

NPDES Compliance Chronic Toxicity Testing of Chevron U.S.A. and Cawelo Water District Effluent

Sample collected December 1, 2008

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Prepared For:

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MONITORING REPORT REVIEW

Engineer	
Compliance	Yes
Date Reviewed	no

December 2008



PACIFIC ECORISK
ENVIRONMENTAL CONSULTING & TESTING

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1. INTRODUCTION

Precision Analytical has contracted Pacific EcoRisk (PER) to perform NPDES compliance toxicity testing evaluations of the acute and chronic toxicity of a Chevron U.S.A. and Cawelo Water District (Chevron/Cawelo) effluent sample. These evaluations consist of performing the following US EPA freshwater acute and chronic toxicity tests:

- 96-hour acute survival test with fathead minnows;
- 96-hour algal growth test with the green alga *Selenastrum capricornutum*;
- 3-brood (6-8-day) survival and reproduction test with the crustacean *Ceriodaphnia dubia*; and
- 7-day survival and growth test with larval fathead minnows (*Pimephales promelas*).

This suite of freshwater acute and chronic toxicity tests was conducted on an effluent sample collected on December 1, 2008. In order to assess the sensitivity of the test organisms to chronic toxic stress, reference toxicant tests were also performed. This report describes the performance and results of these effluent and reference toxicant tests.

2. TOXICITY TEST PROCEDURES

The methods used in conducting these tests followed the guidelines established by the following EPA manuals "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition" (EPA-821-R-02-012). "Short-Term Methods for Estimating the Chronic Effects of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition" (EPA-821-R-02-013).

2.1 Sample Receipt and Handling

On December 1, Precision Analytical staff collected a sample of effluent into an appropriately cleaned sample container. This sample was shipped via overnight delivery, on ice and under chain-of-custody, to the PER 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 this sample are provided in Appendix A.

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

Sample Receipt Date	Sample ID	Temp (°C)	pH	D.O. (mg/L)	Alkalinity (mg/L)	Hardness (mg/L)	Conductivity (µS/cm)	Total Ammonia (mg/L N)
12/2/08	EFF-003	0.7	7.63	9.1	216	83	773	<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 this test. 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% effluent concentration only. Water quality characteristics (pH, dissolved oxygen [D.O.], and conductivity) were determined for each test treatment test solution prior to use in this test.

There were two 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 10 fathead minnows into each replicate beaker. The beakers were placed in a temperature-controlled room at 25°C under a 16L:8D photoperiod.

Each day, each replicate container was examined, and the number of live fish in each was recorded. 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 test, fresh test solutions were prepared and characterized as before, and the fish were fed brine shrimp nauplii. Approximately 2 hrs after feeding, the number of live fish in each replicate was determined and then approximately 80% of the test media in each beaker was carefully poured out and replaced with fresh test solution, after which the "old" water quality characteristics (pH, D.O., and conductivity) were measured on the old test solution that had been discarded from one randomly-selected 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 impairment due to the wastewater; all statistical analyses were performed using the CETIS[®] statistical software (Tide Pool Scientific, McKinleyville, CA).

2.3 Algal Growth Toxicity Testing with *Selenastrum capricornutum*

The chronic algal toxicity test consists of a 96-hr bioassay in which the green alga *Selenastrum capricornutum* is exposed to effluent and the effects on cellular reproduction determined. The specific procedures used in this test are described below.

The Lab Water Control for this test consisted of reverse osmosis, de-ionized (RO/DI) water. The effluent sample was tested at the 100% concentration only. Aliquots of the Lab Water Control

water and the effluent sample were filtered (using sterile 0.45 μm filters) and then spiked with nutrients before use in the algal test, as per EPA guidelines. Routine water quality characteristics (pH, D.O., and conductivity) were measured on these test solutions prior to their use in the test.

There were 4 replicates for each test treatment, each consisting of a 250-mL glass Erlenmeyer flask containing 100 mL of test solution. Each flask was inoculated to an initial cell density of 10,000 cells/mL of *Selenastrum* from an ongoing PER laboratory culture that is maintained in log growth phase. These flasks were loosely capped and randomly positioned within a temperature-controlled room at 25°C, under continuous cool-white fluorescent illumination. Each replicate flask was shaken a minimum of 3 times daily. The temperature and pH were determined daily for the designated "water quality" replicate at each treatment.

After 96 (± 2) hrs exposure, the algal cell density in each replicate flask was determined by spectrophotometric analysis. The resulting cell density data were analyzed to evaluate any impairment of algal growth caused by the effluent; all statistical analyses were performed using the CETIS[®] statistical software.

2.3.1 Reference Toxicant Testing of the *Selenastrum capricornutum*

In order to assess the sensitivity of the *Selenastrum* to toxic stress, a reference toxicant test was performed. The reference toxicant test was performed similarly to the effluent test except that test solutions consisted of "Lab Control" media spiked with NaCl at concentrations of 0.5, 1, 2, 4, and 8 gm/L. The resulting test response data were statistically analyzed to determine key dose-response point estimates (e.g., IC₅₀); all statistical analyses were made using the CETIS[®] software. These response endpoints were then compared to the typical response range established by the mean ± 2 SD of the point estimates generated by the most recent previous reference toxicant tests performed by this lab.

2.4 Survival and Reproduction Toxicity Testing with *Ceriodaphnia dubia*

The short-term chronic *Ceriodaphnia* test consists of exposing individual females to effluent for the length of time it takes for the Lab Control treatment females to produce 3 broods (typically 6-8 days), after which effects on survival and reproduction are evaluated. The specific procedures used in this test are described below.

The Lab Water Control treatment for this test consisted of a mixture of commercial spring waters (80% Arrowhead:20% Evian). The effluent sample was tested at the 100% concentration only. Aliquots of the Lab Water Control water and the effluent sample were used to prepare daily test solutions; for each treatment, 200 mL of test solution was amended with the alga *Selenastrum capricornutum* and Yeast-Cerophyll-Trout Food (YCT) to provide food for the test organisms. "New" water quality characteristics (pH, D.O., and conductivity) were measured on these food-amended test solutions prior to use in this test. Each day of the test, fresh test solutions and a "new" set of replicate cups were prepared and characterized, as before.

There were 10 replicates for each test treatment, each replicate consisting of 15 mL of test solution in a 30-mL plastic cup. This "3-brood" test was initiated by allocating one neonate (<24 hrs old) *Ceriodaphnia*, obtained from ongoing laboratory cultures, into each replicate. The replicate cups were placed into a temperature-controlled room at 25°C, under cool-white fluorescent lighting on a 16L:8D photoperiod.

Each test replicate cup was examined daily, with surviving "original" individual organisms being transferred to the corresponding new cup containing fresh test solution. The contents of each remaining "old" replicate cup were carefully examined, and the number of neonate offspring produced by each original organism was determined, after which "old" water quality characteristics (pH, D.O., and conductivity) were measured for the "old" media from one randomly-selected replicate at each treatment.

After it was determined that $\geq 60\%$ of the *Ceriodaphnia* in the Lab Water Control treatment had produced their third brood of offspring, the test was terminated. The resulting survival and reproduction (number of offspring) data were analyzed to evaluate any impairment(s) caused by the effluent; all statistical analyses were performed using the CETIS[®] statistical software.

2.4.1 Reference Toxicant Testing of the *Ceriodaphnia dubia*

In order to assess the sensitivity of the *Ceriodaphnia* test organisms to toxic stress, a reference toxicant test was performed. The reference toxicant test was performed similarly to the effluent test except that test solutions consisted of "Lab Control" media spiked with NaCl at test concentrations of 250, 500, 1000, 1500 and 2000 mg/L. The resulting test response data were statistically analyzed to determine key dose-response point estimates (e.g., EC₅₀); all statistical analyses were made using the CETIS[®] software. These response endpoints were then compared to the typical response range established by the mean ± 2 SD of the point estimates generated by the most recent previous reference toxicant tests performed by this lab.

2.5 Survival and Growth Toxicity Testing with Larval Fathead Minnows

The chronic fathead minnow test consists of exposing larval fish to effluent for 7 days, after which effects on survival and growth are evaluated. The specific procedures used in this test are described below.

The Lab Water Control treatment for this test consisted of US EPA synthetic moderately-hard water. The effluent sample was tested at the 100% concentration only. "New" water quality characteristics (pH, D.O., and conductivity) were measured on these test solutions prior to use in the test. Each day of the test, fresh test solutions were prepared and characterized as before.

There were 4 replicates at each test treatment, each replicate consisting of 400 mL of test media in a 600-mL glass beaker. This test was initiated by randomly allocating 10 larval fathead

minnows (<48 hrs old) into each replicate. The replicate beakers were placed in a temperature-controlled room at 25°C, under cool-white fluorescent lighting on a 16L:8D photoperiod. The test fish were fed brine shrimp nauplii twice daily.

Each replicate was examined daily, with any dead animals, uneaten food, wastes, and other detritus being removed. The number of live fish in each replicate was determined and then 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 old test water that had been discarded from one randomly-selected replicate at each treatment.

After 7 days exposure, the number of live fish in each replicate beaker was recorded. The fish from each replicate were then carefully euthanized in methanol, rinsed in de-ionized water, and transferred to a pre-dried and pre-tared weighing pan. These fish were then dried at 100°C for >24 hrs and re-weighed to determine the total weight of fish in each replicate; the total weight was then divided by the initial number of fish per replicate (n=10) to determine the "biomass value". The resulting survival and growth ("biomass value") data were analyzed to evaluate any impairment(s) caused by the effluent; all statistical analyses were performed using the CETIS® statistical software.

2.5.1 Reference Toxicant Testing of the Larval Fathead Minnows

In order to assess the sensitivity of the fish to toxic stress, a reference toxicant test was performed. The reference toxicant test was performed similarly to the effluent test, except that test solutions consisted of "Lab Control" media spiked with NaCl at test concentrations of 0.75, 1.5, 3, 6, and 9 gm/L. The resulting test response data were analyzed to determine key dose-response point estimates (e.g., EC50); all statistical analyses were made using the CETIS® software. These response endpoints were then compared to the 'typical response' range established by the mean \pm 2 SD of the point estimates generated by the 20 most recent previous reference toxicant tests performed by this lab.

3. RESULTS

3.1 Acute Effects of the Effluent on Fathead Minnows

The results of this test are summarized in Table 2. There was 100% survival in the Lab Water Control treatment; there was 65% survival in the effluent treatment, which was not significantly less than the Lab Water Control, indicating that there was no acute toxicity to fathead minnows present in the effluent sample.

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

Test Treatment	Mean % Survival
Lab Water Control	100
100% Effluent	65

3.2 Chronic Effects of the Effluent on *Selenastrum capricornutum*

The results of this test are summarized below in Table 3. There was a mean final algal cell density of 2,580,000 cells/mL at the Lab Water Control treatment. There were no significant reductions in algal cell density in the effluent; the NOEC was 100% effluent, resulting in 1.0 TUc (where TUc = 100/NOEC).

The test data and summary of statistical analyses for this test are presented in Appendix C.

Effluent Treatment	Mean Cell Density (cells/mL x 10 ⁶)
Lab Water Control	2.58
100% Effluent	3.69
NOEC =	100% effluent
TUc (where TUc = 100/NOEC) =	1.0

3.2.1 Reference Toxicant Toxicity to *Selenastrum capricornutum*

The results of this test are summarized below in Table 4. There was a mean of 2,490,000 cells/mL in the Lab Control treatment. The IC₅₀ was 1.7 gm/L NaCl.

These reference toxicant test results are consistent with previous *Selenastrum* reference toxicant tests performed in this laboratory, indicating that these organisms were responding to toxic stress in a typical fashion.

The test data and summary of statistical analyses for this test are presented in Appendix D.

NaCl Treatment (gm/L)	Mean Algal Density (cells/mL x 10 ⁶)
Lab Control	2.49
0.5	2.28
1	1.91*
2	1.00*
4	0.16*
8	0*
Summary of Statistics	
IC ₅₀ = 1.7 gm/L NaCl	

* Significantly less than the Lab Control treatment response at p < 0.05.

