3	27	10	10	10	10	10	
24000		4	5	10	10	10	10	10	

GENERAL

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	Sinapic Acid
Test Type	96-Hour Acute Static Renewal
Matrix	Liquid
Test Acceptability	≥ 90% average survival of control
Test Start Date	08/29/24
Test Species	<i>Oncorhynchus mykiss</i>
Organism Batch	TF082224.01
Organism Acquired	8/22/2024
Organism Acclimation	7
Organism Age	19 days from swim up
Test Protocol	TOX 017
Regional Protocol	WDOE WQ-R-95-80
Test Location	Bath 5
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	carbon filtered tap water
Organisms per Replicate	10
Test Chamber Size	1 Gal
Exposure Volume	3.25 L
Feeding Information	None
Test Dissolved Oxygen	> 6
Test Temperature	12 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	6	
Temp	10.5	13.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1300 AR CS
TEST END TIME/INIT:	1255 MARH

REFERENCE TOXICANT TEST ID	LOT #
Sinapic Acid	1512719

Concentrations (µg/L)	
1	Control
2	Acetone Control
3	1500
4	3000
5	6000
6	12000
7	24000

Copy and Past VALUES

Treatment	Rep	Chamber
Control	1	
Control	2	
Control	3	22
Control	4	24
Acetone Co	1	
Acetone Co	2	
Acetone Co	3	10
Acetone Co	4	23
1500	1	9
1500	2	12
1500	3	8
1500	4	18
3000	1	15
3000	2	11
3000	3	14
3000	4	13
6000	1	2
6000	2	3
6000	3	19
6000	4	20
12000	1	21
12000	2	16
12000	3	1
12000	4	7
24000	1	5
24000	2	6
24000	3	17
24000	4	4

CLIENT	Washington Department of Ecology	TEST TYPE	Acute Static Renewal	PROTOCOL	TOX 017
PROJECT	6PPD Alternative Testing	TEST START DATE	8/29/24	PROJECT NUMBER	PG2032
COMPOUND	Sinapic Acid	TEST END DATE	9/2/24	SPECIES	<i>Oncorhynchus mykiss</i>
LOT #	1512719	MATRIX	Liquid	NO. OF ORGANISMS	10

**96-Hour Acute Static Renewal**

**Stock Preparation**

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
28,000,000	100	2800.0

**Day 0 Dilution Preparation**

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)	Total Volume (mLs per rep)
28,000,000	1500	3249.83	0.174	3250
28,000,000	3000	3249.65	0.348	
28,000,000	6000	3249.30	0.696	
28,000,000	12000	3248.61	1.393	
28,000,000	24000	3247.21	2.786	
			<b>Amt. of Acetone (mL)</b>	
	Acetone control	3247.21	2.786	

**Day 2 Dilution Preparation**

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)	Total Volume (mLs renewal stock)
28,000,000	1500	12999.30	0.696	13000
28,000,000	3000	12998.61	1.393	
28,000,000	6000	12997.21	2.786	
28,000,000	12000	12994.43	5.571	
28,000,000	24000	12988.86	11.143	
			<b>Amt. of Acetone (mL)</b>	
	Acetone control	12988.86	11.143	

**Test Dilution Prep**

Date	Balance ID	Water Batch ID	Initials	Comments
8/29/2024	7	CFTW082724.01	Spike AR	AR 08/28/24 Diluent Prep, MARH stock prep 8/29/24
8/31/2024	Volumetric	CFTW082924.01	MARH	Note: Acetone control prepared as stated in alpha-tocopherol datasheet

**Water Quality**

		DO (mg/L)	TEMP (°C)	CONDUCTIVITY	pH	
Concentration (µg/L)		> 6	12 ± 1	(µS/cm)	7.5 ± 1.5	
<b>Day 0 (Stock)</b>	Control	9.7	13.2	176	8.3	
Date	8/29/2024	Acetone Control	9.8	13.3	173	8.3
Time	1239	1500	9.6	13.1	174	8.3
Tech	AR	3000	9.6	12.8	175	8.3
Meter #	7	6000	9.7	12.7	174	8.2
		12000	9.7	13.0	174	8.0
		24000	9.9	12.9	172	7.7
<b>Day 1</b>	Control	8.2	12.2	185	6.8	
Rep 1	Acetone Control	8.6	12.7	181	6.9	
Date	8/30/2024	1500	8.4	12.1	183	6.8
Time	1020	3000	8.7	11.9	184	6.9
Tech	MCK	6000	9.2	12.2	182	7.0
Meter #	8	12000	9.4	12.2	181	7.0
		24000	8.7	11.9	180	7.0
<b>Day 2</b>	Control	8.2	11.6	186	7.6	
Rep 2 (old)	Acetone Control	8.3	12.8	183	7.0	
Date	8/31/2024	1500	8.4	11.8	186	7.5
Time	907	3000	8.7	11.7	186	7.5
Tech	AR	6000	8.5	12.0	185	7.5
Meter #	9	12000	8.6	11.7	185	7.6
		24000	8.7	11.8	183	7.6
<b>Day 2</b>	Control	9.5	13.0	195	7.7	
Renewal Stock	Acetone Control	9.7	12.3	184	7.3	
Date	8/31/2024	1500	9.6	13.4	196	7.4
Time	1202	3000	9.5	12.5	198	7.2
Tech	MARH	6000	9.5	13.3	196	7.2
Meter #	8	12000	9.5	13.3	195	7.1
		24000	9.5	13.4	194	7.0
<b>Day 3</b>	Control	8.0	12.3	191	7.6	
Rep 3	Acetone Control	8.1	12.3	182	7.7	
Date	9/1/2024	1500	8.3	12.1	192	7.7
Time	1140	3000	8.3	11.9	194	7.7
Tech	LG	6000	8.5	12.0	190	7.7
Meter #	7	12000	8.6	12.5	193	7.7
		24000	8.6	11.9	193	7.7
<b>Day 4</b>	Control	8.5	12.8	192	7.7	
Rep 4	Acetone Control	8.6	12.6	182	7.7	
Date	9/2/2024	1500	8.6	12.3	192	7.7
Time	919	3000	8.5	12.0	191	7.7
Tech	EM	6000	8.7	11.8	194	7.7
Meter #	7	12000	8.2	12.0	194	7.7
		24000	8.2	11.8	185	7.6

