AQUA SCIENCE ENVIRONMENTAL TOXICOLOGY SPECIALISTS

ACUTE FATHEAD MINNOW TOXICITY TEST WITH NOVATO SANITARY DISTRICT EFFLUENT

October 2014

Submitted to:

Novato Sanitary District
500 Davidson Street
Novato, CA 94945
Attention: Ken Besnia

Submitted by:

AQUA-Science
630 Cantrill Drive
Davis, CA 95618

October 21, 2014

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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 (10/7/14 – 10/10/14) 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 10/8/14 (Event 14-06). A summary of the test results and water quality parameters are presented in Tables 1 and 2, respectively. Statistical analyses were performed using CETIS™ v1.8.7.7 (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	100	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	25-25	7.8-8.4	7.56-7.88	116-135	722-796	131-142
24-hr Value Range	24-24	6.3-7.3	7.37-7.74	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	5.0	7.5	7.8	8.5	7.4

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 an ideal dose response.

Approved by/Issue date: 10/21/14

Jeff Miller, Ph.D., DABT
President

APPENDIX I

14-06 Raw Data

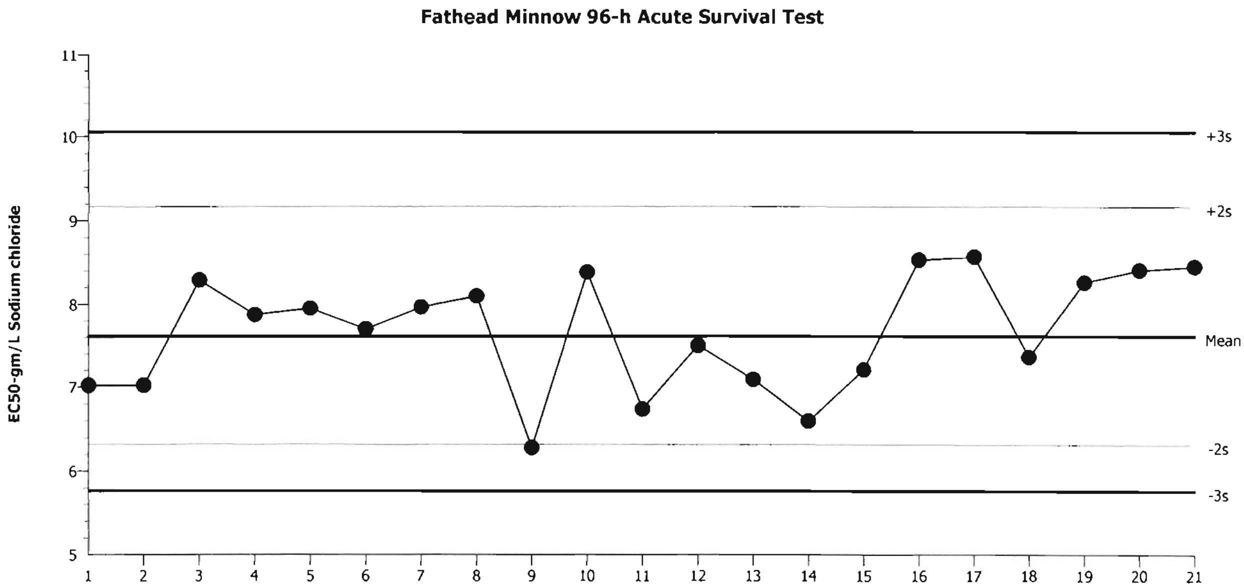
Fathead Minnow

CETIS QC Plot

Report Date: 13 Oct-14 14:40 (1 of 1)

Fathead Minnow 96-h Acute Survival Test | **Aqua-Science**

Test Type:	Survival (96h)	**Organism:**	Pimephales promelas (Fathead Minn	**Material:**	Sodium chloride
Protocol:	EPA/821/R-02-012 (2002)	**Endpoint:**	96h Survival Rate	**Source:**	Reference Toxicant-REF



Mean:	7.614	**Count:**	20	**-2s Warning Limit:**	6.324	**-3s Action Limit:**	5.763
Sigma:	NA	**CV:**	9.73%	**+2s Warning Limit:**	9.168	**+3s Action Limit:**	10.06

