

Bio-Analytical Laboratories (BAL)
ADEQ#88-0630
Project X6229

Bio-Analytical Laboratories' Executive Summary

Permittee: El Dorado Chemical Company
P.O. Box 231
El Dorado, AR 71731

Project #: X6229

Outfall: Outfall 007 (contaminated storm water)

Permit #: AR0000752/ AFIN #70-00040

Contact: Mr. Eddie Pearson

Test Dates: December 5 - 7, 2016

Test Type: 48-hour acute toxicity test using *Pimephales promelas* (EPA 2000.0).
48-hour acute toxicity test using *Daphnia pulex* (EPA 2021.0)

Results:

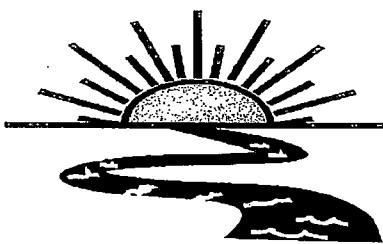
For *Daphnia pulex*:

1. If the NOEC for survival is less than the critical dilution (100.0%), enter a "1"; otherwise, enter a "0" for Parameter No. TEM3D- 0- Pass.
2. Report the NOEC for survival, Parameter TOM3D -100.0%.
3. Report the highest (critical dilution or control) Coefficient of Variation, Parameter TQM3D - 28.17%.

For *Pimephales promelas*:

1. If the NOEC for survival is less than the critical dilution (100.0%), enter a "1"; otherwise, enter a "0" for Parameter No. TEM6C- 0- Pass.
2. Report the NOEC for survival, Parameter TOM6C - 100.0%.
3. Report the highest (critical dilution or control) Coefficient of Variation, Parameter TQM6C - 6.06%.

This report contains a total of 37 pages, including this page. The results pertain only to the samples listed in the chain of custody documents in Appendix A. The information contained within meets the requirements set forth by ADEQ. The chemical data in this report is for monitoring purposes only and should not be reported on discharge monitoring reports.



Bio-Analytical Laboratories

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THE RESULTS OF TWO 48-HOUR ACUTE TOXICITY TESTS FOR OUTFALL 007 AT

EL DORADO CHEMICAL COMPANY
El Dorado, Arkansas

NPDES #AR0000752
AFIN #70-00040

EPA Methods 2000.0 and 2021.0

Project X6229

Test Dates: December 5 - 7, 2016
Report Date: January 5, 2017

Prepared for:
Mr. Eddie Pearson
El Dorado Chemical Company
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El Dorado, AR 71731

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ADEQ #88-0630

BAL
ADEQ #88-0630
Project X6229

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ADEQ #88-0630
Project X6229

1.0 Introduction

Bio-Analytical Laboratories (BAL), Doyline, Louisiana conducted two 48-hour acute toxicity tests for Outfall 007 at El Dorado Chemical Company, El Dorado, Arkansas. The test organisms used were the fathead minnow, *Pimephales promelas* and the cladoceran, *Daphnia pulex*. The purpose of this study is to determine if an appropriately dilute effluent sample adversely affects the survival of the test organism. Toxicity is defined as a statistically significant difference at the 95 percent confidence level between the survival of the test organisms in the critical dilution (the effluent concentration representative of the proportion of effluent in the receiving water during critical low flow or critical mixing conditions) compared to the survival of the test organisms in the control. The test endpoints are the No-Observed-Effect-Concentration (NOEC), which is defined as the highest effluent concentration that is not statistically different from the control, and the 48-hour LC₅₀, the concentration in which 50 percent of the test organisms died.

2.0 Methods and Materials

2.1 Test Methods

All methods followed were according to the latest edition of "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" (EPA-821-R-02-012), "Standard Methods for The Examination of Water and Wastewater. 20th Edition" (APHA 1998. Chemical results using this edition are listed in the report as SM 1997), and BAL's standard operating procedures.

2.2 Test Organisms

The fathead minnows were raised in-house and were approximately ten days old at test initiation. The minnows were acclimated to dilution water hardness prior to testing. The *Daphnia pulex* test organisms were also raised in-house at test temperature and were less than 24 hours old at test initiation. Forty-eight hour reference toxicant tests, using sodium chloride (NaCl), were conducted monthly in order to document organism sensitivity and demonstration of capability.

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2.3 Dilution Water

Soft reconstituted water made per EPA guidelines was used as the dilution water and the control for the acute tests.

2.4 Test Concentrations

The test concentrations used in the test were 100.0, 75.0, 56.0, 50.0, 45.0, and 32.0 percent effluent and a reconstituted water control. The critical dilution was defined as 100.0 percent effluent. The tests were conducted using five replicates of eight animals each for a total of 40 animals per concentration.

2.5 Sample Collection

One composite sample of Outfall 007 was collected by El Dorado Chemical personnel on December 4, 2016 at 2425 hours. Upon completion of collection, the sample was packed in ice and delivered to the laboratory by BAL personnel. The temperature upon arrival was 0.8° Celsius.

2.6 Sample Preparation

Upon arrival, the sample was logged in, given an identification number and refrigerated unless needed. Prior to use, the sample was warmed to 25±1° Celsius. The total residual chlorine level (SM4500-Cl E 1997) was measured in milligrams/Liter (mg/L) with a Capital Controls® amperometric titrator and recorded if present. The total ammonia level was measured in mg/L using a test strip. Dissolved oxygen (SM4500-O G 1997), pH (SM4500-H+ B 1997) and conductivity (SM2510-B 1997) measurements (in mg/L, standard units and umhos/cm, respectively) were taken on the control and each test concentration at test initiation, at each renewal and at test termination. Alkalinity (SM2320-B 1997) and hardness (SM2340-C 1997) levels were measured in mg/L as CaCO₃ on the control and the highest effluent concentration.

2.7 Monitoring of the Tests

The tests were run in a Precision® dual controlled illuminated incubator at a temperature of 25±1° Celsius. An AEMC® data logger was used to monitor diurnal temperature throughout the testing period. Light cycle and intensity were recorded twice a month.

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2.8 Data Analysis

The NOEC and LC₅₀ values were obtained by approved EPA methods of analysis, using the ToxCalc statistical program.

3.0 Results and Discussion

The results of the tests can be found in Table 1. Significant differences in survival were not noted in the critical dilution in both tests after 48 hours of exposure ($p=.05$). The NOEC value for the fathead minnow and *Daphnia pulex* tests was 100.0 percent effluent ($p=.05$). The 48-hour LC₅₀ values for the fathead minnow and *Daphnia pulex* tests could not be calculated because greater than 50.0 percent survival occurred in the 100.0 percent test concentration in both tests. An erratic dose response occurred in the *Daphnia pulex* test, in which several of the lower test dilutions showed significant differences when compared to the control. This was due to a large amount of static electricity in the plastic test cups, which caused a lot of floating daphnids in the water.

Table 1: Results of the 48-hour Acute Definitive Toxicity Tests

Percent Effluent	Percent Survival	
Test Organism	<i>Daphnia pulex</i>	<i>Pimephales promelas</i>
Control	92.5	100.0
32.0	30.0	95.0
45.0	47.5	92.5
50.0	47.5	92.5
56.0	35.0	95.0
75.0	57.5	95.0
100.0	75.0	97.5

The 48-hour reference toxicant test results indicated that the test organisms were within the respective sensitivity range. The graphs of the acute reference toxicant tests can be found in Appendix D.

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4.0 Conclusions

The sample of Outfall 007 collected from El Dorado Chemical Company, El Dorado, Arkansas, on December 4, 2016, was not found to be lethally toxic to the fathead minnow test organisms nor the *Daphnia pulex* test organisms in the 100.0 percent critical dilution after 48 hours of exposure ($p=.05$).

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Project X6229

5.0 References

EPA, 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. EPA-821-R-02-012, Office of Water.

EPA, 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System.
EPA-833-R-00-003, Office of Wastewater Management.

EPA, 2000. Method Guidance and Recommendations for Whole Effluent (WET) Testing. EPA-821-B-00-04, Office of Water

APHA, 1998. Standard Methods for The Examination of Water and Wastewater. 20th Edition.

APPENDIX A
CHAIN-OF-CUSTODY DOCUMENTS



Bio-Analytical Laboratories

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NELAP/LELAP 01975, ADEQ 88-0630, TCEQ T104704278

Laboratory Use Only:

Company: El Dorado Chemical Company		Phone: (870) 863-1484		Analysis:		Project Number: <i>X6229</i>	
Address: 4500 Norwest Ave., El Dorado, AR 71731		Fax: (870) 863-7499		Fecal Coliform		Temp. upon arrival: 0, 8 °C Therm 9 EGB 12/5/16	
Permit #: AR0000752/AFIN 70-00040		Purchase Order:		Acute Ceriodaphnia		9	
Sampler's Signature/Printed Name/Affiliation: <i>D. L. D. / DAVID SARTAIN / EDCC</i>						Acute Mysid	Preservative: (below)
Date Start Date End	Time Start Time End	C	G	# and type of container	Sample Identification	Lab Control Number: <i>C13353</i>	ICP
12-3-16 - 12-4-16	1025 - 2425	X		6 half gallon	007		
Relinquished by/Affiliation: <i>D. L. D. / EDCC</i>		Date:	Time:	Received by/Affiliation: <i>R. B. S.</i>		Date:	Time: 0935
Relinquished by/Affiliation:		Date:	Time:	Received by/Affiliation:		Date:	Time:
Relinquished by/Affiliation: <i>R. B. S.</i>		Date: 12/5/16	Time: 1130	Received by/Affiliation: <i>Chris Bruepp</i>		Date: 12/5/16	Time: 1130
Method of Shipment: <input checked="" type="checkbox"/> Lab <input type="checkbox"/> Bus <input type="checkbox"/> Fed Ex <input type="checkbox"/> DHL <input type="checkbox"/> UPS <input type="checkbox"/> Client <input type="checkbox"/> Other Tracking # _____							
Comments: <i>Tan, no odor. EGB 12/5/16</i>							
COC Rev. 3.0							

**APPENDIX B
RAW DATA SHEETS**

BIO-ANALYTICAL LABORATORIES
ACUTE TOXICITY TEST WATER QUALITY DATA

X6229
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Project# X6229

Client: EDCC/El Dorado Chemical Company

Address: 4500 Northwest Ave El Dorado AR 71731

NPDES#AR0000752 Outfall 007

Technicians: EGB/PC/MM

Test initiated: Date 12/5/16 Time 1800

Test terminated: Date 12/7/16 Time 1633

Test terminated: Date 12/7/16 Time 1505

Dissolved Oxygen Meter: Model # YSI550A Serial #06E2089 AV

pH Meter: Model #Orion 230A+ Serial #015253

Conductivity Meter: Model # FISHER Serial #130168768

Amperometric Titrator: Model #Fischer-Porter Serial #92W445766

Sample Information

Sample ID#	Initial D.O. (mg/L and %)	Aerate? Minutes/ Final D.O.(mg/L & %)	Total Residual Chlorine (mg/L)	Dechlorinated? Amount?	Ammonia (NH3) mg/L	Salinity	Hardness	Alkalinity	Tech
C13353	10.2 138.1%	Y/10.78 97.7%	1.001	NO	3.0	N/A	104.0	24.5	EGB/PC
↓	9.8 180.0%	Y/11.33 92.9%					↓	↓	PC

Dilution Water Information

Dilution Water	ID#	Initial D.O (mg/L & %)	Aerate? Minutes/D.O (mg/L & %)	Total Residual Chlorine (mg/L)	Ammonia (NH3) mg/L	pH	Hardness	Alkalinity	Tech
Soft H2O	3945	N/A	N/A	N/A	N/A	6.9	44.0	28.0	EGB
↓	3946	N/A	N/A	N/A	N/A	6.8	48.0	28.0	↓

