An Evaluation of the Acute Toxicity of the "Chevron Effluent at Cawelo" Wastewater Sample

Sample collected February 5, 2008

Prepared For:

Precision Analytical
321 19th Street
Bakersfield, CA 93301

Prepared By:

Pacific EcoRisk
2250 Cordelia Road
Fairfield, CA 94534

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PACIFIC ECORISK
ENVIRONMENTAL CONSULTING & TESTING

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Table of Contents

Page

1. INTRODUCTION 1
2. ACUTE TOXICITY TEST PROCEDURES 1
   2.1 Receipt and Handling of the Effluent Sample 1
   2.2 Acute Toxicity Testing with Fathead Minnows 1
3. RESULTS 3
   3.1 Acute Effects of the 'Chevron Effluent at Cawelo' Sample on Fathead Minnows 3
4. SUMMARY AND CONCLUSIONS 3
   4.1 QA/QC Summary 3

Appendices

Appendix A Chain-of-Custody Record for the Collection and Delivery of the Effluent Sample

Appendix B Test Data and Summary of Statistics for the Evaluation of the Acute Toxicity of the "Chevron Effluent at Cawelo" Sample to Fathead Minnows

1. INTRODUCTION

In compliance with their NPDES biomonitoring requirements, Precision Analytical has contracted Pacific EcoRisk (PER) to perform an acute toxicity evaluation of an effluent sample. This acute toxicity evaluation consisted of performing the US EPA 96-hr acute toxicity test with fathead minnows (*Pimephales promelas*).

This acute toxicity testing was conducted on the Chevron Effluent at Cawelo sample (identified as "T001A 2/5/08" on the Chain of Custody form) that was collected on February 5, 2008. This report describes the performance and results of this test.

2. ACUTE TOXICITY TEST PROCEDURES

The methods used in conducting this test followed the guidelines established by the EPA manual "Methods for Estimating the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fourth Edition" (EPA/600/4-90/027F).

2.1 Receipt and Handling of the Effluent Samples

On February 5, Precision Analytical staff collected a sample of effluent into an appropriately-cleaned container. This sample was transported that same day, under chain-of-custody, to the PER testing laboratory in Fairfield. Upon receipt at the testing laboratory, aliquots of the sample were collected for analysis of initial water quality characteristics (Table 1), with the remainder of the sample being stored at 4°C except when being used to prepare test solutions. The chain-of-custody record for the collection and delivery of the effluent sample is provided in Appendix A.

Table 1. Initial water quality characteristics of the effluent samples.

Date Sample Received	Sample ID	Temp (°C)	pH	D.O (mg/L)	Alkalinity (mg/L)	Hardness (mg/L)	Conductivity (μS/cm)	Total Ammonia (mg/L N)
2/6/08	T001A 2/5/08	25.7	6.61	6.5	215	91	830	<1.0

2.2 Acute Toxicity Testing with Fathead Minnows

The fathead minnows used in this test were obtained from a commercial supplier (Aquatic Biosystems, Fort Collins, CO). These fish were maintained at 20°C in aerated aquaria containing EPA synthetic moderately-hard water prior to their use in these tests. During this pre-test period, the fish were fed brine shrimp nauplii *ad libitum*.

The Lab Control water for this test consisted of EPA synthetic "moderately-hard" water, prepared by addition of reagent grade chemicals to reverse-osmosis, de-ionized water. The effluent sample was tested at the 100% concentration only. "New" water quality characteristics (pH, dissolved oxygen [D.O.], and conductivity) were determined for the Lab Control and 100% effluent treatment test solutions prior to the start of the test.

There were 2 replicates for each test treatment, each replicate consisting of 400 mL of test solution in a 600-mL glass beaker. The test was initiated by randomly allocating ten 12-day old fathead minnows into each replicate beaker. The beakers were placed in a temperature-controlled room at 20°C under a 16L:8D photoperiod.

Each replicate container was examined daily, and the number of live fish in each was recorded at that time. Routine water quality characteristics (pH, D.O., and conductivity) of the treatment waters were measured and recorded for one randomly-selected replicate per treatment each day.

On Day 2 of the 4-day tests, the test organisms were fed brine shrimp nauplii, and fresh test solutions were prepared and characterized as before. Then, after ~48 hrs exposure, the number of live fish in each replicate was determined after which approximately 80% of the test media in each beaker was carefully poured out and replaced with fresh test solution. "Old" water quality characteristics (pH, D.O., and conductivity) were measured on the discarded test solution from one randomly-selected replicate beaker at each treatment.

After 96 (±2) hrs, the test was terminated and the number of live fish in each replicate beaker was determined. The resulting survival data were analyzed to evaluate any reductions caused by the effluent sample; all statistical analyses were performed using the CETIS® statistical software (Version 1.1.2revL, TidePool Scientific, McKinleyville, CA).