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2013	Oct	16	16:55	7.021	-0.5935	-0.874			16-2831-3166	16-7559-6043
2			16	16:55	7.021	-0.5935	-0.874			18-2274-9965	17-0854-4930
3		Nov	20	15:25	8.29	0.6762	0.9164			12-4846-6076	21-1500-8355
4		Dec	18	16:20	7.873	0.2591	0.3604			07-1161-9505	19-2867-2129
5			18	16:20	7.95	0.3357	0.4647			13-0353-9262	12-1581-1181
6	2014	Jan	15	14:00	7.702	0.0875	0.1231			07-0023-1966	05-6382-5695
7			22	15:00	7.965	0.3511	0.4855			14-1003-3183	00-8812-9129
8		Feb	12	13:20	8.098	0.4844	0.6642			16-6427-6791	15-8617-9843
9			19	13:55	6.281	-1.333	-2.073	(-)		08-8840-5947	00-6690-8022
10		Mar	5	14:20	8.382	0.7677	1.035			21-1601-1544	07-7359-8912
11			12	12:00	6.739	-0.8749	-1.315			05-2345-4416	00-7257-2319
12		Apr	3	15:25	7.5	-0.1141	-0.1626			16-9606-5632	15-1740-5543
13			9	14:30	7.094	-0.5204	-0.7624			14-5074-1802	11-7662-4863
14			30	12:00	6.6	-1.014	-1.54			07-1741-4772	02-2069-9145
15		May	7	15:30	7.209	-0.4051	-0.5887			01-9038-0481	09-5736-8261
16			21	12:15	8.532	0.9179	1.226			18-5881-1423	08-0738-5716
17		Jun	18	14:45	8.569	0.9549	1.272			18-0002-0952	04-6551-9761
18		Jul	16	12:45	7.36	-0.2539	-0.3653			18-2905-4041	03-5059-2153
19		Aug	20	16:30	8.263	0.6487	0.8806			12-0029-1251	17-4673-0915
20		Sep	17	15:15	8.409	0.7946	1.069			03-2107-6984	17-0384-7408
21		Oct	8	13:00	8.452	0.838	1.124			05-0416-8225	18-9477-6667

Analyst: QA:

Fathead Minnow 96-h Acute Survival Test — **Aqua-Science**

Batch ID:	00-3922-3306	**Test Type:**	Survival (96h)	**Analyst:**	C. Walker
Start Date:	08 Oct-14 13:00	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Laboratory Water
Ending Date:	12 Oct-14 13:40	**Species:**	Pimephales promelas	**Brine:**	Not Applicable
Duration:	4d 1h	**Source:**	Aquatox, AR	**Age:**	8d

Sample ID:	19-2100-9156	**Code:**	72804604	**Client:**	Internal Lab
Sample Date:	08 Oct-14	**Material:**	Sodium chloride	**Project:**	Reference Toxicant
Receive Date:		**Source:**	Reference Toxicant		
Sample Age:	13h	**Station:**			

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
19-8676-2947	96h Survival Rate	5	7.5	6.124	7.35%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	gm/L	95% LCL	95% UCL	TU	Method
18-9477-6667	96h Survival Rate	EC5	5.739	4.968	7.35		Linear Interpolation (ICPIN)
		EC10	6.568	4.861	10.3		
		EC15	7.5	4.665	7.991		
		EC20	7.63	7.11	8.098		
		EC25	7.762	7.267	8.208		
		EC40	8.17	7.755	8.542		
		EC50	8.452	8.095	8.772		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
18-9477-6667	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

96h Survival Rate Summary

C-gm/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water	2	1	1	1	1	1	0	0	0.0%	0.0%
1.25		2	1	1	1	1	1	0	0	0.0%	0.0%
2.5		2	1	1	1	1	1	0	0	0.0%	0.0%
5		2	1	1	1	1	1	0	0	0.0%	0.0%
7.5		2	0.85	0.2147	1	0.8	0.9	0.05	0.07071	8.32%	15.0%
10		2	0	0	0	0	0	0	0		100.0%

96h Survival Rate Detail

C-gm/L	Control Type	Rep 1	Rep 2
0	Lab Water	1	1
1.25		1	1
2.5		1	1
5		1	1
7.5		0.8	0.9
10		0	0

Analyst: CW QA: ⊢

Fathead Minnow 96-h Acute Survival Test — Aqua-Science

Analysis ID:	19-8676-2947	Endpoint:	96h Survival Rate	CETIS Version:	CETISv1.8.7
Analyzed:	13 Oct-14 14:40	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	7.35%	5	7.5	6.124	

Dunnett Multiple Comparison Test

Control	vs	C-gm/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Water		1.25	0	2.827	0.116	2	0.8333	CDF	Non-Significant Effect
		2.5	0	2.827	0.116	2	0.8333	CDF	Non-Significant Effect
		5	0	2.827	0.116	2	0.8333	CDF	Non-Significant Effect
		7.5*	5.711	2.827	0.116	2	0.0023	CDF	Significant Effect
		10*	30.59	2.827	0.116	2	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.513427	0.5026854	5	299.6	<0.0001	Significant Effect
Error	0.01006739	0.001677898	6			
Total	2.523494		11			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Distribution	Shapiro-Wilk W Normality	0.5994	0.8025	0.0001	Non-normal Distribution

96h Survival Rate Summary

C-gm/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Water	2	1	1	1	1	1	1	0	0.0%	0.0%
1.25		2	1	1	1	1	1	1	0	0.0%	0.0%
2.5		2	1	1	1	1	1	1	0	0.0%	0.0%
5		2	1	1	1	1	1	1	0	0.0%	0.0%
7.5		2	0.85	0.2147	1	0.85	0.8	0.9	0.05	8.32%	15.0%
10		2	0	0	0	0	0	0	0		100.0%

