

Nautilus Environmental, LLC

## **Toxicity Testing of Chitosan Lactate**

### **Final Report**

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Submitted to:

#### **Cascade EcoSolutions**

3500 188<sup>th</sup> Street SW, Suite 322

Lynnwood, WA 98037

*Washington Laboratory*  
5009 Pacific Hwy East  
Suite 2  
Tacoma, WA 98424

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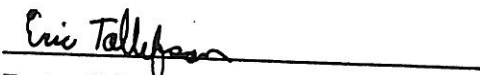
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**SIGNATURE PAGE**

  
Laboratory Manager

  
Project Manager

The results of this study intended for the use of our client, subject to the terms and conditions of their contract with Nautilus Environmental. Any reliance on the data by a third party is at the sole and exclusive risk of that party.

## 1.0 INTRODUCTION

A toxicity testing program was conducted using Chitosan Lactate, as manufactured and distributed by Cascade EcoSolutions, Lynnwood, Washington, in order to meet the requirements specified in Washington Department of Ecology's laboratory guidance document Appendix G titled "Risk Evaluation of Proposed Treatment Chemicals for Stormwater Runoff" (WDOE, 2005).

The program involved conducting a suite of toxicity tests using the product to determine which was the most sensitive species, and to determine the "toxic threshold", defined as the lowest 25% point estimate from the various tests. The tests performed were designated to evaluate effects of the compound in a "juvenile salmonid or other fish rearing habitat" receiving water. The results of the study are provided in this report.

## 2.0 METHODS

An acute toxicity test was conducted using *Daphnia pulex*. This test involved a 48hr exposure of the test organisms during which survival was monitored. Methods for the test are summarized in Table 1.

Chronic toxicity tests were conducted using rainbow trout (*Oncorhynchus mykiss*) and fathead minnows (*Pimephales promelas*). Each of these species was evaluated using a 7-day survival and growth test; methods for these tests are summarized in Tables 2 and 3.

The concentration series for each test was established in discussion with Cascade EcoSolutions and was based on previous testing. For all of the tests, the sample was weighed and placed in a flask of dilution water to achieve the highest concentration. The test solution was mixed on a magnetic stir plate overnight at 25°C until completely dissolved. Serial dilutions were then made from this stock solution. This procedure was repeated daily for all renewal solutions. Both methods used for sample preparation achieved the goal of exposing test organisms to the intended concentrations.

Control water for all of the tests was moderately hard synthetic water, prepared by addition of reagent grade salts into deionized water to achieve a hardness of 80 to 100 mg/L.

Table 1. Summary of test conditions for the 48-hour *Daphnia pulex* acute survival test.

Test organism	<i>Daphnia pulex</i>
Test organism source	In-house cultures
Test organism age at initiation	< 24 hours
Test duration	48 hours
Test solution renewal	None
Feeding	None
Test chamber	30 ml plastic cup
Test solution volume	25 mL
Test temperature	20 ± 1°C
Dilution water	Moderately Hard Synthetic Water
Number of organisms/chamber	5
Number of replicates	4
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test Protocol	EPA-821-R-02-012 (USEPA, 2002a)
Test acceptability criterion for controls	≥ 90% survival
Reference toxicant	Copper sulfate

Table 2. Summary of test conditions for the 7-day rainbow trout survival and growth test.

Test organism	<i>Oncorhynchus mykiss</i>
Test organism source	Thomas Fish Company, Anderson, California
Test organism age at initiation	16 days post hatch, 2 days post swim up
Test duration	7 days
Test solution renewal	Daily
Feeding	<i>Artemia</i> nauplii twice daily
Test chamber	1-L plastic beaker
Test solution volume	500 mL
Test temperature	15 ± 1°C
Dilution water	Moderately Hard Synthetic Water
Number of organisms/chamber	5
Number of replicates	4
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test Protocol	USEPA 2005
Test acceptability criterion for controls	≥ 90% survival
Reference toxicant	Copper chloride

Table 3. Summary of test conditions for the 7-day fathead minnow survival and growth test.

Test organism	<i>Pimephales promelas</i>
Test organism source	Aquatic BioSystems; Fort Collins, Colorado
Test organism age at initiation	<48 hours post hatch
Test duration	7 days
Test solution renewal	Daily
Feeding	<i>Artemia</i> nauplii twice daily
Test chamber	500 mL plastic cup
Test solution volume	250 mL
Test temperature	25 ± 1°C
Dilution water	Moderately Hard Synthetic Water
Number of organisms/chamber	10
Number of replicates	4
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test Protocol	EPA-821-R-02-013 (USEPA, 2002b)
Test acceptability criterion for controls	≥ 80% survival; ≥ 0.25 mg average dry weight
Reference toxicant	Sodium chloride

### 3.0 RESULTS

Results of acute toxicity tests conducted with *D. pulex* are provided in Table 4. Results of definitive tests evaluating survival and growth of rainbow trout and fathead minnows are provided in Table 5.

