FEBRUARY 2013

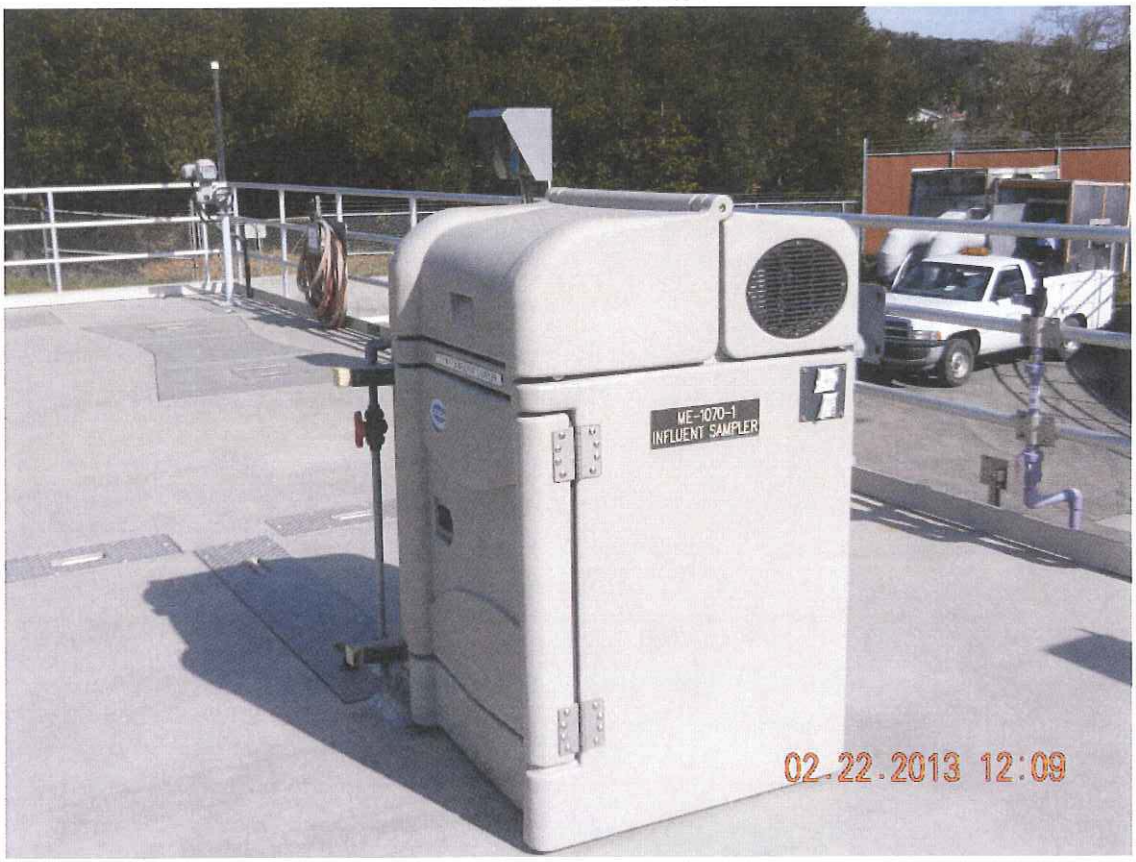
NOVATO SANITARY DISTRICT
VEOLIA WATER WEST OPERATING SERVICES

SELF MONITORING REPORT

SECTION IV

ATTACHMENTS

LAB DATA



ATTACHMENTS
LAB DATA

ACUTE TOXICITY, 96 HOUR – EFFLUENT

CHRONIC TOXICITY – EFFLUENT **(Not Yet Available will submit with March SMR)**

AQUA SCIENCE

ENVIRONMENTAL TOXICOLOGY SPECIALISTS

ACUTE FATHEAD MINNOW TOXICITY TEST WITH NOVATO SANITARY DISTRICT EFFLUENT

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February 20, 2013

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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 (2/12-15/13) 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 ≤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) 5th edition protocol[1]. 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 2/13/13 (Event 13-02). 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

Test Sample	*Survival (%)*	*Survival (NOEC %)*	*PMSD (%)*[a]	*Comments*
Lab Control	95	100	n/a	No mortality was detected with the effluent
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

Parameter @ 100% Effluent	*Temp. (°C)*	*D.O. (mg/L)*	*pH*	*Alkalinity (mg/L)*	*Conductivity (mg/L)*	*Hardness (mg/L)*
Initial Value Range	24-25	8.2-8.5	7.82-8.00	143-150	698-734	120-185
24-hr Value Range	24-25	5.8-6.7	7.60-7.69	n/a	n/a	n/a

[1] 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

Test Endpoint	*NOEC (g/L)*	*LOEC (g/L)*	*EC_{25} (g/L)*	*EC_{50} (g/L)*	*PMSD (%)*
Survival	7.5	10	7.5	8.3	23.8

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 highest concentration only).

Approved by/Issue date: Jeff Miller 2/20/13

Jeff Miller, Ph.D., DABT
President