

Report of Short-Term Chronic Toxicity Testing using the Water Flea (*Ceriodaphnia dubia*)

Project ID: 60147216-408-180

January 2011

Sponsor and Laboratory Information

Sponsor	Pilgrim's Pride, Inc. 19740 US Highway 90 West Live Oak, Florida 32060
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Test Information

Test	Short-Term Chronic (Definitive) under Static-Renewal Conditions
Basis	USEPA (2002), method 1002.0
Test Dates and Time	January 20, 2011 @ 1500 to January 26, 2011 @ 1515
Test Length	6 days
Species	<i>Ceriodaphnia dubia</i>
Test Material	Effluent (24-hour Composite)
Facility/Outfall	Outfall D001
Permit Number	FL0001465
Dilution Water	Moderately Hard Reconstituted Water
Test Concentrations	0 (MH), 6.25, 12.5, 25, 50, and 100%
Permit Compliance	<input type="checkbox"/> Pass <input checked="" type="checkbox"/> Fail

- Results described in this report apply only to the samples submitted to the laboratory and analyzed, as listed in the report
- Test results comply with NELAC standards. Reports are intended to be considered in their entirety; AECOM is not responsible for consequences arising from use of a partial report
- This report contains 5 pages plus 3 appendices

Effluent Collection and Receipt

Sample No.	Field No.	Collection Date & Time	AECOM No.	Date of Receipt	Temp. at Arrival (°C)
1	Not Applicable	01/18/11 @ 1100 to 01/19/11 @ 1100	24466	01/20/11	-0.1 ^a
2	Not Applicable	01/20/11 @ 1308 to 01/21/11 @ 1308	24479	01/22/11	0.0 ^a
3	Not Applicable	01/23/11 @ 1100 to 01/24/11 @ 1100	24481	01/25/11	0.0 ^a

^a No ice present in sample upon arrival

Note: See Appendix A for chain of custody records

Effluent Characterization

Sample No.	pH	Hard. (mg/L) ^a	Alk. (mg/L) ^a	Spec. Cond. (µS/cm)	TRC (mg/L) ^b	NH ₃ -N (mg/L)
1	8.0	368	263	1,120	0.02	<1.0
2	8.1	376	241	1,201	<0.02	<1.0
3	8.1	370	263	1,135	<0.02	<1.0

^a As CaCO₃

^b Total residual chlorine

Initial Dilution/Control Water Characterization

Batch No.	pH	Hard. (mg/L) ^a	Alk. (mg/L) ^a	Spec. Cond. (µS/cm)	TRC (mg/L) ^b	NH ₃ -N (mg/L)
9775	8.2	90	63	304	<0.02	<1.0 ^c

^a As CaCO₃

^b Total residual chlorine

^c Measured in source water

Test Conditions

Type	Static-Renewal Short-term Chronic (Definitive)				
Test Endpoints	Survival and reproduction				
Test Chambers	30-ml plastic cups				
Test Solution Volume	15 ml				
Replicates per Treatment	10				
Organisms per Replicate	1				
Test Temperature	25 ± 1°C (≤ 3°C differential)				
Lighting	Fluorescent, 16 hours light:8 hours dark				
Chamber Placement	Random block according to computer-generated chart				
Aeration?	X	No		Yes	
Test Solution Renewal	Daily				

Test Organism

Species	<i>Ceriodaphnia dubia</i>
Age	<24 hours (all within 8 hours of the same age)
Source	FCETL in house cultures, batch 011911
Acclimation	Test temperature
Feeding	0.2 ml YTC/Algae mix per test chamber daily during the test
Reference Toxicant Testing	Initiated January 3, 2011 using sodium chloride (NaCl)

TEST RESULTS

Biological Data

Treatment (% Effluent)	Percent Survival of <i>Ceriodaphnia dubia</i>							Mean Young per Female ^a	Significant Reduction Relative to Control?	
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7		Surv.	Repr.
0 (Control)	100	100	90	90	90	90	N/A	34.3	N/A	N/A
6.25	100	100	100	100	100	100	N/A	39.6	No	No
12.5	100	100	100	100	100	100	N/A	39.5	No	No
25	100	100	100	100	100	100	N/A	32.7	No	No
50	100	100	100	100 ^b	100	100	N/A	37.1	No	No
100	100	100	100	100	100	100	N/A	16.8	No	Yes
Percent Minimum Significant Difference (PMSD, Reproduction)								22.9-23.5	Acceptable	

^a Mean young per original female. If any 4th broods or higher were produced, they were excluded from calculation of mean young per female and statistical analysis of reproduction.