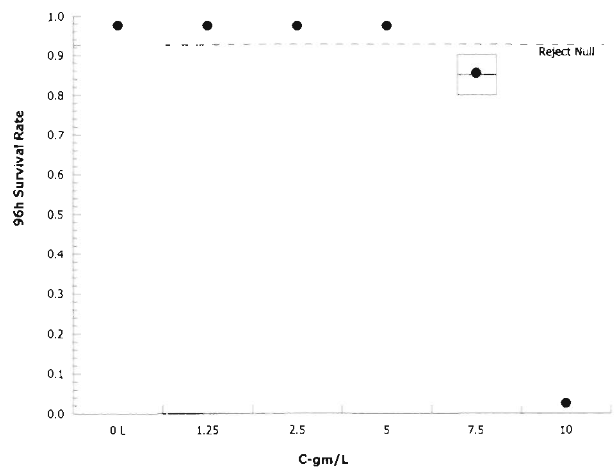
Angular (Corrected) Transformed Summary

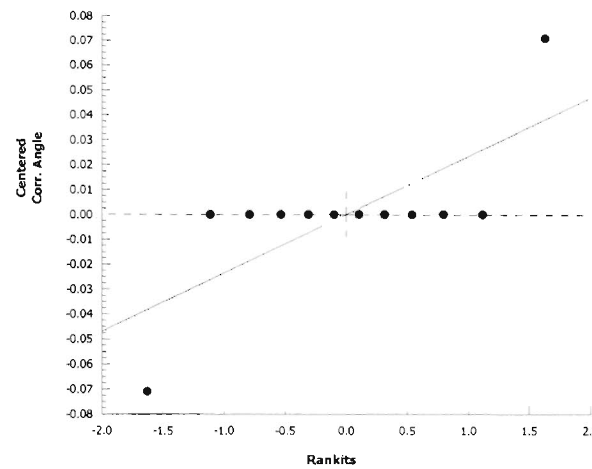
C-gm/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Water	2	1.412	1.409	1.415	1.412	1.412	1.412	0	0.0%	0.0%
1.25		2	1.412	1.409	1.415	1.412	1.412	1.412	0	0.0%	0.0%
2.5		2	1.412	1.409	1.415	1.412	1.412	1.412	0	0.0%	0.0%
5		2	1.412	1.409	1.415	1.412	1.412	1.412	0	0.0%	0.0%
7.5		2	1.178	0.2766	2.08	1.178	1.107	1.249	0.07095	8.52%	16.57%
10		2	0.1588	0.1587	0.1589	0.1588	0.1588	0.1588	0	0.0%	88.76%

Fathead Minnow 96-h Acute Survival Test | **Aqua-Science**

Analysis ID:	19-8676-2947	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Oct-14 14:40	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Report Date: 13 Oct-14 14:40 (p 1 of 1)
Test Code: r5931406 | 05-0416-8225

Fathead Minnow 96-h Acute Survival Test — **Aqua-Science**

Analysis ID:	18-9477-6667	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Oct-14 14:40	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	812801	200	Yes	Two-Point Interpolation

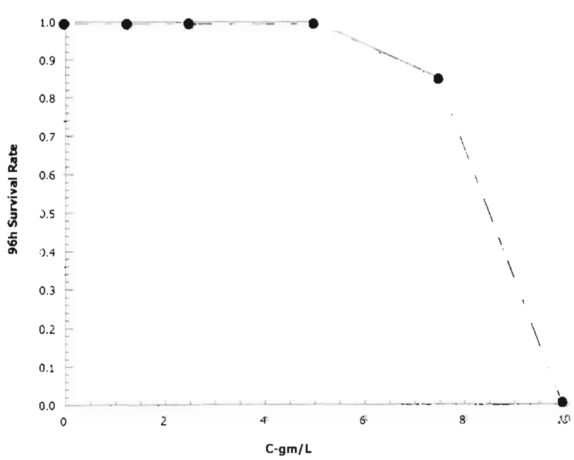
Point Estimates

Level	gm/L	95% LCL	95% UCL
EC5	5.739	4.968	7.35
EC10	6.568	4.861	10.3
EC15	7.5	4.665	7.991
EC20	7.63	7.11	8.098
EC25	7.762	7.267	8.208
EC40	8.17	7.755	8.542
EC50	8.452	8.095	8.772

96h Survival Rate Summary

			Calculated Variate(A/B)								
C-gm/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Water	2	1	1	1	0	0	0.0%	0.0%	20	20
1.25		2	1	1	1	0	0	0.0%	0.0%	20	20
2.5		2	1	1	1	0	0	0.0%	0.0%	20	20
5		2	1	1	1	0	0	0.0%	0.0%	20	20
7.5		2	0.85	0.8	0.9	0.05	0.07071	8.32%	15.0%	17	20
10		2	0	0	0	0	0		100.0%	0	20