**Comments**

v.1

<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	96-Hour Acute Static Renewal	<b>PROTOCOL</b>	TOX 017
<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	8/29/24	<b>PROJECT NUMBER</b>	PG2032
<b>COMPOUND</b>	Sinapic Acid	<b>TEST END DATE</b>	9/2/24	<b>SPECIES</b>	<i>Oncorhynchus mykiss</i>
<b>LOT #</b>	1512719	<b>MATRIX</b>	Liquid	<b>NO. OF ORGANISMS</b>	10

**96-Hour Acute Static Renewal**

**Abbreviation Key:**  
 NB = No Body  
 FB = Found Body  
 ST = Stranded

Concentration (µg/L)	Rep	Day 1			Day 2			Day 3			Day 4			Comments
		Date	08/30/24		Date	08/31/24		Date	09/01/24		Date	09/02/24		
		Time	1033		Time	1202		Time	1201		Time	1255		
		Tech	MCK		Tech	MARH		Tech	LG		Tech	MARH		
Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs			
Control	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
Acetone Control	1	7	3		7	0		6	1		3	3		
	2	9	1		8	1	1 dark	7	1		6	1		
	3	9	1		9	0		9	0		8	1		
	4	8	2		6	2		5	1		5	0		
1500	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
3000	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
6000	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
12000	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
24000	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		

**CETIS Summary Report**

Report Date: 03 Oct-24 15:24 (p 1 of 2)  
 Test Code/ID: Durazone O.m. / 03-2498-1310

**Fish 96-h Acute Survival Test**

EcoAnalysts

Batch ID: 12-7685-7623	Test Type: Survival (96h)	Analyst: Mary Ann Rempel-Hester
Start Date: 05 Sep-24 10:45	Protocol: EPA/821/R-02-012 (2002)	Diluent: Carbon Filtered Municipal Water
Ending Date: 09 Sep-24 10:23	Species: Oncorhynchus mykiss	Brine: Not Applicable
Test Length: 96h	Taxon: Actinopterygii	Source: Thomas Fish Co. Age: 26p <sub>50</sub>
Sample ID: 15-3099-6309	Code: Lot CD1J23P008	Project: 6PPD Alternative Testing 2024
Sample Date: 22 Aug-24 12:00	Material: Durazone 37	Source: Akrochem
Receipt Date: 22 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 13d 23h	Client: Washington Department of Ecology	

**Single Comparison Summary**

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
20-5658-2008	96h Proportion Survived	Unequal Variance t Two-Sample Test	0.0908	Acetone Control passed 96h proportion sur	1

**Multiple Comparison Summary**

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
14-4906-0492	96h Proportion Survived	Steel Many-One Rank Sum Test	136	>136	---	6.72%	1

**Point Estimate Summary**

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
03-6903-9874	96h Proportion Survived	Linear Interpolation (ICPIN)	EC15	>136	---	---	1
			EC20	>136	---	---	
			EC25	>136	---	---	
			EC40	>136	---	---	
			EC50	>136	---	---	

**Test Acceptability**

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits			Overlap	Decision
				Lower	Upper			
03-6903-9874	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
14-4906-0492	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
20-5658-2008	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria	
	96h Proportion Survived	Control Resp	0.95	0.9	<<	Yes	Passes Criteria	

**96h Proportion Survived Summary**

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0	AC	4	0.9500	0.8581	1.0420	0.9000	1.0000	0.0289	0.0577	6.08%	5.00%
		4	0.9500	0.8581	1.0420	0.9000	1.0000	0.0289	0.0577	6.08%	5.00%
8.5		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
17		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
34		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	2.50%
68		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
136		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	2.50%

**96h Proportion Survived Detail**

MD5: AC1C509D879D4185417575F81D42BB89

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
0	AC	0.9000	1.0000	0.9000	1.0000
		0.9000	1.0000	1.0000	0.9000
8.5		1.0000	1.0000	1.0000	1.0000
17		1.0000	1.0000	1.0000	1.0000
34		1.0000	0.9000	1.0000	1.0000
68		1.0000	1.0000	1.0000	1.0000
136		1.0000	1.0000	0.9000	1.0000

# CETIS Summary Report

Report Date: 03 Oct-24 15:24 (p 2 of 2)  
Test Code/ID: Durazone O.m. / 03-2498-1310

Fish 96-h Acute Survival Test

EcoAnalysts

## 96h Proportion Survived Binomials

Conc- $\mu$ g/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	10/10	10/10
0	AC	9/10	10/10	9/10	10/10
8.5		9/10	10/10	10/10	9/10
17		10/10	10/10	10/10	10/10
34		10/10	9/10	10/10	10/10
68		10/10	10/10	10/10	10/10
136		10/10	10/10	9/10	10/10

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 15:24 (p 1 of 1)  
 Test Code/ID: Durazone O.m. / 03-2498-1310

<b>Fish 96-h Acute Survival Test</b>				<b>EcoAnalysts</b>					
<b>Start Date:</b> 05 Sep-24 10:45	<b>Species:</b> Oncorhynchus mykiss			<b>Sample Code:</b> Lot CD1J23P008					
<b>End Date:</b> 09 Sep-24 10:23	<b>Protocol:</b> EPA/821/R-02-012 (2002)			<b>Sample Source:</b> Akrochem					
<b>Sample Date:</b> 22 Aug-24 12:00	<b>Material:</b> Durazone 37			<b>Sample Station:</b>					

