Bio-Analytical Laboratories (BAL) ADEQ#88-0630 Project X5788

Bio-Analytical Laboratories' Executive Summary

Permittee:

El Dorado Chemical Company

P.O. Box 231

El Dorado, AR 71731

Project #:

X5788

Outfall:

Outfall 006 (contaminated storm water)

Permit #:

AR0000752/ AFIN #70-00040

Contact:

Mr. David Sartain

Test Dates:

July 5 - 7, 2015

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 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%.

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-1 Fail.
- 2. Report the NOEC for survival, Parameter TOM3D -0.0%.
- 3.Report the highest (critical dilution or control) Coefficient of Variation, Parameter TQM3D 27.54%.

Note: Due to the lack of available neonates less than 24 hours old, only the control and the 100% effluent dilution was set up in this test.

This report contains a total of 31 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 006 AT

EL DORADO CHEMICAL COMPANY El Dorado, Arkansas

> NPDES #AR0000752 AFIN #70-00040

EPA Methods 2000.0 and 2021.0

Project X5788

Test Dates: July 5 - 7, 2015 Report Date: July 16, 2015

Prepared for: Mr. David Sartain El Dorado Chemical Company P.O. Box 231 El Dorado, AR 71731

Prepared by: Ginger Briggs Bio-Analytical Laboratories P.O. Box 527 Doyline, LA 71023 ADEQ #88-0630

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1.0 Introduction

Bio-Analytical Laboratories (BAL), Doyline, Louisiana conducted two 48-hour acute toxicity tests for Outfall 006 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 seven 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.

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 fathead minnow test were 100.0, 75.0, 56.0, 45.0, 32.0 and 22.0 percent effluent and a reconstituted water control. Due to lack of available neonates, the test concentrations used in the *Daphnia pulex* test were 100.0 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 sample of Outfall 006 was collected by El Dorado Chemical personnel on July 4, 2015, at 1200 hours. Upon completion of collection, the sample was packed in ice and personally delivered to the laboratory. The temperature upon arrival was 3.9° 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^{0} Celsius. The total residual chlorine level (SM4500-Cl D 1997) was measured with a Capital Controls^R amperometric titrator and recorded if present. The total ammonia level was measured using a HACH^R test strip. Dissolved oxygen (SM4500-O G 1997), pH (SM4500-H+ B 1997) and conductivity (SM2510-B 1997) measurements 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 on the control and the highest effluent concentration.

2.7 Monitoring of the Tests

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

2.8 Data Analysis

The NOEC and LC_{50} values 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 the minnow test, but were noted in the *Daphnia pulex* test after 48 hours of exposure (p=.05). The NOEC value for the fathead and *Daphnia pulex* test was 100.0 and zero percent effluent, respectively (p=.05). The 48-hour LC₅₀ values could not be calculated in either test because greater than 50.0 percent survival occurred in each effluent concentration. See Appendix C- Statistical Analyses, for more information.

The original control for the daphnid test was actually invalid (i.e. less than 90 percent survival). The control for Project X5788 (EDCC Outfall 007) was used with this. The daphnids were of the same age and came from the same lot of cultures.

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

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Test Organism	Pimephales promelas	Daphnia pulex
Control	97.5	100.0
22.0	100.0	
32.0	97.5	
45.0	97.5	
56.0	92.5	
75.0	97.5	
100.0	100.0	62.5

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.

4.0 Conclusions

The sample of Outfall 006 collected from El Dorado Chemical Company, El Dorado, Arkansas, on July 4, 2015, was not found to be lethally toxic to the fathead minnow test organisms in the 100.0 percent critical dilution after 48 hours of exposure; however, significant lethal effects were noted in the *Daphnia pulex* test (p=.05). The 48-hour LC₅₀ values could not be calculated because greater than 50.0 percent survival occurred in the effluent dilutions (p=.05).