Graphics



Analyst: QA:

DOSE-RESPONSE RELATIONSHIP REVIEW

Client: **Novato Sanitary District**

Test Material: **Reference Toxicant**

Species: ***P. promelas*** *Endpoint:* **Survival**

Test No.: **14-06** *Test Date:* **10/8/14**

Description of Dose-Response Relationship Type:

- [x] Type 1. *Ideal Dose-Response*. Shows a clear concentration-response relationship.
- [ ] Type 2. *All or Nothing Response*. Shows a transition from no significant effect at one effluent concentration to complete effect at the next concentration.
- [ ] Type 3. *Stimulatory Response at Low Concentration and Detrimental Effects at Higher Concentration*. Shows a stimulatory response - a non-monotonic concentration-response relationship characterized by an increase in response (stimulation at low concentrations) followed by a detrimental effect at higher concentrations.
- [ ] Type 4. *Stimulation at Low Concentration*. Shows a stimulatory response at low concentrations but no significant effect at higher concentrations. Requires data review (see comment section).
- [ ] Type 5. *Interrupted Concentration-Response*. Shows a significant effect bracketed by non-significant effects. Requires data review (see comment section).
- [ ] Type 6. *Interrupted Concentration-Response*. Shows non-significant effect bracketed by significant effects. Requires data review (see comment section).
- [ ] Type 7. *Significant Effects Only at Highest Concentration*. Response characterized by only the highest test concentration producing a significantly different response from control.
- [ ] Type 8. *Significant Effects at All Test Concentrations but Flat Concentration-Response Curve*. All of the test concentrations produce a response that is significantly different than the control response, but a clear concentration-response relationship cannot be determined. Requires data review (see comment section).
- [ ] Type 9. *Significant Effects at All Test Concentrations but Sloped Concentration-Response Curve*. Similar to Type 8 except a concentration-response can be identified at the higher concentrations. Requires data review (see comment section).
- [ ] Type 10. *Inverse Concentration-Response Relationship*. Response characterized by adverse effects decreasing with increasing concentrations (stimulation often encountered in algal growth tests due to nutrient stimulation).

Comments:

Evaluated by Kimberly K. Mull 10/20/14

AQUA-Science

Environmental Toxicology Specialists

MORTALITY AND BEHAVIOR OBSERVATIONS

Test Number:	Novato 14-06	Study Director:	J.L. Miller
Protocol No.:	EPA-821-R-02-012	Technicians:	Walker/McIntyre/Noss/Sanford/Constantinedes/Francis
Test Material:	Novato Effluent		
Test Species:	*Pimephales promelas*	Animal Lot No.:	AQTX: 093014
Initiation Date:	October 8, 2014	Termination Date:	October 12, 2014

Samples @ 100%	Vessel No.	Cumulative Mortality Day 1	Day 2	Day 3	Day 4
Control	A	Ø	Ø	Ø	Ø
	B	Ø	Ø	Ø	Ø
Novato Effluent	A	Ø	Ø	Ø	Ø
	B	Ø	Ø	Ø	Ø
Technician Initials		JN	UC	UC	KM
Observation Time		1210	1125	1130	1330
Observation Date		10/09/14	10/10/14	10/11/14	10/12/14

Comments

n=10 animals/rep

Test Initiation Info:

Time: 1250

Tech: JN

Test Termination Info:

Time: 1330

Tech: KM

Animals Fed:

Day 0	Day 1	Day 2	Day 3
JN	UC	UC	UC
0840	0830	0835	0830
10/08/14	10/09/14	10/10/14	10/11/14

AQUA-Science

Environmental Toxicology Specialists

MORTALITY AND BEHAVIOR OBSERVATIONS

Test Number: Novato 14-06 Study Director: J.L. Miller

Protocol No.: EPA-821-R-02-012 Technicians: Walker/McIntyre/Noss/Sanford/Constantinedes/Francis

Test Material: Reference Toxicant (NaCl) in Laboratory Dilution Water

Test Species: *Pimephales promelas* Animal Lot No.: AQTX: 093014

Initiation Date: October 8, 2014 Termination Date: October 12, 2014

Conc. (g/L)	Vessel No.	Cumulative Mortality Day 1	Day 2	Day 3	Day 4	Comments
Control	A	Ø	Ø	Ø	Ø	n=10 animals/rep
	B	Ø	Ø	Ø	Ø	
1.25	A	Ø	Ø	Ø	Ø	Test Initiation Info:
	B	Ø	Ø	Ø	Ø	Time: 1300
2.5	A	Ø	Ø	Ø	Ø	Tech: FF
	B	Ø	Ø	Ø	Ø	
5	A	Ø	Ø	Ø	Ø	Test Termination Info:
	B	Ø	Ø	Ø	Ø	Time: 1340
7.5	A	Ø	1	1	2	Tech: KW
	B	Ø	1	1	1	
10	A	10				
	B	10				
Technician Initials		FF	UC	UC	KW	
Observation Time		1220	1245	1200	1340	
Observation Date		10/09/14	10/10/14	10/11/14	10/12/14	