The chronic test with fathead minnows was the most sensitive test conducted in this evaluation. The highest concentration with no observed effect (NOEC) was 7.5 mg/L Chitosan Lactate for survival and 15 mg/L Chitosan Lactate for growth. The EC25 for fathead minnow survival and growth was 15.7 mg/L and 17.4 mg/L Chitosan Lactate respectively. The toxic threshold, as defined in WDOE (2003) as the lowest 25% point estimate from the suite of tests, was 15.7 mg/L for fathead minnow survival. For application of the product, WDOE requires a three-fold safety margin between the concentration of the product at discharge and the toxic threshold for the product (WDOE, 2003). Thus, the data supports application of Chitosan Lactate at a rate of 5.23 mg/L. Higher applications rates may be appropriate if concentrations of Chitosan Lactate are reduced as a result of interaction with turbidity.



Table 4. Percent survival of test organisms in acute toxicity tests.

Chitosan Lactate mg/L	Daphnia survival (% ± S.D.)
Control	100.0 ± 0.0
15.0	90.0 ± 11.5
30.0	100.0 ± 0.0
60.0	100.0 ± 0.0
120	95.0 ± 10.0
240	40.0 ± 28.3
<b>Endpoints (mg/L Chitosan Lactate)</b>	
NOEC <sup>1</sup>	120
LOEC <sup>2</sup>	240
LC50 <sup>3</sup>	353
LC25 <sup>4</sup>	138

<sup>1</sup>No Observed Effect Concentration    <sup>2</sup>Lowest Observed Effect Concentration

<sup>3</sup>Predicted concentration to cause 50% mortality in test organisms

<sup>4</sup>Predicted concentration to cause 25% mortality in test organisms

Table 5. Results for 7-day survival and growth tests using rainbow trout and fathead minnows.

Chitosan Lactate (mg/L)	Rainbow trout		Fathead minnow	
	Survival (% ± S.D.)	Biomass (mg ± S.D.)	Survival (% ± S.D.)	Biomass (mg ± S.D.)
Control	100.0 ± 0.0	34.6 ± 1.6	100.0 ± 0.0	0.44 ± 0.04
0.9375	100.0 ± 0.0	34.1 ± 3.3	--	--
1.875	100.0 ± 0.0	34.9 ± 2.0	--	--
3.75	100.0 ± 0.0	35.6 ± 1.4	100.0 ± 0.0	0.45 ± 0.05
7.5	100.0 ± 0.0	35.1 ± 2.0	92.5 ± 9.6	0.38 ± 0.06
15	100.0 ± 0.0	31.2 ± 0.6	82.5 ± 9.6	0.37 ± 0.07
30	95.0 ± 0.10	22.9 ± 3.2	42.5 ± 17.1	0.16 ± 0.08
60	--	--	0.0 ± 0.0	nc
<b>Endpoints (mg/L Chitosan Lactate)</b>				
NOEC	30	15	7.5	15
LOEC	>30	30	15	30
LC50	>30		23.4 (20.1-27.4)	
LC25	>30		15.7 (12.8-18.4)	
IC50 <sup>1</sup>		>30		25.3 (19.9-32.2)
IC25 <sup>2</sup>		24.2 (19.6-29.9)		17.4 (1.46-21.5)

<sup>1</sup> Predicted to cause 50% reduction in growth in test organisms

<sup>2</sup> Predicted to cause 25% reduction in growth in test organisms

#### 4.0 QA/QC

Control performance criteria specified in the corresponding protocols were met for all of the tests. Results of reference toxicant tests are provided in Table 7. The results of the reference toxicant tests indicated that the test organisms were of suitable sensitivity.

Table 6. Results of reference toxicant tests.

Date	Organism	Test type	Effect concentration	Acceptable range
5/29/08	<i>Daphnia pulex</i>	48-hr survival	7.54 µg/L Cu	2.36 - 19.0
6/3/08	Fathead minnow	7-d survival	4.93 g/L NaCl	4.06 - 5.89
		7-d growth	3.85 g/L NaCl	2.90 - 5.58
6/11/08	Rainbow trout	7-d survival	67.6 µg/L Cu	34.4 - 84.7
		7-d growth	63.1 µg/L Cu	36.0 - 86.1

## REFERENCES

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