Report of Toxicity Tests Performed for
Friends of Perdido Bay

Abstract

Personnel at Friends of Perdido Bay collected samples from Perdido Bay, FL. At the request of Friends of Perdido Bay, Hydrosphere Research conducted a chronic bioassay test with the marine algae (*Dunaliella tertiolecta*).

The results are summarized in the accompanying report. This report shall not be reproduced, except in full, without the written approval of the laboratory. The results discussed in this report relate only to the sample as identified on the Chain of Custody form in Appendix A. The Laboratory Bench Sheets and Statistical Results are in Appendix B.

Testing was conducted using generally accepted lab practices and adhered to the standards set forth by the National Environmental Laboratory Accreditation Program (NELAP). The bioassay test conducted with *Dunaliella tertiolecta* is not a NELAP accredited test. Hydrosphere Research believes the results are true and accurate.

06/30/2023

Peter Meyer, Laboratory Director — Date

Introduction

One grab sample was provided by Friends of Perdido Bay. Using these samples, Hydrosphere Research conducted a chronic bioassay test with the algae *D. tertiolecta*.

Materials and Methods

Test Solution Preparation

The grab sample was collected on May 9th, 2023. The sample location was given as Site #2 Perdido Bay, Escambia County. The samples were contained in ½ gallon high density polyethylene containers, which were intact upon arrival. The arrival temperature of the sample met the sample acceptance criteria. The Chain of Custody forms for this sample is attached hereto in Appendix A.

The water quality values fell into expected ranges for pH, salinity, and dissolved oxygen. All other chemical characterization data for the effluent sample upon arrival in the laboratory is provided on the Sample Data Bench Sheet in Appendix B.

The sample arrived at a salinity of 10.8‰. No further adjustments were made to the samples salinity.

Site specifics for toxics sampling May 2023

Site #1 - Elevenmile Creek, just north of Kingsfield Road Bridge 30°34' 26.36N, 87°19' 17.80W

Site #2 - Upper Perdido Bay, just off the beach 30°24' 50.30 N, 87°22' 18.35" W

Sampling was done with a bucket grab of surface water

A summary of the sample received, and Hydrosphere's identification number is presented in Table 1. Sample Information below.

Table 1. Sample Information

Location ID	Date	Time	Hydrosphere ID
Site #2 Perdido Bay, Escambia County	5/9/2023	09:00	23057-A

Test Organisms

The test organism used in this study was the saltwater algae (*Dunaliella tertiolecta*). The algae are cultured in-house at Hydrosphere Research.

Test Methods

There is no 96-hour method for the saltwater algae (*Dunaliella tertiolecta*). EPA method 1003.0 for the 96-hour freshwater algae was adapted to the saltwater species. The summary of the test methods used are described in the following table:

Table 2. Summary of Test Methods

96-Hour *Dunaliella tertiolecta* Chronic	
Test method	EPA-821-R-02-013, Method 1003.0 - Adapted
Test type	Static non-renewal
Test duration	96 hours
Renewal	NA
Temperature	25 ± 1 °C
Light quality	"Cool white" fluorescent
Light intensity	86 8.6 (µE/m^2/s)
Photoperiod	Continuous illumination
Test chamber size	250 mL
Test solution volume	50 mL
Age of test organism	4-7 days
Initial cell density in test chambers:	5,000 cells/mL
No. of replicate chambers per concentration	4
Shaking rate	Twice daily by hand
Aeration	None
Dilution water	saltwater algae growth medium
Test concentrations	5 and a control
Endpoint	Growth (cell counts)
Physical / Chemical Measurements	pH and Temperature daily
Test acceptability	Mean cell density not currently established for this method adaptation. Variability (CV%) among control replicates less than or equal to 20%

All statistical calculations were made using CETIS® (Tidepool Scientific Software, McKinleyville, CA). The sample statistical results are in Appendices A and B.

The bioassay tests were performed at Hydrosphere Research, 11842 Research Circle, Alachua, FL 32615; telephone number (386) 462-7889. The laboratory is NELAP certified by the State of Florida Department of Health and Rehabilitation Services (E82295).

Results

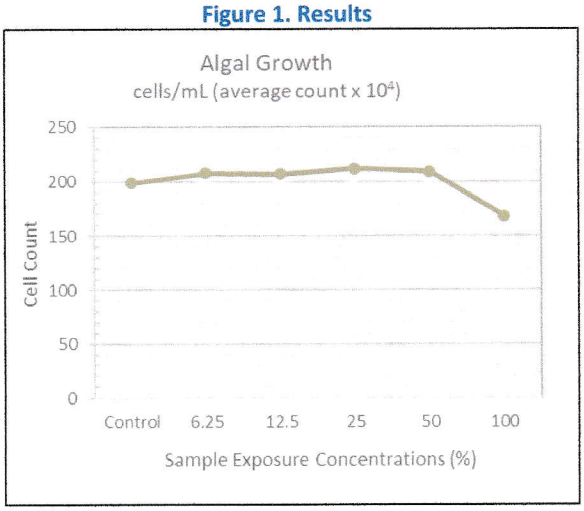
Water quality values remained within acceptable limits during the test periods. The bioassay tests were acceptable tests based on controls and test conditions specified in the methods. Copies of the relevant laboratory raw data pertaining to the toxicity tests are provided in Appendix B.

The results of the *Dunaliella tertiolecta* chronic effect concentration test is summarized in the tables and figures below.

Table 3. Results

Algal Growth	
Sample (%)	Cells/mL (average count x 10^4)
Control	198.6
6.25	207.9
12.5	207.2
25	212.0
50	209.4
100	168.0
NOEC	100%
IC_{25}	>100%
An "*", if present, indicates a statistically difference when compared to the control.	