3.3 Chronic Effects of the Effluent on *Ceriodaphnia dubia*

The results for this test are summarized below in Table 5. There was 100% survival and a mean of 25.7 offspring per female at the Lab Water Control treatment. There were no significant reductions in survival in the effluent; the survival NOEC was 100% effluent, resulting in 1.0 TUc (where $TUc = 100/NOEC$). There was a significant reduction in reproduction in the effluent; the reproduction NOEC was <100% effluent, resulting in >1.0 TUc.

The test data and summary of statistical analyses for this test are presented in Appendix E.

Effluent Treatment	% Survival	Reproduction (# neonates/female)
Lab Water Control	100	25.7
100% Effluent	100	6.5*
NOEC =	100% effluent	<100% effluent
TUc (where $TUc = 100/NOEC$) =	1.0	>1.0

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

3.3.1 Reference Toxicant Toxicity to *Ceriodaphnia dubia*

Results of this test are summarized below in Table 6. There was 100% survival and a mean of 24.9 neonates per female at the Lab Control treatment. The survival EC₅₀ was 1610 mg/L NaCl, and the reproduction IC₅₀ was 953 mg/L NaCl.

The reference toxicant test survival results were consistent with the reference toxicant test database, indicating that these test organisms were responding to toxic stress in a typical fashion.

The test data and the summary of statistical analyses for this test are presented in Appendix F.

Treatment (mg/L NaCl)	% Survival	Reproduction (# neonates/female)
Lab Control	100	24.9
250	100	26.8
500	90	19.0*
1000	90	12.3*
1500	80	1.9*
2000	0.1*	0*
Summary of Key Statistics		
Survival EC ₅₀ or Reproduction IC ₅₀ =	1610 mg/L NaCl	953 mg/L NaCl

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

3.4 Chronic Effects of the Effluent on Fathead Minnows

The results of this test are summarized below in Table 7. There was 100% survival and a mean 'biomass value' of 0.64 mg at the Lab Water Control treatment. There were significant reductions in survival in the effluent; the NOEC was <100% effluent, resulting in >1.0 TUC (where TUC = 100/NOEC).

The test data and the summary of statistical analyses for this test are presented in Appendix G.

Effluent Treatment	Mean % Survival	Mean Fish Biomass Value (mg)
Lab Water Control	100	0.64
100% Effluent	7.5*	0.02
NOEC =	<100% effluent	<100% effluent
TUC (where TUC = 100/NOEC) =	>1.0	>1.0

* Significantly less than the Lab Water Control treatment response at $p < 0.05$.

3.4.1 Reference Toxicant Toxicity to Fathead Minnows

The results of this test are summarized below in Table 8. There was 95% survival and a mean biomass value of 0.79 mg at the Lab Control treatment. The survival EC₅₀ was 3.84 gm/L NaCl and the growth IC₅₀ was 2.77 gm/L NaCl.

These reference toxicant test results are consistent with previous fathead minnow reference toxicant tests performed in this laboratory, indicating that these organisms were responding to toxic stress in a typical fashion.

The test data and summary of statistical analyses for this test are presented in Appendix H.

NaCl Treatment (gm/L)	% Survival	Mean Fish Biomass Value (mg)
Lab Control	95	0.79
0.75	100	0.72
1.5	92.5	0.63*
3	72.5*	0.35
6	12.5*	0.04
9	0*	0
Summary of Statistics		
Survival EC ₅₀ or Growth IC ₅₀ =	3.8 gm/L NaCl	2.8 gm/L NaCl

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

4. SUMMARY AND CONCLUSIONS

Acute Effects of the Chevron/Cawelo Effluent on Fathead Minnows

There was 65% survival, indicating that the effluent was acutely toxic to fathead minnows.

Chronic Effects of Chevron/Cawelo Effluent on *Selenastrum capricornutum*

There were no significant reductions in algal growth in the effluent sample; the NOEC was 100% effluent, resulting in 1.0 chronic Toxic Units (TUc).

Chronic Effects of Chevron/Cawelo Effluent on *Ceriodaphnia dubia*

There were significant reductions in reproduction in the effluent sample. The NOEC was <100% effluent resulting in >1.0 TUc (where TUc = 100/NOEC).

Chronic Effects of Chevron/Cawelo Effluent on Fathead Minnows

There were significant reductions in survival in the effluent sample. The NOEC was <100% effluent resulting in >1.0 TUc (where TUc = 100/NOEC).

4.1 QA/QC Summary

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

Negative Lab Control – The biological responses in the Lab Water Control treatments for these tests were within acceptable limits.

Positive Control – The results of the concurrent reference toxicant tests were consistent with the previous reference toxicant tests performed for these species in our lab, indicating that the test organisms used in the current tests were responding to toxic stress in a typical and consistent fashion.

Concentration Response Relationships – There were valid concentration-response relationships for the reference toxicant tests, which were determined to be acceptable for this testing.

Appendix A

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

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Appendix B

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

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CETIS Summary Report

Report Date: 13 Dec-08 12:25 (p 1 of 1)
 Test Code: 15-7930-4300/31124

Acute Fish Survival Test										Pacific EcoRisk	
Test Run No: 11-1850-1013	Test Type: Survival (96h)			Analyst: John Jirasritumrong							
Start Date: 02 Dec-08 15:00	Protocol: EPA/600/4-90/027F (1991)			Diluent: Not Applicable							
Ending Date: 06 Dec-08 13:00	Species: Pimephales promelas			Brine: Not Applicable							
Duration: 94h	Source: Aquatic Biosystems, CO			Age: 1							
Sample No: 11-7191-8560	Code: Eff			Client: Precision Analytical							
Sample Date: 01 Dec-08 14:10	Material: Effluent			Project: 14208							
Receive Date: 02 Dec-08 11:00	Source: Precision Analytical										
Sample Age: 25h (0.7 °C)	Station: EFF-003										
Comparison Summary											
Analysis No	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
12-8754-9863	96h Survival Rate	100	>100	N/A	34.6%	1	Equal Variance t Two-Sample Test				
96h Survival Rate Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	2	1	1	1	1	1	0	0	0.0%	0.0%
100		2	0.65	0.571	0.729	0.5	0.8	0.0387	0.212	32.6%	35.0%
96h Survival Rate Detail											
Conc-%	Control Type	Rep 1	Rep 2								
0	Control	1	1								
100		0.8	0.5								

CETIS Analytical Report

Report Date: 13 Dec-08 12:25 (p 1 of 1)
 Test Code: 15-7930-4300/31124

Acute Fish Survival Test			Pacific EcoRisk		
Analysis No: 12-8754-9863	Endpoint: 96h Survival Rate	CETIS Version: CETISv1.6.5			
Analyzed: 13 Dec-08 12:25	Analysis: Parametric-Two Sample	Official Results: Yes			

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)		C > T	Not Run	100	>100	N/A	1	34.6%

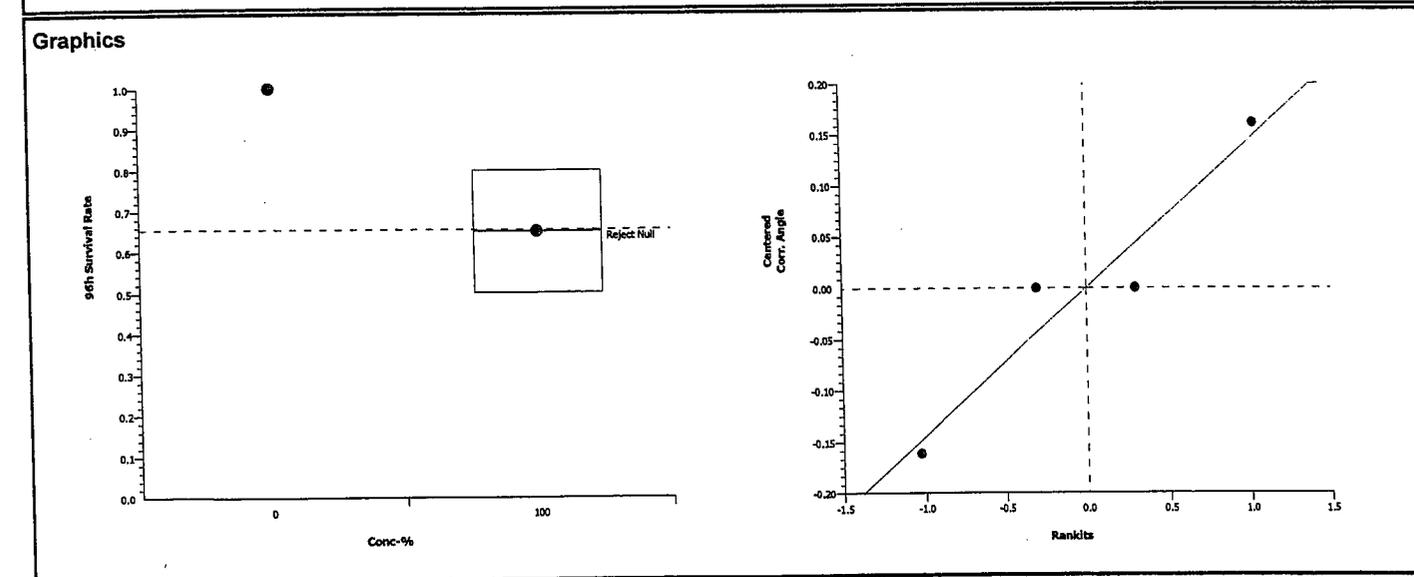
Equal Variance t Two-Sample Test							
Control	vs	Conc-%	Test Stat	Critical	MSD	P-Value	Decision(5%)
Control		100	2.9	2.92	0.47	0.0507	Non-Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Between	0.2169162	0.2169162	1	8.38	0.1010	Non-Significant Effect
Error	0.05176171	0.02588085	2			
Total	0.26867794245482	0.24279708787799	3			

ANOVA Assumptions						
Attribute	Test	Test Stat	Critical	P-Value	Decision(1%)	
Variances	Mod Levene Equality of Variance	65500	98.5	0.0000	Unequal Variances	
Distribution	Shapiro-Wilk Normality	0.945		0.6830	Normal Distribution	

96h Survival Rate Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	2	1	1	1	1	1	0	0	0.0%	0.0%
100		2	0.65	0.569	0.731	0.5	0.8	0.0394	0.212	32.6%	35.0%

Angular (Corrected) Transformed Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	2	1.41	1.41	1.41	1.41	1.41	0	0	0.0%	0.0%
100		2	0.946	0.86	1.03	0.785	1.11	0.0422	0.228	24.0%	33.0%



96 Hour Acute Fathead Minnow Toxicity Test

Client: Precision Analytical

Organism Log #: 4281 Age: < 24 hrs

Test Material: Effluent

Organism Supplier: ABS

Test ID#: 31124 Project #: 14208

Control/Diluent: EPAMH

Test Date: 12/2/07 Randomization: 4.2.7

Control Water Batch: 1149

Feeding To Time: 1400 Initials: JL

Treatment	Temp (°C)	pH		D.O. (mg/L)		Conductivity (µS/cm)		# Live Organisms		SIGN-OFF
		new	old	new	old	new	old	Rep A	Rep B	
Control	25.7	8.30		8.2		283		10	10	Date: 12/2/08 Sample ID: 20977 Test Solution Prep: JLR New WQ: D6V Initiation Time: 1500 Initiation Signoff: JL
100%	25.7	7.83		9.1		791		10	10	
Meter ID	11A	PH11		DO14		EC01				
Control	25.8	-	7.76	-	6.4	-		10	10	Date: 12/3/08 Sample ID: 20977 Test Solution Prep: JLR New WQ: - Renewal Time: 1020 Renewal Signoff: JLR Old WQ: SL
100%	25.5	-	8.51	-	7.3	-		10	10	
Meter ID	11A	-	PH03	-	DO10	-				
Control	25.6	8.09	8.23	8.4	7.8	282		10	10	Date: 12/4/08 Sample ID: 20977 Test Solution Prep: JLR New WQ: SL Renewal Time: 1115 Renewal Signoff: JLR Old WQ: DAP
100%	25.6	7.79	8.40	9.6	7.3	794		10	10	
Meter ID	11A	PH12	PH11	DO14	DO10	EC05				
Control	25.6	8.16	7.86	7.9	6.6	275		10	10	Date: 12/5/08 Sample ID: 20977 Test Solution Prep: JLR New WQ: DAP Renewal Time: 1300 Renewal Signoff: EKR Old WQ: DAP
100%	25.6	7.90	8.44	9.2	6.5	783		10	8	
Meter ID	11A	PH03	PH12	DO10	DO14	EC04				
Control	25.5		7.48		7.0		314	10	10	Date: 12/6/08 Termination Time: 1300 Termination Signoff: JLR Old WQ: 8M
100%	25.5		8.00		6.9		788	8	5	
Meter ID	11A		PH03		DO12		EC01			

