



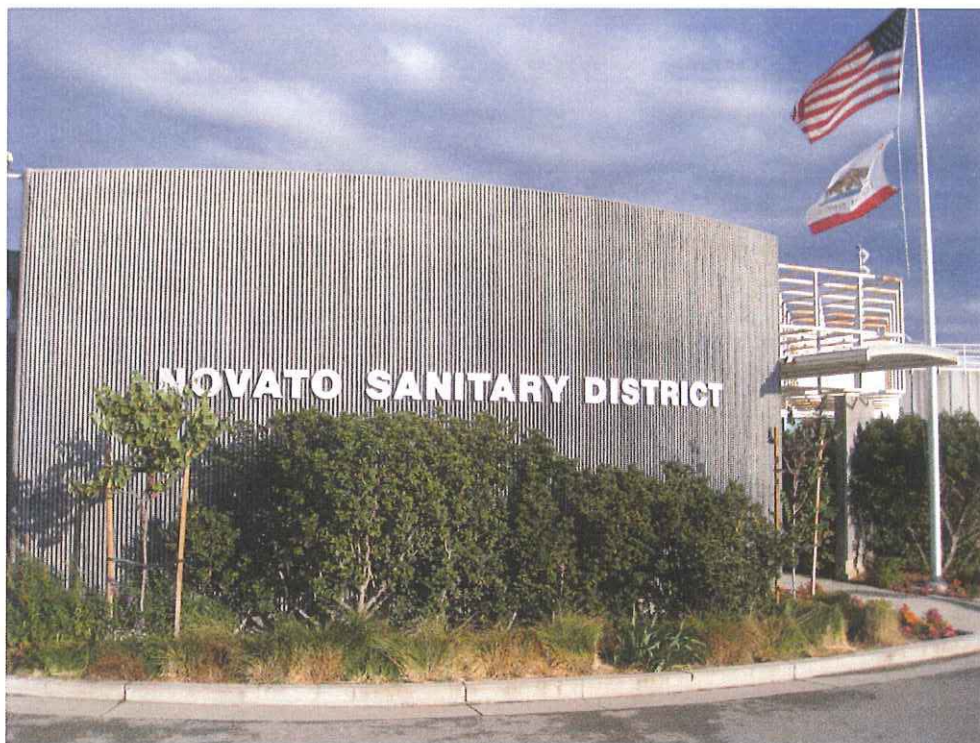
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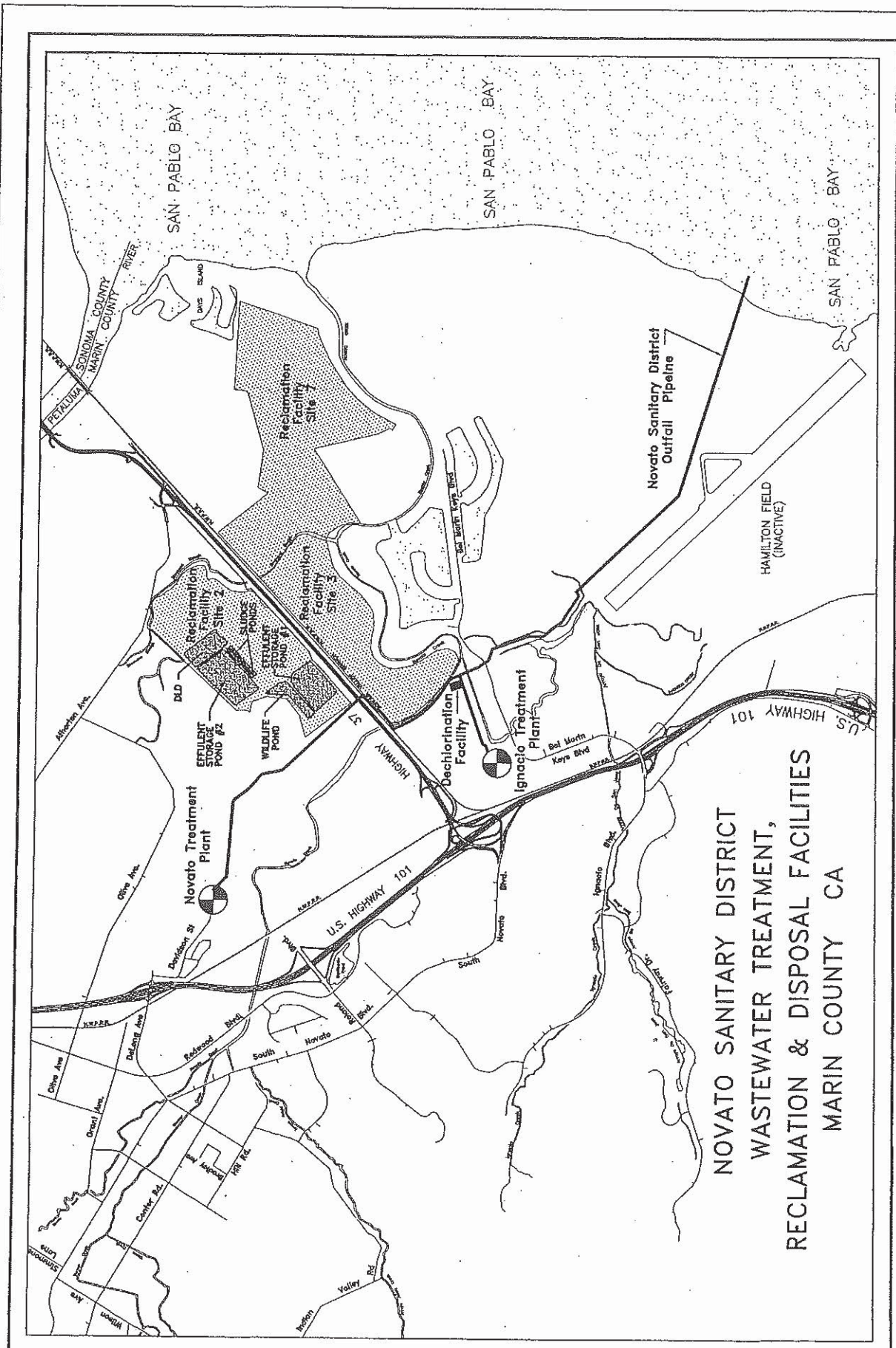
**NOVATO SANITARY DISTRICT  
VEOLIA WATER WEST OPERATING SERVICES**