^b One test organism was killed due to technician error and was excluded from analysis of survival and reproduction.
Note: See Appendix B for copies of laboratory data sheets

Data Analysis and Test Endpoints

Biological Endpoint	Statistical Endpoint	Value (% Effluent)
Survival	NOEC	100
	LOEC	>100
	IC ₂₅	>100
Reproduction	NOEC	50
	LOEC	100
	IC ₂₅	67.90

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

ChV = Chronic Value

IC₂₅ = Threshold Inhibition Concentration

Note: Analyses completed using, where appropriate, USEPA Linear Interpolation Method v 2.0 (Norberg-King 1993) and Toxstat v 3.5 (WEST, Inc. and Gulley 1996)

Physical and Chemical Data

Treatment (% Effluent)	pH		Dissolved Oxygen (mg/L)		Conductivity (μS/cm)		Temperature (°C)	
	Low	High	Low	High	Low	High	Low	High
0 (Control)	8.2	8.3	6.5	7.0	301	326	25	26
100	8.0	8.7	6.4	6.9	1,120	1,201	25	26
All Treatments	8.0	8.7	≥6.2		NA		25 ^a	26 ^a
							22.6 ^b	25.0 ^b

^a Temperature in test solutions

^b Continuous temperature in the environmental chamber

Reference Toxicant Test Results for *C. dubia*

IC ₂₅	AECOM/FCETL Historical 95% Control Limits	
	Low	High
472	149	606

Note: Values are expressed as mg/L of chloride.

See Appendix C for reference toxicant summary and control chart.

References

Norberg-King, T. J.. 1993. A linear interpolation method for sublethal toxicity: the inhibition concentration (ICp) approach (version 2.0). National Effluent Toxicity Assessment Center Technical Report 03-93, U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

USEPA. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Fourth Edition. EPA-821-R-02-013.

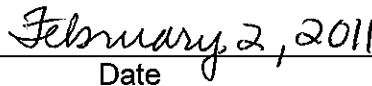
WEST, Inc. and D.D. Gulley. 1996. Toxstat Version 3.5. Western EcoSystems Technology, Inc., Cheyenne, WY.

Statement of Quality Assurance

The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures, and that the resulting data and report meet the requirements of the NELAC standards. This report is an accurate reflection of the raw data.



Quality Assurance Unit



Date



Data Analyst



Date

APPENDIX A
Chain of Custody Records

APPENDIX B

Test Data

TOXICITY DATA PACKAGE COVER SHEET

Test Type: Chronic
Test Substance: Effluent
Dilution Water Type: Mod Hard
Concurrent Control Water Type: NA
Date and Time Test Began: 01/20/11 @ 1500
Protocol Number: CD4FL.WER408.005

Project Number: 60147216-408-180
Species: Ceriodaphnia dubia
Organism Lot or Batch Number: 011911
Age: <24 hr (< 24 hr) Supplier: FCETL
Date and Time Test Ended: 1/26/11 @ 1515
Investigator(s): BP/AMM/SS/CW

Background Information

Type of Test: Static-Renewal
Test Temperature: 25 ± 1 °C
Test Solution Vol.: 15 ml
Length of Test: 3 broods
Type of Food and Quantity per Chamber: 0.2 ml YTC/ALG

pH control?: Yes No
If yes, give % CO₂: N/A
Env. Chmbr/Bath #: 21 Test Chmbrs: 30-ml cups
Number of Replicates per Treatment: 10
Number of Organisms per Replicate: 1
Feeding Frequency: 1 x Daily

Test Substance Characterization Parameters and Frequency:

Hardness: Sx Receipt Alkalinity: Sx Receipt NH₃: Sx Receipt TRC: Sx Receipt
pH: Daily Conductivity: Daily and Test Termination*

Photoperiod: 16 h light : 8 h dark Light Intensity: 50-100 ft-c

Test Concentrations (Volume:Volume): MH, 6.25, 12.5, 25, 50 and 100% effluent

Agency Summary Sheet(s)?: FDEP

Reference Toxicant Data: Test Dates: 1/3/11 to 1/9/11 IC₂₅: 472 mg/L Cl⁻
Hist. 95% Control Limits: 149 to 606 Method for Determining Ref. Tox. Value: Linear Interpolation

Special Procedures and Considerations:

*Conductivity measured in dilution water and 100% effluent at test termination
D.O. maintained ≥ 4.0 mg/L