Appendix C

Test Data and Summary of Statistics for the Evaluation of the Chronic Toxicity of Chevron/Cawelo Effluent to *Selenastrum capricornutum*



CETIS Summary Report

Report Date: 13 Dec-08 11:57 (p 1 of 1)
 Test Code: 14-6504-1382/31120

Algal Growth Test Pacific EcoRisk

Test Run No: 18-5958-7318	Test Type: Cell Growth	Analyst: John Jirasritumrong
Start Date: 02 Dec-08 14:30	Protocol: EPA/600/4-91/002 (1994)	Diluent: Not Applicable
Ending Date: 06 Dec-08 09:35	Species: Selenastrum capricornutum	Brine: Not Applicable
Duration: 91h	Source: In-House Culture	Age: 6

Sample No: 11-7191-8560	Code: Eff	Client: Precision Analytical
Sample Date: 01 Dec-08 14:10	Material: Effluent	Project: 14208
Receive Date: 02 Dec-08 11:00	Source: Precision Analytical	
Sample Age: 24h (0.7 °C)	Station: EFF-003	

Comparison Summary							
Analysis No	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
10-4080-7230	Cell Density	100	>100	N/A	14.0%	1	Equal Variance t Two-Sample Test

Cell Density Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	4	2.58E+6	2.45E+6	2.72E+6	2.34E+6	3.11E+6	6.56E+4	3.59E+5	13.9%	0.0%
100		4	3.69E+6	3.65E+6	3.72E+6	3.55E+6	3.76E+6	1.71E+4	9.36E+4	2.54%	-42.8%

Cell Density Detail						
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	
0	Control	2.34E+6	3.11E+6	2.37E+6	2.51E+6	
100		3.72E+6	3.72E+6	3.55E+6	3.76E+6	

CETIS Analytical Report

Report Date: 13 Dec-08 11:57 (p 1 of 1)
 Test Code: 14-6504-1382/31120

Algal Growth Test Pacific EcoRisk

Analysis No: 10-4080-7230 Endpoint: Cell Density CETIS Version: CETISv1.6.5
 Analyzed: 13 Dec-08 11:56 Analysis: Parametric-Two Sample Official Results: Yes

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Untransformed		C > T	Not Run	100	>100	N/A	1	14.0%

Equal Variance t Two-Sample Test

Control	vs	Conc-%	Test Stat	Critical	MSD	P-Value	Decision(5%)
Control		100	-5.95	1.94	361000	0.9990	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Between	2.44205E+12	2.44205E+12	1	35.4	0.0010	Significant Effect
Error	4.1375E+11	68958330000	6			
Total	2.8557998817E+12	2.5110082109E+12	7			

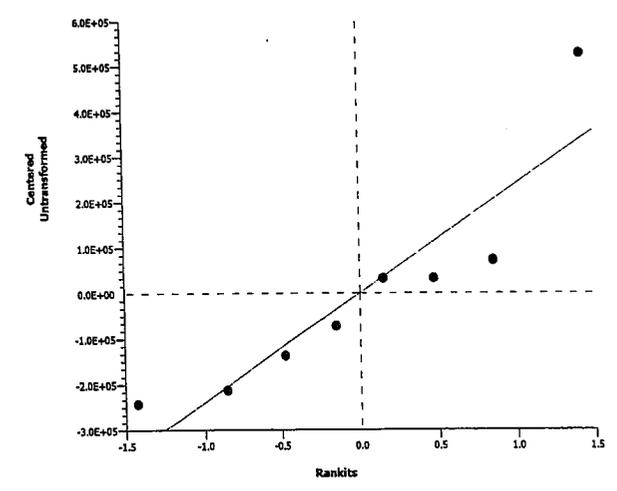
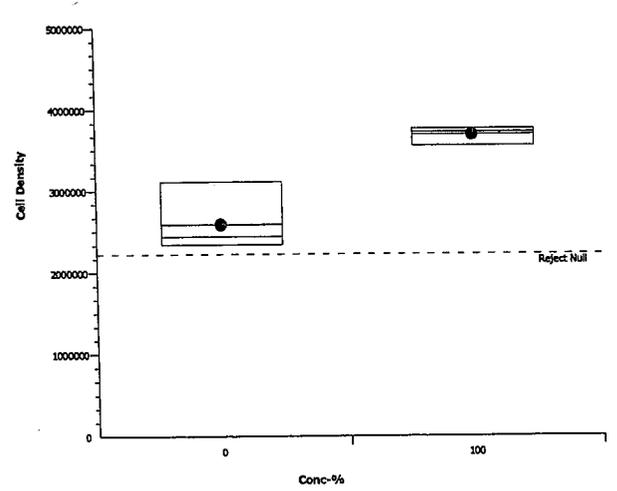
ANOVA Assumptions

Attribute	Test	Test Stat	Critical	P-Value	Decision(1%)
Variances	Variance Ratio F	14.7	47.5	0.0533	Equal Variances
Distribution	Shapiro-Wilk Normality	0.846		0.0874	Normal Distribution

Cell Density Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	4	2.58E+6	2.45E+6	2.72E+6	2.34E+6	3.11E+6	6.67E+4	3.59E+5	13.9%	0.0%
100		4	3.69E+6	3.65E+6	3.72E+6	3.55E+6	3.76E+6	1.74E+4	9.36E+4	2.54%	-42.8%

Graphics



Selenastrum capricornutum Algal Toxicity Test Data Sheet

Client: Precision Analytical Material: Effluent
 Test Start Date: 12/2/08 Test ID #: 31120 Project #: 14208
 Test End Date: 12/6/08 Control/Diluent: Algal Medium Location: R451
 Batch #: R451

Test Treatment	Temp (°C)	pH	D.O. (mg/L)	Conductivity (µS/cm)	Sign-Off
Control	25.3	7.59	8.4	115	Date: 12/2/08
100%	25.3	7.74	9.3	870	Sample ID #: 20977
					Test Solution Prep: <u>SN</u>
					New WQ: <u>D60</u>
					Inoculation Time: <u>1430</u>
Meter ID	33	PH4	D014	ECO1	Inoculation Signoff: <u>SN</u>
Control	25.0	7.55			Date: 12/3/08
100%	25.0	8.39			WQ Time: 1215
Meter ID	33	PH12			WQ Signoff: <u>NW</u>
Control	25.0	8.15			Date: 12/4/08
100%	25.0	8.65			WQ Time: 0903
Meter ID	33	PH11			WQ Signoff: <u>NZ</u>
Control	25.0	9.92			Date: 12/5/08
100%	25.0	9.31			WQ Time: 1005
Meter ID	33	PH03			WQ Signoff: <u>SN</u>
Control	25.1	10.04	12.2	119	Date: 12/6/08
100%	25.1	9.63	18.8	792	WQ Time: 0935
Meter ID	33	PH11	D010	ECO1	WQ Signoff: <u>SN</u>

Initial Count: 10,000 cells/mL Termination Time: 1300 Enumerating Scientist: SN

Treatment	Cell Density (cells/mL x 10 ⁶)				Mean Cell Density (cells/mL x 10 ⁶)
	Rep A	Rep B	Rep C	Rep D	
Control	2.34	3.11	2.37	2.51	2.58
100%	3.72	3.72	3.55	3.76	3.69
This datasheet has been reviewed for completeness and consistency with Test Acceptability Criteria and/or other issues of concern.					
Control Mean Density (cells/mL x 10 ⁶)		% CV	Date:	Time:	Signoff:
2.58		13.9	12/6/08	1605	160

Initial Test Conditions	Alkalinity	Hardness	Light Intensity (ftc)
	221	96	387

Appendix D

Test Data and Summary of Statistics for the Reference Toxicant Evaluation of the *Selenastrum capricornutum*

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CETIS Summary Report

Report Date: 07 Dec-08 15:56 (p 1 of 1)
 Test Code: 02-2084-1959/31076

Algal Growth Test				Pacific EcoRisk
Test Run No: 21-4695-2013	Test Type: Cell Growth	Analyst: Rivian Villanueva		
Start Date: 02 Dec-08 14:30	Protocol: EPA/821/R-02-013 (2002)	Diluent: Laboratory Water		
Ending Date: 06 Dec-08 13:30	Species: Selenastrum capricornutum	Brine: Not Applicable		
Duration: 95h	Source: In-House Culture	Age: 6		
Sample No: 11-6973-5217	Code: NaCl	Client: Reference Toxicant		
Sample Date: 02 Dec-08 14:30	Material: Sodium chloride	Project: 14178		
Receive Date: 02 Dec-08 14:30	Source: Reference Toxicant			
Sample Age: N/A (25.3 °C)	Station: In House			

Comparison Summary

Analysis No	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
13-6240-2904	Cell Density	0.5	1	0.707	8.74%		Steel Many-One Rank Test

Point Estimate Summary

Analysis No	Endpoint	Level	Conc-mg/	95% LCL	95% UCL	TU	Method
14-7036-3443	Cell Density	IC2.5	0.145	0.0374	0.81		Linear Interpolation (ICPIN)
		IC5	0.29	0.0747	0.824		
		IC10	0.546	0.169	0.921		
		IC15	0.716	0.316	1.08		
		IC20	0.885	0.556	1.2		
		IC25	1.04	0.76	1.3		
		IC40	1.45	1.24	1.64		
		IC50	1.73	1.58	1.87		

Cell Density Summary

Conc-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	4	2.49E+6	2.42E+6	2.56E+6	2.22E+6	2.65E+6	3.43E+4	1.88E+5	7.54%	0.0%
0.5		4	2.28E+6	2.21E+6	2.34E+6	2.08E+6	2.45E+6	3.11E+4	1.70E+5	7.48%	8.63%
1		4	1.91E+6	1.86E+6	1.96E+6	1.70E+6	1.98E+6	2.53E+4	1.38E+5	7.26%	23.4%
2		4	9.96E+5	9.79E+5	1.01E+6	9.34E+5	1.03E+6	7.87E+3	4.31E+4	4.33%	60.0%
4		4	1.57E+5	1.54E+5	1.60E+5	1.47E+5	1.65E+5	1.45E+3	7.93E+3	5.06%	93.7%
8		4	0.00E+0		100.0%						

Cell Density Detail

Conc-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Control	2.52E+6	2.22E+6	2.65E+6	2.57E+6
0.5		2.19E+6	2.08E+6	2.45E+6	2.38E+6
1		1.98E+6	1.98E+6	1.70E+6	1.97E+6
2		9.98E+5	1.03E+6	1.02E+6	9.34E+5
4		1.47E+5	1.61E+5	1.65E+5	1.54E+5
8		0.00E+0	0.00E+0	0.00E+0	0.00E+0

Selenastrum capricornutum Algal Toxicity Test Water Quality Data

Client: Reference Toxicant Test ID #: 31076 Shelf Zone #: R451
 Test Material: NaCl Project #: 14178 Control/Diluent: Algal Medium