Test Species Information

Test Species Info.	Species: O. pullex ID#: 5A-C1-S1	Species: P. promelas ID#: 5A-C1-M016	Species: ID#:	Species: ID#:
Age	<24 hours	10 days		
Test Container Size	30 ml	250 ml		
Test volume	20 ml	200 ml		
Feeding: Type	Algae/YCT	Artemia		
Amount	>2.0 hrs.prior to initiation	>2.0 hrs prior to initiation		
Aeration?	N/A	N/A		
Condition of survivors	Fair	Good		

Comments:

BIO-ANALYTICAL LABORATORIES ACUTE TOXICITY TEST SURVIVAL AND WATER QUALITY DATA

Project# 1600

Test started: Date 12/5/16

Time 1600

client EDCC

Test ended: Date 12/11/14

Time 150

Sample Description 001

Test Species D. pulex
SDI 0.000 96 hour

ID# BAL Q1-51

Sample Description: Ohour PC 24hour PC
Technician: ~~H. A. T.~~ 24 hour 1908

72hour 96hour
521mm 86hour

Time: Ohour 1000 24hour 1-0
(a) Ohour 24/3 24hour 24.4

72hour _____ 96hour _____
72hour _____ 96hour _____

Temperature (°C): 0hour 15.0 24hour 16.0

2 72hour _____ 96hour _____

ACUTE2 Rev 1.0 2

Chemistry Tech
prerenewal/postrenewal

~~PC~~ ~~PC~~ ~~PC~~ ~~PC~~ ~~PC~~ ~~PC~~

ACUTE2 Rev 1.0 Combined controls from X60228 & X60229. Static electricity causing organisms to stick to sides of cup. EIB 12/7/16

BIO-ANALYTICAL LABORATORIES ACUTE TOXICITY TEST SURVIVAL AND WATER QUALITY DATA

Project# X60229

Test started: Date 12/5/16

Time 1600

Client EJCC

Test ended: Date 12/7/16

Time 1505

Sample Description 007

Test Species Daphnia

ID# BALQ1-S1

Technician: Ohour PC 24hour PC 48hour PC

72hour 96hour

Time: Ohour 1600 24hour 900 48hour 1505

72hour 96hour

Temperature (°C): Ohour 24.5 24hour 24.4 48hour 24.6

72hour 96hour

Test Dilution	Replicate	Test Salinity	# Live Organisms					Dissolved Oxygen						pH						Conductivity						
			0 hr	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96				
0/0																										
45.0	1	NA	8	7	5			8.1	7.8	7.8	7.8	7.8	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3		
	2		8	8	7																					
	3		8	8	2																					
	4		8	7	2																					
	5		8	8	3																					
50.0	1		8	8	1			8.1	7.8	7.8	7.8	7.8	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	
	2		8	8	3																					
	3		8	8	5																					
	4		8	8	5																					
	5		8	8	5																					
Chemistry Tech prerenewal/postrenewal			TC X PC						TC X PC						TC X PC						TC X PC					

BIO-ANALYTICAL LABORATORIES ACUTE TOXICITY TEST SURVIVAL AND WATER QUALITY DATA

Project# X6229
Client EDCC

Sample Description 007
Technician: Ohour PC 24hour TC 48hour PC
Time: Ohour 1600 24hour 1900 48hour 1505
Temperature (°C): Ohour 14.8 24hour 14.4 48hour 14.6 72hour 14.6 96hour 14.6

Test started: Date 12/5/16 Time 1600
Test ended: Date 12/7/16 Time 1505
Test Species D. pulex ID# BALQ1-S

Test Dilution	Replicate	Test salinity	# Live Organisms					Dissolved Oxygen					pH					Conductivity				
			0 hr	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
0/0																						
50.0	1	N/A	8	8	4			8.1	7.9	7.8	7.3	7.3	7.3	7.3	7.3	7.3	7.3	359	329	405		
	2	(8	8	6																	
	3	(8	8	2																	
	4	S	8	8	2																	
	5)	8	8	0																	
75.0	1		8	8	2			8.0	7.9	7.8	7.3	7.3	7.3	7.3	7.3	7.3	7.3	451	426	400		
	2		8	8	5																	
	3		8	8	5																	
	4		8	8	4																	
	5		8	8	7																	
Chemistry Tech prerenewal/postrenewal			TC/FC/RC					TC/FC/RC					TC/FC/RC									

BIO-ANALYTICAL LABORATORIES ACUTE TOXICITY TEST SURVIVAL AND WATER QUALITY DATA

Project# A609

Test started: Date 12/5/16

Time 1000

Client EJCC

Test ended: Date 12/11/04

Time 1505

Sample Description 007 - 20

Test Species SQUID

ID# BAUQ1-S1

Sample Description PC Technician: Ohour PC 24hour PC

72hour _____ 96hour _____
501 hour _____ 96hour _____

Technician: Ohour 24hour
Time: Ohour 1600 24hour 1900

72hour _____ 96hour _____

Temperature ($^{\circ}\text{C}$): 0hour 24.8 24hour 24.4

72hour _____ 96hour _____

Test: DA-Daphnid Acute Test

Test ID: X6229DP

Species: DP-Daphnia pulex

Protocol: EPAAW02-EPA/821/R-02-012

Sample ID: AR0000752007

Sample Type: EFF2-Industrial

Start Date: 12/5/2016

End Date: 12/7/2016

Lab ID: 880630

Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
106	8	3	32						
107	30	5	75						
108	12	2	45						
109	9	4	32						
110	16	1	50						
111	5	5	D-Control						
112	7	2	32						
113	17	2	50						
114	6	1	32						
115	18	3	50						
116	3	3	D-Control						
117	32	2	100						
118	19	4	50						
119	13	3	45						
120	25	5	56						
121	27	2	75						
122	24	4	56						
123	28	3	75						
124	34	4	100						
125	29	4	75						
126	20	5	50						
127	14	4	45						
128	33	3	100						
129	31	1	100						
130	23	3	56						
131	26	1	75						
132	10	5	32						
133	22	2	56						
134	35	5	100						
135	15	5	45						
136	11	1	45						
137	2	2	D-Control						
138	21	1	56						
139	4	4	D-Control						
140	1	1	D-Control						

Comments:

BIO-ANALYTICAL LABORATORIES ACUTE TOXICITY TEST SURVIVAL AND WATER QUALITY DATA

Project# X6229

Client EDCC

Sample Description 007

Technician: Ohour

Time: 1800

Temperature (°C): 24.6

Test started: Date 11/5/16 Time 1800

Test ended: Date 11/7/16 Time 1133

Test Species PomeloS ID# BAL 112016

Technician: PC 24hour PC 48hour PC 72hour PC 96hour

Time: 1800 24hour 1000 48hour 1133 72hour 9600 96hour

Temperature (°C): 24.6 24hour 24.4 48hour 24.6 72hour 24.6 96hour

Test Dilution	Replicate	Test Salinity	# Live Organisms					Dissolved Oxygen						pH						Conductivity					
			0 hr	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
0/0			8	8	8			83	74	74	74	74	74	74	74	74	74	74	74	74	74	74	74	74	
0.5	1	N/A	8	8	8			83	74	74	74	74	74	74	74	74	74	74	74	74	74	74	74	74	
	2		8	8	8																				
	3		8	8	8																				
	4		8	8	8																				
	5		8	8	8																				
32.0	1		8	8	8			8.1	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	
	2		8	8	8																				
	3		8	7	7																				
	4		8	8	7																				
	5		8	8	8																				
Chemistry Tech prerenewal/postrenewal																									
TC TC TC TC TC TC TC TC																									

BIO-ANALYTICAL LABORATORIES ACUTE TOXICITY TEST SURVIVAL AND WATER QUALITY DATA

Project# X6004

Test started: Date 12/5/16 Time 1800

Client EJCC

Test ended: Date 12/7/16 Time 11:03

Sample Prescription 007 PC

Test Species P. promelas ID# BAR 11216

Sample Description 100 Technician: Ohour PC 24hour PC

72hour _____ 96hour _____

Technician: Chirag 24hour 1000
Time: Ohour 1000 24hour 1000

72hour _____ 96hour _____
72hour _____ 96hour _____

Temperature ($^{\circ}\text{C}$): 0hour 14.6 24hour 14.4

72hour _____ 96hour _____

BIO-ANALYTICAL LABORATORIES ACUTE TOXICITY TEST SURVIVAL AND WATER QUALITY DATA

Project# X6229

Client EDCC

Sample Description 007

Technician: ohour TC 24hour TC 48hour TC
 Time: ohour 500 24hour 1000 48hour 1035

Temperature (°C): ohour 24.6 24hour 24.4 48hour 24.6

Test started: Date 12/5/16

Time 1800

Test ended: Date 12/7/16

Time 1433

Test Species PomeloAS ID# BAL112010

Test Dilution	Replicate	Test Salinity	# Live Organisms					Dissolved Oxygen					pH					Conductivity				
			0 hr	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
0/0			8	8	8			81	75	72	70	68	75	71	68	62	85	83	81	75	73	69
56.0	1	N/A	8	8	8																	
	2	(8	7	6																	
	3	(8	8	8																	
	4	(8	8	8																	
	5	(8	8	8																	
75.0	1		8	8	7			8.0	76	71	67	62	7.3	71	67	62	75	74	71	67	62	57
	2		8	8	8																	
	3		8	8	8																	
	4		8	8	8																	
	5		8	8	7																	
Chemistry Tech prerenewal/postrenewal								TC	TC	TC	TC	TC	TC	TC	TC	TC	TC	TC	TC	TC	TC	TC

BIO-ANALYTICAL LABORATORIES ACUTE TOXICITY TEST SURVIVAL AND WATER QUALITY DATA

Project# 1000

Test started: Date 12/5/16 Time 1800

Time (800)

Client: EDCC

Test ended: Date 12/7/16 Time 163

Time 1035

Sample Description 001

Test Species *Platypterus* ID# BAU 0001

Sample Description: Technician: Ohour 24hour

72hour _____ 96hour _____
72hours _____ 96hours _____

Time: Ohour 1800 24hour 1800

72hour _____ 96hour _____
72hour _____ 96hour _____

Temperature ($^{\circ}\text{C}$): 0hour 24.1 24hour 24.7

72hour _____ 96hour _____

ACUTE2 Rev 1.0

Test: AC-Acute Fish Test

Test ID: X6229PP

Species: PP-Pimephales promelas

Protocol: EPAA 91-EPA/600/4-90/027F

Sample ID: AR0000752007

Sample Type: EFF2-Industrial

Start Date: 12/5/2016

Lab ID: 880630

End Date: 12/7/2016

Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
71	6	1		32					
72	31	1		100					
73	12	2		45					
74	3	3	D-Control						
75	21	1		56					
76	28	3		75					
77	30	5		75					
78	7	2		32					
79	18	3		50					
80	10	5		32					
81	35	5		100					
82	1	1	D-Control						
83	8	3		32					
84	5	5	D-Control						
85	25	5		56					
86	13	3		45					
87	11	1		45					
88	27	2		75					
89	19	4		50					
90	23	3		56					
91	22	2		56					
92	20	5		50					
93	15	5		45					
94	17	2		50					
95	24	4		56					
96	16	1		50					
97	9	4		32					
98	33	3		100					
99	4	4	D-Control						
100	2	2	D-Control						
101	14	4		45					
102	26	1		75					
103	34	4		100					
104	32	2		100					
105	29	4		75					

Comments:

APPENDIX C
STATISTICAL ANALYSES

Daphnid Acute Test-48 Hr Survival

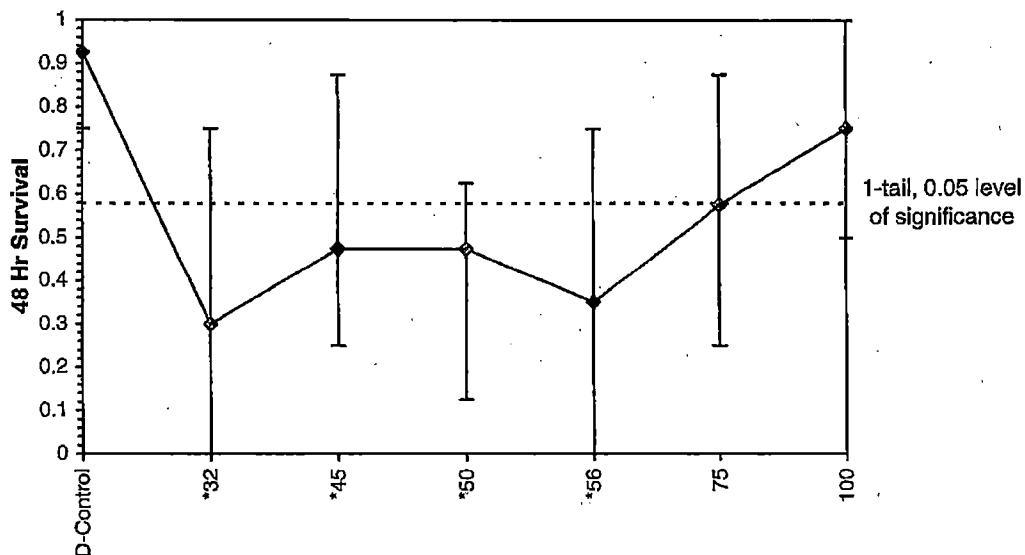
Start Date: 12/5/2016 Test ID: X6229DP Sample ID: AR0000752
 End Date: 12/7/2016 Lab ID: ADEQ880630 Sample Type: EFF2-Industrial
 Sample Date: 12/4/2016 Protocol: EPAAW02-EPA/821/R-02-01 Test Species: DP-Daphnia pulex
 Comments:

Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	1.0000	0.8750	0.7500
*32	0.0000	0.1250	0.2500	0.3750	0.7500
*45	0.6250	0.8750	0.2500	0.2500	0.3750
*50	0.1250	0.3750	0.6250	0.6250	0.6250
*56	0.5000	0.7500	0.2500	0.2500	0.0000
75	0.2500	0.6250	0.6250	0.5000	0.8750
100	1.0000	1.0000	0.5000	0.5000	0.7500

Conc-%	Transform: Arcsin Square Root						1-Tailed			
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	0.9250	1.0000	1.2872	1.0472	1.3931	12.116	5			
*32	0.3000	0.3243	0.5538	0.1777	1.0472	59.474	5	4.179	2.409	0.4227
*45	0.4750	0.5135	0.7655	0.5236	1.2094	38.467	5	2.973	2.409	0.4227
*50	0.4750	0.5135	0.7511	0.3614	0.9117	32.459	5	3.054	2.409	0.4227
*56	0.3500	0.3784	0.6115	0.1777	1.0472	53.223	5	3.850	2.409	0.4227
75	0.5750	0.6216	0.8684	0.5236	1.2094	28.548	5	2.386	2.409	0.4227
100	0.7500	0.8108	1.0808	0.7854	1.3931	28.166	5	1.176	2.409	0.4227

Auxiliary Tests		Statistic		Critical		Skew	Kurt			
Shapiro-Wilk's Test indicates normal distribution ($p > 0.05$)		0.96258		0.934		0.14907	-0.75			
Bartlett's Test indicates equal variances ($p = 0.87$)		2.45091		16.8119						
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		.1	0.34295	0.37209	0.33845	0.077	0.003	6, 28
Treatments vs D-Control										

Dose-Response Plot



Test: DA-Daphnid Acute Test

Test ID: X6229DP

Species: DP-Daphnia pulex

Protocol: EPAAW02-EPA/821/R-02-012

Sample ID: AR0000752

Sample Type: EFF2-Industrial

Start Date: 12/5/2016

End Date: 12/7/2016

Lab ID: ADEQ880630

Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
	1	1	D-Control	8	8	8			
	2	2	D-Control	8	8	8			
	3	3	D-Control	8	8	8			
	4	4	D-Control	8	7	7			
	5	5	D-Control	8	6	6			
	6	1	32	8	8	0			
	7	2	32	8	8	1			
	8	3	32	8	7	2			
	9	4	32	8	8	3			
	10	5	32	8	6	6			
	11	1	45	8	7	5			
	12	2	45	8	8	7			
	13	3	45	8	8	2			
	14	4	45	8	7	2			
	15	5	45	8	8	3			
	16	1	50	8	8	1			
	17	2	50	8	8	3			
	18	3	50	8	8	5			
	19	4	50	8	8	5			
	20	5	50	8	8	5			
	21	1	56	8	8	4			
	22	2	56	8	8	6			
	23	3	56	8	8	2			
	24	4	56	8	8	2			
	25	5	56	8	8	0			
	26	1	75	8	8	2			
	27	2	75	8	8	5			
	28	3	75	8	8	5			
	29	4	75	8	8	4			
	30	5	75	8	8	7			
	31	1	100	8	8	8			
	32	2	100	8	8	8			
	33	3	100	8	8	4			
	34	4	100	8	8	4			
	35	5	100	8	8	6			

Comments: Static electricity in the cups caused a lot of floaters in the test

Acute Fish Test-48 Hr Survival

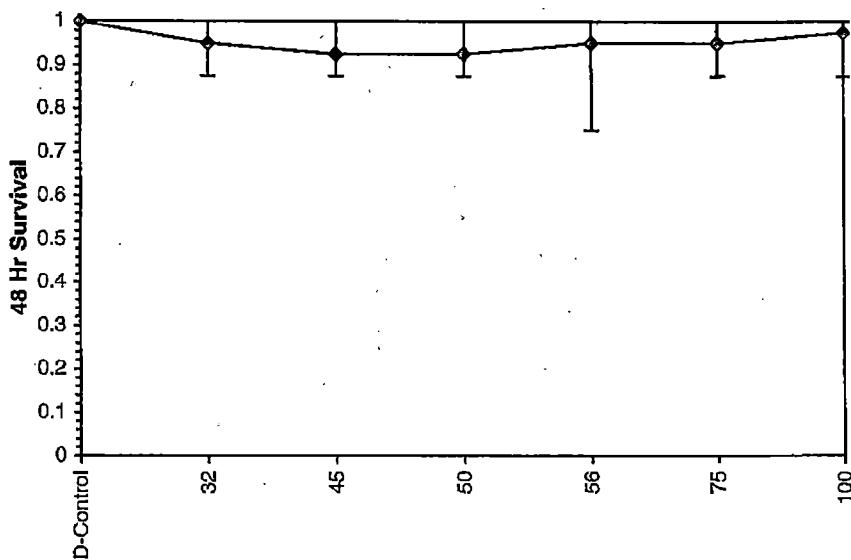
Start Date:	12/5/2016	Test ID:	X6229PP	Sample ID:	AR0000752
End Date:	12/7/2016	Lab ID:	ADEQ880630	Sample Type:	EFF2-Industrial
Sample Date:	12/4/2016	Protocol:	EPAAW02-EPA/821/R-02-01	Test Species:	PP-Pimephales promelas
Comments:					

Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000
32	1.0000	1.0000	0.8750	0.8750	1.0000
45	0.8750	1.0000	0.8750	1.0000	0.8750
50	0.8750	1.0000	0.8750	1.0000	0.8750
56	1.0000	0.7500	1.0000	1.0000	1.0000
75	0.8750	1.0000	1.0000	1.0000	0.8750
100	1.0000	0.8750	1.0000	1.0000	1.0000

Conc-%	Transform: Arcsin Square Root						Rank Sum	1-Tailed Critical
	Mean	N-Mean	Mean	Min	Max	CV%		
D-Control	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5	
32	0.9500	0.9500	1.3196	1.2094	1.3931	7.623	5	22.50 16.00
45	0.9250	0.9250	1.2829	1.2094	1.3931	7.841	5	20.00 16.00
50	0.9250	0.9250	1.2829	1.2094	1.3931	7.841	5	20.00 16.00
56	0.9500	0.9500	1.3239	1.0472	1.3931	11.684	5	25.00 16.00
75	0.9500	0.9500	1.3196	1.2094	1.3931	7.623	5	22.50 16.00
100	0.9750	0.9750	1.3564	1.2094	1.3931	6.055	5	25.00 16.00

Auxiliary Tests		Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.05$)		0.89022	0.934	-0.9302	0.70118
Equality of variance cannot be confirmed					
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	
Steel's Many-One Rank Test	100	>100		1	
Treatments vs D-Control					

Dose-Response Plot



Test: AC-Acute Fish Test
 Species: PP-Pimephales promelas
 Sample ID: AR0000752
 Start Date: 12/5/2016 End Date: 12/7/2016

Test ID: X6229PP
 Protocol: EPAAW02-EPA/821/R-02-012
 Sample Type: EFF2-Industrial
 Lab ID: ADEQ880630

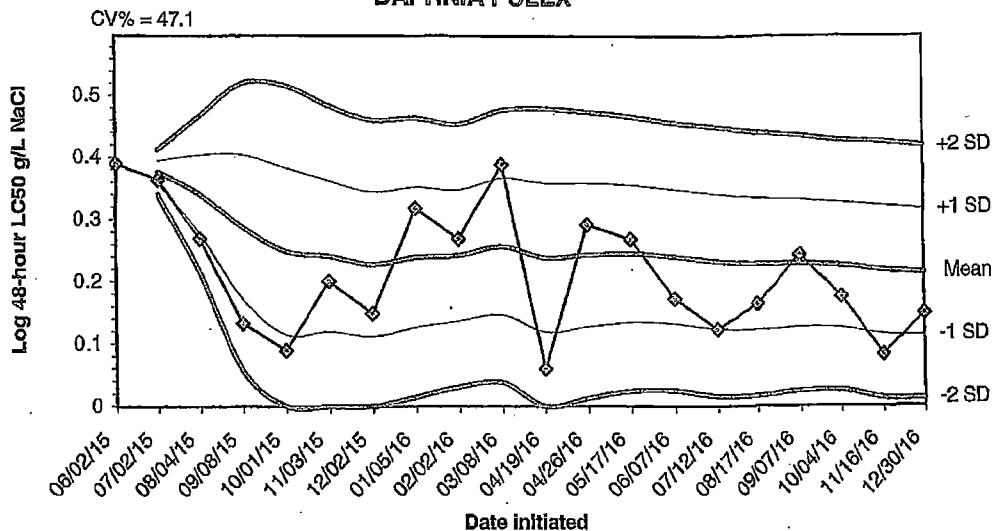
Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
1	1	1	D-Control	8	8	8			
2	2	2	D-Control	8	8	8			
3	3	3	D-Control	8	8	8			
4	4	4	D-Control	8	8	8			
5	5	5	D-Control	8	8	8			
6	1	32		8	8	8			
7	2	32		8	8	8			
8	3	32		8	7	7			
9	4	32		8	8	7			
10	5	32		8	8	8			
11	1	45		8	8	7			
12	2	45		8	8	8			
13	3	45		8	8	7			
14	4	45		8	8	8			
15	5	45		8	7	7			
16	1	50		8	8	7			
17	2	50		8	8	8			
18	3	50		8	7	7			
19	4	50		8	8	8			
20	5	50		8	7	7			
21	1	56		8	8	8			
22	2	56		8	7	6			
23	3	56		8	8	8			
24	4	56		8	8	8			
25	5	56		8	8	8			
26	1	75		8	8	7			
27	2	75		8	8	8			
28	3	75		8	8	8			
29	4	75		8	8	8			
30	5	75		8	8	7			
31	1	100		8	8	8			
32	2	100		8	8	7			
33	3	100		8	8	8			
34	4	100		8	8	8			
35	5	100		8	8	8			

Comments:

EVB
12/26/16

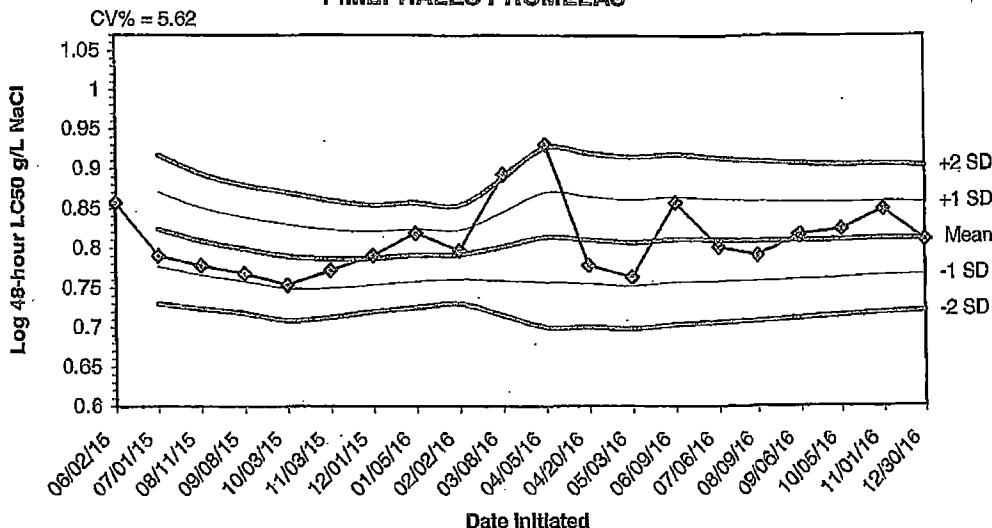
APPENDIX D
QUALITY ASSURANCE CHARTS

**2016 48 HOUR ACUTE REFERENCE TOXICANT TEST RESULTS -
DAPHNIA PULEX**



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
06/02/15	0.3892					
07/02/15	0.3636	0.3764	0.3583	0.3403	0.3945	0.4125
08/04/15	0.2695	0.3408	0.2777	0.2147	0.4038	0.4668
09/08/15	0.1335	0.2890	0.1733	0.0576	0.4046	0.5203
10/01/15	0.0899	0.2491	0.1151	0.0000	0.3832	0.5172
11/03/15	0.2014	0.2412	0.1197	0.0000	0.3626	0.4841
12/02/15	0.1492	0.2281	0.1119	0.0000	0.3442	0.4604
01/05/16	0.3181	0.2393	0.1271	0.0149	0.3515	0.4637
02/02/16	0.2695	0.2427	0.1372	0.0318	0.3481	0.4535
03/08/16	0.3892	0.2573	0.1477	0.0380	0.3670	0.4766
04/19/16	0.0607	0.2394	0.1197	0.0000	0.3592	0.4789
04/26/16	0.2923	0.2438	0.1287	0.0135	0.3590	0.4742
05/17/16	0.2695	0.2458	0.1353	0.0248	0.3563	0.4668
06/07/16	0.1732	0.2406	0.1327	0.0248	0.3485	0.4565
07/12/16	0.1239	0.2328	0.1246	0.0163	0.3411	0.4494
08/17/16	0.1644	0.2286	0.1226	0.0166	0.3346	0.4406
09/07/16	0.2430	0.2294	0.1267	0.0240	0.3321	0.4348
10/04/16	0.1761	0.2264	0.1260	0.0256	0.3269	0.4273
11/16/16	0.0828	0.2189	0.1159	0.0129	0.3219	0.4249
12/30/16	0.1492	0.2154	0.1139	0.0125	0.3169	0.4183

**2016 48 HOUR ACUTE REFERENCE TOXICANT TEST RESULTS -
PIMEPHALES PROMELAS**



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
06/02/15	0.8573					
07/01/15	0.7910	0.8242	0.7772	0.7303	0.8711	0.9180
08/11/15	0.7782	0.8088	0.7663	0.7238	0.8513	0.8938
09/08/15	0.7679	0.7986	0.7583	0.7180	0.8389	0.8792
10/03/15	0.7536	0.7896	0.7493	0.7090	0.8299	0.8701
11/03/15	0.7723	0.7867	0.7500	0.7133	0.8234	0.8601
12/01/15	0.7910	0.7873	0.7538	0.7202	0.8209	0.8544
01/05/16	0.8189	0.7913	0.7583	0.7253	0.8243	0.8573
02/02/16	0.7973	0.7919	0.7610	0.7301	0.8229	0.8538
03/08/16	0.8932	0.8021	0.7587	0.7154	0.8454	0.8887
04/05/16	0.9309	0.8188	0.7572	0.7007	0.8703	0.9269
04/20/16	0.7789	0.8109	0.7560	0.7012	0.8657	0.9206
05/03/16	0.7642	0.8073	0.7532	0.6991	0.8614	0.9155
06/09/16	0.8573	0.8109	0.7572	0.7035	0.8645	0.9182
07/06/16	0.8014	0.8102	0.7584	0.7067	0.8620	0.9138
08/09/16	0.7910	0.8090	0.7588	0.7085	0.8593	0.9095
09/06/16	0.8169	0.8095	0.7608	0.7121	0.8582	0.9069
10/05/16	0.8241	0.8103	0.7629	0.7156	0.8577	0.9050
11/01/16	0.8488	0.8123	0.7655	0.7186	0.8592	0.9061
12/30/16	0.8102	0.8122	0.7666	0.7210	0.8578	0.9035

**APPENDIX E
AGENCY FORMS**

Acute Forms
Daphnia pulex Survival

Permittee: El Dorado Chemical - Outfall 007

NPDES Permit Number: AR0000752/ AFIN 70-00040

Composite Collected From: 12/3/16 To: 12/4/16
From:

Test Initiated: 12/5/16

Dilution Water Used: Receiving Water Reconstituted Water

Dilution Series Results - Percent Survival

TIME OF READING	REP	0	32.0	45.0	50.0	56.0	75.0	100.0
24-hour	A	100.0	100.0	87.5	100.0	100.0	100.0	100.0
	B	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	C	100.0	87.5	100.0	100.0	100.0	100.0	100.0
	D	87.5	100.0	87.5	100.0	100.0	100.0	100.0
	E	75.0	75.0	100.0	100.0	100.0	100.0	100.0
48-hour	A	100.0	0.0	62.5	12.5	50.0	25.0	100.0
	B	100.0	12.5	87.5	37.5	75.0	62.5	100.0
	C	100.0	25.0	25.0	62.5	25.0	62.5	50.0
	D	87.5	37.5	25.0	62.5	25.0	50.0	50.0
	E	75.0	75.0	37.5	62.5	0.0	87.5	75.0
	Mean	92.5	30.0	47.5	47.5	35.0	57.5	75.0

1. Dunnett's Procedure or Steel's Many-One Rank Test as appropriate: Is the mean survival at 48 hours significantly different ($p=.05$) than the control survival for the % effluent corresponding to:

a.) LOW FLOW OR CRITICAL DILUTION (100.0%) YES NO
b.) $\frac{1}{2}$ LOW FLOW OR 2X CRITICAL DILUTION (N/A%) YES / NO

2. Enter percent effluent corresponding to the LC_{50} below:

LC_{50} = N/A % effluent

95 % confidence limits:

Method of LC_{50} calculation:

3. If you answered NO to 1.a) enter (P) otherwise enter (F) P
4. Enter response to item 3 on DMR Form, parameter TEM3D
5. If you answered NO to 1.b) enter (P) otherwise enter (F): N/A
6. Enter response to item 5 on DMR Form, parameter TFM3D

Biomonitoring
Daphnia pulex 48 hour Acute Static Renewal
Chemical Parameters Chart*

Permittee: El Dorado Chemical - Outfall 007

NPDES Number: AR0000752/ AFIN 70-00040

Contact: Eddie Pearson

Analyst: Carter

Sample Collected	From:	Date 12/3/16	Time 1025
	To:	Date 12/4/16	Time 2425
Test Begin		Date 12/5/16	Time 1600
Test End		Date 12/7/16	Time 1505

Parameter	D.O.			Temperature			Alkalinity			Hardness			pH			
	Dilut./Time	0hrs.	24hrs	48hrs	0hrs	24hrs	48hrs	0hrs	24hrs	48hrs	0hrs	24hrs	48hrs	0hrs	24hrs	48hrs
0	8.3	8.2	7.8	24.0	24.4	24.6	28.0			44.0				7.4	7.5	7.3
32.0	8.2	8.2	7.8	24.0	24.4	24.6								7.3	7.3	7.3
45.0	8.1	8.2	7.8	24.0	24.4	24.6								7.3	7.3	7.4
50.0	8.1	8.2	7.8	24.0	24.4	24.6								7.3	7.3	7.4
56.0	8.1	7.2	7.8	24.0	24.4	24.6								7.3	7.3	7.3
75.0	8.0	8.1	7.8	24.0	24.4	24.6								7.3	7.2	7.4
100.0	7.9	8.2	7.8	24.0	24.4	24.6	24.0			104.0				7.3	7.3	7.3

*This Form is to be submitted with each DMR.

Alkalinity and hardness to be reported as mg/l CaCO₃

Acute Forms
Pimephales promelas Survival

Permittee: El Dorado Chemical - Outfall 007

NPDES Permit Number: AR0000752/ AFIN 70-00040

Composite Collected From: 12/3/16 To: 12/4/16
From: To:

Test Initiated: 12/5/16

Dilution Water Used: Receiving Water Reconstituted Water

Dilution Series Results - Percent Survival

TIME OF READING	REP	0	32.0	45.0	50.0	56.0	75.0	100.0
24-hour	A	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	B	100.0	100.0	100.0	100.0	87.5	100.0	100.0
	C	100.0	87.5	100.0	87.5	100.0	100.0	100.0
	D	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	E	100.0	100.0	87.5	87.5	100.0	100.0	100.0
48-hour	A	100.0	100.0	87.5	87.5	100.0	87.5	100.0
	B	100.0	100.0	100.0	100.0	75.0	100.0	87.5
	C	100.0	87.5	87.5	87.5	100.0	100.0	100.0
	D	100.0	87.5	100.0	100.0	100.0	100.0	100.0
	E	100.0	100.0	87.5	87.5	100.0	87.5	100.0
	Mean	100.0	95.0	92.5	92.5	95.0	95.0	97.5

1. Dunnett's Procedure or Steel's Many-One Rank Test as appropriate: Is the mean survival at 48 hours significantly different ($p=.05$) than the control survival for the % effluent corresponding to:

a.) LOW FLOW OR CRITICAL DILUTION (100.0%) YES NO
b.) $\frac{1}{2}$ LOW FLOW OR 2X CRITICAL DILUTION (N/A %) YES NO

2. Enter percent effluent corresponding to the LC_{50} below:

$LC_{50} =$ N/A % effluent

95 % confidence limits:

Method of LC_{50} calculation:

- 3. If you answered NO to 1.a) enter (P) otherwise enter (F) P**
- 4. Enter response to item 3 on DMR Form, parameter TEM3D**
- 5. If you answered NO to 1.b) enter (P) otherwise enter (F): N/A**
- 6. Enter response to item 5 on DMR Form, parameter TFM3D**

Biomonitoring
Pimephales promelas 48 hour Acute Static Renewal
Chemical Parameters Chart*

Permittee: El Dorado Chemical - Outfall 007

NPDES Number: AR0000752/ AFIN 70-00040

Contact: Eddie Pearson

Analyst: Carter

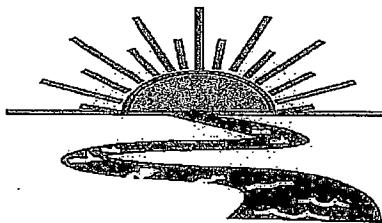
Sample Collected	From:	Date 12/3/16	Time 1025
	To:	Date 12/4/16	Time 2425
Test Begin		Date 12/5/16	Time 1800
Test End		Date 12/7/16	Time 1633

Parameter	D.O.			Temperature			Alkalinity			Hardness			pH			
	Dilut./Time	0hrs.	24hrs	48hrs	0hrs	24hrs	48hrs	0hrs	24hrs	48hrs	0hrs	24hrs	48hrs	0hrs	24hrs	48hrs
0	8.3	8.2	7.8	24.6	24.4	24.6	28.0			44.0				7.4	7.5	7.2
32.0	8.2	8.2	7.4	24.6	24.4	24.6								7.3	7.3	7.2
45.0	8.1	8.2	7.4	24.6	24.4	24.6								7.3	7.3	7.2
50.0	8.1	8.2	7.4	24.6	24.4	24.6								7.3	7.3	7.2
56.0	8.1	7.2	7.4	24.6	24.4	24.6								7.3	7.3	7.2
75.0	8.0	8.1	7.4	24.6	24.4	24.6								7.3	7.2	7.2
100.0	7.9	8.2	7.3	24.6	24.4	24.6	24.0			104.0				7.3	7.3	7.2

*This Form is to be submitted with each DMR.