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

3240 Spurgin Road Post Office Box 527 Doyline, LA 71023 (318) 745-277: 1-800-259-124 Fax: (318) 745-277:

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

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APPENDIX B RAW DATA SHEETS

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3749			- . E		7.0	56.0	24.0	EB
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APPENDIX C STATISTICAL ANALYSES

X5788

				Dap	hnid Acute To	est-48 Hr Survival	Page 20 c	of 31
Start Date: End Date: Sample Date: Comments:	7/5/2015 7/7/2015 7/4/2015		Lab ID:	X5788DP ADEQ880 EPAAW02		Sample ID: Sample Type: 02-01 Test Species:	AR0000752-006 EFF2-Industrial DP-Daphnia pulex	
Conc-%	1	2	3	4	5			
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000			
100	0.6050	0.0750	0.7500	O COEO	0.0500			

-			Tra	ansform:	Arcsin Sc	uare Roo	1	Rank	1-Tailed
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
D-Control	1.0000	1.0000	1.3931	1,3931	1.3931	0.000	5		
*100	0.6250	0.6250	0.9207	0.5236	1.2094	27.541	5	15.00	19.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test Indicates non-normal distribution (p <= 0.05)	0.75303	0.842	-1.0509	4.23212
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)				
Wilcoxon Two-Sample Test Indicates significant differences				
Treatments vs D-Control				

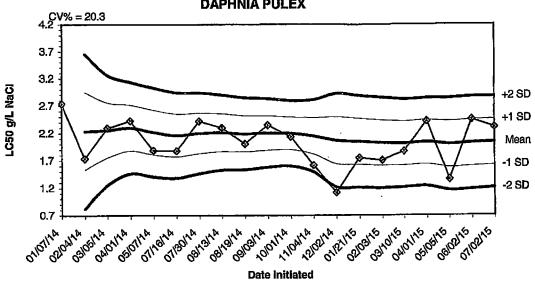
	-						
Start Date:	7/5/2015		Test ID:	X5788PP		Sample ID:	AR0000752-006
End Date:	7/7/2015	1	Lab ID:	ADEQ880	330	Sample Type:	EFF2-Industrial
Sample Date:	7/4/2015		Protocol:	EPAAW02	-EPA/821/F	I-02-01 Test Species:	PP-Pimephales promelas
Comments:						<u></u>	
Conc-%	1	2	3	4	5		
D-Control	1.0000	1.0000	1.0000	1.0000	0.8750		
22	1.0000	1.0000	1.0000	1.0000	1.0000		
32	1.0000	1.0000	0.8750	1.0000	1.0000		
45	1.0000	0.8750	1.0000	1.0000	1.0000		
56	0.7500	1.0000	0.8750	1.0000	1.0000		
75	1.0000	1.0000	1.0000	0.8750	1.0000		
100	1.0000	1.0000	1.0000	1.0000	1.0000		

			Tra	ansform:	Arcsin Sc	quare Roof	ł i	Rank	1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	
D-Control	0.9750	1.0000	1.3564	1.2094	1.3931	6.055	5			
22	1.0000	1.0256	1.3931	1.3931	1.3931	0.000	5	30.00	16.00	
32	0.9750	1.0000	1.3564	1.2094	1.3931	6.055	5	27.50	16.00	
45	0.9750	1.0000	1:3564	1.2094	1.3931	6.055	5	27.50	16.00	
56	0.9250	0.9487	1.2872	1.0472	1.3931	12.116	5	24.50	16.00	
75	0.9750	1.0000	1.3564	1.2094	1.3931	6.055	5	27.50	16.00	
100	1.0000	1.0256	1.3931	1.3931	1.3931	0.000	5	30.00	16.00	

Auxiliary Tests	-	•••			Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates nor	-normal dis	stribution (p <= 0.05)	•	0.77154	0.934	-1.5047	2.13305
Equality of variance cannot be co	nfirmed							
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TŲ				
Steel's Many-One Rank Test	100	>100		1				
Treatments vs D-Control								