Conc-µg/L	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Survival 72h	Survival 96h	Notes
0	AC	1	11	10	9	9	9	9	
0	AC	2	5	10	10	10	10	10	
0	AC	3	8	10	9	9	9	9	
0	AC	4	18	10	10	10	10	10	
0	D	1	3	10	10	10	10	10	
0	D	2	6	10	10	10	10	10	
0	D	3	19	10	10	10	10	10	
0	D	4	13	10	10	10	10	10	
8.5		1	23	10	10	9	9	9	
8.5		2	2	10	10	10	10	10	
8.5		3	14	10	10	10	10	10	
8.5		4	26	10	10	9	9	9	
17		1	22	10	10	10	10	10	
17		2	12	10	10	10	10	10	
17		3	17	10	10	10	10	10	
17		4	1	10	10	10	10	10	
34		1	21	10	10	10	10	10	
34		2	10	10	10	9	9	9	
34		3	27	10	10	10	10	10	
34		4	20	10	10	10	10	10	
68		1	25	10	10	10	10	10	
68		2	28	10	10	10	10	10	
68		3	24	10	10	10	10	10	
68		4	4	10	10	10	10	10	
136		1	16	10	10	10	10	10	
136		2	9	10	10	10	10	10	
136		3	7	10	10	9	9	9	
136		4	15	10	10	10	10	10	

GENERAL

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	Durazone 37
Test Type	96-Hour Acute Static Renewal
Matrix	Liquid
Test Acceptability	≥ 90% average survival of control
Test Start Date	09/05/24
Test Species	<i>Oncorhynchus mykiss</i>
Organism Batch	TF082224.01
Organism Acquired	8/22/2024
Organism Acclimation	14
Organism Age	26 days from swimup
Test Protocol	TOX 017
Regional Protocol	WDOE WQ-R-95-80
Test Location	Bath 5
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	carbon filtered tap water
Organisms per Replicate	10
Test Chamber Size	5 L
Exposure Volume	4 L
Feeding Information	None
Test Dissolved Oxygen	> 6
Test Temperature	12 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	6	
Temp	10.5	13.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1045 CS/EM
TEST END TIME/INIT:	1023 TW

REFERENCE TOXICANT TEST ID	LOT #
Durazone 37	CD1J23P008

Concentrations (µg/L)	
1	Control
2	Acetone Control
3	8.5
4	17
5	34
6	68
7	136

Copy and Past VALUES

Treatment	Rep	Chamber
Control	1	
Control	2	
Control	3	
Control	4	22
Acetone Co	1	23
Acetone Co	2	25
Acetone Co	3	24
Acetone Co	4	10
9	1	9
9	2	12
9	3	8
9	4	18
17	1	15
17	2	11
17	3	14
17	4	13
34	1	2
34	2	3
34	3	19
34	4	20
68	1	21
68	2	16
68	3	1
68	4	7
136	1	5
136	2	6
136	3	17
136	4	4

CLIENT	Washington Department of Ecology	TEST TYPE	Acute Static Renewal	PROTOCOL	TOX 017
PROJECT	6PPD Alternative Testing	TEST START DATE	9/5/24	PROJECT NUMBER	PG2032
COMPOUND	Durazone 37	TEST END DATE	9/9/24	SPECIES	<i>Oncorhynchus mykiss</i>
LOT #	CD1J23P008	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Static Renewal

Stock Preparation

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
560,000	50	28.0

Day 0 Dilution Preparation

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)	Total Volume (mLs per rep)
560,000	8.5	3249.95	0.049	3250
560,000	17	3249.90	0.099	
560,000	34	3249.80	0.197	
560,000	68	3249.61	0.395	
560,000	136	3249.21	0.789	
	Acetone control	3249.21	0.789	

Day 2 Dilution Preparation

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)	Total Volume (mLs renewal stock)
560,000	8.5	12999.80	0.197	13000
560,000	17	12999.61	0.395	
560,000	34	12999.21	0.789	
560,000	68	12998.42	1.579	
560,000	136	12996.84	3.157	
	Acetone control	12996.84	3.157	

Test Dilution Prep

Date	Balance ID	Water Batch ID	Initials	Comments
9/5/2024	7	CFTW090324.01	EM	Diluent prepared on 9/4/24, spiked with toxicant on 9/5/24 - EM
9/7/2024	Volumetric	CFTW090324.01	MARH	

Water Quality

		DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH		
	Concentration (µg/L)	> 6	12 ± 1		7.5 ± 1.5		
<b>Day 0 (Stock)</b>	Control	9.4	13.2	179	7.8		
Date	9/5/2024	Acetone Control	9.4	13.4	179	8.0	
Time	1017		8.5	9.3	13.4	179	8.1
Tech	EM		17	9.4	13.4	178	8.2
Meter #	8		34	9.4	13.4	179	8.2
			68	9.4	13.4	179	8.2
			136	9.3	13.4	172	8.1
<b>Day 1</b>	Control	7.8	11.3	187	7.4		
Rep 1	Acetone Control	8.1	11.3	186	7.2		
Date	9/6/2024	8.5	8.1	10.6	187	7.6	
Time	1423	17	8.5	10.8	188	7.7	
Tech	TVL	34	7.5	11.2	187	7.7	
Meter #	9	68	7.5	11.2	186	7.7	
		136	8.3	11.3	180	7.7	
<b>Day 2</b>	Control	7.7	10.7	181	7.5		
Rep 2 (old)	Acetone Control	8.5	12.6	185	7.6		
Date	9/7/2024	8.5	8.4	11.8	184	7.7	
Time	834	17	8.1	12.0	183	7.6	
Tech	MCK	34	8.2	12.1	184	7.6	
Meter #	7	68	8.9	11.9	185	7.7	
		136	7.5	11.1	176	7.6	
<b>Day 2</b>	Control	10.9	10.8	156	7.5		
Renewal Stock	Acetone Control	10.8	11.7	165	7.8		
Date	9/7/2024	8.5	10.7	11.7	165	7.7	
Time	916	17	10.8	11.8	159	7.8	
Tech	MARH	34	10.7	11.7	164	7.8	
Meter #	9	68	10.8	11.3	163	7.7	
		136	10.8	11.3	163	7.9	
<b>Day 3</b>	Control	7.9	13.0	166	7.3		
Rep 3	Acetone Control	7.5	13.2	170	7.5		
Date	9/8/2024	8.5	7.5	13.2	172	7.5	
Time	1345	17	7.9	13.4	169	7.6	
Tech	LG	34	7.5	13.5	166	7.6	
Meter #	9	68	6.3	12.3	168	7.5	
		136	8.0	12.9	167	7.5	
<b>Day 4</b>	Control	7.9	12.7	176	7.5		
Rep 4	Acetone Control	6.4	11.8	174	7.5		
Date	9/9/2024	8.5	5.9	11.8	177	7.5	
Time	816	17	5.6	11.8	173	7.5	
Tech	TW	34	6.5	11.6	178	7.5	
Meter #	9	68	5.6	11.8	174	7.5	
		136	6.1	11.9	173	7.5	