Animals Fed:

	Day 0	Day 2
Technician Initials	JN	UC
Observation Time	0840	0835
Observation Date	10/08/14	10/10/14

AQUA-Science
Environmental Toxicology Specialists

DOSE PREPARATION SHEET

10/08/14 **Novato 14-06**

96 Hr. Flow-Through Acute

Pimephales promelas

Test concentrations: **Lab Control, 100% Effluent**

Reference Toxicant concentrations: **0, 1.25, 2.5, 5, 7.5, and 10 g/L NaCl**

Effluent = 0.5L of a grab sample of **Novato Effluent**

Dilution water = 2x carbon filtered R/O well water with EPA salts to EPAMH specifications.

Sample Conc. (100%)	Amount Sample (mL)	Dilution Water (mL)	Total (mL)
Lab Control	0	500	500
Novato Effluent	500	0	500

REFERENCE TOXICANT: 10 g/L NaCl Std.

Sample Conc. (g/L)	Amount ref tox (mL)	Dilution Water (mL)	Total (mL)
0	0	1000	500
1.25	63	QS to 500 mL	500
2.5	125	QS to 500 mL	500
5	250	QS to 500 mL	500
7.5	375	QS to 500 mL	500
10	500	0	500

All surface waters filtered through 60 µm screen

R/T Dilution water = same as effluent dilution water.

2 replicates/concentration: 10 animals/replicate

Test Day	Day 0	Day 1*	Day 2	Day 3*
Technician	[illegible]	[illegible]	[illegible]	[illegible]
Time (effluent/RT)	1220/1010	1200	1125/0905	1115
Date	10/08/14	10/09/14	10/10/14	10/11/14

*Reference toxicant solutions made up on days 0 and 2 only.

AQUA-Science

Environmental Toxicology Specialists

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	Novato 14-06	Study Director:	J.L. Miller
Protocol No.:	EPA-821-R-02-012	Technicians:	Walker/McIntyre/Noss/Sanford/Constantinedes/Francis
Test Material:	Novato Effluent		
Test Species:	*Pimephales promelas*	Animal Lot No.:	AQTX: 093014
Initiation Date:	October 8, 2014	Termination Date:	October 12, 2014

Sample @ 100%	OBSERVATIONS Day (0) Date: 10/08/14 Temperature (°C)	Dissolved Oxygen*	pH^^	Alkalinity **	Conductivity ^	Water Hardness ~	24 Hour Obsv. Temp.	D.O.*	pH^^
Lab Control	25	8.1	8.16	58	303	81	24	8.2	8.02
Novato Effluent	25	8.0	7.56	135	727	131	24	7.3	7.64
							Final Flow Rate:		
							4.8 mL/min.		
						Tech Init/Date	JN 10/09/14		

UNIT INSTRUMENTATION LEGEND

*=Dissolved oxygen (mg/L): Meter ID 05

^^= pH: Meter ID 08

^=Conductivity/Salinity (µmohs): Meter ID 06

**Alkalinity (mg/L CaCO3); HACH Test Kit

~=Water Hardness (mg/L CaCO3); HACH Test Kit

ADDITIONAL COMMENTS:

Effluent Date: 10/07/14

Initial Flow Rate: 5.0 mL/min.

Lab Control = 2x carbon filtered reverse osmosis water at EPA moderately hard level using EPA salts.
Control Water ID = R/O EPAMH #155

All surface waters filtered through a 60 µm screen daily

Technician: [signature] Date: 10/08/14

AQUA-Science

Environmental Toxicology Specialists

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	Novato 14-06	Study Director:	J.L. Miller
Protocol No.:	EPA-821-R-02-012	Technicians:	Walker/McIntyre/Noss/Sanford/Constantinedes/Francis
Test Material:	Novato Effluent		
Test Species:	*Pimephales promelas*	Animal Lot No.:	AQTX: 093014
Initiation Date:	October 8, 2014	Termination Date:	October 12, 2014

Sample @ 100%	Temperature (°C)	Dissolved Oxygen*	pH^^	Alkalinity **	Conductivity ^	Water Hardness ~	24 Hour Obsv. Temp.	D.O.*	pH^^
	OBSERVATIONS Day (1) Date: 10/09/14								
Lab Control	25	8.3	8.04	58	298	81	24	7.7	8.11
Novato Effluent	25	8.4(101.0)	7.43	130	742	140	24	6.7	7.24
							Final Flow Rate:		
							4.7 mL/min.		
						Tech Init/Date	JM 10/10/14		

UNIT INSTRUMENTATION LEGEND

*=Dissolved oxygen (mg/L): Meter ID 05

^^= pH: Meter ID 08

^=Conductivity/Salinity (μmohs): Meter ID 06

**Alkalinity (mg/L CaCO3); HACH Test Kit

~=Water Hardness (mg/L CaCO3); HACH Test Kit

ADDITIONAL COMMENTS:

Effluent Date: 10/08/14

Initial Flow Rate: 5.0 mL/min.