Study Director Initials: K3N Date: 1/20/11

TEST SUBSTANCE USAGE LOG

Project Number: 60147216-408-180

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	24466	24479	24481	
Test Substance Collection Date and time	From: 1/18/11 @ 1100 To: 1/19/11 @ 1100	From: 1/20/11 @ 1308 To: 1/21/11 @ 1308	From: 1/23/11 @ 1100 To: 1/24/11 @ 1100	From: @ To: @
Sample Type (Grab or Comp)	Comp	Comp	Comp	
Date Test Substance Received	1/20/11	1/22/11	1/25/11	
Dilution Water Number				
QWP or HOPII or dilutions	9775	9775	9775	
Conductance Control Water RW	NA	NA	NA	
Date(s) Used	01/20/11 1/21/11	1/22/11 1/23/11 01/24/11	1/25/11	

Preparation of Test Solutions

Test Substance % Conc.	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)
MH	0	200	200						
6.25%	12.5	187.5	200						
12.5%	25	175	200						
25%	50	150	200						
50%	100	100	200						
100%	200	0	200						
Total	387.5	812.5	1200						
Initial / Date	BP 01/20/11								
Initial / Date	Amc 1/21/11								
Initial / Date	JS 1/22/11								
Initial / Date	JS 1/23/11								
Initial / Date	BP 01/24/11								
Initial / Date	on 1/25/11								
Initial / Date									
Initial / Date									

CERIODAPHNIA DUBIA
CHRONIC BIOLOGICAL DATA

Q13: 9/12/11

Project Number: 60147216-408-180

Number of Neonates Produced and Survival of Original Organisms

Conc.	Day	A	B	C	D	E	F	G	H	I	J	Total	Mean	Remarks
MH	1	0	0	0	0	0	0	0	0	0	0	0		survival 90%
	2	0	0	0	0	0	0	0	0	0	0	0		
	3	0	0	6/1	4/1	5/1	5/1	10/1 X	4/1	0	0	31		
	4	2/1	8/1	0	0	0	0		0	6/1	1/1	17		
	5	14/2	18/2	12/2	13/2	10/2	10/2		15/2	13/2	12/2	117		
	6	19/3	22/3	23/3	20/3	22/3	16/3		19/3	22/3	15/3	178		
	7													
	8													
Total		35	48	41	37	37	31	6	39	41	28	343	34.3	
6.25%	1	0	0	0	0	0	0	0	0	0	0	0		100%
	2	0	0	0	0	0	0	0	0	0	0	0		
	3	6/1	7/1	7/1	4/1	7/1	0	0	7/1	8/1	8/1	54		
	4	0	1/1	1/1	1/1	0	6/1	6/1	0	0	0	15		1 LARGE NEO
	5	15/2	15/2	14/2	11/2	16/2	11/2	16/2	10/2	13/2	13/2	134		
	6	22/3	19/3	22/3	14/3	19/3	21/3	20/3	16/3	21/3	19/3	193		
	7													
	8													
Total		43	42	44	30	42	38	42	33	42	40	396	39.6	
12.5%	1	0	0	0	0	0	0	0	0	0	0	0		100%
	2	0	0	0	0	0	0	0	0	0	0	0		
	3	0	0	7/1	3/1	8/1	7/1	0	6/1	7/1	0	38		
	4	8/1	9/1	0	0	0	0	8/1	0	0	7/1	32		
	5	10/2	16/2	13/2	16/2	11/2	12/2	10/2	13/2	15/2	16/2	126		
	6	20/3	18/3	19/3	20/3	21/3	22/3	19/3	17/3	22/3	21/3	199		
	7													
	8													
Total		38	43	33	33	40	41	37	36	44	44	395	39.5	
Day:	1	2	3	4	5	6	7	8	Key to symbols:					
Date:	1/21/11	1/22/11	1/23/11	1/24/11	1/25/11	1/26/11			X = Original organism died.					
Time:	1145	1120	1325	1030	1120	1515			M = Male					
Initials:	AM	AS	AS	BS	CN	CN								