Comments

CLIENT	Washington Department of Ecology	TEST TYPE	96-Hour Acute Static Renewal	PROTOCOL	TOX 017
PROJECT	6PPD Alternative Testing	TEST START DATE	9/5/24	PROJECT NUMBER	PG2032
COMPOUND	Durazone 37	TEST END DATE	9/9/24	SPECIES	<i>Oncorhynchus mykiss</i>
LOT #	CD1J23P008	MATRIX	Liquid	NO. OF ORGANISMS	10

**Abbreviation Key:**

NB = No Body  
 FB = Found Body  
 ST = Stranded

**96-Hour Acute Static Renewal**

Concentration (µg/L)	Rep	Day 1			Day 2			Day 3			Day 4			Comments
		Date	09/06/24		Date	09/07/24		Date	09/08/24		Date	09/09/24		
		Time	1444		Time	920		Time	1346		Time	1023		
		Tech	TVL		Tech	MARH		Tech	LG		Tech	TW		
Control	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
Acetone Control	1	9	1		9	0		9	0		9	0		
	2	10	0		10	0		10	0		10	0		
	3	9	1		9	0		9	0		9	0		
	4	10	0		10	0		10	0		10	0		
8.5	1	10	0		9	1		9	0		9	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		9	0	1NB,1LOE	9	0		9	0		
17	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
34	1	10	0		10	0		10	0		10	0		
	2	10	0		9	1		9	0		9	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
68	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
136	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		9	1		9	0		9	0		Dead fish on Day 2 being eaten by another fish
	4	10	0		10	0		10	0		10	0		

**CETIS Summary Report**

Report Date: 03 Oct-24 15:15 (p 1 of 2)  
 Test Code/ID: Irganox O.m. / 19-2679-4009

**Fish 96-h Acute Survival Test**

EcoAnalysts

Batch ID: 18-3100-9137	Test Type: Survival (96h)	Analyst: Mary Ann Rempel-Hester
Start Date: 05 Sep-24 13:08	Protocol: EPA/821/R-02-012 (2002)	Diluent: Carbon Filtered Municipal Water
Ending Date: 09 Sep-24 11:10	Species: Oncorhynchus mykiss	Brine: Not Applicable
Test Length: 94h	Taxon: Actinopterygii	Source: Thomas Fish Co. Age: 26p SU
Sample ID: 05-7594-5957	Code: Lot C2PNC-GN	Project: 6PPD Alternative Testing 2024
Sample Date: 13 Aug-24 12:00	Material: Irganox 1076	Source: TCI America
Receipt Date: 13 Aug-24 12:00	CAS (PC):	Station:
Sample Age: 23d 1h	Client: Washington Department of Ecology	

**Single Comparison Summary**

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
08-7976-1042	96h Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	0.5000	Acetone Control passed 96h proportion sur	1

**Multiple Comparison Summary**

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
03-3306-2594	96h Proportion Survived	Steel Many-One Rank Sum Test	340000	>340000	---	8.56%	1

**Point Estimate Summary**

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
03-8566-8995	96h Proportion Survived	Linear Interpolation (ICPIN)	EC15	>340000	---	---	1
			EC20	>340000	---	---	
			EC25	>340000	---	---	
			EC40	>340000	---	---	
			EC50	>340000	---	---	

**Test Acceptability**

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
03-3306-2594	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
03-8566-8995	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
08-7976-1042	96h Proportion Survived	Control Resp	1	0.9	<<	Yes	Passes Criteria
	96h Proportion Survived	Control Resp	0.975	0.9	<<	Yes	Passes Criteria

**96h Proportion Survived Summary**

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
0	AC	4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	2.50%
21250		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
42500		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
85000		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
170000		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
340000		4	0.9000	0.6750	1.1250	0.7000	1.0000	0.0707	0.1414	15.71%	10.00%

**96h Proportion Survived Detail**

MD5: 91435818B5292256A239D15E2E24EA2C

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
0	AC	1.0000	1.0000	0.9000	1.0000
21250		1.0000	1.0000	1.0000	1.0000
42500		1.0000	1.0000	1.0000	1.0000
85000		1.0000	1.0000	1.0000	1.0000
170000		1.0000	1.0000	1.0000	1.0000
340000		0.9000	1.0000	0.7000	1.0000

# CETIS Summary Report

Report Date: 03 Oct-24 15:15 (p 2 of 2)  
Test Code/ID: Irganox O.m. / 19-2679-4009

Fish 96-h Acute Survival Test

EcoAnalysts

## 96h Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	10/10	10/10
0	AC	10/10	10/10	9/10	10/10
21250		10/10	10/10	10/10	10/10
42500		10/10	10/10	10/10	10/10
85000		10/10	10/10	10/10	10/10
170000		10/10	10/10	10/10	10/10
340000		9/10	10/10	7/10	10/10

**CETIS Test Data Worksheet**

Report Date: 03 Oct-24 15:15 (p 1 of 1)  
 Test Code/ID: Irganox O.m. / 19-2679-4009

<b>Fish 96-h Acute Survival Test</b>				<b>EcoAnalysts</b>					
<b>Start Date:</b>	05 Sep-24 13:08	<b>Species:</b>	Oncorhynchus mykiss	<b>Sample Code:</b>	Lot C2PNC-GN				
<b>End Date:</b>	09 Sep-24 11:10	<b>Protocol:</b>	EPA/821/R-02-012 (2002)	<b>Sample Source:</b>	TCI America				
<b>Sample Date:</b>	13 Aug-24 12:00	<b>Material:</b>	Irganox 1076	<b>Sample Station:</b>					