Lab Control = 2x carbon filtered reverse osmosis water at EPA moderately hard level using EPA salts.
Control Water ID = R/O EPAMH # 155

All surface waters filtered through a 60 μm screen daily

Technician: [signature] Date: 10/09/14

AQUA-Science

Environmental Toxicology Specialists

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	Novato 14-06	Study Director:	J.L. Miller
Protocol No.:	EPA-821-R-02-012	Technicians:	Walker/McIntyre/Noss/Sanford/Constantinedes/Francis
Test Material:	Novato Effluent		
Test Species:	*Pimephales promelas*	Animal Lot No.:	AQTX: 093014
Initiation Date:	October 8, 2014	Termination Date:	October 12, 2014

	OBSERVATIONS Day (2) Date: 10/10/14						24 Hour Obsv.		
Sample @ 100%	Temperature (°C)	Dissolved Oxygen*	pH^^	Alkalinity **	Conductivity ^	Water Hardness ~	Temp.	D.O.*	pH^^
Lab Control	25	7.8	8.43	58	300	81	24	7.7	8.15
Novato Effluent	25	8.0	7.88	127	796	139	24	6.6	7.72
							Final Flow Rate: 4.8 mL/min.		
						Tech Init/Date	WE 10/11/14		

UNIT INSTRUMENTATION LEGEND

*=Dissolved oxygen (mg/L): Meter ID 05

^^= pH: Meter ID 08

^=Conductivity/Salinity (µmohs): Meter ID 06

**Alkalinity (mg/L CaCO3); HACH Test Kit

~=Water Hardness (mg/L CaCO3); HACH Test Kit

ADDITIONAL COMMENTS:

Effluent Date: 10/09/14

Initial Flow Rate: 5.0 mL/min.

Lab Control = 2x carbon filtered reverse osmosis water at EPA moderately hard level using EPA salts.
Control Water ID = R/O EPAMH #155

All surface waters filtered through a 60 µm screen daily

Technician: [signature] Date: 10/10/14

AQUA-Science

Environmental Toxicology Specialists

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	Novato 14-06	Study Director:	J.L. Miller
Protocol No.:	EPA-821-R-02-012	Technicians:	Walker/McIntyre/Noss/Sanford/Constantinedes/Francis
Test Material:	Novato Effluent		
Test Species:	*Pimephales promelas*	Animal Lot No.:	AQTX: 093014
Initiation Date:	October 8, 2014	Termination Date:	October 12, 2014

	OBSERVATIONS Day (3) Date:			10/11/14			24 Hour Obsv.		
Sample @ 100%	Temperature (°C)	Dissolved Oxygen*	pH^^	Alkalinity **	Conductivity ^	Water Hardness ~	Temp.	D.O.*	pH^^
Lab Control	24	8.0	8.47	58	295	81	24	7.7	7.79
Novato Effluent	25	7.8	7.80	116	794	142	24	6.3	7.37
							Final Flow Rate:		
							4.8 mL/min.		
						Tech Init/Date	KW 10/12/14		

UNIT INSTRUMENTATION LEGEND

*=Dissolved oxygen (mg/L): Meter ID 05

^^= pH: Meter ID 08

^=Conductivity/Salinity (μmohs): Meter ID 06

**Alkalinity (mg/L CaCO3); HACH Test Kit

~=Water Hardness (mg/L CaCO3); HACH Test Kit

ADDITIONAL COMMENTS:

Effluent Date: 10/10/14

Initial Flow Rate: 5.0 mL/min.

Lab Control = 2x carbon filtered reverse osmosis water at EPA moderately hard level using EPA salts.
Control Water ID = R/O EPAMH #155

All surface waters filtered through a 60 μm screen daily

Technician: [signature] Date: 10/11/14

AQUA-Science

Environmental Toxicology Specialists

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	Novato 14-06	Study Director:	J.L. Miller
Protocol No.:	EPA-821-R-02-012	Technicians:	Walker/McIntyre/Noss/Sanford/Constantinedes/Francis
Test Material:	Reference Toxicant (NaCl) in Laboratory Dilution Water		
Test Species:	*Pimephales promelas*	Animal Lot No.:	AQTX: 093014
Initiation Date:	October 8, 2014	Termination Date:	October 12, 2014