Alkalinity and hardness to be reported as mg/l CaCO₃

APPENDIX F
REPORT QUALITY ASSURANCE FORM



Bio-Analytical Laboratories

3240 Spurgin Road
Post Office Box 527
Doyline, LA 71023

(318) 745-2772
1-800-259-1246
Fax: (318) 745-2773

REPORT QUALITY ASSURANCE FORM

Client: Eldorado Chemical

Project#: X6229

Chain of Custody Documents Checked by: EB/12-20-16
Technician/Date

Raw Data Documents Checked by: EB/12-20-16
Technician/Date

Statistical Analysis Package Checked by: EB/12-20-16
Quality Manager/Date

Quality Control Data Checked by: EB/12-20-16 / 1/3/17
Quality Manager/Date

Report Checked by: EB 1/5/17
Quality Manager/Date

I certify that this document was prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. The information contained in this document, to the best of my knowledge, is true, accurate and complete.

Erin L. Brugg BS 1/5/17
Quality Manager Date

No part of this work may be altered in any form or by any means without written permission from Bio-Analytical Laboratories.

Report Rev. 3.0



November 22, 2016
Control No. 207100-1R
Page 1 of 32

November 22, 2016

Test Results of
Fourth Quarter
Chronic
Biomonitoring Testing
for
Outfall 010
El Dorado, AR

Control No. 207100-1R

Revised to correct Appendix B

Prepared for:

Mr. Eddie Pearson
El Dorado Chemical Company
4500 North West Avenue
El Dorado, AR 71730

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322



November 22, 2016
Control No. 207100-1R
Page 2 of 32

El Dorado Chemical Company
ATTN: Mr. Eddie Pearson
4500 North West Avenue
El Dorado, AR 71730

Re.: Chronic utilizing *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
Outfall 010 - El Dorado, AR
NPDES Permit No. AR0000752

Dear Mr. Eddie Pearson:

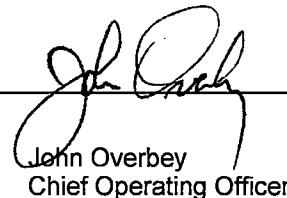
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the Chief Operating Officer or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1000.0 Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: The No Observable Effects Concentration (NOEC) for survival occurred at 2.1 % effluent, which is above the critical dilution of 1.6 %. The NOEC for growth occurred at 2.1 % effluent, which is above the critical dilution of 1.6 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at 2.1 % effluent, which is above the critical dilution of 1.6 %. The NOEC for reproduction occurred at 2.1 % effluent, which is above the critical dilution of 1.6 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Ceriodaphnia dubia test.**

AMERICAN INTERPLEX CORPORATION



A handwritten signature in black ink, appearing to read 'John Overby', is written over a horizontal line. Below the signature, the name 'John Overby' is printed in a smaller, sans-serif font, followed by the title 'Chief Operating Officer'.



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I. Control Acceptance Criteria
Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.269	PASS
Control Growth CV < or = 40%	7.14	PASS
Growth Minimum Significant Difference 12 to 30%	13.0	PASS
Critical Dilution CV < or = 40%	13.1	PASS

Centrodeaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	30.4	PASS
Control CV < or = 40% per Surviving Female	17.8	PASS
Reproduction Minimum Significant Difference 13 to 47%	18.4	PASS
Critical Dilution CV < or = 40%	18.5	PASS

II. Outlined Report
A. Introduction

1. Permit Number: AR0000752
2. Test Requirements: Test Methods 1000.0 and 1002.0
3. Receiving Stream:

B. Source of Effluent/Dilution Water

1. Effluent Samples:
 - a. Sampling Point: Outfall 010
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.5	8.2	8.8
pH (standard units)	7.3	7.5	7.6
Alkalinity (mg/l as CaCO ₃)	21	32	37
Hardness (mg/l as CaCO ₃)	50	51	47
Conductivity (umhos/cm)	570	580	560
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.31	1.1	0.69

2. Dilution Water Samples: Synthetic Soft Water #4374

- a. Dates Prepared:
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.0	8.1	8.2
pH (standard units)	7.6	7.6	7.4
Alkalinity (mg/l as CaCO ₃)	32	31	31
Hardness (mg/l as CaCO ₃)	48	48	48
Conductivity (umhos/cm)	170	160	190
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods

1. Test methods used:

Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Methods 1000.0 and 1002.0, Fathead Minnow Survival and Growth and *Ceriodaphnia dubia* Survival and Reproduction.

2. Endpoint: No Observable Effects Concentration (NOEC)

3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated: November 8, 2016 at 1355
Date & Time Test Terminated: November 15, 2016 at 0750
Type & Volume of Test Chamber: 500 ml disposable beaker
Volume of Sample: 250 ml
Number of Organisms per replicate: 8
Number of Replicates per dilution: 5

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated: November 8, 2016 at 1350
Date & Time Test Terminated: November 15, 2016 at 1130
Type & Volume of Test Chamber: 30 ml disposable beaker
Volume of Sample: 15 ml
Number of Organisms per replicate: 1
Number of Replicates per dilution: 10

4. Acclimation of test organisms: Obtained from in-house cultures

5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms

1. Scientific Name

- a. Test 1000.0 *Pimephales promelas*
- b. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Pimephales promelas (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's. The survival data was then analyzed using Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC).

Fathead minnow growth data was analyzed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's test. Dunnett's Test was used to determine the No Observable Effects Concentration (NOEC) for growth.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and Bartlett's test and analyzed with Dunnett's Test to determine the No Observable Effects Concentration (NOEC) for Reproduction. Dunnett's Test was used to calculate the PMSD.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Pimephales promelas (Fathead minnow)

Chronic reference tests are performed monthly.

A chronic reference test was performed on October 14, 2016 at 1445 to October 18, 2016 at 1040

The results were as follows: (Control No. 206268-1.)

Survival LC-50: 2528 mg/l

Growth IC-25: 1434 mg/l

Growth PMSD: 5.65

Ceriodaphnia dubia

Chronic reference tests are performed monthly.

A chronic reference test was performed on October 14, 2016 at 1330 to October 21, 2016 at 1345

The results were as follows: (Control No. 206268-2.)

Survival LC-50: 1853 mg/l

Growth IC-25: 1055 mg/l

Growth PMSD: 18.8

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	101	2.95
pH	SM 4500-H+ B	101	0.145
Conductivity	EPA 120.1	102	3.28

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: November 8, 2016

Age: <24 hours

Source: In-house culture

Water Chemistry Record:

Alkalinity: 57-64 mg/l

Hardness: 80-100 mg/l

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: November 8, 2016

Age: <24 hours

Source: In-house culture

Water Chemistry Record:

Alkalinity: 57-64 mg/l

Hardness: 80-100 mg/l

Temperature: 25 deg.C

VII. Results Summary *Pimephales promelas*, Fathead minnow Larval Survival and Growth Test -- Method 1000.0

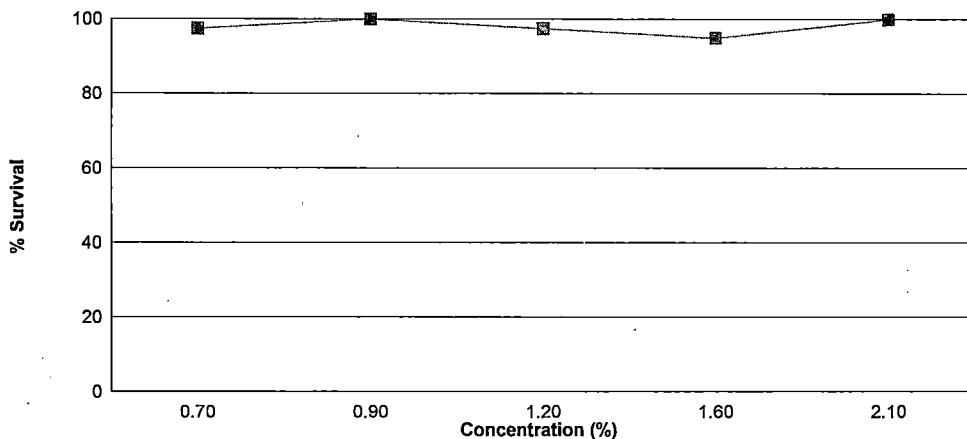
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 0.7 %, 0.9 %, 1.2 %, 1.6 %, 2.1 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 1.6 % effluent.

The test was initiated on November 8, 2016 at 1355 and continued through November 15, 2016 at 0750. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 2.1 % effluent
- b.) NOEC growth = 2.1 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.269
0.7 %	97.5	0.273
0.9 %	100	0.271
1.2 %	97.5	0.306
1.6 %	95.0	0.288
2.1 %	100	0.297

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

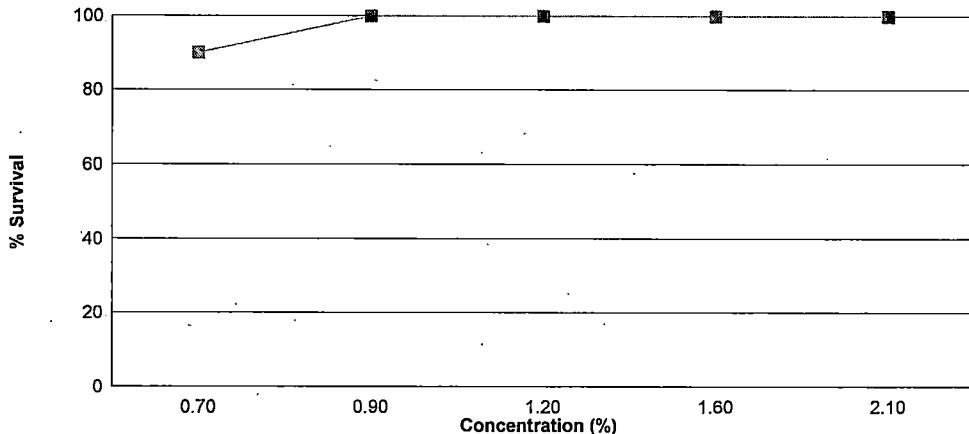
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 0.7 %, 0.9 %, 1.2 %, 1.6 %, 2.1 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 1.6 % effluent.