Reference Toxicant Test Treatment (g/L NaCl)	Temp (°C)	pH	D.O. (mg/L)	Conductivity (µS/cm)	Sign-Off
Lab Water Control	25.3	7.59	8.4	100	Date: 12/2/08
0.5	25.3	7.82	8.4	1134	Test Solution Prep: <i>sn</i>
1	25.3	7.72	8.4	2109	New WQ: <i>DGW</i>
2	25.3	7.66	8.4	3930	Innoculation Time: 1430
4	25.3	7.89	8.4	7530	Innoculation Signoff: <i>sn</i>
8	25.3	7.54	8.4	14510	
Meter ID:	33	pH4	DO14	EC01	
Lab Water Control	25.0	7.53			Date: 12/3/08
0.5	25.0	7.47			WQ Time: 1210
1	25.0	7.43			WQ Signoff: <i>NW</i>
2	25.0	7.37			
4	25.0	7.29			
8	25.0	7.21			
Meter ID:	33	pH12			
Lab Water Control	25.0	8.30			Date: 12/4/08
0.5	25.0	8.43			WQ Time: 0900
1	25.0	8.34			WQ Signoff: <i>AR</i>
2	25.0	8.13			
4	25.0	7.94			
8	25.0	7.73			
Meter ID:	33	pH11			
Lab Water Control	25.0	9.55			Date: 12/5/08
0.5	25.0	9.63			WQ Time: 1000
1	25.0	9.45			WQ Signoff: <i>DAP</i>
2	25.0	8.50			
4	25.0	8.39			
8	25.0	7.42			
Meter ID:	33	pH03			
Lab Water Control	25.0	9.92	11.4	112	Date: 12/6/08
0.5	25.0	9.97	11.5	1140	Termination Time: 1330
1	25.0	9.76	10.9	2098	Termination Signoff: <i>sn</i>
2	25.0	9.51	10.0	3900	WQ Time: 0940
4	25.0	8.94	8.8	7430	WQ Signoff: <i>SM</i>
8	25.0	8.09	7.6	14370	
Meter ID:	33	pH11	DO10	EC01	

Initial Test Conditions			
Target: 16g NaCl in 2 L	Alkalinity	Hardness	Light Intensity (ftc)
Actual: 16.02	11	14	387

***Selenastrum capricornutum* Cell Density Enumeration Data**

Client: Reference Toxicant Initial Count: 10,000 cells/mL
 Test Material: NaCl Enumerating Scientist: SW
 Test Start Date: 12/2/08 Start Time: 1430 Test ID #: 31076
 Test End Date: 12/4/08 End Time: 1330 Project #: 14178

Treatment	Rep A	Rep B	Rep C	Rep D	Mean
Lab Water Control (w/EDTA)	2.52	2.22	2.65	2.57	2.49
0.5	2.19	2.08	2.45	2.38	2.28
1	1.98	1.98	1.70	1.97	1.91
2	0.998	1.03	1.02	0.934	0.996
4	0.147	0.161	0.165	0.154	0.157
8	0.000	0.000	0.000	0.000	0.000
This datasheet has been reviewed for completeness and consistency with Test Acceptability Criteria and/or other issues of concern.	Control Mean Density (cells/mL x 10 ⁶)	% CV	Date:	Time:	Signoff:
	2.49	7.5	12/6/08	1605	KO

Appendix E

Test Data and Summary of Statistics for the Evaluation of the Chronic Toxicity of Chevron/Cawelo Effluent to *Ceriodaphnia dubia*



CETIS Summary Report

Report Date: 13 Dec-08 12:20 (p 1 of 1)
 Test Code: 02-0380-2363/31121

Ceriodaphnia Survival and Reproduction Test							Pacific EcoRisk				
Test Run No: 01-8687-8107	Test Type: Reproduction-Survival (7d)	Analyst: John Jirasritumrong									
Start Date: 02 Dec-08 15:00	Protocol: EPA/821/R-02-013 (2002)	Diluent: Not Applicable									
Ending Date: 09 Dec-08 11:40	Species: Ceriodaphnia dubia	Brine: Not Applicable									
Duration: 6d 21h	Source: In-House Culture	Age: 1									
Sample No: 11-7191-8560	Code: Eff	Client: Precision Analytical									
Sample Date: 01 Dec-08 14:10	Material: Effluent	Project: 14208									
Receive Date: 02 Dec-08 11:00	Source: Precision Analytical										
Sample Age: 25h (0.7 °C)	Station: EFF-003										
Comparison Summary											
Analysis No	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
05-7884-6967	7d Survival Rate	100	>100	N/A	N/A	1	Fisher Exact Test				
06-6416-7365	Reproduction	<100	100	N/A	11.5%	>1	Equal Variance t Two-Sample Test				
Point Estimate Summary											
Analysis No	Endpoint	Level	Conc-%	95% LCL	95% UCL	TU	Method				
12-3949-1682	Reproduction	IC2.5	3.35	2.95	3.84	29.9	Linear Interpolation (ICPIN)				
		IC5	6.69	5.9	7.69	14.9					
		IC10	13.4	11.8	15.4	7.47					
		IC15	20.1	17.7	23.1	4.98					
		IC20	26.8	23.6	30.7	3.74					
		IC25	33.5	29.5	38.4	2.99					
		IC40	53.5	47.2	61.5	1.87					
IC50	66.9	59	76.9	1.49							
7d Survival Rate Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	10	1	1	1	1	1	0	0	0.0%	0.0%
100		10	1	1	1	1	1	0	0	0.0%	0.0%
Reproduction Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	10	25.7	24.6	26.8	22	31	0.531	2.91	11.3%	0.0%
100		10	6.5	4.8	8.2	0	14	0.831	4.55	70.0%	74.7%
7d Survival Rate Detail											
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Control	1	1	1	1	1	1	1	1	1	1
100		1	1	1	1	1	1	1	1	1	1
Reproduction Detail											
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Control	27	24	24	25	23	22	24	29	31	28
100		5	10	0	7	14	13	4	3	3	6

CETIS Analytical Report

Report Date: 13 Dec-08 12:20 (p 1 of 1)
 Test Code: 02-0380-2363/31121

Ceriodaphnia Survival and Reproduction Test Pacific EcoRisk

Analysis No: 06-6416-7365 Endpoint: Reproduction CETIS Version: CETISv1.6.5
 Analyzed: 13 Dec-08 12:19 Analysis: Parametric-Two Sample Official Results: Yes

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Untransformed		C > T	Not Run	<100	100	N/A	>1	11.5%

Equal Variance t Two-Sample Test

Control	vs	Conc-%	Test Stat	Critical	MSD	P-Value	Decision(5%)
Control		100*	11.2	1.73	2.96	0.0000	Significant Effect

ANOVA Table

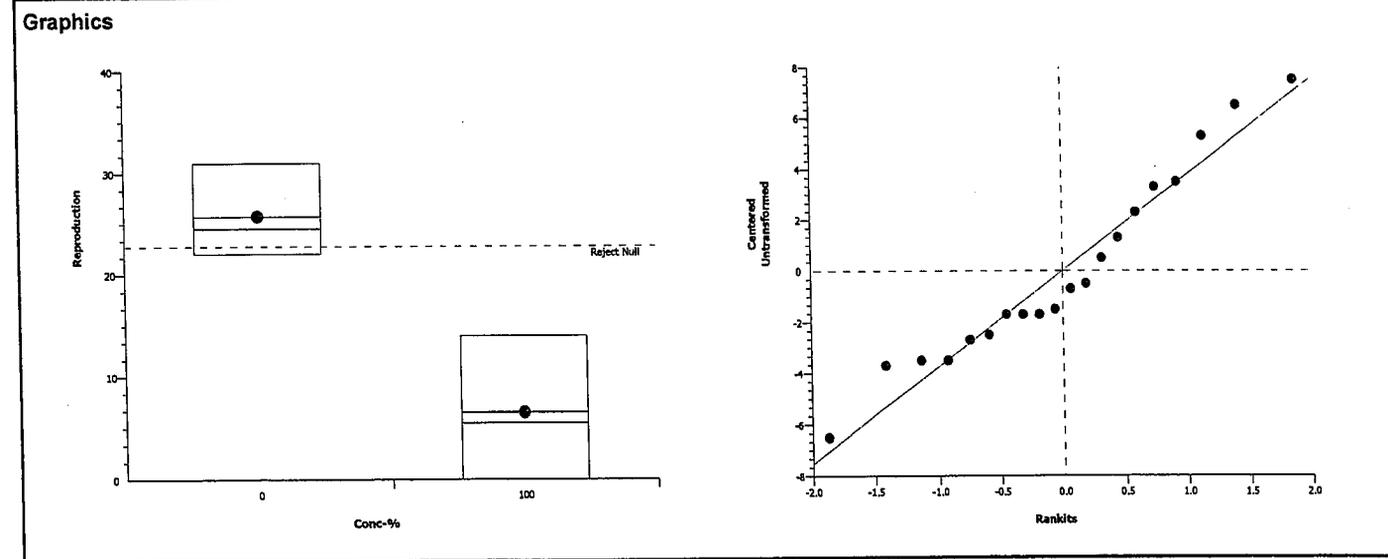
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Between	1843.2	1843.2	1	126	0.0000	Significant Effect
Error	262.6	14.58889	18			
Total	2105.79995727539	1857.78884029388	19			

ANOVA Assumptions

Attribute	Test	Test Stat	Critical	P-Value	Decision(1%)
Variances	Variance Ratio F	2.45	6.54	0.1980	Equal Variances
Distribution	Shapiro-Wilk Normality	0.95		0.3640	Normal Distribution

Reproduction Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	10	25.7	24.6	26.8	22	31	0.54	2.91	11.3%	0.0%
100		10	6.5	4.77	8.23	0	14	0.845	4.55	70.0%	74.7%



CETIS Analytical Report

Report Date: 13 Dec-08 12:20 (p 1 of 1)
 Test Code: 02-0380-2363/31121

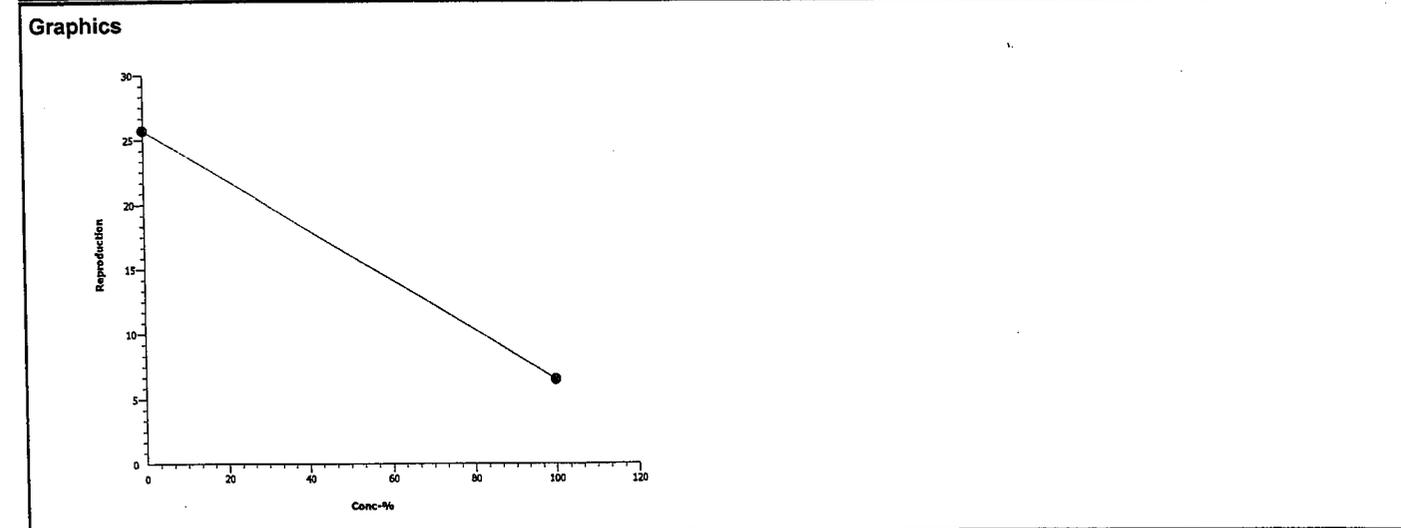
Ceriodaphnia Survival and Reproduction Test			Pacific EcoRisk
Analysis No: 12-3949-1682	Endpoint: Reproduction	CETIS Version: CETISv1.6.5	
Analyzed: 13 Dec-08 12:19	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes	