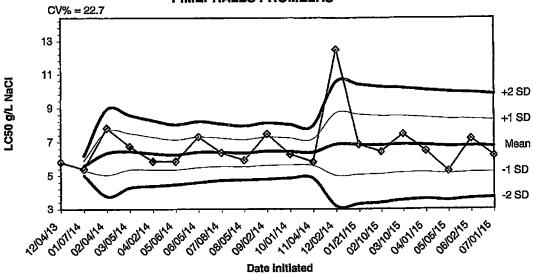
APPENDIX D QUALITY ASSURANCE CHARTS

2015 48-HOUR ACUTE REFERENCE TOXICANT TEST RESULTS USING DAPHNIA PULEX



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
01/07/14	2.7400					1
02/04/14	1.7400	2.2400	1.5329	0.8258	2.9471	3.6542
03/05/14	2.3000	2.2600	1.7588	1.2576	2.7612	3.2624
04/01/14	2.4300	2.3025	1.8845	1.4666	2.7205	3.1384
05/07/14	1.8900	2.2200	1.8137	1.4075	2.6263	3.0325
07/16/14	1.8800	2.1633	1.7744	1.3854	2.5523	2.9413
07/30/14	2.4200	2.2000	1.8319	1.4638	2.5681	2.9362
08/13/14	2.3000	2.2125	1.8699	1.5272	2.5551	2.8978
08/19/14	2.0100	2.1900	1.8625	1.5349	2.5175	2.8451
09/03/14	2.3500	2.2060	1.8931	1.5802	2.5189	2.8318
10/01/14	2.1400	2.2000	1.9025	1.6050	2.4975	2.7950
11/04/14	1.6200	2.1517	1.8223	1.4929	2.4811	2.8105
12/02/14	1.1200	2.0723	1.6465	1.2206	2.4981	2.9240
01/21/15	1.7500	2.0493	1.6312	1.2131	2.4674	2.8855
02/03/15	1.7100	2.0267	1.6144	1.2021	2.4390	2.8513
03/10/15	1.8700	2.0169	1.6166	1.2164	2.4171	2.8174
04/01/15	2.4200	2.0406	1.6409	1.2412	2.4403	2.8399
05/05/15	1.3600	2.0028	1.5832	1.1635	2.4224	2.8420
06/02/15	2.4500	2.0263	1.6058	1.1853	2.4468	
07/02/15	2.3100	2.0405	1.6263	1.2121	2,4547	2.8689

2015 48-HOUR ACUTE REFERENCE TOXICANT TEST RESULTS USING PIMEPHALES PROMELAS



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
12/04/13	5.8100					
01/07/14	5.4000	5.6050	5.3151	5.0252	5.8949	6.1848
02/04/14	7.8200	6.3433	5.0482	3.7530	7.6385	8.9336
03/05/14	6.7500	6.4450	5.3681	4.2913	7.5219	8.5987
04/02/14	5.8600	6.3280	5.3594	4.3908	7.2966	8.2652
05/06/14	5.8600	6.2500	5.3628	4.4757	7.1372	8.0243
06/05/14	7.3100	6.4014	5.4979	4,5944	7.3050	8.2085
07/08/14	6.3700	6.3975	5.5609	4.7243	7.2341	8.0707
08/05/14	5.9200	6.3444	5.5459	4.7473	7.1430	7.9416
09/02/14	7.4800	6.4580	5.6238	4.7897	7.2922	8.1263
10/01/14	6.2800	6.4418	5.6486	4.8555	7.2350	8.0282
11/04/14	5.8100	6.3892	5.6112	4.8333	7.1671	7.9450
12/02/14	(6.8592	5.0079	3,1567	8.7105	10.5618
01/21/15	6.8500	6.8586	5.0799	3,3013	8.6372	10.4159
02/10/15	6.4200	6.8293	5.1116	3,3940	8.5470	10.2647
03/10/15	7.4800	6.8700	5.2026	3.5352	8.5374	10.2048
04/01/15	6.4800	6.8471	5.2298	3.6126	8.4643	10.0815
05/05/15		6.7606	5.1493	3.5380	8.3718	9.9831
06/02/15	1	6.7837	5.2146	3.6454	8.3528	9.9220
07/01/15		6.7535	5.2203	3.6870	8,2867	9.8200

APPENDIX E AGENCY FORMS

Acute Forms Daphnia pulex Survival

Permittee: El Dorado Chemical - Outfall 006

NPDES Permit Number: AR0000752/ AFIN 70-00040

Composite Collected

From: 7/4/15

To: 7/4/15

From:

To:

Test Initiated: 6/18/15

Dilution Water Used:

Receiving Water

X Reconstituted Water

Dilution Series Results - Percent Survival

Committee and an arrangement of the second control of the second c	REP	LEADING	100.0				
24-hour	A	100.0	62.5				
	В	100.0	87.5				
	С	100.0	75.0				
	D	100.0	62.5				
	E	100.0	25.0				
48-hour	A	100.0	62.5				
	В	100.0	87.5			<u> </u>	
	C	100.0	75. 0				
	D	100.0	62.5				
	E	100.0	25.0				
	Mean	100.0	62.5		_		

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%) X YES NO b.)½ 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 LC₅₀ calculation:

- 3. If you answered NO to 1.a) enter (P) otherwise enter (F) F
- 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 006 NPDES Number: AR0000752/ AFIN 70-00040

Contact: David Sartain Analyst: Briggs, Rose

Sample Collected

From:

Date 7/04/15

Date 7/04/15 To:

Time 0800 Time 1200

Test Begin Test End

Date 7/05/15 Date 7/07/15 Time 1130 Time 1043

Parameter		DiO.			lemperatur	e ////		Alkalinity			Hardness			pH.	
Dilut/Time	Service of the servic	3台小校公司的第三人称	多数是是一种的特别的现象	Honor Commence of the Commence	24tirs	48lirs.	¥0lirs	24hrs.	48hrs	Ohrs.	24hrs.	48hrs		24hrs	The state of the s
0	8.0	8.3	8.1	25.5	25.5	25.5	28.0	24.0		48.0	56.0		7.2	7.3	7.3
100.0	7.6	7.8	7.9	25.5	25.5	25.5	20.0			88.0			6.5	6.6	6.9
															
									,						<u> </u>
						_				<u> </u>					

^{*}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 006

NPDES Permit Number: AR0000752/ AFIN 70-00040

Composite Collected

From: 7/4/15

To: 7/4/15

From:

To:

Test Initiated: 6/18/15

Dilution Water Used:

Receiving Water

X Reconstituted Water

Dilution Series Results - Percent Survival

Daniel Color	unon bei	Notebook of the West	NATIONAL CONTRACTOR	Cent Bu	12000	\$7823 CA	D8. 40.55	
TIME OF READING	REP	Ö	22.0	32.0	45:0	56.0	75:0	100.0
24-hour	A	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	В	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	С	100.0	100.0	100.0	100.0	87.5	100.0	100.0
	D	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	E	100.0	100.0	100.0	100.0	100.0	100.0	100.0
48-hour	A	100.0	100.0	100.0	100.0	75.0	100.0	100.0
	В	100.0	100.0	100.0	87.5	100.0	100.0	100.0
	С	100.0	100.0	87.5	100.0	87.5	100.0	100.0
	D	100.0	100.0	100.0	100.0	100.0	87.5	100.0
	E	87.5	100.0	100.0	100.0	100.0	100.0	100.0
	Mean	97.5	100.0	97.5	97.5	92.5	97.5	100.0

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

YES

X NO NO

b.)½ LOW FLOW OR 2X CRITICAL DILUTION (N/A%)

 $LC_{50} =$

2. Enter percent effluent corresponding to the LC_{50} below: N/A% effluent

95 % confidence limits:

Method of LC_{50} calculation:

- 3. If you answered NO to 1.a) enter (P) otherwise enter (F) F
- 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 006 NPDES Number: AR0000752/ AFIN 70-00040

Contact: David Sartain Analyst: Briggs, Rose

Sample Collected Fro

From:

Date 7/04/15

Time 0800

To:

Date 7/04/15 Date 7/05/15 Time 1200 Time 1000

Test Begin Test End

Date 7/07/15

Time 1120

Parameter		D.Θ.			lemperatur	e-		Alkalinity			Hardness		zera i 🖃	ρΗ	
Dilut/Time.	∂0hrs.	24ins.	48hrs.	Ohrs	24hrs	48hrs	Ohrs	24hrs	48hrs	Ohrs'	24hrs.	48hrs	Ohrs	:24hrs	48hrs
0	8.0	8.3	7.7	24.9	24.9	24.9	28.0	24.0		48.0	56.0		7.2	7.3	7.2
22.0	7.9	8.2	7.7	24.9	24.9	24.9						[7.1	7.1	7.1
32.0	7.8	8.1	7.6	24.9	24.9	24.9							7.0	7.0	7.2
45.0	7.9	8.0	7.5	24.9	24.9	24.9				, , , , , , , , , , , , , , , , , , , ,			6.8	6.9	7.1
56.0	7.8	7.9	7.5	24.9	24.9	24.9							6.8	6.9	7.1
75.0	7.9	7.9	7.5	24.9	24.9	24.9							6.8	6.8	7.0
100.0	7.6	7.8	7.5	24.9	24.9	24.9	20.0			88.0			6.5	6.6	6.9

^{*}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: El Dorado Chemical 006 y 57 88
P.C 7/16/15
Project#: X × 5788
• · · ·
Chain of Custody Documents Checked by: RC, 7/16/15
Technician/Date
Raw Data Documents Checked by: PC 7/16/15
Technician/Date
Statistical Analysis Package Checked by: EB 711015
Quality Manager/Date
Quality Control Data Checked by: EGB 7 110 5
Quality Manager/Date
Report Checked by: EGB 7/10/15
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.
Child Burio B5 Thulis
Quality Manager Date

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Report Rev. 3.0