3. RESULTS

3.1 Acute Effects of the "Chevron Effluent at Cawelo" Sample on Fathead Minnows

The results of this test are summarized in Table 2. There was 100% survival in the Lab Control treatment; there was complete mortality in the "Chevron Effluent at Cawelo" sample, which was significantly less than the Lab Control, indicating that this effluent was acutely toxicity to fathead minnows.

The test data and summary of statistics for this test are presented in Appendix B.

Table 2. Acute effects of the "Chevron Effluent at Cawelo" sample on fathead minnows.

Test Treatment	Mean % Survival
Lab Control	100
100% Chevron Effluent at Cawelo	**0***

* Significantly less than the Lab Control treatment response ($p < 0.05$).

4. SUMMARY AND CONCLUSIONS

The results of this test indicated that the "Chevron Effluent at Cawelo" sample was acutely toxic to fathead minnows.

4.1 QA/QC Summary

Test Conditions - Test conditions (pH, D.O., temperature, etc.) were all within acceptable limits for these tests. All analyses were performed according to laboratory Standard Operating Procedures.

Negative Control - The biological responses for the test organisms at the Lab Control treatment were within acceptable limits.

Appendix A

Chain-of-Custody Record for the Collection and Delivery of the Effluent Sample

Precision Analytical

Chain of Custody Form

Page _____ of _____

Client Information	Client Instructions	Lab No:
Company: PRESICION ANALYTICAL		
Address: 321 19TH ST		
City: BAKERSFIELD State: CA Zip: 93301		
Report To: STEVE HARRIS Tel: 661-330-7537 Fax: 661-323-1684 Email:		
Report To: Tel: Fax: Email:		

Report Delivery ("x" where applicable)						Project ID:
Fax		Email		US Post		

Log-In/Project Billing Information (lab use only)	Matrix Codes: (column below)
Log-In Date:	AQ = Aqueous
Logged-In By:	W = Water S = Solid
Field Sampling: hours miles	G = Gas M = Multi-phasic
Precision Analytical Quote No:	A = Air O = Oil
Client P.O. No:	F = Filter U = Unknown

Temperature Readings:	°C	°F
Cooler (Inside)		
Bacteriological Container		
VOA Vial		
Other:		

Preserved: Y = Yes N = No

Container: P = Plastic G = Glass

Priority Codes: (column below)	
IM = Immediate	24 = 24 hour
48 = 48 hour	N = 10 day

Sample No.	Sample Description	Matrix Code	Date Sampled	Time Sampled	Analysis Requested: 96 HR STATIC-RENEWAL	Analysis Requested: ACUTE TEST (48 HR)	Analysis Requested: WITH FAT HEAD MINN.	Analysis Requested: (100%)	Priority Code	Sampled By	Preserved (Y/N)	Container (P/G)
1	TOOLA 2/5/08		2/5/08	4:30	X					DAVID HARRIS	N	P
2	VWOOLA 2/5/08		2/5/08	4:30	X					DAVID HARRIS	N	P
3	BOOLA 2/5/08		2/5/08	4:30	X					ROB VICKERY	N	P

1. Relinquished By:	Date: 2/5/08	Time: 4:35	Received By:	Date: 2/5/08	Time: 1640
2. Relinquished By:	Date: 2/5/08	Time: 4:55	Received By:	Date: 2/5/08	Time: 1640
3. Relinquished By:	Date:	Time:	Received By:	Date: 2-6-08	Time: 1005

Precision Analytical, 321 19th Street, Bakersfield, CA, 93301-4905, Tel: 661 323 1682, Fax 661 323 1684 or 661 323 1682, Email: sharris@bak.rr.com

Appendix B

Test Data and Summary of Statistics for the Evaluation of the Acute Toxicity of the "Chevron Effluent at Cawelo" Sample to Fathead Minnows

CETIS Test Summary

Report Date: 13 Feb-08 2:25 PM
Test Link: 11-7775-0340/27290

Acute Fish Survival Test — **Pacific EcoRisk**

Test No:	10-3385-0560	**Test Type:**	Survival (96h)	**Duration:**	94h
Start Date:	06 Feb-08 05:12 PM	**Protocol:**	EPA/600/4-90/027F (1991)	**Species:**	Pimephales promelas
Ending Date:	10 Feb-08 03:40 PM	**Dil Water:**	Not Applicable	**Source:**	Aquatic Biosystems, CO
Setup Date:	06 Feb-08 05:12 PM	**Brine:**	Not Applicable		