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	453528	280	Yes	Two-Point Interpolation

Point Estimates						
Level	Conc-%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC2.5	3.35	2.95	3.84	29.9	26	33.9
IC5	6.69	5.9	7.69	14.9	13	17
IC10	13.4	11.8	15.4	7.47	6.51	8.48
IC15	20.1	17.7	23.1	4.98	4.34	5.65
IC20	26.8	23.6	30.7	3.74	3.25	4.24
IC25	33.5	29.5	38.4	2.99	2.6	3.39
IC40	53.5	47.2	61.5	1.87	1.63	2.12
IC50	66.9	59	76.9	1.49	1.3	1.7

Reproduction Summary			Calculated Variate						
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	10	25.7	22	31	0.531	2.91	11.3%	0.0%
100		10	6.5	0	14	0.831	4.55	70.0%	74.7%

Reproduction Detail											
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Control	27	24	24	25	23	22	24	29	31	28
100		5	10	0	7	14	13	4	3	3	6



CETIS Analytical Report

Report Date: 13 Dec-08 12:20 (p 1 of 1)
 Test Code: 02-0380-2363/31121

Ceriodaphnia Survival and Reproduction Test Pacific EcoRisk

Analysis No: 05-7884-6967 Endpoint: 7d Survival Rate CETIS Version: CETISv1.6.5
 Analyzed: 13 Dec-08 12:19 Analysis: Single 2x2 Contingency Table Official Results: Yes

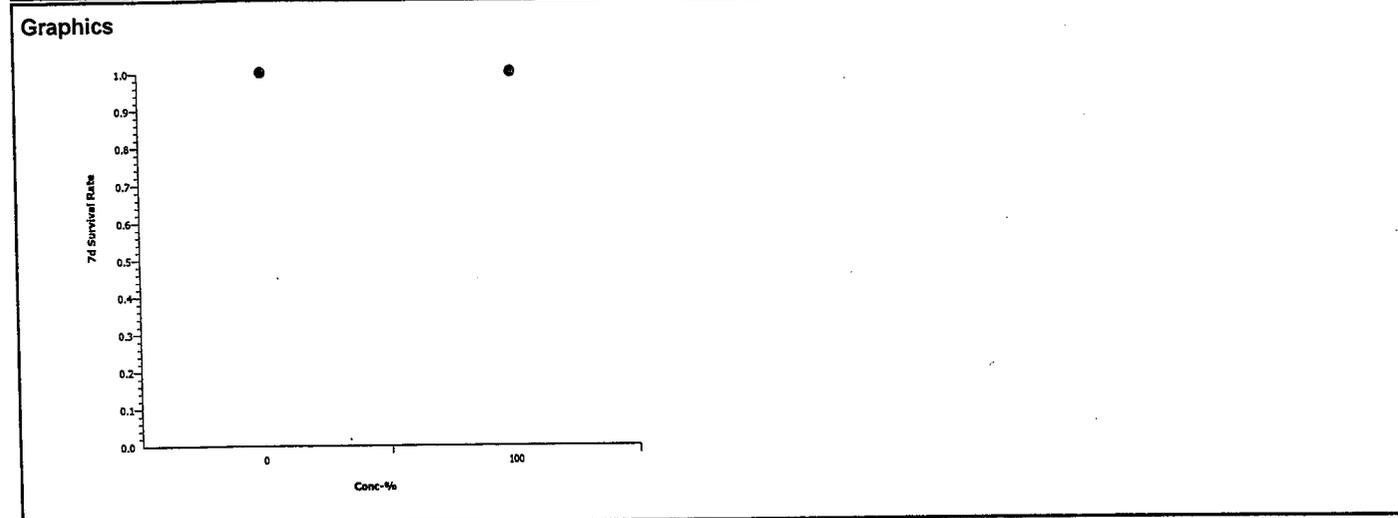
Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Untransformed		C > T	Not Run	100	>100	N/A	1	N/A

Fisher Exact Test

Control	vs	Conc-%	Test Stat	P-Value	Decision(0.05)
Control		100	1	1	Non-Significant Effect

Data Summary

Conc-%	Control Type	No-Resp	Resp	Total
0	Control	10	0	10
100		10	0	10



Short-Term Chronic 3-Brood *Ceriodaphnia dubia* Survival & Reproduction Test Data

Client: Precision Analytical Material: Effluent Test Date: 12/2/08
 Project #: 14208 Test ID #: 31121 Control Water: Lab Water (80:20)

Day	pH		D.O.		Temp (°C)	Survival / Reproduction										SIGN-OFF	
	New	Old	New	Old		A	B	C	D	E	F	G	H	I	J		
0	8.01		7.7		25.8	0	0	0	0	0	0	0	0	0	0	0	Date: 12/1/08 Time: 15:00 Sol'n Prep: <u>SW</u> New WQ: <u>DEU</u> Test Loading: <u>SW</u>
1	8.35	8.40	8.7	7.4	25.6	0	0	0	0	0	0	0	0	0	0	0	Date: 12/5/08 Time: 09:45 Sol'n Prep: <u>SW</u> New WQ: <u>NW</u> Old WQ: <u>NW</u> Counts: <u>SW</u>
2	8.20	8.33	8.4	6.3	25.6	0	0	0	0	0	0	0	0	0	0	0	Date: 12/4/08 Time: 10:45 Sol'n Prep: <u>SW</u> New WQ: <u>SL</u> Old WQ: <u>AR</u> Counts: <u>SW</u>
3	8.41	8.41	8.6	7.4	25.3	0	0	0	0	0	0	0	0	0	0	0	Date: 12/5/08 Time: 12:30 Sol'n Prep: <u>SW</u> New WQ: <u>SW</u> Old WQ: <u>AR</u> Counts: <u>SW</u>
4	8.21	8.28	8.7	8.2	25.5	6	5	3	7	5	4	4	7	5	5	5	Date: 12/6/08 Time: 11:15 Sol'n Prep: <u>AR</u> New WQ: <u>DEU</u> Old WQ: <u>SW</u> Counts: <u>SW</u>
5	8.22	8.26	8.2	8.2	25.5	0	0	0	0	0	0	0	0	0	0	0	Date: 12/7/08 Time: 09:45 Sol'n Prep: <u>SW</u> New WQ: <u>SW</u> Old WQ: <u>SL</u> Counts: <u>SW</u>
6	8.22	8.23	9.0	8.0	25.5	8	9	6	7	7	8	9	10	10	10	10	Date: 12/8/08 Time: 09:45 Sol'n Prep: <u>SW</u> New WQ: <u>AR</u> Old WQ: <u>NW</u> Counts: <u>SW</u>
7	—	8.36 8.60	—	7.6 8.78	—	13	10	13	11	11	10	11	15	14	13	13	Date: 12/9/08 Time: 17:40 Sol'n Prep: <u>SW</u> New WQ: <u>—</u> Old WQ: <u>NW</u> Counts: <u>SW</u>
8																	Date: <u>—</u> Time: <u>—</u> Sol'n Prep: <u>—</u> New WQ: <u>—</u> Old WQ: <u>—</u> Counts: <u>—</u>
Total =						27	24	24	25	23	22	24	29	31	28	28	X = 25.7
Day	pH		D.O.		Cond. (µS/cm)	Survival / Reproduction											
	New	Old	New	Old		A	B	C	D	E	F	G	H	I	J		
0	7.83		8.5		797	0	0	0	0	0	0	0	0	0	0	0	
1	7.88	8.72	10.1	7.3	800	0	0	0	0	0	0	0	0	0	0	0	
2	7.86	8.72	9.2	6.0	796	0	0	0	0	0	0	0	0	0	0	0	
3	7.95	8.75	9.3	7.2	781	0	0	0	0	0	0	0	0	0	0	0	
4	7.96	N/M	8.8	N/M	793	0	0	0	0	0	0	0	0	0	0	0	
5	7.85	8.65	9.8	7.7	800	0	0	0	0	0	0	0	0	0	0	0	
6	7.93	8.63	10.4	7.5	789	9	5	0	6	8	4	4	0	3	2	2	
7	—	8.40 8.78	—	7.6 7.6	878 869	0	5	0	7	8	9	0	3	0	6	6	
8						5	10	0	7	14	13	4	3	3	6	6	X = 6.5

Appendix F

Test Data and Summary of Statistics for the Reference Toxicant Evaluation of the *Ceriodaphnia dubia*



CETIS Summary Report

Report Date: 13 Dec-08 11:05 (p 1 of 2)
 Test Code: 06-5742-0613/30601

Ceriodaphnia Survival and Reproduction Test Pacific EcoRisk

Test Run No: 16-3703-3026	Test Type: Reproduction-Survival (7d)	Analyst: John Jirasritumrong
Start Date: 02 Dec-08 16:00	Protocol: EPA/821/R-02-013 (2002)	Diluent: Laboratory Water
Ending Date: 08 Dec-08 14:15	Species: Ceriodaphnia dubia	Brine: Not Applicable
Duration: 5d 22h	Source: In-House Culture	Age: 1

Sample No: 18-1405-3335	Code: NaCl	Client: Reference Toxicant
Sample Date: 02 Dec-08 16:00	Material: Sodium chloride	Project: 13695
Receive Date: 02 Dec-08 16:00	Source: Reference Toxicant	
Sample Age: N/A (25.6 °C)	Station: In House	

Comparison Summary

Analysis No	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
16-1093-3141	7d Survival Rate	1500	2000	1730	N/A		Fisher Exact/Bonferroni-Holm Test
05-5809-4074	Reproduction	250	500	354	20.1%		Steel Many-One Rank Test

Point Estimate Summary

Analysis No	Endpoint	Level	Conc-mg/	95% LCL	95% UCL	TU	Method
18-6989-3485	7d Survival Rate	EC2.5	506	N/A	N/A		Linear Regression (MLE)
		EC10	755	N/A	N/A		
		EC15	873	N/A	N/A		
		EC20	979	N/A	N/A		
		EC25	1080	N/A	N/A		
		EC40	1380	N/A	N/A		
08-5513-1195	Reproduction	IC2.5	274	75.6	328		Linear Interpolation (ICPIN)
		IC5	297	151	407		
		IC10	344	263	531		
		IC15	392	307	585		
		IC20	439	355	638		
		IC25	486	387	704		
		IC40	760	473	951		
IC50	953	774	1080				

7d Survival Rate Summary

Conc-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	10	1	1	1	1	1	0	0	0.0%	0.0%
250		10	1	1	1	1	1	0	0	0.0%	0.0%
500		10	0.9	0.782	1	0	1	0.0577	0.316	35.1%	10.0%
1000		10	0.9	0.782	1	0	1	0.0577	0.316	35.1%	10.0%
1500		10	0.8	0.643	0.957	0	1	0.077	0.422	52.7%	20.0%
2000		10	0.1	0	0.218	0	1	0.0577	0.316	316.0%	90.0%

Reproduction Summary

Conc-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	10	24.9	23	26.8	15	29	0.945	5.17	20.8%	0.0%
250		10	26.8	24.7	28.9	16	31	1.01	5.53	20.6%	-7.63%
500		10	19	16.2	21.8	5	27	1.35	7.41	39.0%	23.7%
1000		10	12.3	11	13.6	9	19	0.65	3.56	28.9%	50.6%
1500		10	1.9	1.36	2.44	0	4	0.265	1.45	76.3%	92.4%
2000		10	0	0	0	0	0	0	0		100.0%