Figure 1. Results



Quality Assurance

All phases of the study including, but not limited to, sample handling and storage, glassware preparation, test organism culturing/acquisition and acclimation, test organism handling during test, and maintaining appropriate test conditions were conducted per the applicable method. No known deviations were noted during the study.

All chemicals were certified products used before expiration dates (where applicable). All identification, service, and calibration information pertaining to laboratory instruments is recorded in calibration and maintenance logbooks. The bioassay tests were acceptable tests based on control performance and test conditions.

Standard Reference Toxicant Test Results

The results for the standard reference toxicant tests are in Appendix C which includes the control charts, raw data and statistics.

Summary and Conclusions

The sample provided, Site #2 Perdido Bay, Escambia County produced a 96-hour IC_{25} of >100% and a NOEC of 100% for the *Dunaliella tertiolecta* test species.

Temperature and pH remained within the limits established in the test methods.

No unusual observations or deviations from standard test protocol were noted. No unusual qualitative test organism behaviors were observed in the test exposures. These test results only relate to the samples described in this report.

References

U.S. Environmental Protection Agency. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms*. Fourth Edition. EPA-821-R-02-013. October 2002. Method 1003.0. – Adapted to saltwater.

Handbook of Analytical Quality Control in Water and Wastewater Laboratories. EPA-600/4-79-019. March 1979.

Chemical and physical parameters reported herein were determined by methods described in *Methods for Chemical Analysis of Water and Waste*. EPA 600/4-79-020. March 1983.

Appendix C.

EcoAnalysts, Port Gamble, WA
Toxicity Test Results using the bivalve *Mytilus galloprovincialis*
Conducted May 2023

ACRONYMS AND ABBREVIATIONS

EPA	Environmental Protection Agency
LC_{25}/EC_{25}	Lethal/ Effect Concentration to 25% of test population
LC_{50}/EC_{50}	Lethal/ Effect Concentration that results in a 50% reduction in survival
LOEL	Lowest Observed Effect Level
mg/L	Milligrams per liter
µg/L	Micrograms per liter
NOEL	No Observed Effect Level
PMSD	Percent Minimum Significant Difference
QM	Quality manual
SOP	Standard operation procedure
WDOE	Washington Department of Ecology

1. EXECUTIVE SUMMARY

EcoAnalysts conducted toxicity testing with samples collected from two different sites; Site 1 and Site 2. The results of the toxicity testing are contained in this report. Results of potential toxicity of Site 1 and Site 2 followed test acceptability criteria from Washington Department of Ecology (WDOE).

Table 1-1 indicates that Site 2 was less toxic than Site 1.

Table 1-1. Toxicity Test Results Summary.

Test		NOEL (%)	LOEL (%)	LC_{25}/EC_{25} (%)	LC_{50}/EC_{50} (%)
Site 1	Bivalve Proportion Survived	100	>100	>100	>100
	Bivalve Proportion Normal	25	50	40.9	61.8
Site 2	Bivalve Proportion Survived	100	>100	>100	>100
	Bivalve Proportion Normal	100	>100	>100	>100

NOEL = No Observed Effect Level
LOEL = Lowest Observed Effect Level
LC_{25}/EC_{25} = Lethal/Effect Concentration to 25% of test population
LC_{50}/EC_{50} = Lethal/Effect Concentration to 50% of test population

2. RESULTS

2.1 Sample Collection/ Receipt

Friends of Perdido Bay personnel collected one sample from Site 1 and one sample Site 2 on May 2, 2023. The samples were received at the EcoAnalysts Port Gamble laboratory on the day following collection. Temperatures upon receipt ranged from 0.0 – 0.6°C. Additional sample conditions are summarized in Table 2-1. The effluent samples were held in a walk-in cold room at 4 ± 2 °C in the dark until utilized for testing.

Table 2-1. Sample Conditions Upon Receipt.

Sample	Site 1	Site 2
Laboratory ID	P230503.01	P230503.02
Date/Time sampled	5/2/23; 1000	5/2/23; 0900
Date/Time received	5/3/23; 1115	5/3/23; 1115
Dissolved Oxygen (mg/L) **Recommended: >4.0 mg/L**	10.1	9.3
Temperature (°C) **Recommended: 0 – 6°C**	0.5-0.6	0.0-0.4
pH (units) **Recommended: 6 – 9**	7.8	7.7
Salinity (ppt)	0.57	11.2
Total Free Chlorine (mg/L)	0.04	0.02
Total Ammonia (mg/L)	0.00	0.00

Due to the low salinity of the samples (0.57 ppt and 11.2 ppt for site 1 and site 2, respectively), the samples were salted to bring the salinity within the test range (30 ± 2 ppt). A salt control was added to the test to evaluate any effects of salting on the toxicity of the samples.

2.2 *Mytilus galloprovincialis* Test Results

Two chronic toxicity tests with *M.* galloprovincialis, conducted on sample "Site 1" and "Site 2", were initiated on May 3rd, 2023. Site 1 was validated by 73.7% combined proportion normal (number normal/stocking density) and 16.7% Percent Minimum Significant Difference (PMSD) in the laboratory control, meeting the test acceptability criteria of ≥70% combined normal shell development and <25% PMSD (Table 2-2). Site 2, with 66.8% combined proportion normal, was slightly below the acceptability criterion of ≥70% combined normal shell development but did meet acceptability criteria of <25% PMSD with a PMSD of 10.6%. However, the salt control for Site 2 was validated by 73.4% combined proportion normal and 9.7% PMSD (stats not printed) (Table 2-3).

Concentrations of 6.25, 12.5, 25, 50, and 100% effluent were prepared utilizing laboratory water. Water quality parameters were within the acceptable limits throughout the duration of the 48-hour static test for both Site 1 and Site 2. A summary of test conditions is provided in Table 2-4.