CW 1/26/11 E

CERIODAPHNIA DUBIA
CHRONIC BIOLOGICAL DATA

DPG 1/28/11

Project Number: 60147216-408-180

Number of Neonates Produced and Survival of Original Organisms

Conc.	Day	A	B	C	D	E	F	G	H	I	J	Total	Mean	Remarks
25%	1	0	0	0	0	0	0	0	0	0	0	0		100%.
	2	0	0	0	0	0	0	0	0	0	0	0		
	3	7/1	5/1	8/1	6/1	5/1	6/1	6/1	0	7/1	0	50		
	4	0	0	0	0	0	0	0	6/1	0	6/1	12		
	5	10/2	13/2	12/2	14/2	12/2	12/2	14/2	13/2	12/2	11/2	123		
	6	14/3	15/3	20/3	19/3	18/3	18/3	19/3	0	18/3	1/2	142 1520		
	7													
	8													
Total		31	33	40	39	35	36	39	19	37	18	327	32.7	
50%	1	0	0	0	0	0	0	0	0	0	0	0		100%.
	2	0	0	0	0	0	0	0	0	0	0	0		
	3	6/1	6/1	6/1	6/1	6/1	6/1	5/1	5/1	6/1	0	52		
	4	0	0	11/2	0	0	0	0	1/1	0	8/1 X	12		ALAB & NEO V.T.E.
	5	14/2	17/2	0	11/2	15/2	11/2	14/2	13/2	11/2		106		
	6	17/3	18/3	21/3	20/3	16/3	17/3	20/3	18/3	17/3		164		
	7													
	8													
Total		37	41	38	37	37	34	39	37	34	TE	334	37.1	n=9
100%	1	0	0	0	0	0	0	0	0	0	0	0		100%.
	2	0	0	0	0	0	0	0	0	0	0	0		
	3	0	0	4/1	5/1	6/1	0	0	0	7/1	0	22		
	4	2/1	2/1	0	0	0	0	3/1	4/1	0	6/1	17		
	5	10/2	6/2	5/2	1/2*	7/2	0*	8/2	10/2	11/2	8/2	66		*under-neos-N
	6	11/3	9/3	11/3	0*	12/3	0*	0	9/3	11/3	0	63		*under-neos-NC
	7													
	8													
Total		23	17	20	6	25	0	11	23	29	14	168	16.8	
Day:	1	2	3	4	5	6	7	8	Key to symbols: NC = not counted					
Date:	1/21/11	1/22/11	1/23/11	1/24/11	1/25/11	1/26/11			X = Original organism died.					
Time:	1145	1120	1305	1030	1120	1515			M = Male					
Initials:	Am	AS	AS	BP	ew	ew								

DPG 1/21/11 E @ CW 1/26/11 E

CHRONIC CHEMICAL DATA (INITIAL)

Project Number:	60147216-408-180
Test Species:	<i>Ceriodaphnia dubia</i>

%	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: MH									All Conc.	
pH	8.2	8.3	8.2	8.2	8.2	8.2			16	
D.O. (mg/L)	6.9	6.8	6.8	6.9	7.0	6.9			5	
Temp. (°C)	25	25	25	25	25	25			D53	
Cond. (µS/cm)	304	301	321	326	313	308			15	
Hard. (mg/L)	90		376			90			Titr	
Alk. (mg/L)	63		241			63			Titr	
TRC (mg/L) 1/20/11	<0.02		<0.02						20	
NH ₃ (mg/L)	<1.0								HA#1	
Conc.: 6.25%										
pH	8.2	8.3	8.2	8.2	8.2	8.2				
D.O. (mg/L)	6.9	6.8	6.8	6.9	7.0	6.9				
Temp. (°C)	*	*	*	*	*	*				
Cond. (µS/cm)	347	348	361	354	360	353				
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH ₃ (mg/L)										
Conc.: 12.5%										
pH	8.2	8.2	8.2	8.2	8.2	8.2				
D.O. (mg/L)	6.9	6.8	6.8	6.9	7.0	6.8				
Temp. (°C)	*	*	*	*	*	*				
Cond. (µS/cm)	405	402	417	406	418	402				
Conc.: 25%										
pH	8.2	8.2	8.2	8.2	8.2	8.2				
D.O. (mg/L)	6.9	6.7	6.8	6.9	6.9	6.8				
Temp. (°C)	*	*	*	*	*	*				
Cond. (µS/cm)	502	503	537	520	520	510				
Date:	1/20/11	1/21/11	1/22/11	1/23/11	01/24/11	1/25/11				
Time:	1445	1135	1100	1255	1005	1050				
Initials:	BP	Am	BS	AS	BP	CW				

Note: Hardness, alkalinity, TRC, and NH₃ data appearing on this page have been transcribed from the wet chemistry log
 FCETL QA Form No. 084.

*Dilution/control water and effluent were brought to 25°C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25°C.