CETIS Summary Report

Report Date:

13 Dec-08 11:05 (p 2 of 2)

Test Code:

06-5742-0613/30601

Ceriodaphnia Survival and Reproduction Test											Pacific EcoRisk
7d Survival Rate Detail											
Conc-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Control	1	1	1	1	1	1	1	1	1	1
250		1	1	1	1	1	1	1	1	1	1
500		1	1	1	1	0	1	1	1	1	1
1000		1	1	1	1	1	1	1	1	1	0
1500		0	1	1	1	1	1	1	1	0	1
2000		0	0	0	1	0	0	0	0	0	0
Reproduction Detail											
Conc-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Control	27	28	29	26	29	24	27	15	16	28
250		28	31	29	30	31	17	28	29	16	29
500		27	14	24	23	5	22	24	13	12	26
1000		19	9	10	9	15	17	13	10	11	10
1500		0	4	3	2	2	0	3	3	0	2
2000		0	0	0	0	0	0	0	0	0	0

Short-Term Chronic 3-Brood *Ceriodaphnia dubia* Survival & Reproduction Test Data

Test Date: 12/2/08

Client: Reference Toxicant Material: Sodium Chloride Control Water / Diluent: Lab Water (80:20)

Project #: 13695 Test ID #: 30601

Day	pH		D.O.		Temp (°C)	Survival / Reproduction										SIGN-OFF
	New	Old	New	Old		A	B	C	D	E	F	G	H	I	J	
0	8.32		7.8		25.6	0	0	0	0	0	0	0	0	0	0	Date: 12/2/08 Time: 16:00 Sol'n Prep: SLR New WQ: 4% Test Loading: JL
1	8.18	8.34	8.6	7.7	25.8	0	0	0	0	0	0	0	0	0	0	Date: 12/3/08 Time: 10:50 Sol'n Prep: SLR New WQ: 1% Old WQ: SL Counts: 200
2	8.19	8.03	8.4	7.5	25.6	0	0	0	0	0	0	0	0	0	0	Date: 12/4/08 Time: 14:15 Sol'n Prep: SLR New WQ: SL Old WQ: 0.5% Counts: 200
3	8.26	8.09	8.3	8.6	25.4	6	7	7	5	7	6	6	0	6	6	Date: 12/5/08 Time: 17:35 Sol'n Prep: SLR New WQ: 0.5% Old WQ: 0.5% Counts: 200
4	8.17	8.12	8.5	8.1	25.5	0	0	0	0	0	0	0	5	0	0	Date: 12/16/08 Time: 11:30 Sol'n Prep: SLR New WQ: 0.5% Old WQ: 0.5% Counts: 200
5	8.13	8.37	8.4	7.5	25.5	9	9	9	10	11	9	9	11	11	11	Date: 12/16/08 Time: 11:45 Sol'n Prep: SLR New WQ: 0.5% Old WQ: 0.5% Counts: 200
6	8.28	8.26	8.4	6.8	25.4	12	12	13	11	11	9	12	0	11	11	Date: 12/30/08 Time: 14:15 Sol'n Prep: SLR New WQ: 0.5% Old WQ: 0.5% Counts: 200
7																Date: Time: Sol'n Prep: Counts:
8																Date: Time: Sol'n Prep: Counts:
Total =						27	28	29	26	29	24	27	15	16	28	X = 24.9
Day	pH		D.O.		Cond. (µS/cm)	Survival / Reproduction										Total =
	New	Old	New	Old		A	B	C	D	E	F	G	H	I	J	
0	8.26		8.2		742	0	0	0	0	0	0	0	0	0	0	
1	8.16	8.23	8.5	7.5	735	0	0	0	0	0	0	0	0	0	0	
2	8.10	8.14	8.1	7.4	701	0	0	0	0	0	0	0	0	0	0	
3	8.25	8.18	8.2	8.5	704	5	6	6	6	6	6	6	0	6	6	
4	8.13	8.20	8.5	8.1	736	0	0	0	0	0	0	0	0	0	0	
5	8.00	8.32	8.2	7.5	748	10	11	11	10	12	11	10	10	11	11	
6	8.24	8.24	8.1	5.4	684	13	14	12	14	13	0	12	13	0	12	
7																
8																
Total =						28	31	29	30	31	17	28	29	14	29	X = 26.8

Short-Term Chronic 3-Brood Ceriodaphnia dubia Survival & Reproduction Test Data

Test Date: 12/2/08

Client: Reference Toxicant: Sodium Chloride Material: Control Water / Diluent: Lab Water (80:20)

Project #: 13695 Test ID #: 30601

Day	pH		D.O.		Cond. (µS/cm)	Survival / Reproduction										Total =		
	New	Old	New	Old		A	B	C	D	E	F	G	H	I	J			
0	8.24		8.0		1254	0	0	0	0	0	0	0	0	0	0	0	0	
1	8.17	8.15	8.1	7.5	1347	0	0	0	0	0	0	0	0	0	0	0	0	
2	8.10	8.19	8.2	7.5	1196	0	0	0	0	0	0	0	0	0	0	0	0	
3	8.24	8.26	8.3	8.4	1258	5	0	5	6	5	4	7	5	0	5	0	5	
4	8.10	8.19	8.0	7.9	1272	0	4	0	0	0	0	0	0	4	0	0	11	
5	8.19	8.27	8.3	7.4	1259	9	10	10	9	-	9	8	8	0	8	10		
6	8.23	8.21	7.9	5.7	1224	13	0	9	8	-	9	0	0	0	8	10		
7																		
8																		
Total =						27	14	24	23	15	22	24	13	12	26			X = 19

Day	pH		D.O.		Cond. (µS/cm)	Survival / Reproduction										Total =		
	New	Old	New	Old		A	B	C	D	E	F	G	H	I	J			
0	8.21		8.0		2249	0	0	0	0	0	0	0	0	0	0	0	0	
1	8.12	8.15	8.3	7.5	2291	0	0	0	0	0	0	0	0	0	0	0	0	
2	8.10	8.20	8.2	7.4	2115	0	0	0	0	0	0	0	0	0	0	0	0	
3	8.22	8.27	8.2	8.2	2205	1	0	0	2	3	3	2	0	0	4			
4	8.02	8.26	9.4	7.9	2165	5	2	4	0	0	0	0	3	4	0			
5	8.18	8.25	8.0	7.4	2226	8	7	6	3	5	7	7	7	0	6			
6	8.21	8.17	7.8	5.6	2243	5	0	0	4	7	7	6	0	0	0			
7																		
8																		
Total =						19	9	10	9	15	17	13	10	11	10			X = 12.3

500 mg/L

1000 mg/L

Appendix G

Test Data and Summary of Statistics for the Evaluation of the Chronic Toxicity of Chevron/Cawelo Effluent to Fathead Minnows



CETIS Summary Report

Report Date: 13 Dec-08 11:48 (p 1 of 1)
 Test Code: 09-1826-6088/31122

Fathead Minnow 7-d Larval Survival and Growth Test Pacific EcoRisk

Test Run No: 09-0573-3298	Test Type: Growth-Survival (7d)	Analyst: John Jirasritumrong
Start Date: 02 Dec-08 15:00	Protocol: EPA/821/R-02-013 (2002)	Diluent: Laboratory Water
Ending Date: 09 Dec-08 09:55	Species: Pimephales promelas	Brine: Not Applicable
Duration: 6d 19h	Source: Aquatic Biosystems, CO	Age: 1

Sample No: 11-7191-8560	Code: Eff	Client: Precision Analytical
Sample Date: 01 Dec-08 14:10	Material: Effluent	Project: 14208
Receive Date: 02 Dec-08 11:00	Source: Precision Analytical	
Sample Age: 25h (0.7 °C)	Station: EFF-003	

Comparison Summary

Analysis No	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
07-1689-7050	7d Survival Rate	<100	100	N/A	5.56%	>1	Wilcoxon Rank Sum Two-Sample Test
15-9400-2255	Mean Dry Biomass-mg	<100	100	N/A	7.19%	>1	Equal Variance t Two-Sample Test

Point Estimate Summary

Analysis No	Endpoint	Level	Conc-%	95% LCL	95% UCL	TU	Method
08-3594-9209	Mean Dry Biomass-mg	IC2.5	2.56	2.49	2.63	39	Linear Interpolation (ICPIN)
		IC5	5.12	4.98	5.27	19.5	
		IC10	10.2	9.96	10.5	9.76	
		IC15	15.4	14.9	15.8	6.5	
		IC20	20.5	19.9	21.1	4.88	
		IC25	25.6	24.9	26.3	3.9	
		IC40	41	39.9	42.2	2.44	
IC50	51.2	49.8	52.7	1.95			

7d Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	0.075	0.0563	0.0937	0	0.1	0.00913	0.05	66.7%	92.5%

Mean Dry Biomass-mg Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	4	0.638	0.621	0.655	0.586	0.685	0.00832	0.0456	7.14%	0.0%
100		4	0.0155	0.0109	0.0201	0	0.03	0.00226	0.0124	79.8%	97.6%

7d Survival Rate Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Control	1	1	1	1
100		0.1	0.1	0	0.1

Mean Dry Biomass-mg Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Control	0.586	0.615	0.666	0.685
100		0.018	0.014	0	0.03

CETIS Analytical Report

Report Date: 13 Dec-08 11:48 (p 1 of 2)
 Test Code: 09-1826-6088/31122

Fathead Minnow 7-d Larval Survival and Growth Test Pacific EcoRisk

Analysis No: 15-9400-2255 Endpoint: Mean Dry Biomass-mg CETIS Version: CETISv1.6.5
 Analyzed: 13 Dec-08 11:45 Analysis: Parametric-Two Sample Official Results: Yes

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Untransformed		C > T	Not Run	<100	100	N/A	>1	7.19%

Equal Variance t Two-Sample Test

Control	vs	Conc-%	Test Stat	Critical	MSD	P-Value	Decision(5%)
Control		100*	26.4	1.94	0.0459	0.0000	Significant Effect

ANOVA Table

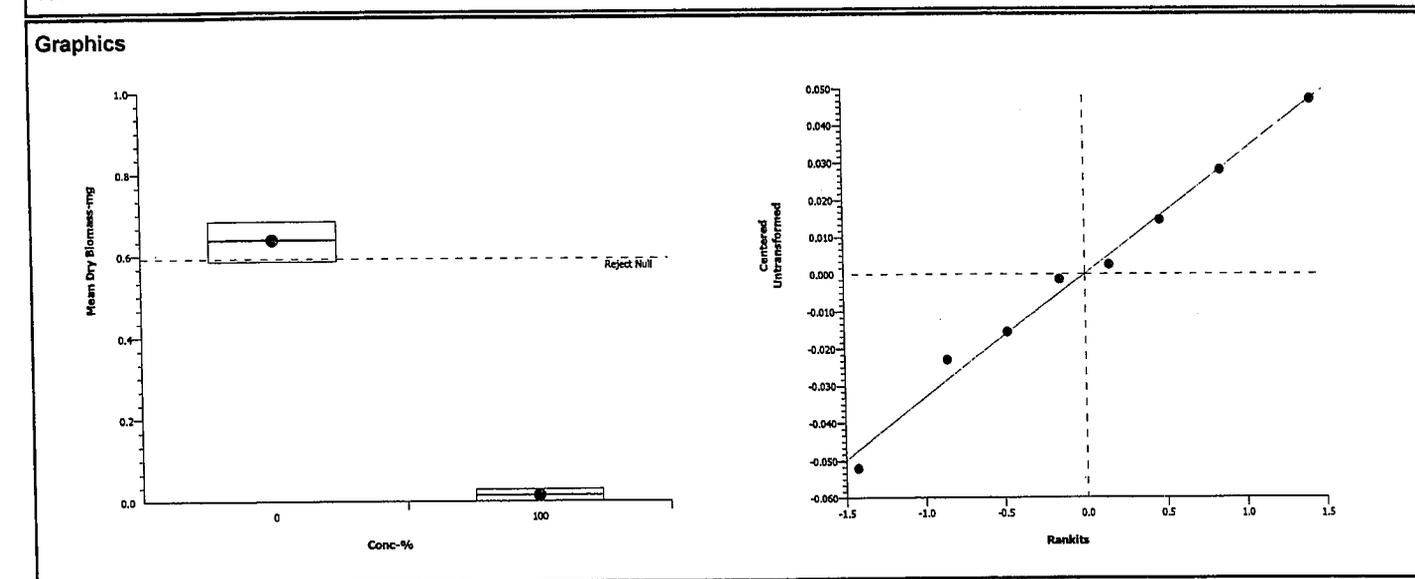
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Between	0.7750112	0.7750112	1	696	0.0000	Significant Effect
Error	0.006684968	0.001114161	6			
Total	0.78169620921835	0.77612540277187	7			