Conc-µg/L	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Survival 72h	Survival 96h	Notes
0	AC	1	10	10	10	10	10	10	
0	AC	2	5	10	10	10	10	10	
0	AC	3	28	10	9	9	9	9	
0	AC	4	6	10	10	10	10	10	
0	D	1	8	10	10	10	10	10	
0	D	2	3	10	10	10	10	10	
0	D	3	12	10	10	10	10	10	
0	D	4	15	10	10	10	10	10	
21250		1	17	10	10	10	10	10	
21250		2	27	10	10	10	10	10	
21250		3	16	10	10	10	10	10	
21250		4	25	10	10	10	10	10	
42500		1	4	10	10	10	10	10	
42500		2	13	10	10	10	10	10	
42500		3	7	10	10	10	10	10	
42500		4	23	10	10	10	10	10	
85000		1	9	10	10	10	10	10	
85000		2	11	10	10	10	10	10	
85000		3	22	10	10	10	10	10	
85000		4	18	10	10	10	10	10	
170000		1	21	10	10	10	10	10	
170000		2	14	10	10	10	10	10	
170000		3	24	10	10	10	10	10	
170000		4	1	10	10	10	10	10	
340000		1	20	10	10	10	9	9	
340000		2	19	10	10	10	10	10	
340000		3	26	10	10	9	8	7	
340000		4	2	10	10	10	10	10	

GENERAL

Client	Washington Department of Ecology
Project	6PPD Alternative Testing
Project Number	PG2032
Toxicant	Irganox 1076
Test Type	96-Hour Acute Static Renewal
Matrix	Liquid
Test Acceptability	≥ 90% average survival of control
Test Start Date	09/05/24
Test Species	<i>Oncorhynchus mykiss</i>
Organism Batch	TF082224.01
Organism Acquired	8/22/2024
Organism Acclimation	14
Organism Age	26 days from swimup
Test Protocol	TOX 017
Regional Protocol	WDOE WQ-R-95-80
Test Location	Bath 5
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	carbon filtered tap water
Organisms per Replicate	10
Test Chamber Size	5 L
Exposure Volume	4 L
Feeding Information	None
Test Dissolved Oxygen	> 6
Test Temperature	12 ± 1
Conductivity	
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	6	
Temp	10.5	13.4
Conductivity		
pH	6	9

TEST START TIME/INIT:	1308 CS/EM
TEST END TIME/INIT:	1110 TW

REFERENCE TOXICANT TEST ID	LOT #
Irganox 1076	C2PNC-GN

Concentrations (µg/L)	
1	Control
2	Acetone Control
3	21250
4	42500
5	85000
6	170000
7	340000

Copy and Past VALUES

Treatment	Rep	Chamber
Control	1	
Control	2	
Control	3	22
Control	4	
Acetone Co	1	24
Acetone Co	2	25
Acetone Co	3	10
Acetone Co	4	23
21250	1	9
21250	2	12
21250	3	8
21250	4	18
42500	1	15
42500	2	11
42500	3	14
42500	4	13
85000	1	2
85000	2	3
85000	3	19
85000	4	20
170000	1	21
170000	2	16
170000	3	1
170000	4	7
340000	1	5
340000	2	6
340000	3	17
340000	4	4

CLIENT	Washington Department of Ecology	TEST TYPE	96-Hour Acute Static Renewal	PROTOCOL	TOX 017
PROJECT	6PPD Alternative Testing	TEST START DATE	9/5/24	PROJECT NUMBER	PG2032
COMPOUND	Irganox 1076	TEST END DATE	9/9/24	SPECIES	Oncorhynchus mykiss
LOT #	C2PNC-GN	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Static Renewal

Stock Preparation

Target Stock Concentration (µg/L)	Volume of Acetone (mLs)	Amt. of Toxicant (mg)
136,000,000	250	34000.0

Day 0 Dilution Preparation

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)	Total Volume (mLs per rep)
136,000,000	21250	3249.49	0.508	3250
136,000,000	42500	3248.98	1.016	
136,000,000	85000	3247.97	2.031	
136,000,000	170000	3245.94	4.063	
136,000,000	340000	3241.88	8.125	
			<b>Amt. of Acetone (mL)</b>	
	Acetone control	3241.88	8.125	

Day 2 Dilution Preparation

Stock Solution (µg/L)	Target Concentration (µg/L)	Volume of Diluent (mLs)	Amt. of Toxicant (mL)	Total Volume (mLs renewal stock)
136,000,000	21250	12997.97	2.031	13000
136,000,000	42500	12995.94	4.063	
136,000,000	85000	12991.88	8.125	
136,000,000	170000	12983.75	16.250	
136,000,000	340000	12967.50	32.500	
			<b>Amt. of Acetone (mL)</b>	
	Acetone control	12967.50	32.500	

Test Dilution Prep

Date	Balance ID	Water Batch ID	Initials	Comments
9/5/2024	7	CFTW083024.01	EM	Diluent prepared on 9/4/24, spiked with toxicant on 9/5/24 - EM
9/7/2024	Volumetric	CFTW090324.01	MARH	Film on surface of treatment chambers