① CW 1/26/11 NA

CHRONIC CHEMICAL DATA (INITIAL)

Project Number:	60147216-408-180
Test Species:	<i>Ceriodaphnia dubia</i>

%	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: 50%									All Conc.	
pH	8.1	8.1	8.1	8.1	8.1	8.1				
D.O. (mg/L)	6.9	6.7	6.7	6.7	6.9	6.8				
Temp. (°C)	*	*	*	*	*	*				
Cond. (µS/cm)	709	709	745	742	743	722				
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.: 100%										
pH	8.0	8.0	8.1	8.1	8.1	8.1				
D.O. (mg/L)	6.9	6.7	6.7	6.6	6.9	6.7				
Temp. (°C)	25	25	25	25	25	25				
Cond. (µS/cm)	1120	1129	1201	1196	1197	1135				
Hard. (mg/L)	308		376			370				
Alk. (mg/L)	263		241			263				
TRC (mg/L)	0.02		<0.02			<0.02				
NH ₃ (mg/L)	<1.0		<1.0			<1.0				
Date:	01/20/11	1/21/11	1/22/11	1/23/11	01/24/11	1/25/11				
Time:	1445	1135	1100	1255	1005	1050				
Initials:	BP	Am	AS	AS	BP	ew				

Note: Hardness, alkalinity, TRC, and NH₃ data appearing on this page have been transcribed from the wet chemistry log FCETL QA Form No. 084.

*Dilution/control water and effluent were brought to 25°C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25°C.

005 1/22/11 E

CHRONIC CHEMICAL DATA (FINAL)

Project Number:	60147216-408-180
Test Species:	<i>Ceriodaphnia dubia</i>

%	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.: MH						* 324			All Conc.	* conductivity
pH	8.2	8.3	8.3	8.2	8.2	8.2			16	
D.O. (mg/L)	6.5	6.6	6.7	6.9	6.8	6.5			5	
Temp (°C)	25	25	26	26	26	26			046	
Conc.: 6.25%										
pH	8.3	8.4	8.3	8.2	8.3	8.3				
D.O. (mg/L)	6.4	6.4	6.5	6.6	6.7	6.3				
Temp (°C)	26	26	26	25	26	25				
Conc.: 12.5%										
pH	8.4	8.4	8.5	8.3	8.4	8.4				
D.O. (mg/L)	6.5	6.6	6.6	6.7	6.7	6.5				
Temp (°C)	26	26	26	26	26	26				
Conc.: 25%										
pH	8.5	8.5	8.5	8.4	8.5	8.5				
D.O. (mg/L)	6.4	6.3	6.7	6.7	6.5	6.5				
Temp (°C)	25	25	26	26	26	26				
Conc.: 50%										
pH	8.6	8.6	8.6	8.5	8.6	8.6				
D.O. (mg/L)	6.2	6.3	6.4	6.8	6.4	6.2				
Temp (°C)	26	26	26	26	26	26				
Conc.: 100%						* 1124				* conductivity
pH	8.7	8.7	8.7	8.6	8.7	8.7				
D.O. (mg/L)	6.5	6.5	6.6	6.7	6.7	6.4				
Temp (°C)	26	25	26	26	26	26				
Conc.:										
pH										
D.O. (mg/L)										
Temp (°C)										
Date:	1/20/11	1/20/11	1/23/11	01/24/11	1/25/11	1/26/11				
Time:	1150	1125	1325	1040	1125	1525				
Initials:	Am	AB	AB	BP	en	en				

0801/23/11 2

DAILY TOXICITY TEST LOG

Project Number:	60147216-408-180
Test Species:	<i>Ceriodaphnia dubia</i>

General Comments	Neonates obtained from <u>011111 HTA + HTB</u> cerio monoculture board (Green marked cups) <u>Random chart: Sheldon</u>	Feeding 0.2 ml YTC/ALG Daily	Initials/Date
Test Day 0	Test Solution Mixed at: <u>1440</u> Test Organisms Added at: <u>1500</u>	Fed @ <u>1450</u>	<u>BP</u> <u>01/20/11</u>
Test Day 1	CT = <u>24.2</u> °C Range = <u>22.6 - 25.0</u> °C	Fed @ <u>1130</u>	<u>Am</u> <u>1/21/11</u>
Test Day 2	CT = <u>24.2</u> °C Range = <u>22.6 - 25.0</u> °C	Fed @ <u>1100</u>	<u>AS</u> <u>1/22/11</u>
Test Day 3	CT = <u>24.0</u> °C Range = <u>22.6 - 24.6</u> °C	Fed @ <u>1315</u>	<u>AS</u> <u>1/23/11</u>
Test Day 4	CT = <u>24.0</u> °C Range = <u>22.6 - 24.6</u> °C	Fed @ <u>1005</u>	<u>BP</u> <u>01/24/11</u>
Test Day 5	CT = <u>24.0</u> °C Range = <u>22.6 - 25.0</u> °C	Fed @ <u>1055</u>	<u>CW</u> <u>1/25/11</u>
Test Day 6	CT = <u>24.0</u> °C Range = <u>22.6 - 24.6</u> °C	Fed @ <u>None</u>	<u>CW</u> <u>1/26/11</u>
Test Day 7	CT = °C Range = °C	Fed @	
Test Day 8	CT = °C Range = °C		