**SELF MONITORING REPORT**

**SECTION III**

**ATTACHMENTS**





NOVATO SANITARY DISTRICT  
WASTEWATER TREATMENT,  
RECLAMATION & DISPOSAL FACILITIES  
MARIN COUNTY CA









ENVIRONMENTAL TOXICOLOGY SPECIALISTS

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## **ACUTE FATHEAD MINNOW TOXICITY TEST WITH NOVATO SANITARY DISTRICT EFFLUENT**

**Submitted to:**

**Bob Adamson**  
Novato Sanitary District  
500 Davidson Street  
Novato, CA 94945

**Submitted by:**

**AQUA-Science**  
630 Cantrill Drive  
Davis, CA 95618  
ELAP Certificate No. 2205  
Expires: 1/31/2013

**April 4, 2012**

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## ACUTE FATHEAD MINNOW TOXICITY TEST WITH NOVATO SANITARY DISTRICT EFFLUENT

### 1.0 EXECUTIVE SUMMARY

Novato Sanitary District final effluent (E-002) was tested using the acute *Pimephales promelas* (fathead minnow) toxicity flow-through test protocol. The 96-hour fathead minnow bioassay did not detect significant mortality when compared to the control. This test met all protocol and QA/QC requirements.

### 2.0 INTRODUCTION

AQUA-Science (Davis, CA) was retained by the Novato Sanitary District to perform a 96-hour acute flow-through fathead minnow (*Pimephales promelas*) survival toxicity test with final effluent E-002.

### 3.0 MATERIALS AND METHODS

#### 3.1 Sample Collection and Transport

A 24-hour composite effluent sample was collected daily by Novato Sanitary District personnel in cubitainers and delivered on wet ice to AQUA-Science via courier. Appropriate chain-of-custody procedures were employed during collection and transport.