Water Quality

	Concentration (µg/L)	DO (mg/L)	TEMP (°C)	CONDUCTIVITY (µS/cm)	pH	
		> 6	12 ± 1		7.5 ± 1.5	
<b>Day 0 (Stock)</b>	Control	9.3	12.8	185	8.0	
Date	9/5/2024	Acetone Control	9.5	12.9	183	8.1
Time	1251	21250	9.3	12.7	184	8.2
Tech	CS	42500	9.3	12.7	184	8.2
Meter #	9	85000	9.3	12.7	184	8.3
		170000	9.4	12.8	183	8.3
		340000	9.5	12.9	183	8.3
<b>Day 1</b>	Control	8.0	11.1	188	7.4	
Rep 1	Acetone Control	8.2	10.6	187	7.5	
Date	9/6/2024	21250	8.6	11.0	188	7.7
Time	1408	42500	8.2	10.9	188	7.7
Tech	TVL	85000	8.0	10.8	188	7.7
Meter #	9	170000	7.4	10.9	188	7.7
		340000	6.7	10.9	187	7.7
<b>Day 2</b>	Control	7.7	10.7	181	7.5	
Rep 2 (old)	Acetone Control	8.6	12.3	184	7.6	
Date	9/7/2024	21250	8.7	11.6	185	7.7
Time	810	42500	8.5	10.9	186	7.7
Tech	MCK	85000	8.2	11.8	185	7.6
Meter #	7	170000	7.4	11.4	186	7.7
		340000	7.0	11.0	185	7.6
<b>Day 2</b>	Control	10.9	10.8	156	7.5	
Renewal Stock	Acetone Control	10.8	12.1	160	7.8	
Date	9/7/2024	21250	10.7	11.9	164	7.7
Time	1144	42500	10.7	11.9	157	7.7
Tech	MARH	85000	10.6	12.1	168	7.7
Meter #	9	170000	10.2	12.7	162	7.7
		340000	9.8	13.0	160	7.9
<b>Day 3</b>	Control	7.9	13.0	166	7.3	
Rep 3	Acetone Control	7.9	12.8	166	7.3	
Date	9/8/2024	21250	8.0	12.2	167	7.4
Time	1408	42500	8.1	12.2	163	7.5
Tech	LG	85000	8.0	12.4	177	7.5
Meter #	9	170000	7.0	12.6	164	7.5
		340000	7.2	12.8	169	7.6
<b>Day 4</b>	Control	7.9	12.7	176	7.5	
Rep 4	Acetone Control	6.9	12.0	174	7.4	
Date	9/9/2024	21250	6.3	11.2	174	7.5
Time	816	42500	6.9	11.4	172	7.5
Tech	TW	85000	6.7	11.4	177	7.5
Meter #	9	170000	7.1	11.2	170	7.6
		340000	6.8	10.6	174	7.6

Comments

Stock prep was 34 g in 250 mL acetone. Stock concentration is 136,000,000 ug/L.

v.1

<b>CLIENT</b>	Washington Department of Ecology	<b>TEST TYPE</b>	96-Hour Acute Static Renewal	<b>PROTOCOL</b>	TOX 017
<b>PROJECT</b>	6PPD Alternative Testing	<b>TEST START DATE</b>	9/5/24	<b>PROJECT NUMBER</b>	PG2032
<b>COMPOUND</b>	Irganox 1076	<b>TEST END DATE</b>	9/9/24	<b>SPECIES</b>	<i>Oncorhynchus mykiss</i>
<b>LOT #</b>	C2PNC-GN	<b>MATRIX</b>	Liquid	<b>NO. OF ORGANISMS</b>	10

**96-Hour Acute Static Renewal**

**Abbreviation Key:**  
 NB = No Body  
 FB = Found Body  
 ST = Stranded

Concentration (µg/L)	Rep	Day 1			Day 2			Day 3			Day 4			Comments
		Date	09/06/24		Date	09/07/24		Date	09/08/24		Date	09/09/24		
		Time	1412		Time	1139		Time	1409		Time	1110		
		Tech	TVL		Tech	MARH		Tech	LG		Tech	TW		
Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs			
Control	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
Acetone Control	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	9	1		9	0		9	0		9	0		
	4	10	0		10	0		10	0		10	0		
21250	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
42500	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
85000	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
170000	1	10	0		10	0		10	0		10	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		10	0		10	0		10	0		
	4	10	0		10	0		10	0		10	0		
340000	1	10	0		10	0		9	1		9	0		
	2	10	0		10	0		10	0		10	0		
	3	10	0		9	1		8	1		7	1		
	4	10	0		10	0		10	0		10	0		

## **APPENDIX B**

### **AVAILABLE CERTIFICATES OF ANALYSIS**



## Technical Data Sheet

### 6PPD

**Technical Name:** N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine  
**CAS #:** 793-24-8  
**Appearance:** Brown to Violet

**Technical Parameters:**

	Min	Max
Assay	96.0%	
Heat Loss @70 C		0.40%
% Ash		0.10%
Melt Point C	44	50

Other information:

Solubility: Well soluble in organic solvents and rubbers. Insoluble in hexane, petrol and water

Kumanox 13 (6PPD) It is a low-viscous liquid at temperatures above 50 C.

The above information has been compiled from what we believe to be credible sources. To our knowledge the information is accurate and reliable, however, it is not guaranteed. Any recommendations issued by HB Chemical personnel or literature is derived from experience and by no means should be taken as fact or construed as a recommendation to violate of any law, regulation or patent. It is the users responsibility to determine the suitability of any HB supplied material in their application. The individual conditions of each customer are well outside of our control and we cannot be held liable for its functionality and use. Please contact our office should you need specific information beyond what is supplied above. As with all Chemical usage safety precautions beyond the stated are highly recommended.

1665 Enterprise Parkway, Twinsburg, OH, 44087 Tel: (330) 920-8023 Fax: (330) 920-0971

Lot# 5221031128

Item Number	T2764	Lot Number	1NG0212
Item	(+)-alpha-Tocopherol	CAS Number	59-02-9
Molecular Formula	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	Molecular Weight	430.71

TEST	SPECIFICATION		RESULT
	MIN	MAX	
MIXED ISOMERS (D-alpha-TOCOPHEROL)	APPROX. 670 mg/g		808 mg/g
APPEARANCE			CLEAR YELLOW VISCOUS LIQUID
RETEST DATE			01-JUN-2026
DATE OF MANUFACTURE			04-OCT-2022
REANALYZED ON			24-JUN-2024

Certificate of Analysis Results Entered By:

HBERMUDEZ  
Hernan Bermudez  
29-JUL-24 11:01:57

Certificate of Analysis Results Approved By:

SHANSEN  
Selin Hansen  
30-JUL-24 15:47:51

Spectrum Chemical Mfg Corp  
14422 South San Pedro Street  
Gardena 90248 CA



All pharmaceutical ingredients are tested using current edition of applicable pharmacopeia.

Read and understand label and SDS before handling any chemicals. All Spectrum's chemicals are for manufacturing, processing, repacking or research purposes by experienced personnel only. It is the customer's responsibility to provide adequate hazardous material training and ensure that appropriate Personal Protective Equipment (PPE) is used before handling any chemical.