Sample No:	09-4202-9586	**Code:**	12967	**Client:**	Precision Analytical
Sample Date:	05 Feb-08 04:30 PM	**Material:**	Effluent	**Project:**	NPDES
Receive Date:	06 Feb-08 10:05 AM	**Source:**	Precision Analytical		
Sample Age:	25h (25.7 °C)	**Station:**	TOO1A		

Comparison Summary

Analysis	Endpoint	NOEL	LOEL	ChV	PMSD	Method
13-5332-3889	96h Proportion Survived	<100	100	N/A	N/A	Fisher Exact

96h Proportion Survived Summary

Conc-%	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Lab Water	2	1.00000	1.00000	1.00000	0.00000	0.00000	0.00%
100		2	0.00000	0.00000	0.00000	0.00000	0.00000	0.00%

96h Proportion Survived Detail

Conc-%	Control Type	Rep 1	Rep 2
0	Lab Water	1.00000	1.00000
100		0.00000	0.00000

CETIS Analysis Detail

Acute Fish Survival Test — **Pacific EcoRisk**

Endpoint	Analysis Type	Sample Link	Control Link	Date Analyzed	Version
96h Proportion Survived	Comparison	11-7775-0340	11-7775-0340	13 Feb-08 2:25 PM	CETISv1.1.2

Method	Alt H	Data Transform	Zeta	NOEL	LOEL	Toxic Units	ChV	PMSD
Fisher Exact	C > T	Untransformed		<100	100		N/A	

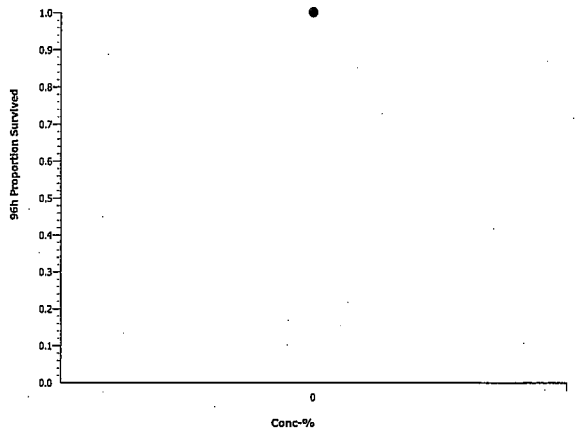
Group Comparisons

Control	vs	Conc-%	Statistic	P-Value	Decision(0.05)
Lab Water		100	0.00000	0.00000	Significant Effect

Data Summary

Conc-%	Control Type	Non-Responders	Responders	Total Observed
0	Lab Water	20	0	20
100		0	20	20

Graphics



96 Hour Acute Fathead Minnow Toxicity Test

Client: **Precision Analytical** Organism Log #: 3709 Age: 12 days

Test Material: FOOLA DE T001A Organism Supplier: ABS

Test ID#: 27290 Project #: 12967 Control: EPAMH

Test Date: 2-6-08 Control Water Batch: 1079

Feeding T_0 Time: 1140 Initials: AB Feeding T46-hr Time: 8130 Initials: CB

Treatment	Temp (°C)	pH new	pH old	D.O. (mg/L) new	D.O. (mg/L) old	Conductivity (µS/cm) new	Conductivity (µS/cm) old	# Live Organisms Rep A	# Live Organisms Rep B	SIGN-OFF
Control	19.2	8.94		9.2		330		10	10	Date: 2-6-08 Sample ID: T001C 18937 Test Solution Prep: DC
100%	19.2	7.78		9.3		813		10	10	New WQ: HTA Initiation Time: 1712 Initiation Signoff: AB
Meter ID	10A	PH11		DO12		EC03				
Control	19.6		8.08		8.3		286	10	10	Date: 2/7/08 Count Time: 9:47 Count Signoff: KO
100%	19.6		7.63		8.3		818	8	10	Old WQ: YC
Meter ID	10A		PH12		DO14		EC04			
Control	19.4	7.99	8.39	8.6	8.5	298	302	10	10	Date: 2/8/08 Sample ID: 18937 Test Solution Prep: CB
100%	19.4	7.30	8.21	8.8	8.3	817	835	6	7	New WQ: YC Renewal Time: 11:35 Renewal Signoff: KO
Meter ID	10A	PH03	PH03	DO10	DO10	EC01	EC01			Old WQ: HTA
Control	19.3		8.34		8.6		353	10	10	Date: 2/9/08 Count Time: 9:15 Count Signoff: KO
100%	19.3		7.65		8.3		834	0	0	Old WQ: KZ
Meter ID	10A		PH11		DO14		EC04			
Control	19.1		8.26		8.3		336	10	10	Date: 2-10-08 Termination Time: 1540 Termination Signoff: DC
100%	—		—		—		—	—	—	Old WQ: HM
Meter ID	10A		PH11		DO14		EC01			