Toxstat version 3.5, Study # 60147216-408-180
 Pilgrim's Pride
 Ceriodaphnia dubia Chronic
 Summary of Reproduction, per original female

Page 9 of 12

CW 1/27/11

QAR GRC 1/28/11

File: 408180RO.dat

Transform:

NO TRANSFORMATION

Summary Statistics on Data

TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	MH	10	6.0000	48.0000	34.3000
2	6.25	10	30.0000	44.0000	39.6000
3	12.5	10	33.0000	44.0000	39.5000
4	25	10	18.0000	40.0000	32.7000
5	50	9	34.0000	41.0000	37.1111
6	100	10	0.0000	29.0000	16.8000

File: 408180RO.dat

Transform:

NO TRANSFORMATION

Summary Statistics on Data

TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	MH	129.5667	11.3827	3.5995	33.1858
2	6.25	21.3778	4.6236	1.4621	11.6758
3	12.5	13.1667	3.6286	1.1475	9.1863
4	25	63.7889	7.9868	2.5256	24.4244
5	50	4.8611	2.2048	0.7349	5.9411
6	100	82.6222	9.0897	2.8744	54.1052

Pilgrim's Pride

CW 11/27/11

Ceriodaphnia dubia Chronic

Determination of NOEC, LOEC for Reproduction, per Original female

CW 11/28/11

File: 408180RO.dat

Transform:

NO TRANSFORMATION

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.9530	14.2780	22.5380	14.2780	3.9530
OBSERVED	6	8	23	21	1

Chi-Square = 9.2005 (p-value = 0.0563)
 Critical Chi-Square = 13.277 (alpha = 0.01, df = 4)
 = 9.488 (alpha = 0.05, df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

Note: too many replicates to run Shapiro-Wilk's Test

File: 408180RO.dat

Transform:

NO TRANSFORMATION

Bartlett's Test for Homogeneity of Variance

Calculated B1 statistic = 25.4478 (p-value = 0.0001)

Data FAIL B1 homogeneity test at 0.01 level. Try another transformation.

Critical B = 15.0863 (alpha = 0.01, df = 5)
 = 11.0705 (alpha = 0.05, df = 5)

Using Average Degrees of Freedom
 (Based on average replicate size of 9.83)

Calculated B2 statistic = 23.7786 (p-value = 0.0002)

Data FAIL B2 homogeneity test at 0.01 level. Try another transformation.

File: 408180RO.dat

Transform:

NO TRANSFORMATION

Wilcoxon's Rank Sum Test w/ Bonferroni Adjustment

Ho: Control < Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	REPS	SIG 0.05
1	MH	34.3000				
2	6.25	39.6000	127.00	74	10	
3	12.5	39.5000	121.50	74	10	
4	25	32.7000	95.00	74	10	
5	50	37.1111	91.50	61	9	
6	100	16.8000	64.50	74	10	*

Critical values are 1 tailed (k = 5)

on 11/27/11

GA: JNE/28/11

File: 408180RO.dat

Transform:

NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	3648.0721	729.6144	13.6469
Within (Error)	53	2833.5889	53.4639	
Total	58	6481.6610		

(p-value = 0.0000)

Critical F = 3.3841 (alpha = 0.01, df = 5,53)
 = 2.3894 (alpha = 0.05, df = 5,53)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

File: 408180RO.dat

Transform:

NO TRANSFORMATION

Bonferroni t-Test - TABLE 1 OF 2

Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	t STAT	SIG 0.05
1	MH	34.3000	34.3000		
2	6.25	39.6000	39.6000	-1.6208	
3	12.5	39.5000	39.5000	-1.5902	
4	25	32.7000	32.7000	0.4893	
5	50	37.1111	37.1111	-0.8367	
6	100	16.8000	16.8000	5.3517	*