# INDOFINE Chemical Company, Inc.

121 Stryker Lane, Bldg. 30, Suite 1 • Hillsborough, NJ 08844 • U.S.A.

Phone: (908) 359-6778 • FAX: (908) 359-1179

website: [www.indofinechemical.com](http://www.indofinechemical.com)

e-mail: [chemical@indofinechemical.com](mailto:chemical@indofinechemical.com)

## CERTIFICATE OF ANALYSIS

Catalog Number: 01-1354

Product Name: **3,5-DIMETHOXY-4-HYDROXYCINNAMIC ACID**  
(Sinapinic acid)

Cas Number: [530-59-6]

Lot Number: 1512719

Chemical Formula: C<sub>11</sub>H<sub>12</sub>O<sub>5</sub>

Molecular Weight: 224.21

Melting Point: 199-201°C

Appearance: Cream powder

Solubility: Soluble in DMSO

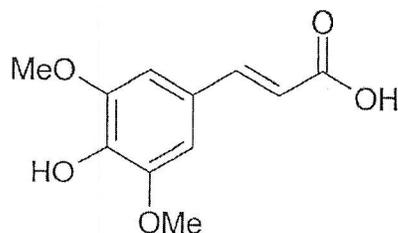
TLC: Chloroform:Methanol (9:1)

Moisture(KF): 0.34%

NMR Spectrum: Enclosed (conforms to the structure)

Storage: Store in a cool, dry place

Purity(HPLC): 99.84%





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Phone: (908) 359-6778 • FAX: (908) 359-1179

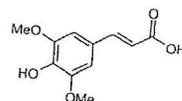
website: [www.indofinechemical.com](http://www.indofinechemical.com)

e-mail: [chemical@indofinechemical.com](mailto:chemical@indofinechemical.com)

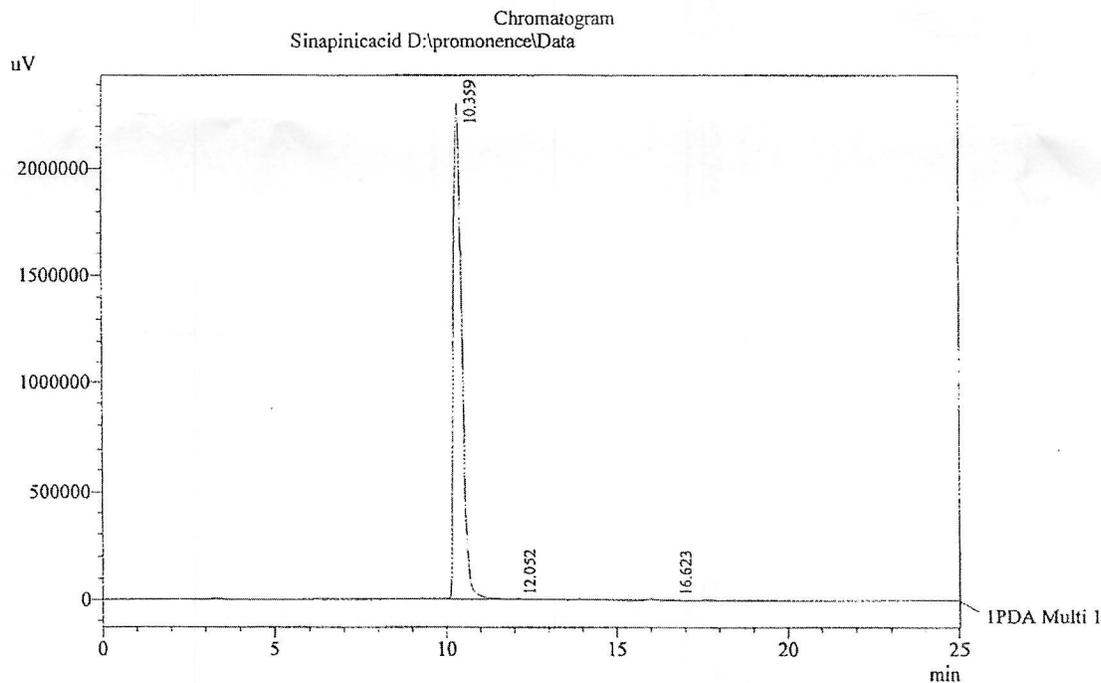
## HPLC ANALYSIS

Acquired by : Admin  
Sample Name : Sinapinicacid  
Sample ID : 2.35 kg  
Injection Volume : 20 uL  
Data Filename : 041215B.lcd  
Method Filename : Linear Gradient.M.lcm

Catalog No.: 01-1354  
Product Name: Sinapinic acid  
Lot No.: 1512719



Column : Gemini-C(18), (250mm x4.6mm 5.0μ)  
Flow : 1.000 mL/min.  
Mobile Phase : A( 0.05% TFA in Water) B(Acetonitrile)  
0.01 90 10  
12.0 10 90  
20.0 10 90  
23.0 90 10  
25.0 90 10  
Diluent :Acetonitrile



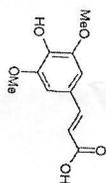
PeakTable

PDA Ch1 240nm 4nm

Peak#	Name	Ret. Time	Area	Area %	Relative Retention Time
1	Sinapinic acid	10.359	38688579	99.83	1.00
2	RT:12.052	12.052	27937	0.07	1.16
3	RT:16.623	16.623	36020	0.09	1.60
Total			38752536	100.00	



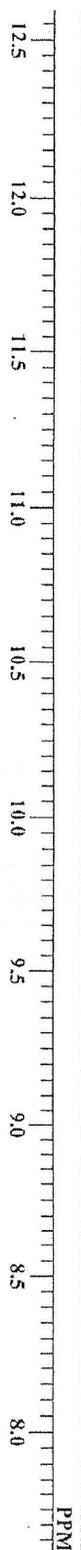
**INDOFINE Chemical Company, Inc.**  
 121 STRYKER LANE  
 BLDG. 30, SUITE 1  
 HILLSBOROUGH, NJ 08844