The test was initiated on November 8, 2016 at 1350 and continued through November 15, 2016 at 1130. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 2.1 % effluent
- b.) NOEC reproduction = 2.1 % effluent



Summary of the 7-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	30.4
0.7 %	90.0	27.4
0.9 %	100	31.5
1.2 %	100	33.4
1.6 %	100	30.9
2.1 %	100	31.4



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Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: November 8, 2016 at 1355
Date and Time Test Terminated: November 15, 2016 at 0750

Concentration Replicate	Number of Survivors						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	8	8	8	8	8	8
	B	8	8	8	8	8	8
	C	8	8	8	8	8	8
	D	8	8	8	8	8	8
	E	7	8	8	8	8	8
0.7 %	A	8	8	8	8	7	7
	B	8	8	8	8	8	8
	C	8	8	8	8	8	8
	D	8	8	8	8	8	8
	E	8	8	8	8	8	8
0.9 %	A	8	8	8	8	8	8
	B	8	8	8	8	8	8
	C	8	8	8	8	8	8
	D	8	8	8	8	8	8
	E	8	8	8	8	8	8
1.2 %	A	8	8	7	7	7	7
	B	8	8	8	8	8	8
	C	8	8	8	8	8	8
	D	8	8	8	8	8	8
	E	8	8	8	8	8	8
1.6 %	A	8	8	8	8	7	7
	B	8	8	8	8	8	8
	C	8	8	7	7	7	7
	D	8	8	8	8	8	8
	E	8	8	8	8	8	8
2.1 %	A	8	8	8	8	8	8
	B	8	8	8	8	8	8
	C	8	8	8	8	8	8
	D	8	8	8	8	8	8
	E	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: November 8, 2016 at 1355
 Test Terminated: November 15, 2016 at 0750

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.91947	.92157	0.00210	8	0.262
	B	.92573	.92796	0.00223	8	0.279
	C	.92623	.92815	0.00192	8	0.240
	D	.91994	.92226	0.00232	8	0.290
	E	.91836	.92057	0.00221	8	0.276
0.7 %	A	.92069	.92270	0.00201	8	0.251
	B	.92208	.92453	0.00245	8	0.306
	C	.92177	.92384	0.00207	8	0.259
	D	.92282	.92501	0.00219	8	0.274
	E	.92015	.92235	0.00220	8	0.275
0.9 %	A	.91636	.91824	0.00188	8	0.235
	B	.92101	.92313	0.00212	8	0.265
	C	.92293	.92506	0.00213	8	0.266
	D	.92637	.92863	0.00226	8	0.282
	E	.92676	.92923	0.00247	8	0.309
1.2 %	A	.93036	.93267	0.00231	8	0.289
	B	.92831	.93071	0.00240	8	0.300
	C	.91847	.92098	0.00251	8	0.314
	D	.92380	.92637	0.00257	8	0.321
	E	.92213	.92457	0.00244	8	0.305
1.6 %	A	.92098	.92299	0.00201	8	0.251
	B	.92068	.92292	0.00224	8	0.280
	C	.92201	.92405	0.00204	8	0.255
	D	.92069	.92326	0.00257	8	0.321
	E	.92275	.92542	0.00267	8	0.334
2.1 %	A	.92484	.92710	0.00226	8	0.282
	B	.91951	.92187	0.00236	8	0.295
	C	.92775	.93019	0.00244	8	0.305
	D	.92737	.92989	0.00252	8	0.315
	E	.92278	.92507	0.00229	8	0.286



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Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: November 8, 2016 at 1350

Date and Time Test Terminated: November 15, 2016 at 1130

Day	Concentration: Control										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	5	0	0	5	10	0.500
4	4	4	6	7	0	4	4	0	4	8	41	10	4.10
5	0	11	0	11	4	7	0	9	10	0	52	10	5.20
6	13	14	12	0	10	11	9	13	13	14	109	10	10.9
7	16	1	15	19	15	14E	13	17E	18E	18	97	10	9.70
8													
TOTAL	33	30	33	37	29	22	26	27	27	40	304	10	30.4

E = Excluded fourth brood neonates

Day	Concentration: 0.7 %										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	1	1	10	0.100
4	7	5	8	9	3	5	6	3	2	5	53	10	5.30
5	0	0	7	11	0	0	8	10	X	5	41	9	4.56
6	13	10	0	0	6	11	0	14	X	8	62	9	6.89
7	17	15	19	17	13	16	16	11E	X	4	117	9	13.0
8													
TOTAL	37	30	34	37	22	32	30	27	2	23	274	10	27.4

E = Excluded fourth brood neonates

Day	Concentration: 0.9 %										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	1	0	0	0	0	0	0	0	0	1	10	0.100
4	8	3	7	6	7	7	6	4	5	6	59	10	5.90
5	0	11	13	9	0	12	13	0	0	10	68	10	6.80
6	12	12	0	0	11	0	0	7	10	0	52	10	5.20
7	18	12E	18	16	13	12	15	9	15	19	135	10	13.5
8													
TOTAL	38	27	38	31	31	31	34	20	30	35	315	10	31.5

E = Excluded fourth brood neonates

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: November 8, 2016 at 1350

Date and Time Test Terminated: November 15, 2016 at 1130

Day	Concentration: 1.2 %										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	7	6	7	6	2	5	6	4	7	6	56	10	5.60
5	0	0	12	0	8	0	9	8	10	14	61	10	6.10
6	13	13	0	9	13	13	0	14	0	0	75	10	7.50
7	19	18	15	18	16E	18	15	14E	20	19	142	10	14.2
8													
TOTAL	39	37	34	33	23	36	30	26	37	39	334	10	33.4

E = Excluded fourth brood neonates

Day	Concentration: 1.6 %										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	3	0	0	0	0	3	0	0	6	10	0.600
4	6	4	0	6	2	6	6	0	5	0	35	10	3.50
5	0	10	10	13	9	11	13	7	0	8	81	10	8.10
6	14	13	15	0	13	1	2	11	7	13	89	10	8.90
7	14	11E	16E	18	12E	17	17	13E	19	13	98	10	9.80
8													
TOTAL	34	27	28	37	24	35	38	21	31	34	309	10	30.9

E = Excluded fourth brood neonates

Day	Concentration: 2.1 %										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	2	5	8	6	4	6	6	9	6	56	10	5.60
5	10	11	14	6	12	11	11	13	12	15	115	10	11.5
6	12	13	0	0	1	13	0	0	0	0	39	10	3.90
7	12E	0	14	14	15	14E	17	16	11	17	104	10	10.4
8													
TOTAL	26	26	33	28	34	28	34	35	32	38	314	10	31.4

E = Excluded fourth brood neonates

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Group	Identification	Transformation of Data		Transform: Arc Sin(Square Root(Y))
		Rep	Value	
1	Control	1	1.00000	1.39310
1	Control	2	1.00000	1.39310
1	Control	3	1.00000	1.39310
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
2	0.7 %	1	0.87500	1.20940
2	0.7 %	2	1.00000	1.39310
2	0.7 %	3	1.00000	1.39310
2	0.7 %	4	1.00000	1.39310
2	0.7 %	5	1.00000	1.39310
3	0.9 %	1	1.00000	1.39310
3	0.9 %	2	1.00000	1.39310
3	0.9 %	3	1.00000	1.39310
3	0.9 %	4	1.00000	1.39310
3	0.9 %	5	1.00000	1.39310
4	1.2 %	1	0.87500	1.20940
4	1.2 %	2	1.00000	1.39310
4	1.2 %	3	1.00000	1.39310
4	1.2 %	4	1.00000	1.39310
4	1.2 %	5	1.00000	1.39310
5	1.6 %	1	0.87500	1.20940
5	1.6 %	2	1.00000	1.39310
5	1.6 %	3	0.87500	1.20940
5	1.6 %	4	1.00000	1.39310
5	1.6 %	5	1.00000	1.39310
6	2.1 %	1	1.00000	1.39310
6	2.1 %	2	1.00000	1.39310
6	2.1 %	3	1.00000	1.39310
6	2.1 %	4	1.00000	1.39310
6	2.1 %	5	1.00000	1.39310

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
D = 0.09449 W = 0.7601 Critical W = 0.9 Critical W = 0.927		(alpha = 0.01, N = 30) (alpha = 0.05, N = 30)

Data FAIL normality test (alpha = 0.01).

Steel's Many-One Rank Test			Transform: Arc Sin(Square Root(Y))		
Ho:Control < Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	0.7 %	25.00	16.00	5.00	
3	0.9 %	27.50	16.00	5.00	
4	1.2 %	25.00	16.00	5.00	
5	1.6 %	22.50	16.00	5.00	
6	2.1 %	27.50	16.00	5.00	

Critical values are 1 tailed (k=5)



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Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
D = 0.01325 W = 0.9736 Critical W = 0.9 Critical W = 0.927	(alpha = 0.01, N = 30) (alpha = 0.05, N = 30)

Data PASS normality test (alpha = 0.01).

Bartlett's Test for Homogeneity of Variance	No Transformation
Calculated B1 statistic = 6.432 Critical B = 15.086	(alpha = 0.01, df = 5)

Data PASS B1 homogeneity test at 0.01 level.

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table					No Transformation
SOURCE	DF	SS	MS	F	
Between	5	0.005723	0.001145	2.072	
Within (Error)	24	0.01326	0.0005525		
Total	29	0.01898			
Critical F = 3.9 (alpha = 0.01, df = 5,24) 2.62 (alpha = 0.05, df = 5,24)					
Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)					

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	0.2694	0.2694		
2	0.7 %	0.273	0.273	-0.2422	
3	0.9 %	0.2714	0.2714	-0.1345	
4	1.2 %	0.3058	0.3058	-2.449	
5	1.6 %	0.2882	0.2882	-1.265	
6	2.1 %	0.2966	0.2966	-1.83	
Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)					

Dunnett's Test - Table 2 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	5			
2	0.7 %	5	0.03508	13	-0.0036
3	0.9 %	5	0.03508	13	-0.002
4	1.2 %	5	0.03508	13	-0.0364
5	1.6 %	5	0.03508	13	-0.0188
6	2.1 %	5	0.03508	13	-0.0272

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
0.7 %	9	1	10
Total	19	1	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 9. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
0.9 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
1.2 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
1.6 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
2.1 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	0.7 %	10	1	
2	0.9 %	10	0	
3	1.2 %	10	0	
4	1.6 %	10	0	
5	2.1 %	10	0	



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Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
D = 0.1258 D* = 0.987 Critical D* = 1.035 Data PASS normality test (alpha = 0.01).	(alpha = 0.01, N = 60)

Bartlett's Test for Homogeneity of Variance	No Transformation
Calculated B1 statistic = 9.522 Critical B = 15.086 Data PASS B1 homogeneity test at 0.01 level.	(alpha = 0.01, df = 5)

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table					No Transformation
SOURCE	DF	SS	MS	F	
Between	5	193.3	38.66	0.9501	
Within (Error)	54	2197	40.69		
Total	59	2390			
Critical F = 3.38 (alpha = 0.01, df = 5,54) 2.38 (alpha = 0.05, df = 5,54)					
Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)					

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	30.4	30.4		
2	0.7 %	27.4	27.4	1.052	
3	0.9 %	31.5	31.5	-0.3856	
4	1.2 %	33.4	33.4	-1.052	
5	1.6 %	30.9	30.9	-0.1753	
6	2.1 %	31.4	31.4	-0.3505	
Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,54)					

Dunnett's Test - Table 2 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	0.7 %	10	6.59	21.7	3
3	0.9 %	10	6.59	21.7	-1.1
4	1.2 %	10	6.59	21.7	-3
5	1.6 %	10	6.59	21.7	-0.5
6	2.1 %	10	6.59	21.7	-1

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Dunnett's Test for PMSD Calculation (excluding deaths if applicable)

	ANOVA Table			No Transformation
SOURCE	DF	SS	MS	F
Between	5	64.73	12.95	0.4638
Within (Error)	53	1480	27.92	
Total	58	1545		
Critical F = 3.39 (alpha = 0.01, df = 5,53) 2.39 (alpha = 0.05, df = 5,53)				
Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)				

Dunnett's Test - Table 1 of 2			No Transformation	
Ho:Control<Treatment				
Group	Identification	Transformed Mean	Mean In Original Units	T Stat
1	Control	30.4	30.4	
2	0.7 %	30.222	30.222	0.07332
3	0.9 %	31.5	31.5	-0.4655
4	1.2 %	33.4	33.4	-1.27
5	1.6 %	30.9	30.9	-0.2116
6	2.1 %	31.4	31.4	-0.4232
Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,53) WARNING - Unequal replicate sizes. Critical values assuming equal replicate sizes have been used.				