Bonferroni t critical value = 2.3988 (1 Tailed, alpha = 0.05, df = 5,53)

Bonferroni t-Test - TABLE 2 OF 2

Ho: Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	MH	10			
2	6.25	10	7.8440	22.9	-5.3000
3	12.5	10	7.8440	22.9	-5.2000
4	25	10	7.8440	22.9	1.6000
5	50	9	8.0589	23.5	-2.8111
6	100	10	7.8440	22.9	17.5000

IC25
Study # 60147216-408-180
Pilgrims Pride
Ceriodaphnia dubia Chronic
IC25 of Reproduction

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CV 11/27/11

QA: JMC 1/28/11

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	6.25	12.5	25	50	100
Response 1	35	43	38	31	37	23
Response 2	48	42	43	33	41	17
Response 3	41	44	39	40	38	20
Response 4	37	30	33	39	37	6
Response 5	37	42	40	35	37	25
Response 6	31	38	41	36	34	0
Response 7	6	42	37	39	39	11
Response 8	39	33	36	19	37	23
Response 9	41	42	44	37	34	29
Response 10	28	40	44	18		14

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: Effluent

Test Start Date: 01/20/11 Test Ending Date: 01/26/11

Test Species: Ceriodaphnia dubia

Test Duration: 6 days

DATA FILE: 408180R.icp

OUTPUT FILE: 408180R.i25

Conc. ID	Number Replicates	Concentration % Effluent	Response Means	Std. Dev.	Pooled Response
1	10	0.000	34.300	11.383	37.800
2	10	6.250	39.600	4.624	37.800
3	10	12.500	39.500	3.629	37.800
4	10	25.000	32.700	7.987	34.789
5	9	50.000	37.111	2.205	34.789
6	10	100.000	16.800	9.090	16.800

The Linear Interpolation Estimate: 67.8979 Entered P Value: 25

Number of Resamplings: 80

The Bootstrap Estimates Mean: 67.6760 Standard Deviation: 4.7627

Original Confidence Limits: Lower: 59.4494 Upper: 77.4953

Resampling time in Seconds: 0.05 Random Seed: 126945681

APPENDIX C

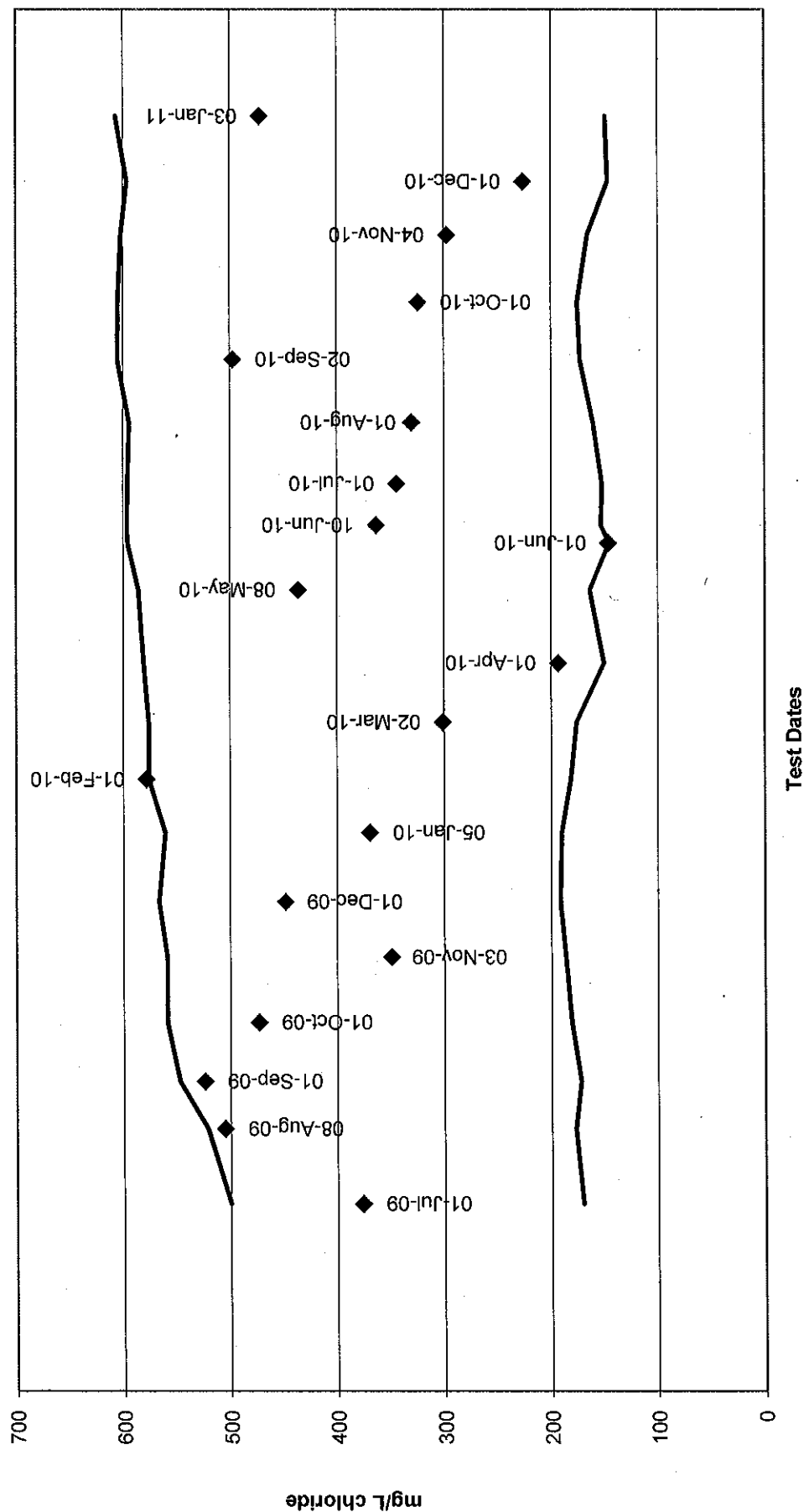
Reference Toxicant Summary and Control Chart