Catalog No.: 01-1354  
 Product Name: Sinapinic acid  
 Lot No.: 1512719

SPAR/C in MeOH) 1H NMR DMSO-d6  
 Analyzed By: KNR

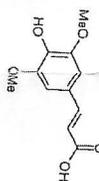
12.145  
 8.927



DFILE SPAR-C in MeOH) 1H.als  
 COMNT SPAR/C in MeOH) 1H NMR DMSO  
 OBNVC 1H  
 EXMOD NON  
 OBFRO 399.65 MHz  
 OBSRT 124.00 KHz  
 OBFIN 10500.00 Hz  
 POINT 16384  
 FREQU 10000.00 Hz  
 SCANS 16  
 ACQIM 1.6384 sec  
 PD 2.0000 sec  
 PW1 6.00 usec  
 IRNUC 1H  
 CTEMP 18.5 c  
 SLVNT DMSO  
 EXREF 0.00 ppm  
 BF 0.30 Hz  
 RGAIN 16



**INDOFINE Chemical Company, Inc.**  
 121 STRYKER LANE  
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 HILLSBOROUGH, NJ 08844



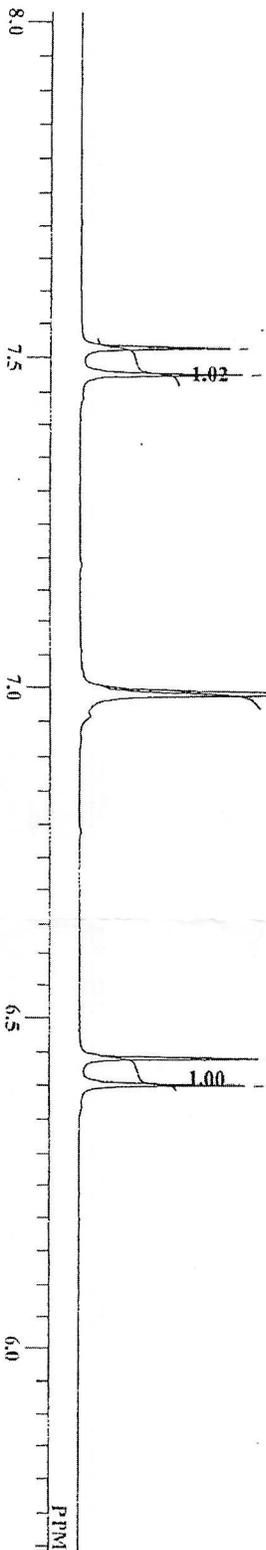
Catalog No.: 01-1354  
 Product Name: Sinapinic acid  
 Lot No.: 1512719

SPA(R/C in MeOH) 1H NMR DMSO-d6  
 Analyzed By: KNR

7.514  
 7.475

6.991

6.441  
 6.401



DFILE	SPA(R-C in MeOH) 1H.als
COMNT	SPA(R/C in MeOH) 1H NMR DMS
ORNUC	1H
EXMOD	NON
ORRQ	399.65 MHz
ORSET	124.00 KHz
ORFIN	10500.00 Hz
POINT	16384
FREQU	10000.00 Hz
SCANS	16
ACQTM	1.6384 sec
PD	2.0000 sec
PV1	6.00 usec
IRNUC	1H
CTEMP	18.5 c
SLVNT	DMSO
EXREF	0.00 ppm
BF	0.30 Hz
RGAIN	16

AKROCHEM CORPORATION  
3770 EMBASSY PARKWAY  
AKRON, OH 44333-8367  
(330) 535-2100

800-321-2260 NATIONWIDE 330-762-0815 FAX

-----  
COVER SHEET  
-----

Date : 8/22/24  
Time : 15:17:23

Name : 8000-00  
EXAMPLE CUSTOMER COA

Akrochem Corporation  
3770 Embassy Parkway  
Akron OH 44333

Attn : QA

Memo No. : 0000  
Ship Date: 8/22/24

From : Quality Assurance Department

Notes : 1 Page(s) To Follow, Not Including This Cover Sheet.  
Please Call If All Pages Are Not Received.

1 COPY(S) RETAIN

AT TIME OF SHIPMENT

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3770 EMBASSY PARKWAY  
AKRON, OH 44333-8367  
(330) 535-2100

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\* CERTIFICATE OF ANALYSIS \*

Page 1

8000-00  
EXAMPLE CUSTOMER COA  
DURAZONE 37 PASTILLES  
St # : 3928  
Cust Code :

B/L #	Date Shipped	Cust. P.O. #	Date Cert.
0000	8/22/24	SAMPLE	8/22/24

Qty : 1 LOT # : CD1J23P008  
Mfg Date : 9/24/21  
Exp Date : 9/24/25

WE CERTIFY THAT THE MATERIAL DESCRIBED HEREON COMPLIES  
WITH THE SI GROUP SPECIFICATION AS SHOWN BELOW.  
Analysis :

Test			Limits	Results
FINAL MELT POINT	DEGREES C	Min.	55.0	68.0
		Max.	73.0	
HEAT LOSS	PERCENT	Max.	0.50	0.20
ASH	PERCENT	Max.	0.40	0.00
DURAZONE 37 ASSAY	PERCENT	Min.	90.0	90.0
		Max.	100.0	

  
QUALITY ASSURANCE DEPT.  
AKROCHEM CORPORATION



## Certificate of Analysis

08/13/2024(JST)

TOKYO CHEMICAL INDUSTRY CO.,LTD.

T-PLUS Nihonbashi-Kodemmacho

16-12 Nihonbashi-kodemmacho, Chuo-ku, Tokyo 103-0001, Japan

Chemical Name: Stearyl 3-(3,5-Di- <i>tert</i> -butyl-4-hydroxyphenyl)propionate		
Product Number: D1644 CAS RN: 2082-79-3	Lot: C2PNC	

Tests	Results	Specifications
Appearance	White crystal	White to Almost white powder to crystal
Purity(GC)	98.5 %	min. 98.0 %
Melting point	51.8 °C	50.0 to 55.0 °C

TCI Lot numbers are 4-5 characters in length. Characters listed after the first 4-5 characters are control numbers for internal purpose only.

The contents of the specifications are subject to change without advance notice. The specification values displayed here are the most up to date values. There may be cases where the product labels display a different specification, however, the product quality still meets the latest specification.

### Customer Service:

TCI AMERICA

Tel: +1-800-423-8616 / +1-503-283-1681

Fax: +1-888-520-1075 / +1-503-283-1987

E-mail: Sales-US@TCIchemicals.com

Takuya Nishioka  
Quality Assurance Department Manager