#### 3.2 Sample Receipt

Water quality measurements including temperature, dissolved oxygen (DO), and pH were recorded on chain-of-custody form at sample receipt. Samples were stored in the dark at  $\leq 4$  °C until used for bioassays.

#### 3.3 Water Quality Measurements

Temperature was continuously recorded in all bioassay test chambers with a Dickson pen recorder (Model ICT855, Addison, IL). DO (YSI Model 550A, Yellow Springs, OH), pH (Beckman 240, Fulton, CO), and temperature (calibrated digital thermometer; Central Co., Friendswood, TX) were measured in initial and 24-hour test solutions at change-out. Conductivity (WTW Model 330, Ft. Myers, FL), alkalinity (Hach Model AL-DT calorimetric test, Hach Co., Loveland, CO), and hardness (Hach HA-DT colorimetric test) were measured in the initial test solutions.

### 3.4 Fathead Minnow Toxicity Tests

The 96-hour acute flow-through fathead minnow bioassays were conducted in accordance with the U.S. Environmental Protection Agency (USEPA) 5<sup>th</sup> edition protocol<sup>1</sup>. Fathead minnows were obtained from Aquatox, Inc. (Hot Springs, AK), and were maintained in EPA moderately hard (EPAMH) water until tested at 7 days old. The effluent was tested using 2 replicates of 10 fish each in 400 mL plastic beakers containing 250 mL of test solutions. The effluent was continuously delivered to the test chambers using a 4-channel peristaltic pump at a flow rate of 5 mL/min, which provided a total of approximately 29 test chamber volumes per day. The effluent flow-rate was measured daily. Fish were fed *Artemia* nauplii daily. Tests were conducted at 25 ± 2 °C with a 16 hour light:8 hour dark photoperiod. Mortality was recorded daily.

### 3.5 Reference Toxicant Tests

A concurrent reference toxicant test was conducted with this species. Sodium chloride was the reference toxicant material used for the fathead minnows (control, 1.25, 2.5, 5, 7.5, and 10 g/L).

## 4.0 RESULTS

The acute fathead minnow flow-through toxicity test was initiated on 3/14/12 (Event 12-03). A summary of the test results and water quality parameters are presented in Tables 1 and 2, respectively. Statistical analyses were performed using CETIS™ v1.8.0.13 (Tidepool Scientific, McKinleyville, CA). A summary of the reference toxicant test results is presented in Table 3. All raw data is found in Appendix I.

**Table 1. Summary of Acute Fathead Minnow Mortality**

<i>Test Sample</i>	<i>Survival (%)</i>	<i>Survival (NOEC %)</i>	<i>PMSD (%)<sup>a</sup></i>	<i>Comments</i>
Lab Control	90	100	n/a	No mortality was detected
E-002 (100%)	100	100	b	

a PMSD = percent minimum significant difference

b Value not calculated since there was 100% survival

**Table 2. Summary of Water Quality Parameters**

<i>Parameter @ 100% Effluent</i>	<i>Temp. (°C)</i>	<i>D.O. (mg/L)</i>	<i>pH</i>	<i>Alkalinity (mg/L)</i>	<i>Conductivity (mg/L)</i>	<i>Hardness (mg/L)</i>
Initial Value Range	25-26	7.6-8.0	7.72-7.86	120-165	558-716	140-175
24-hr Value Range	25-25	4.4-5.6	7.37-7.44	n/a	n/a	n/a

<sup>1</sup> Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fifth Edition. October 2002. EPA 821-R-02-012.




**Table 3. Summary of Reference Toxicant Results**

<i>Test Endpoint</i>	<i>NOEC (g/L)</i>	<i>LOEC (g/L)</i>	<i>EC<sub>25</sub> (g/L)</i>	<i>EC<sub>50</sub> (g/L)</i>	<i>PMSD (%)</i>
Survival	7.5	10.0	8.0	8.6	n/a

The effluent (E-002) did not cause any mortality in the 96-hour acute flow-through fathead minnow aquatic bioassay. The reference toxicant test was within normal limits for this laboratory, and produced a Type 7 dose response (significant effect at the highest concentration only).