Conc. (g/L)	OBSERVATIONS Day (0) Date: 10/08/14						48 Hour Obsv.		
	Temperature (°C)	Dissolved Oxygen*	pH^^	Alkalinity **	Conductivity ^	Water Hardness ~	Temp.	D.O.*	pH^^
Lab Control	25	8.1	8.16	60	307	81	24	7.4	7.90
1.25	25	8.2	8.09	70①	2580	90①	24	7.2	7.82
2.5	25	8.3	8.03	-	4700	-	24	7.3	7.75
5	25	8.3	7.97	80①	8930	100①	24	6.9	7.69
7.5	25	8.3	7.91	-	12980	-	24	7.2	7.66
10	25	8.3	7.87	90①	16930	110①	24②	7.[illegible]②	7.55②
					Tech Init/Date		JLC	10/10/14	

UNIT INSTRUMENTATION LEGEND

*=Dissolved oxygen (mg/L): Meter ID 05

^^= pH: Meter ID 08

^=Conductivity/Salinity (µmohs): Meter ID 06

**Alkalinity (mg/L CaCO3); HACH Test Kit

~=Water Hardness (mg/L CaCO3); HACH Test Kit

ADDITIONAL COMMENTS: ① MEASUREMENTS TAKEN W/ 10mL SAMPLE VOLUME 10/08/14 CLC
② 24 hr water quality taken due to total mortality FF 10/09/14

Lab Control = 2x carbon filtered reverse osmosis water at EPA moderately hard level using EPA salts.
Control Water ID = R/O EPAMH #150

All surface waters filtered through a 60 µm screen daily

Technician: [signature] Date: 10/08/14

AQUA-Science

Environmental Toxicology Specialists

WATER QUALITY REPORT FOR AQUATIC BIOASSAYS

Test Number:	Novato 14-06	Study Director:	J.L. Miller
Protocol No.:	EPA-821-R-02-012	Technicians:	Walker/McIntyre/Noss/Sanford/Constantinedes/Francis
Test Material:	Reference Toxicant (NaCl) in Laboratory Dilution Water		
Test Species:	*Pimephales promelas*	Animal Lot No.:	AQTX: 093014
Initiation Date:	October 8, 2014	Termination Date:	October 12, 2014

	OBSERVATIONS Day (2) Date: 10/10/14						48 Hour Obsv.		
Conc. (g/L)	Temperature (°C)	Dissolved Oxygen*	pH^^	Alkalinity **	Conductivity ^	Water Hardness ~	Temp.	D.O.*	pH^^
Lab Control	25	7.8	7.59	58	295	81	24	7.4	7.72
1.25	25	7.9	7.69	60①	2520	90①	24	7.6	7.69
2.5	25	7.9	7.73	-	4660	-	24	7.4	7.69
5	25	8.0	7.69	70①	8830	100①	24	7.4	7.68
7.5	25	8.1	7.65	-	12870	-	24	7.8	7.68
10	—	—	—	—	—	—	—	—	—
					Tech Init/Date		KM 10/12/14		

UNIT INSTRUMENTATION LEGEND

*=Dissolved oxygen (mg/L): Meter ID 05

^^= pH: Meter ID 08

^=Conductivity/Salinity (µmohs): Meter ID 06

**Alkalinity (mg/L CaCO3); HACH Test Kit

~=Water Hardness (mg/L CaCO3); HACH Test Kit

ADDITIONAL COMMENTS: ① MEASUREMENTS TAKEN W/ 10mL SAMPLE VOLUME AC 10/10/14

Lab Control = 2x carbon filtered reverse osmosis water at EPA moderately hard level using EPA salts.
Control Water ID = R/O EPAMH #155

All surface waters filtered through a 60 µm screen daily

Technician: [signature] Date: 10/10/14

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Environmental Toxicology Specialists

NaCl Reference Toxicant Preparation

Date: 10/07/14

Pimephales Promelas
10 g/L NaCl Reference Toxicant
Novato 14-06

Measured 18.75 L R/O EPAMH Number: 150

To achieve 10 g/L NaCl: 10 g/L x 18.75 L = 187.5 g NaCl required.

Weighed:

171.09
10.24
2.26

183.59 g NaCl added to 18.75 L of R/O EPAMH # 150 to achieve 10 g/L NaCl

Salts weighed on Sartorius AC210S scale
Final Salinity Measurement: 10.0 ‰
Salinity monitored using Beckman Coulter pH:470 conductivity meter (EC ID: 06)

Technician: [signature] Date: 10/07/14

AQUATOX, INC.