Dunnett's Test - Table 2 of 2			No Transformation		
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	0.7 %	9	5.608	18.4	0.178
3	0.9 %	10	5.459	18	-1.1
4	1.2 %	10	5.459	18	-3
5	1.6 %	10	5.459	18	-0.5
6	2.1 %	10	5.459	18	-1

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Effluent Conc.: Control	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.6	8.1	7.8	8.2	8.3
	Final *1	7.3	7.2	7.4	7.8	8.2	7.8
	Final *2	8.0	7.4	7.9	8.4	8.4	8.1
pH, units	Initial	7.6	7.5	7.6	7.5	7.4	7.5
	Final *1	7.3	7.5	7.2	7.3	7.2	7.0
	Final *2	8.0	7.9	7.9	7.9	7.4	7.1
Alkalinity, mg CaCO ₃ /l	32	NA	31	NA	31	NA	NA
Hardness, mg CaCO ₃ /l	48	NA	48	NA	48	NA	NA
Conductivity, umhos/cm	170	160	160	180	190	230	140
Res. Chlorine, mg/l	<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 0.7 %	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.6	8.0	7.8	8.0	8.0
	Final *1	7.2	7.0	7.0	7.3	7.9	7.6
	Final *2	7.8	7.4	8.2	8.0	8.4	8.1
pH, units	Initial	7.6	7.6	7.6	7.6	7.4	7.3
	Final *1	7.3	7.5	7.1	7.2	7.1	7.0
	Final *2	8.1	7.9	8.0	7.9	7.4	7.2

Effluent Conc.: 0.9 %	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.6	7.6	8.0	7.8	7.8	7.8
	Final *1	7.3	7.0	6.7	7.2	7.9	7.7
	Final *2	8.0	7.5	8.2	8.7	8.2	8.1
pH, units	Initial	7.6	7.6	7.6	7.6	7.4	7.3
	Final *1	7.4	7.4	7.1	7.3	7.2	7.0
	Final *2	8.1	8.0	8.0	8.0	7.4	7.2

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Effluent Conc.: 1.2 %	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.8	8.1	7.8	7.5	8.0
	Final *1	7.1	7.1	7.0	7.5	8.0	7.6
	Final *2	7.9	7.4	8.1	8.4	8.6	7.7
pH, units	Initial	7.7	7.6	7.7	7.6	7.5	7.3
	Final *1	7.4	7.5	7.2	7.3	7.2	7.0
	Final *2	8.1	7.9	8.0	7.9	7.4	7.2

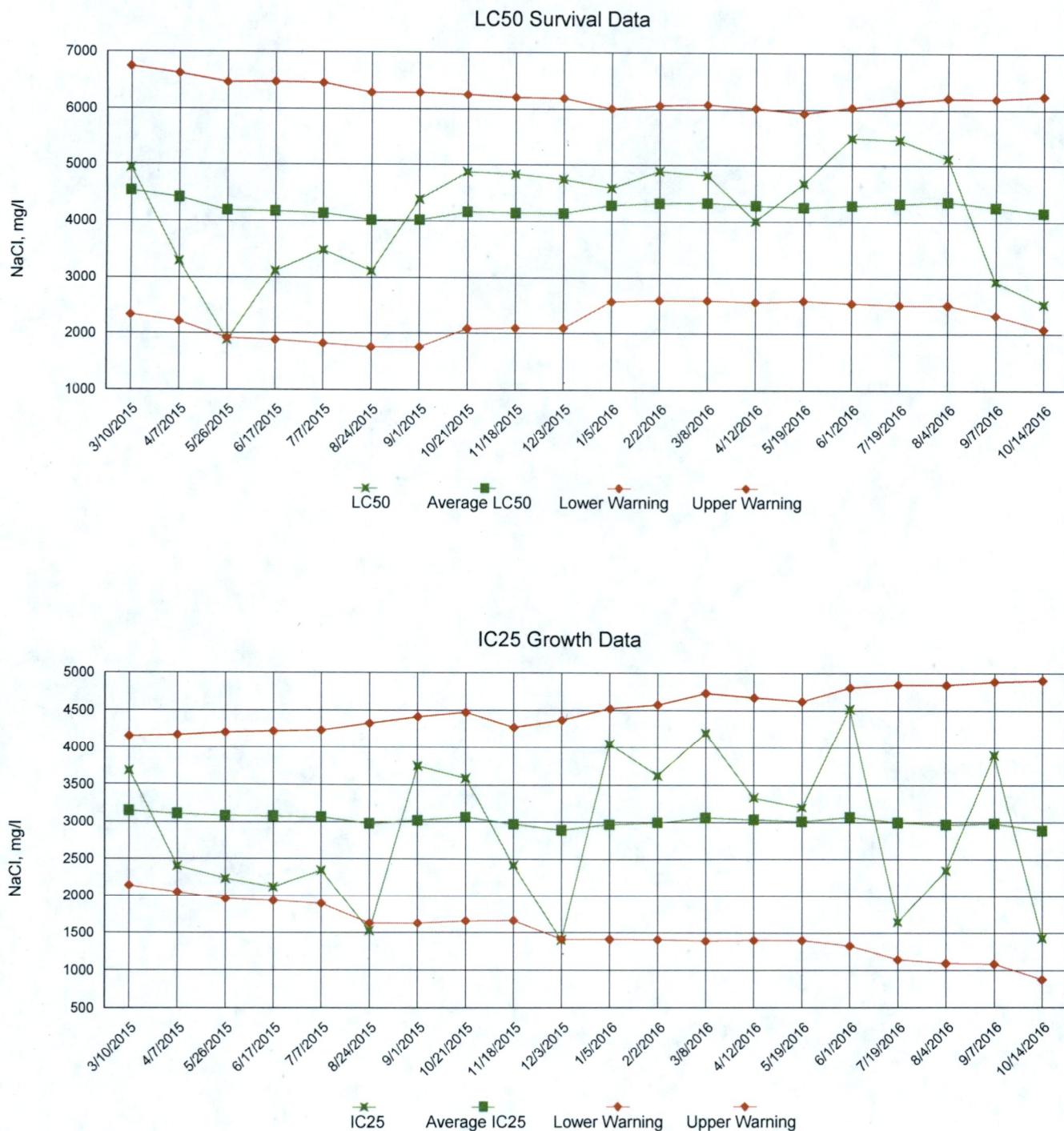
Effluent Conc.: 1.6 %	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.7	8.0	7.8	7.9	8.1
	Final *1	7.5	7.1	7.0	7.5	8.2	7.9
	Final *2	7.9	7.2	8.1	7.8	8.4	8.2
pH, units	Initial	7.6	7.6	7.7	7.6	7.5	7.3
	Final *1	7.4	7.5	7.2	7.3	7.2	7.0
	Final *2	8.2	7.9	8.0	7.9	7.4	7.2
Alkalinity, mg CaCO ₃ /l	29	NA	33	NA	32	NA	NA
Hardness, mg CaCO ₃ /l	46	NA	59	NA	32	NA	NA
Conductivity, umhos/cm	170	160	190	180	140	160	140
Res. Chlorine, mg/l	<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 2.1 %	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.8	7.9	8.0	8.2	8.2
	Final *1	7.5	7.3	7.1	7.3	8.4	7.8
	Final *2	7.9	7.8	8.1	8.0	8.1	8.2
pH, units	Initial	7.7	7.7	7.7	7.6	7.5	7.2
	Final *1	7.4	7.5	7.2	7.3	7.2	7.0
	Final *2	8.1	7.9	8.1	8.0	7.4	7.8

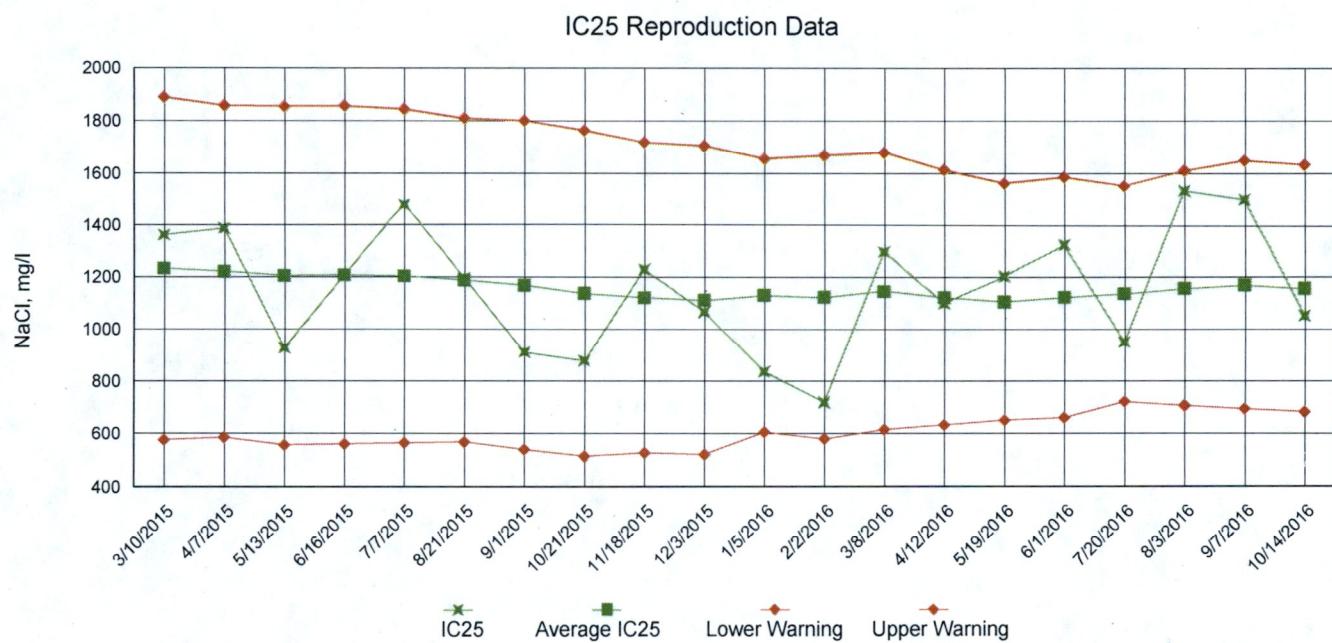
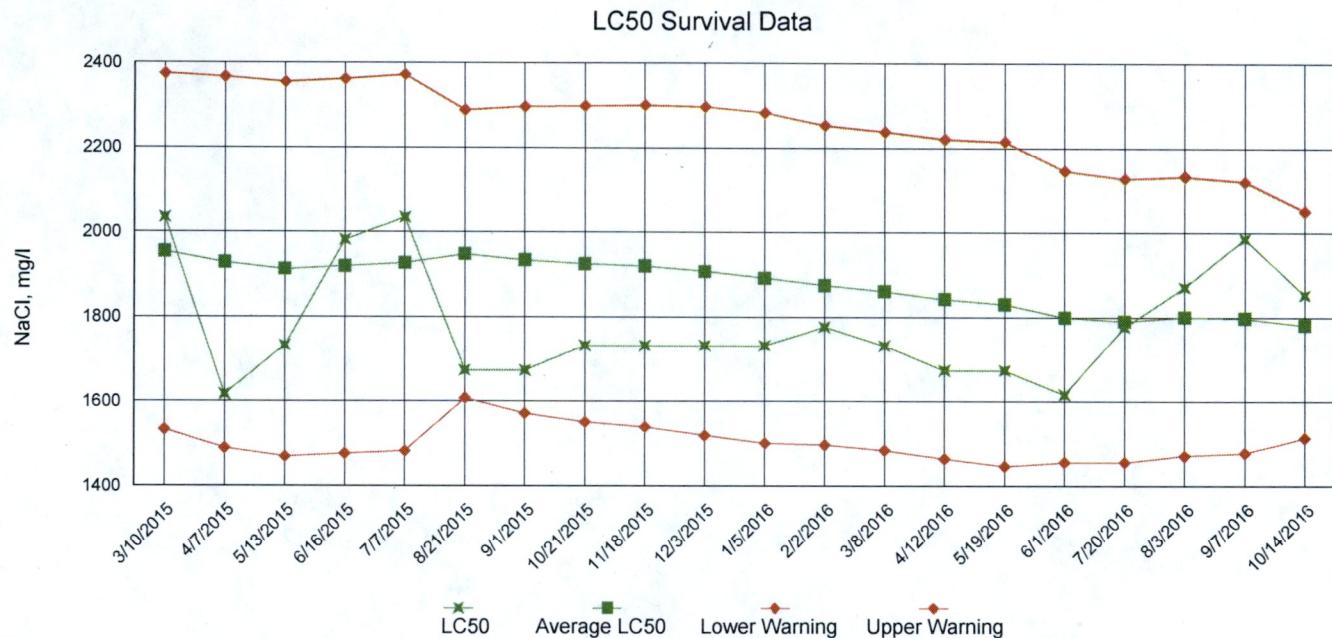
 *1 = data from the *Pimephales promelas* (Fathead Minnow) test