Approved by/Issue date:

 4/4/12  
Jeff Miller, Ph.D., DABT  
President



*Environmental Toxicology Specialists*

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**EFFLUENT TOXICITY TESTS FOR ASSESSING COMPLIANCE  
WITH NPDES CHRONIC TOXICITY LIMITS**

**NOVATO SANITARY DISTRICT  
WASTEWATER TREATMENT PLANT**

***SUBMITTED TO:***

***Bob Adamson***  
Novato Sanitary District  
500 Davidson Street  
Novato, CA 94945

***SUBMITTED BY:***

***AQUA-Science***  
630 Cantrill Drive  
Davis, CA 95618

**April 4, 2012**

## EFFLUENT TOXICITY TESTS FOR ASSESSING COMPLIANCE WITH NOVATO SANITARY DISTRICT WASTEWATER TREATMENT PLANT NPDES CHRONIC TOXICITY LIMITS

### 1.0 CLIENT INFORMATION

<b>Client:</b>	<b>Novato Sanitary District</b>
<b>Address:</b>	500 Davidson Street Novato, CA 94945
<b>Contact:</b>	<b>Bob Adamson</b>
<b>Phone/Fax:</b>	(415) 892-1694 / (415) 898-2279

### 2.0 BIOTOXICITY TESTING REQUIREMENTS

<b>Test Type:</b>	<b>7-Day <i>Ceriodaphnia dubia</i> Chronic Survival and Reproduction Test</b>
<b>Test Frequency:</b>	<b>Quarterly</b>
<b>Test Protocol:</b>	<b>EPA 821-R-02-013</b>
<b>Dilution Series:</b>	<b>0, 6.25, 12.5, 25, 50, and 100% Effluent E-002</b> <b>Antibiotic treatment (control &amp; 100% effluent)</b> <b>Concurrent Reference Toxicant Series</b>

### 3.0 CURRENT TEST INFORMATION

<b>Event No.:</b>	<b>First Quarter, 2012 (2012-03)</b>
<b>Test Sample:</b>	<b>Daily composite samples: 3/13-19/12</b>
<b>Test Initiation:</b>	<b>3/13/12</b>
<b>Test Completion:</b>	<b>3/19/12</b>

### 4.0 SUMMARY OF RESULTS

Based on the  $EC_{25}$ , the effluent produced significant reproductive effects in the chronic *Ceriodaphnia* toxicity test [45.5 TUc (100/ $EC_{25}$ )]. Antibiotic addition with the 100% effluent eliminated the toxicity in this test, indicating the toxicity was caused by bacteria. The concurrent reference toxicant test for this species was within the acceptable range, and all protocol and QA/QC requirements were met.



## 5.0 RESULTS OF INDIVIDUAL TESTS

### 5.1 Effluent

#### 5.1.1 Current Effluent Test Data

Sample Concentration (%)	% Survival	Reproduction (neonates/female)	QA/QC Requirements Met:
Lab Control	100	27.0	<ul style="list-style-type: none"> <li>≥80% survival in controls</li> <li>average neonates/female in controls ≥15</li> <li>60% of surviving control females produced at least three broods</li> </ul>
12.5	100	14.4*	
25	80	14.2*	
50	70	13.4*	
75	100	15.9*	
100	70	19.4*	

\* = significantly different than control ( $p < 0.05$ )

#### 5.1.2 Current Effluent Test Results

Test Endpoint <sup>a</sup>		NOEC (%)	LOEC (%)	EC <sub>25</sub> (%)	EC <sub>50</sub> (%)	PMSD (%) <sup>b</sup>
Survival	% Effluent	100	> 100	77.2	> 100	c
	TUc	1.0	n/a	1.3	< 1.0	
Reproduction	% Effluent	< 6.25	6.25	2.2	> 100	30
	TUc	> 16.0	n/a	45.5	< 1.0	