AECOM / FCETL *Ceriodaphnia dubia* Chronic Ref Tox at 25C

IC25

In-House Organisms

July 2009 through January 2011



FILE IS CERIO CHRONIC 60147216-804-097

REFERENCE TOXICANT DATA FOR IN-HOUSE CERIODAPHNIA CHRONICS

SODIUM CHLORIDE - EXPRESSED AS MG/L CL-

AECOM ENVIRONMENT FORT COLLINS ENVIRONMENTAL TOXICOLOGY LABORATORY

GRAPH		ACCEPTABLE RANGE							
DATE	BATCH	IC25	MEAN	SD	LOW	HIGH	%CV	PMSD	MSD
01-Jun-09	053109	348	336.05	83.00	170.04	502.06	24.70	13.2	4.58
01-Jul-09	063009	376	335.05	82.36	170.33	499.77	24.58	33.8	11.77
08-Aug-09	080709	505	349.30	85.98	177.33	521.27	24.62	16.0	5.46
01-Sep-09	083109	524	359.90	93.85	172.20	547.60	26.08	18.2	6.49
01-Oct-09	093009	473	370.05	94.59	180.87	559.23	25.56	26.1	7.99
03-Nov-09	110209	349	372.65	93.18	186.29	559.01	25.00	21.2	7.29
01-Dec-09	113009	448	379.00	93.80	191.40	566.60	24.75	16.1	5.92
05-Jan-10	010410	369	375.35	92.63	190.08	560.62	24.68	17.6	5.98
01-Feb-10	013110	578	378.60	98.66	181.28	575.92	26.06	27.0	9.12
02-Mar-10	030110	301	376.15	100.01	176.13	576.17	26.59	11.4	3.85
01-Apr-10	033110	193	365.40	107.67	150.07	580.73	29.47	21.9	6.95
08-May-10	050710	436	374.45	105.48	163.48	585.42	28.17	31.0	9.76
01-Jun-10	053110	146	370.50	112.61	145.28	595.72	30.39	20.9	6.51
10-Jun-10	060910	363	374.40	110.83	152.74	596.06	29.60	21.1	6.97
01-Jul-10	063010	344	373.75	110.98	151.80	595.70	29.69	18.1	4.96
01-Aug-10	073110	330	377.10	108.44	160.22	593.98	28.76	26.1	7.05
02-Sep-10	090110	497	388.75	108.17	172.42	605.08	27.82	22.8	7.40
01-Oct-10	093010	324	389.80	107.39	175.02	604.58	27.55	26.1	7.95
04-Nov-10	110310	297	383.65	109.08	165.50	601.80	28.43	31.0	8.05
01-Dec-10	113010	226	371.35	112.41	146.53	596.17	30.27	35.9	11.16
03-Jan-11	010211	472	377.55	114.45	148.64	606.46	30.32	36.0	10.29