 *2 = data from the *Ceriodaphnia dubia* test

Appendix A4: Test 1000.0
 Chronic Reference Toxicant, *Pimephales promelas* (Fathead Minnow)



Appendix A4: Test 1002.0
 Chronic Reference Toxicant, *Ceriodaphnia dubia*



Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

Permittee: El Dorado Chemical Company

NPDES No.: AR0000752

Date and Time Test Initiated: November 8, 2016 at 1355

Date and Time Test Terminated: November 15, 2016 at 0750

Dilution water used: Synthetic Soft Water #4374

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers					Mean percent survival			CV%
	A	B	C	D	E	24 hr	48 hr	7 days	
Control	100	100	100	100	100	97.5	100	100	0.00
0.7 %	87.5	100	100	100	100	100	100	97.5	5.73
0.9 %	100	100	100	100	100	100	100	100	0.00
1.2 %	87.5	100	100	100	100	100	100	97.5	5.73
1.6 %	87.5	100	87.5	100	100	100	100	95.0	7.21
2.1 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.262	0.279	0.240	0.290	0.276	0.269	7.14
0.7 %	0.251	0.306	0.259	0.274	0.275	0.273	7.71
0.9 %	0.235	0.265	0.266	0.282	0.309	0.271	9.96
1.2 %	0.289	0.300	0.314	0.321	0.305	0.306	4.05
1.6 %	0.251	0.280	0.255	0.321	0.334	0.288	13.1
2.1 %	0.282	0.295	0.305	0.315	0.286	0.297	4.58

CV = Coefficient of variation = standard deviation * 100 / mean

Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

1. Steel's Many-One Rank Test:

Is the mean survival significantly different ($p=0.05$) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(1.6 %)	<input type="checkbox"/> YES	<input checked="" type="checkbox"/> X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<input type="checkbox"/> YES	<input type="checkbox"/> NO

2. Dunnett's Test:

Is the mean dry weight (growth) significantly different ($p=0.05$) than the control's dry weight (growth) for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(1.6 %)	<input type="checkbox"/> YES	<input checked="" type="checkbox"/> X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<input type="checkbox"/> YES	<input type="checkbox"/> NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 0 (TLP6C)

4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 0 (TGP6C)

5. NOEC Pimephales Lethality: 2.1 % (TOP6C)

6. LOEC Pimephales Lethality: 2.1 % (TXP6C)

7. NOEC Pimephales Sublethality: 2.1 % (TPP6C)

8. LOEC Pimephales Sublethality: 2.1 % (TYP6C)

9. Coefficient of variation for Pimephales growth: 13.1 (TQP6C)



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Appendix B: Test 1000.0

CHRONIC TOXICITY SUMMARY FORM
Pimephales promelas (Fathead minnow)
CHEMICAL PARAMETERS CHART

PERMITTEE: El Dorado Chemical Company
NPDES NO.: AR0000752
CONTACT: Mr. Eddie Pearson
ANALYST: 280, 310, 314

Test Initiated: DATE: November 8, 2016 TIME: 1355
Test Terminated: DATE: November 15, 2016 TIME: 0750

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.6	8.1	7.8	8.2	8.3	8.6
Final	7.3	7.2	7.4	7.8	8.2	7.8	7.2
pH Initial	7.6	7.5	7.6	7.5	7.4	7.5	6.9
Final	7.3	7.5	7.2	7.3	7.2	7.0	7.0

DILUTION 0.7 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.6	8.0	7.8	8.0	8.0	8.3
Final	7.2	7.0	7.0	7.3	7.9	7.6	7.2
pH Initial	7.6	7.6	7.6	7.6	7.6	7.4	7.3
Final	7.3	7.5	7.1	7.2	7.1	7.0	7.0

DILUTION 0.9 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	7.6	8.0	7.8	7.8	7.8	8.3
Final	7.3	7.0	6.7	7.2	7.9	7.7	7.6
pH Initial	7.6	7.6	7.6	7.6	7.4	7.3	7.0
Final	7.4	7.4	7.1	7.3	7.2	7.0	7.2

DILUTION 1.2 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.8	8.1	7.8	7.5	8.0	8.6
Final	7.1	7.1	7.0	7.5	8.0	7.6	7.3
pH Initial	7.7	7.6	7.7	7.6	7.5	7.3	7.0
Final	7.4	7.5	7.2	7.3	7.2	7.0	7.1

DILUTION .1.6 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	7.7	8.0	7.8	7.9	8.1	8.4
Final	7.5	7.1	7.0	7.5	8.2	7.9	7.4
pH Initial	7.6	7.6	7.7	7.6	7.5	7.3	7.0
Final	7.4	7.5	7.2	7.3	7.2	7.0	7.2

DILUTION 2.1 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.8	7.9	8.0	8.2	8.2	8.4
Final	7.5	7.3	7.1	7.3	8.4	7.8	8.9
pH Initial	7.7	7.7	7.7	7.6	7.5	7.2	7.1
Final	7.4	7.5	7.2	7.3	7.2	7.0	7.2

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
21	50	570	<0.05	010 07-NOV-16
32	51	580	<0.05	010 09-NOV-16
37	47	560	<0.05	010 11-NOV-16

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	48	170	<0.05	Synthetic Soft Water #4374
31	48	160	<0.05	Synthetic Soft Water #4394

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: El Dorado Chemical Company

NPDES No.: AR0000752

Date and Time Test Initiated: November 8, 2016 at 1350

Date and Time Test Terminated: November 15, 2016 at 1130

Dilution water used: Synthetic Soft Water #4374

PERCENT SURVIVAL

Time of Reading	Control	0.7 %	0.9 %	Percent Effluent 1.2 %	1.6 %	2.1 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
7 day	100	90.0	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	0.7 %	0.9 %	Percent Effluent 1.2 %	1.6 %	2.1 %
A	33	37	38	39	34	26
B	30	30	27	37	27	26
C	33	34	38	34	28	33
D	37	37	31	33	37	28
E	29	22	31	23	24	34
F	22	32	31	36	35	28
G	26	30	34	30	38	34
H	27	27	20	26	21	35
I	27	2	30	37	31	32
J	40	23	35	39	34	38
Mean per Adult	30.4	27.4	31.5	33.4	30.9	31.4
Mean per Surviving Adult	30.4	30.2	31.5	33.4	30.9	31.4
CV %	17.8	18.1	17.0	16.4	18.5	13.2

CV = Coefficient of variation = standard deviation * 100 / mean
(calculated based on young produced by surviving females)

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

1. Fisher's Exact Test:

Is the mean survival significantly different ($p=0.05$) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(1.6 %)	<input type="checkbox"/> YES	<input checked="" type="checkbox"/> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<input type="checkbox"/> YES	<input type="checkbox"/> NO

2. Dunnett's Test:

Is the mean number of young produced per female significantly different ($p=0.05$) than the control's number of young per female for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(1.6 %)	<input type="checkbox"/> YES	<input checked="" type="checkbox"/> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<input type="checkbox"/> YES	<input type="checkbox"/> NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 0 (TLP3B)

4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 0 (TGP3B)

5. NOEC Ceriodaphnia Lethality: 2.1 % (TOP3B)

6. LOEC Ceriodaphnia Lethality: 2.1 % (TXP3B)

7. NOEC Ceriodaphnia Sublethality: 2.1 % (TPP3B)

8. LOEC Ceriodaphnia Sublethality: 2.1 % (TYP3B)

9. Coefficient of variation for Ceriodaphnia Reproduction: 18.5 (TQP3B)



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Appendix B: Test 1002.0

CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
 CHEMICAL PARAMETERS CHART

PERMITTEE: El Dorado Chemical Company
 NPDES NO.: AR0000752
 CONTACT: Mr. Eddie Pearson
 ANALYST: 280, 310, 314

Test Initiated: DATE: November 8, 2016 TIME: 1350
 Test Terminated: DATE: November 15, 2016 TIME: 1130

DILUTION	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.6	8.1	7.8	8.2	8.3	8.6
Final	8.0	7.4	7.9	8.4	8.4	8.1	7.6
pH Initial	7.6	7.5	7.6	7.5	7.4	7.5	6.9
Final	8.0	7.9	7.9	7.9	7.4	7.1	7.9

DILUTION	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.6	8.0	7.8	8.0	8.0	8.3
Final	7.8	7.4	8.2	8.0	8.4	8.1	7.9
pH Initial	7.6	7.6	7.6	7.6	7.6	7.4	7.0
Final	8.1	7.9	8.0	7.9	7.4	7.2	7.8

DILUTION	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	7.6	8.0	7.8	7.8	7.8	8.3
Final	8.0	7.5	8.2	8.7	8.2	8.1	7.6
pH Initial	7.6	7.6	7.6	7.6	7.4	7.3	7.0
Final	8.1	8.0	8.0	8.0	7.4	7.2	7.8

DILUTION	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.8	8.1	7.8	7.5	8.0	8.6
Final	7.9	7.4	8.1	8.4	8.6	8.1	7.7
pH Initial	7.7	7.6	7.7	7.6	7.5	7.3	7.0
Final	8.1	7.9	8.0	7.9	7.4	7.2	7.8

DILUTION	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	7.7	8.0	7.8	7.9	8.1	8.4
Final	7.9	7.2	8.1	7.8	8.4	8.2	7.5
pH Initial	7.6	7.6	7.7	7.6	7.5	7.3	7.0
Final	8.2	7.9	8.0	7.9	7.4	7.2	7.8

DILUTION	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.8	7.9	8.0	8.2	8.2	8.4
Final	7.9	7.8	8.1	8.0	8.1	8.2	7.6
pH Initial	7.7	7.7	7.7	7.6	7.5	7.2	7.1
Final	8.1	7.9	8.1	8.0	7.4	7.4	7.8

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
21	50	570	<0.05	010 07-NOV-16
32	51	580	<0.05	010 09-NOV-16
37	47	560	<0.05	010 11-NOV-16

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	48	170	<0.05	Synthetic Soft Water #4374
31	48	160	<0.05	Synthetic Soft Water #4394

ORIGIN ID:ELDA (870) 863-1400
EDDIE PEARSON
ELDORADO CHEMICAL COMPANY
4500 NORTH WEST AVE

ELDORADO, AR 71730
UNITED STATES US

SHIP DATE: 23JAN17
ACTWGT: 4.00 LB
CAD: 5887030/NET3850

BILL SENDER

TO LAYNE PEMBERTON
ADEQ - WATER ENFORCEMENT
5301 NORTHSHERE DRIVE

NORTH LITTLE ROCK AR 72118

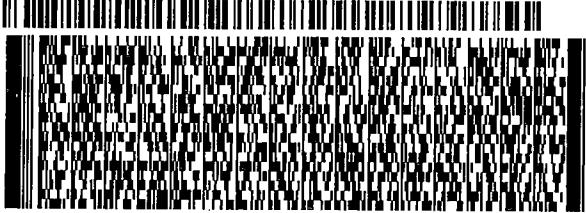
(501) 682-0664

REF:

INV:

PO:

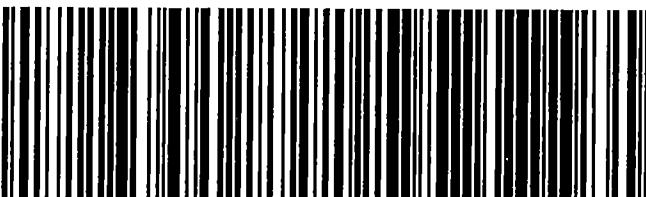
DEPT:



TUE - 24 JAN 10:30A
PRIORITY OVERNIGHT

TRK# 7782 4844 7760
0201

X2 LITA 72118
AR-US LIT



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