a Cetis™ v. 1.8.0.13 was used to calculate test endpoint

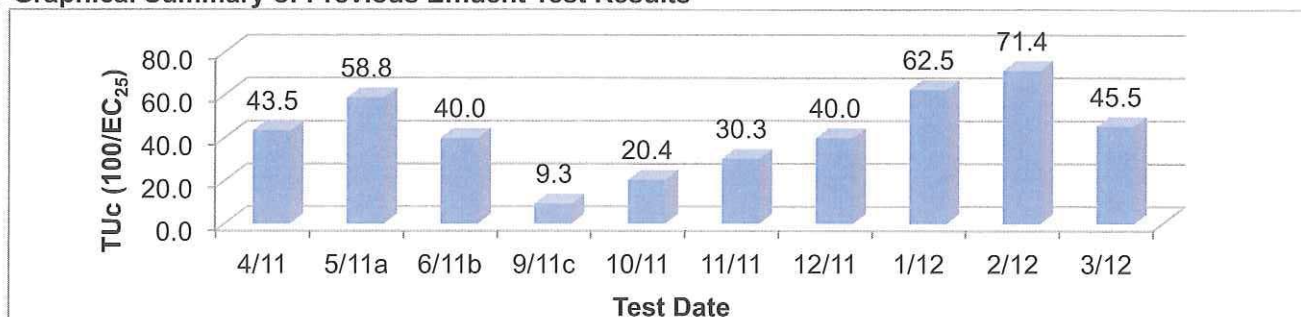
b PMSD = Percent Minimum Significant Difference

c Value could not be calculated due to statistical method used

#### 5.1.3 Tabular Summary of Effluent Test Results (most sensitive endpoint - Section 5.1.2)

Test Date	4/11	5/11a	6/11b	9/11c	10/11	11/11	12/11	1/12	2/12	3/12
EC <sub>25</sub> (%)	2.3	1.7	2.5	10.7	4.9	3.3	2.5	1.6	1.4	2.2
NOEC (%)	<6.25	< 6.25	< 25	6.3	12.5	< 6.25	< 6.25	< 6.25	< 6.25	< 6.25
TU (100/EC <sub>25</sub> )	43.5	58.8	40.0	9.3	20.4	30.3	40.0	62.5	71.4	45.5

#### 5.1.4 Graphical Summary of Previous Effluent Test Results



a TIE on composite of 4/18-24/11 samples; 5 replicates/concentration

b TIE - 5 replicates/concentration

c TIE - 10 replicates/concentration

#### 5.1.5 Comments

Based on the EC<sub>25</sub>, the effluent produced significant reproductive effects (45.5 TUc; 100/EC<sub>25</sub>), and produced a Type 8 dose response (significant effects at all test concentrations but flat dose-response curve).

## 5.2 Reference Toxicant

### 5.2.1 Current Reference Toxicant Test Results

Test Endpoint <sup>a</sup>	NOEC (g/L)	LOEC (g/L)	EC <sub>25</sub> (g/L)	EC <sub>50</sub> (g/L)	PMSD (%) <sup>b</sup>
Survival	2.0	4.0	2.3	2.8	c
Reproduction	0.25	0.50	0.4	0.8	17

a Cetis™ v. 1.8.0.13 was used to calculate test endpoint

b PMSD = percent minimum significant difference

c The PMSD could not be calculated due to the statistical method used

### 5.2.2 Comments

The reference toxicant test was within normal limits for this laboratory, and produced a Type 7 (significant effects only at the highest concentration) for the survival endpoint, and an ideal dose response for the reproduction endpoint. Appendix I shows the laboratory reference control chart for *C. dubia* reproduction.

## 5.3 Antibiotic Treatment

### 5.3.1 Individual Test Data

Sample Concentration (%)	Antibiotics	% Survival	Reproduction (neonates/female)
Antibiotic Control	Maracyn™ & Maracyn™ Two	100	27.6
100		90	32.3


### 5.3.2 Comments

Antibiotic addition eliminated the effluent toxicity, indicating the effluent toxicity was caused by bacteria.

## 6.0 TESTING FACILITY

<b>AQUA-Science</b> 630 Cantrill Drive Davis, CA 95618 (530) 753-5456	California Department of Health Services ELAP Certification No. 2205 (1/31/13)  File Reference: Novato (2012-03)
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Approved By/Issue Date:

 4/4/12  
Jeffrey L. Miller, Ph.D., DABT