ANOVA Assumptions

Attribute	Test	Test Stat	Critical	P-Value	Decision(1%)
Variances	Variance Ratio F	13.6	47.5	0.0598	Equal Variances
Distribution	Shapiro-Wilk Normality	0.994		0.9990	Normal Distribution

Mean Dry Biomass-mg Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	4	0.638	0.621	0.655	0.586	0.685	0.00846	0.0456	7.14%	0.0%
100		4	0.0155	0.0108	0.0202	0	0.03	0.0023	0.0124	79.8%	97.6%



CETIS Analytical Report

Report Date: 13 Dec-08 11:48 (p 2 of 2)
 Test Code: 09-1826-6088/31122

Fathead Minnow 7-d Larval Survival and Growth Test				Pacific EcoRisk			
Analysis No: 07-1689-7050	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.6.5					
Analyzed: 13 Dec-08 11:44	Analysis: Nonparametric-Two Sample	Official Results: Yes					

Data Transform	Zeta	Alt Hyp	Monte Carlo	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)		C > T	Not Run	<100	100	N/A	>1	5.56%

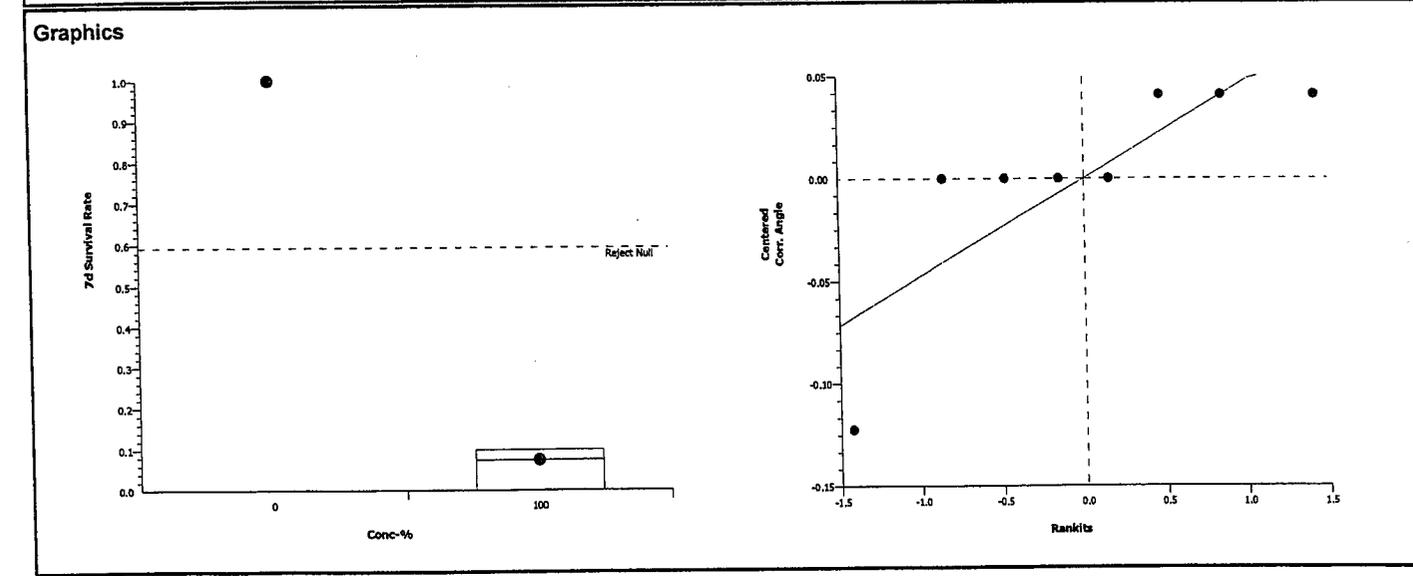
Wilcoxon Rank Sum Two-Sample Test							
Control	vs	Conc-%	Test Stat	Critical	Ties	P-Value	Decision(5%)
Control		100*	10		0	0.0143	Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(5%)
Between	2.558359	2.558359	1	771	0.0000	Significant Effect
Error	0.0199195	0.003319917	6			
Total	2.57827840559185	2.56167882424779	7			

ANOVA Assumptions						
Attribute	Test	Test Stat	Critical	P-Value	Decision(1%)	
Variances	Mod Levene Equality of Variance	1	13.7	0.3560	Equal Variances	
Distribution	Shapiro-Wilk Normality	0.706		0.0027	Non-normal Distribution	

7d Survival Rate Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	0.075	0.056	0.094	0	0.1	0.00928	0.05	66.7%	92.5%

Angular (Corrected) Transformed Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	4	1.41	1.41	1.41	1.41	1.41	0	0	0.0%	0.0%
100		4	0.281	0.25	0.312	0.159	0.322	0.0151	0.0815	29.0%	80.1%



CETIS Analytical Report

Report Date: 13 Dec-08 11:48 (p 1 of 1)
 Test Code: 09-1826-6088/31122

Fathead Minnow 7-d Larval Survival and Growth Test			Pacific EcoRisk
Analysis No: 08-3594-9209	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.6.5	
Analyzed: 13 Dec-08 11:47	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes	

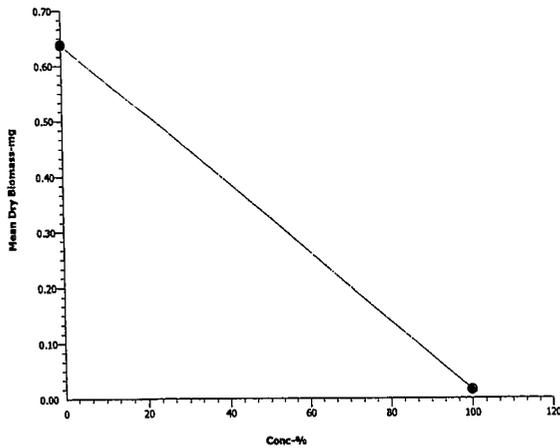
Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	7607236	280	Yes	Two-Point Interpolation

Point Estimates						
Level	Conc-%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC2.5	2.56	2.49	2.63	39	38	40.1
IC5	5.12	4.98	5.27	19.5	19	20.1
IC10	10.2	9.96	10.5	9.76	9.49	10
IC15	15.4	14.9	15.8	6.5	6.33	6.69
IC20	20.5	19.9	21.1	4.88	4.74	5.02
IC25	25.6	24.9	26.3	3.9	3.8	4.01
IC40	41	39.9	42.2	2.44	2.37	2.51
IC50	51.2	49.8	52.7	1.95	1.9	2.01

Mean Dry Biomass-mg Summary			Calculated Variate							
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	Diff%	
0	Control	4	0.638	0.586	0.685	0.00832	0.0456	7.14%	0.0%	
100		4	0.0155	0	0.03	0.00226	0.0124	79.8%	97.6%	

Mean Dry Biomass-mg Detail						
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	
0	Control	0.586	0.615	0.666	0.685	
100		0.03	0.018	0.014	0	

Graphics



7 Day Chronic Fathead Minnow Toxicity Test Test Data

Client: Precision Analytical
 Test Material: Effluent
 Test ID#: 31122 Project #: 14208
 Test Date: 12/2/08 Randomization: 427

Organism Log#: 4281 Age: 24hrs
 Organism Supplier: ABS
 Control/Diluent: EPAMH
 Control Water Batch: 1149

Treatment (%)	Temp (°C)	pH		D.O. (mg/L)		Conductivity (µS/cm)	# Live Organisms				SIGN-OFF
		new	old	new	old		A	B	C	D	
Lab Water	25.7	8.30		8.2		283	10	10	10	10	Date: 12/2/08
100%	25.7	7.83		9.1		791	10	10	10	10	Sample ID: 20977
											Test Solution Prep: JLR
											New WQ: DW
											Initiation Time: 1500
											Initiation Signoff: JL
Meter ID:	11A	PH11		DO14		EC01					
Lab Water	25.8	8.05	7.76	8.1	6.4	281	10	10	10	10	Date: 12/3/08
100%	25.8	7.78	8.51	10.2	7.3	799	10	10	10	10	Sample ID: 20977
											Test Solution Prep: JLR
											New WQ: NW
											Renewal Time: 1020
											Renewal Signoff: JLR
											Old WQ: SL
Meter ID:	11A	PH03	PH03	DO10	DO10	EC05					
Lab Water	25.6	8.09	8.23	8.4	7.8	282	10	10	10	10	Date: 12/9/08
100%	25.6	7.79	8.40	9.6	7.3	794	10	10	10	10	Sample ID: 20977
											Test Solution Prep: JLR
											New WQ: SL
											Renewal Time: 1115
											Renewal Signoff: JLR
											Old WQ: BAP
Meter ID:	11A	PH12	PH11	DO14	DO10	EC05					
Lab Water	25.6	8.16	7.86	7.9	6.6	275	10	10	10	10	Date: 12/5/08
100%	25.6	7.90	8.44	9.2	6.5	783	9	10	8	9	Sample ID: 20977
											Test Solution Prep: JPC
											New WQ: BAP
											Renewal Time: 1300
											Renewal Signoff: BAK
											Old WQ: DAY
Meter ID:	11A	PH03	PH12	DO10	DO14	EC04					

7 Day Chronic Fathead Minnow Toxicity Test Test Data

Client: Precision Analytical Organism Log#: 4281 Age: < 24 hrs
 Test Material: Effluent Organism Supplier: ABS
 Test ID#: 31122 Project #: 14208 Control/Diluent: EPAMH
 Test Date: 12/2/08 Randomization: 4.2.7 Control Water Batch: 1149

Treatment (%)	Temp (°C)	pH		D.O. (mg/L)		Conductivity (µS/cm)	# Live Organisms				SIGN-OFF
		new	old	new	old		A	B	C	D	
Lab Water	25.5	8.20	7.48	7.5	7.0	314	10	10	10	10	Date: 12/6/08
100%	25.5	7.94	8.00	7.8	6.9	788	8	5	8	8	Sample ID: 20977
							7	8	5	8	Test Solution Prep: JPC
											New WQ: JPC
											Renewal Time: 1110
											Renewal Signoff: JPC
											Old WQ: JPC
Meter ID:	11A	PH11	PH03	DO10	DO12	EC01					
Lab Water	25.6	8.07	8.18	8.8	7.5	25320	10	10	10	10	Date: 12/7/08
100%	25.6	7.91	8.38	9.3	7.7	788	2	4	0	1	Sample ID: 20977
											Test Solution Prep: JPC
											New WQ: JPC
											Renewal Time: 1000
											Renewal Signoff: JPC
											Old WQ: SL
Meter ID:	11A	PH12	PH11	DO14	DO10	EC05					
Lab Water	25.4	8.11	8.20	9.2	7.0	322	10	10	10	10	Date: 12/8/08
100%	25.4	7.96	8.55	10.2	6.8	795	2	3	-	1	Sample ID: 20977
											Test Solution Prep: JPC
											New WQ: JPC
											Renewal Time: 1015
											Renewal Signoff: JPC
											Old WQ: JPC
Meter ID:	11A	PH11	PH12	DO14	DO10	EC01					
Lab Water	25.3		8.09		7.1	328	10	10	10	10	Date: 12/9/08
100%	25.3		8.37		6.8	807	1	1	-	1	Termination Time: 0955
											Termination Signoff: RV
											Old WQ: RV
Meter ID:	11A		PH12		DO10	EC04					