416 TWIN POINTS ROAD
HOT SPRINGS, ARKANSAS 71913
501-520-0560

TEST ORGANISM HISTORY

DATE SHIPPED 10/16/14 CLIENT Aqua Science Ocala

Purchase Order #:

SPECIES: Pimephales promelas

Quantity Shipped: 200

Age: 7 days on 10/7/14

Brood Stock Source: Anderson Farms, AR

Culture Water: Groundwater

Hardness (Mg/l CaCO3): 160

Dissolved Oxygen (Mg/1): 8.1

Temperature (°C): 25.1°C

Feeding: Arte

Comments: log-in 10/07/14 by FF @ 1045
T = 23.1°C
DO = 19.8 mg/L (218%)

Shipped Via: Federal Express UPS Overnight Shuttle

Packaged By:

Bill Hall Printers 3171

14-06 Raw Data

Chain-of-Custody Forms

AQUA-SCIENCE CHAIN OF CUSTODY

AQUA-Science Environmental Toxicology Consultants 630 Cantrill Dr.; Davis, CA 95618 (530) 753-5456 aquasci@aol.com	Client: Novato Sanitary District		Contact Name: Ken Besnia	
	Address: 500 Davidson Street, Novato CA 94945			
	Billing Address: SAME		P.O. No.:	
	Phone No.: 415- 892-1694 ext. 105		Fax No.: ( ) 415-892-1694	

Project No.:	Project Name: Acute Toxicity Flow Through, 5th Edition: OCT 2014 + Chronic Toxicity OCT 2014	Sampler (Print Name): Jessica Bena (Sign Name): JBena

Date Sampled	Time Sampled	Matrix	Container Type/ Amt.	Preservative	Sample Description/ Site	Lab No.	Grab or Comp	Comments
10/7/2014	800	AQ	3 @ 2.5 Gallons	none	Novato Effluent: E-002		Comp	Temp. 2.9 ° C
								T: 4.1
								DO: 10.2
								PH: 8.45

Relinquished by:	Date/ Time:	Received by:	Date/ Time:
JBena	10-7-14 /0851	[illegible]	10/07/14 8:12
[illegible]	10/07/14 920	[illegible]	10/07/14 0920

AQUA-SCIENCE CHAIN OF CUSTODY

AQUA-Science	Client: **Novato Sanitary District**	Contact Name: **Ken Besnia**	
Environmental Toxicology Consultants	Address: 500 Davidson Street, Novato CA 94945		
630 Cantrill Dr.; Davis, CA 95618	Billing Address: SAME		P.O. No.:
(530) 753-5456 aquasci@aol.com	Phone No.: 415- 892-1694 ext. 105	Fax No.: ( ) 415-892-1694	

Project No.:	Project Name: Acute Toxicity Flow Through, 5th Edition: OCT 2014 + Chronic Toxicity OCT 2014	Sampler (Print Name): Jessica Bena (Sign Name): JBena

Date Sampled	Time Sampled	Matrix	Container Type/ Amt.	Preservative	Sample Description/ Site	Lab No.	Grab or Comp	Comments
10/8/2014	800	AQ	3 @ 2.5 Gallons	none	Novato Effluent: E-002		Comp	Temp. 2.8 °C
								TEMP: 8.8°C
								pH: 7.67
								DO: 9.0 mg/L

Relinquished by:	Date/ Time:	Received by:	Date/ Time:
JBena	10-8-14, 0816	[illegible signature]	8:16.
[illegible signature]	10-08-14 9:35	[illegible signature]	10/08/14 09:35

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AQUA-SCIENCE CHAIN OF CUSTODY

AQUA-Science	Client: Novato Sanitary District	Contact Name: Ken Besnia	
Environmental Toxicology Consultants	Address: 500 Davidson Street, Novato CA 94945		
630 Cantrill Dr.; Davis, CA 95618	Billing Address: SAME		P.O. No.:
(530) 753-5456 aquasci@aol.com	Phone No.: 415- 892-1694 ext. 105	Fax No.: ( ) 415-892-1694	

Project No.:	Project Name: Acute Toxicity Flow Through, 5th Edition: OCT 2014 + Chronic Toxicity OCT 2014	Sampler (Print Name): Jessica Bena (Sign Name): JBena

Date Sampled	Time Sampled	Matrix	Container Type/ Amt.	Preservative	Sample Description/ Site	Lab No.	Grab or Comp	Comments
10/9/2014	800	AQ	3 @ 2.5 Gallons	none	Novato Effluent: E-002		Comp	Temp. 3.3 °C
								T= 6.7
								DO 8.7 mg/L
								PH 7.30

Relinquished by:	Date/ Time:	Received by:	Date/ Time:
JBena	10-9-14 / 8:20	[illegible]	10/9/14 8:20
[illegible]	10/9/14 9:45	[illegible]	10/09/14 0945

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Date Sampled	Time Sampled	Matrix	Container Type/ Amt.	Preservative	Sample Description/ Site	Lab No.	Grab or Comp	Comments
10/10/2014	800	AQ	3 @ 2.5 Gallons	none	Novato Effluent: E-002		Comp	Temp. 2.6 °C
								TEMP: 5.0°C
								pH: 7.5
								DO: 8.4 mg/L

Relinquished by:	Date/ Time:	Received by:	Date/ Time:
JBena	101014	[illegible]	10/10/14. 8:16
[illegible] 10/10/14	9:30	[illegible]	10/10/14 0930

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