Appendix H

Test Data and Summary of Statistics for the Reference Toxicant Evaluation of the Fathead Minnows



CETIS Summary Report

Report Date: 13 Dec-08 10:45 (p 1 of 2)
 Link/Link Code: 07-6954-9892/31123

Chronic Larval Fish Survival and Growth Test				Pacific EcoRisk
Test Run No: 07-7413-2235	Test Type: Growth-Survival (7d)	Analyst: John Jirasritumrong		
Start Date: 02 Dec-08 16:00	Protocol: EPA/821/R-02-013 (2002)	Diluent: Laboratory Water		
Ending Date: 09 Dec-08 10:45	Species: Pimephales promelas	Brine: Not Applicable		
Duration: 6d 19h	Source: Aquatic Biosystems, CO	Age: 1		

Sample No: 05-8333-0148	Code: NaCl	Client: Reference Toxicant
Sample Date: 02 Dec-08 16:00	Material: Sodium chloride	Project: 14209
Receive Date: 02 Dec-08 16:00	Source: Reference Toxicant	
Sample Age: N/A (25.4 °C)	Station: In House	

Comparison Summary						
Analysis No	Endpoint	NOEL	LOEL	TOEL	PMSD	Method
01-5025-9706	7d Survival Rate	1.5	3	2.12	12.8%	Dunnett's Multiple Comparison Test
13-5890-8674	Mean Dry Biomass-mg	0.75	1.5	1.06	16.5%	Dunnett's Multiple Comparison Test

Point Estimate Summary						
Analysis No	Endpoint	Effect-%	Conc-g/L	95% LCL	95% UCL	Method
14-4996-3988	7d Survival Rate	2.5	1.87	1.25	2.35	Linear Regression (MLE)
		10	2.4	1.77	2.87	
		15	2.63	2.01	3.09	
		20	2.82	2.21	3.28	
		25	3	2.41	3.46	
		40	3.5	2.95	3.97	
08-2004-9885	Mean Dry Biomass-mg	2.5	0.206	0.0384	1.36	Linear Interpolation (ICPIN)
		5	0.411	0.0769	1.67	
		10	0.812	0.16	2.11	
		15	1.17	0.271	2.17	
		20	1.51	0.481	2.23	
		25	1.72	0.814	2.36	

7d Survival Rate Summary											
Conc-g/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	4	0.95	0.928	0.972	0.9	1	0.0105	0.0577	6.08%	0.0%
0.75		4	1	1	1	1	1	0	0	0.0%	-5.26%
1.5		4	0.925	0.889	0.961	0.8	1	0.0175	0.0957	10.4%	2.63%
3		4	0.725	0.689	0.761	0.6	0.8	0.0175	0.0957	13.2%	23.7%
6		4	0.125	0.0892	0.161	0	0.2	0.0175	0.0957	76.6%	86.8%
9		4	0	0	0	0	0	0	0		100.0%

Mean Dry Biomass-mg Summary											
Conc-g/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Control	4	0.787	0.763	0.811	0.706	0.86	0.0118	0.0644	8.19%	0.0%
0.75		4	0.715	0.684	0.746	0.624	0.811	0.0153	0.0836	11.7%	9.12%
1.5		4	0.631	0.585	0.678	0.479	0.784	0.0228	0.125	19.7%	19.7%
3		4	0.35	0.329	0.371	0.293	0.415	0.0103	0.0562	16.1%	55.5%
6		4	0.0353	0.0255	0.045	0	0.058	0.00478	0.0262	74.3%	-95.5%
9		4	0	0	0	0	0	0	0		100.0%

7 Day Chronic Fathead Minnow Reference Toxicant Test Data

Client: Reference Toxicant Organism Log#: 4281 Age: 224 hrs.
 Test Material: Sodium Chloride Organism Supplier: ABS
 Test ID#: 31123 Project #: 14209 Control/Diluent: EPAMH
 Test Date: 12/2/08 Randomization: 4.6.5 Control Water Batch: 1149

Treatment (g/L)	Temp (°C)	pH		D.O. (mg/L)		Conductivity (µs/cm)	# Live Organisms				SIGN-OFF
		New	Old	New	Old		A	B	C	D	
Control	25.8	8.36		8.4		290	10	10	10	10	Date: 12/2/08
0.75	25.8	8.26		8.4		1777	10	10	10	10	Test Solution Prep: SLR
1.5	25.8	8.21		8.0		3200	10	10	10	10	New WQ: YK
3	25.8	8.12		8.5		5980	10	10	10	10	Initiation Time: 1600
6	25.8	8.06		8.0		11150	10	10	10	10	Initiation Signoff: J
9	25.8	8.00		8.0		16130	10	10	10	10	
Meter ID	PH11A	PH		DO14		ECO1					
Control	25.1	8.05	7.86	7.8	7.7	274	10	10	10	10	Date: 12/3/08
0.75	25.7	7.98	7.73	8.1	7.1	1732	10	10	10	10	Test Solution Prep: JL
1.5	25.7	7.94	7.81	8.4	8.0	3140	10	10	10	10	New WQ: YK
3	25.7	7.90	7.80	8.5	8.1	5850	10	10	10	10	Renewal Time: 1100
6	25.7	7.85	7.78	8.4	8.0	10990	9	10	10	9	Renewal Signoff: J
9	25.7	7.80	7.73	8.5	7.8	16110	0	0	0	0	Old WQ: NW
Meter ID	11A	PH12	PH12	DO12	DO12	ECO4					
Control	25.6	8.73	8.27	6.8	7.3	274	10	10	10	10	Date: 12/4/08
0.75	25.6	8.47	8.13	6.0	7.1	1745	10	10	10	10	Test Solution Prep: JW
1.5	25.6	8.37	8.05	6.0	7.0	3410	10	9	10	10	New WQ: JR
3	25.6	8.26	8.02	6.5	7.0	6110	10	10	10	10	Renewal Time: 1145
6	25.6	8.16	7.93	6.0	7.0	11380	7	8	8	6	Renewal Signoff: SLR
9	-	-	-	-	-	-	-	-	-	-	Old WQ: OAP
Meter ID	11A	PH11	PH11	DO10	DO10	ECO4					
Control	25.5	8.22	7.96	8.2	6.5	287	10	10	9	10	Date: 12/5/08
0.75	25.5	8.12	7.75	8.2	6.5	1834	10	10	10	10	Test Solution Prep: JL
1.5	25.5	8.08	7.74	8.1	6.8	3180	10	9	10	10	New WQ: JWP
3	25.5	8.02	7.76	8.2	6.8	6120	10	10	9	10	Renewal Time: 1430
6	25.5	7.99	7.68	8.2	6.9	11350	5	6	7	3	Renewal Signoff: ERK
9	-	-	-	-	-	-	-	-	-	-	Old WQ: OGU
Meter ID	11A	PH12	PH12	DO14	DO14	ECO5					

7 Day Chronic Fathead Minnow Reference Toxicant Test Data

Client: Reference Toxicant
 Test Material: Sodium Chloride
 Test ID#: 31123 Project #: 14209
 Test Date: 12/2/08 Randomization: 4.6.5.

Organism Log#: 4281 Age: 248 hrs
 Organism Supplier: ABS
 Control/Diluent: EPAMH
 Control Water Batch: 1149

Treatment (g/L)	Temp (°C)	pH		D.O. (mg/L)		Conductivity (µs/cm)	# Live Organisms				SIGN-OFF
		new	old	new	old		A	B	C	D	
Control	25.4	8.11	7.89	8.4	7.1	312	10	10	9	9	Date: 12/6/08
0.75	25.4	8.07	7.71	8.4	6.9	1752	10	10	10	10	Test Solution Prep: J
1.5	25.4	8.05	7.73	8.4	7.1	3170	10	9	10	10	New WQ: JAP
3	25.4	8.01	7.70	8.5	7.1	5880	9	9	8	10	Renewal Time: 1145
6	25.4	7.97	7.69	8.4	7.0	11080	4	4	4	3	Renewal Signoff: JLR
9	-	-	-	-	-	-	-	-	-	-	Old WQ: JAP
Meter ID	11A	PH12	PH12	DO14	DO14	EC04					
Control	25.4	8.08	8.05	8.4	7.2	315	10	10	9	9	Date: 12/7/08
0.75	25.4	8.07	7.95	8.3	7.2	1842	10	10	10	10	Test Solution Prep: SM
1.5	25.4	8.00	7.84	8.3	7.1	3190	10	9	10	10	New WQ: SL
3	25.4	8.04	7.83	8.2	7.2	5930	9	9	8	10	Renewal Time: 1130
6	25.4	7.99	7.75	8.2	7.2	11150	4	3	3	1	Renewal Signoff: JPL
9	-	-	-	-	-	-	-	-	-	-	Old WQ: SL
Meter ID	11A	PH11	PH11	DO10	DO10	EC01					
Control	25.4	8.15	7.94	8.0	7.1	322	10	10	9	9	Date: 12/8/08
0.75	25.4	8.10	7.75	8.1	6.8	1918	10	10	10	10	Test Solution Prep: SM
1.5	25.4	8.06	7.72	7.9	6.6	3310	10	8	10	10	New WQ: JM
3	25.4	8.02	7.71	7.8	6.6	6030	8	6	7	9	Renewal Time: 1145
6	25.4	7.98	7.69	7.6	6.6	11380	2	2	2	1	Renewal Signoff: JPL
9	-	-	-	-	-	-	-	-	-	-	Old WQ: MJM
Meter ID	11A	PH12	PH12	DO14	DO10	EC05					
Control	25.3		7.83		7.2	332	10	10	9	9	Date: 12/9/08
0.75	25.3		7.79		7.0	1904	10	10	10	10	Termination Time: 1045
1.5	25.3		7.79		7.1	3290	10	8	9	10	Termination Signoff: J
3	25.3		7.75		7.1	5940	8	6	7	8	Old WQ: JAP
6	25.3		7.83		7.3	11360	2	2	1	0	
9	-						-	-	-	-	
Meter ID	11A		PH12		DO10	EC04					

Fathead Minnow Dry Weight Data Sheet

Client: Reference Toxicant Test ID #: 31123 Project # 14209
 Sample: Sodium Chloride Tare Weight Date: 12/3/08 Sign-off: DAP
 Test Date: 12/2/08 Final Weight Date: 12-10-08 Sign-off: MC

Pan ID	Concentration	Replicate	Initial Pan Weight (mg)	Final Pan Weight (mg)	Initial # of Organisms	Biomass Value (mg)
1	Control	A	157.07	165.67	10	0.860
2		B	171.76	179.83	10	0.807
3		C	175.54	182.60	10	0.706
4		D	176.77	184.51	10	0.774
5	0.75	A	172.78	180.89	10	0.811
6		B	175.91	182.15	10	0.624
7		C	172.17	178.88	10	0.671
8		D	167.01	174.55	10	0.754
9	1.5	A	168.24	174.47	10	0.623
10		B	171.13	175.92	10	0.479
11		C	156.95	163.35	10	0.640
12		D	169.52	177.36	10	0.784
13	3	A	165.26	169.41	10	0.415
14		B	164.51	167.44	10	0.293
15		C	168.14	171.92	10	0.378
16		D	164.43	167.58	10	0.315
17	6	A	161.18	161.70	10	0.052
18		B	164.26	164.84	10	0.058
19		C	154.08	154.39	10	0.031
20		D	192.60	—	10	—
21	9	A	178.05	—	10	—
22		B	174.64	—	10	—
23		C	162.12	—	10	—
24		D	170.50	—	10	—
QA1			170.88	170.90		
QA2			179.61	179.63		
QA3			171.75	171.78		
Balance ID:			ID#1	1		