



### 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
openies:	Live Oak, Florida 32060
Project Officer	Mr. Joseph Jenkins (386) 208-0308
Testing Facility	AECOM Environment Fort Collins Environmental Toxicology Laboratory 4303 West LaPorte Ave. Fort Collins, CO 80521 Fax: (970) 490-2963 State of Florida NELAP Laboratory ID: E87972
Study Director	Rami B. Naddy, Ph.D. (970) 416-0916 email: rami.naddy@aecom.com
Report Author	Christina Needham (970) 416-0916 email: <a href="mailto:christina.needham@aecom.com">christina.needham@aecom.com</a>

#### **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	Ceriodaphnia dubia
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	PassX_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

**AECOM Environment** 60147216-408-180

#### **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 <sup>a</sup>
2	Not Applicable	01/20/11 @ 1308 to 01/21/11 @ 1308	24479	01/22/11	0.0 <sup>a</sup>
3	Not Applicable	01/23/11 @ 1100 to 01/24/11 @ 1100	24481	01/25/11	0.0 <sup>a</sup>

<sup>&</sup>lt;sup>a</sup> No ice present in sample upon arrival

Note: See Appendix A for chain of custody records

#### **Effluent Characterization**

Sample No.	рН	Hard. (mg/L) <sup>a</sup>	Alk. (mg/L) <sup>a</sup>	Spec. Cond. (μS/cm)	TRC (mg/L) <sup>b</sup>	NH <sub>3</sub> -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

#### **Initial Dilution/Control Water Characterization**

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

#### **Test Conditions**

Туре	Static-Renewal Short-term Chronic (Definitive)				
Test Endpoints	Survival a	nd reproduction			
Test Chambers	30-ml plas	tic 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 b	lock according to comp	uter-genera	ited chart	
Aeration?	X	No		Yes	
Test Solution Renewal	Daily				

<sup>&</sup>lt;sup>a</sup> As CaCO<sub>3</sub>
<sup>b</sup> Total residual chlorine

<sup>&</sup>lt;sup>a</sup> As CaCO<sub>3</sub>
<sup>b</sup> Total residual chlorine

<sup>&</sup>lt;sup>c</sup> Measured in source water

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#### **Test Organism**

Species	Ceriodaphnia dubia
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	ent Survival of <i>Ceriodaphnia dubia</i> ent Survival of <i>Ceriodaphnia dubia</i> Mean Young per Female <sup>a</sup> Significan Reductior Relative to						iction ive to	
,	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	remaie	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 <sup>b</sup>	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	Acce	ptable		

<sup>&</sup>lt;sup>a</sup> Mean young per original female. If any 4<sup>th</sup> broods or higher were produced, they were excluded from calculation of mean young per female and statistical analysis of reproduction.

<sup>b</sup> 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

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#### **Data Analysis and Test Endpoints**

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

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

ChV = Chronic Value

 $IC_{25}$  = 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)	рН		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 Tractments	0.0	0.0		0.0	NA		25 <sup>a</sup>	26 <sup>a</sup>
All Treatments	8.0	8.7	≥(	6.2	I IN	A	22.6 <sup>b</sup>	25.0 <sup>b</sup>

<sup>&</sup>lt;sup>a</sup> Temperature in test solutions

#### Reference Toxicant Test Results for C. dubia

10	<b>AECOM/FCETL Historical 95% Control Limits</b>				
IC <sub>25</sub>	Low	High			
472	149	606			

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

See Appendix C for reference toxicant summary and control chart.

<sup>&</sup>lt;sup>b</sup> Continuous temperature in the environmental chamber

#### 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.

Misting Needhar February 2,2011
Data Analyst Date

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#### **APPENDIX A**

**Chain of Custody Records** 

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# CHAIN OF CUSTODY RECORD

A=COM		CH/	VIN OF CUS	CHAIN OF CUSTODY RECORD	ZD			Pag
Client Project Name:	Project Location:	ation:	SIC		Analysis Requested	ednested	Container Type Per Plastic A – Amber Glass G – Clear Glass	!
Project Number 726-408	Field Logbook No.:	ook No.:	·				V – VOA Vial O – Other E – Encore	4 – NaOH, 4° 5 – NaOH/ZnAc, 4°
Sampler (Print Name)/(Affiliation):	Chain of C	Chain of Custody Tape Nos.:						6 - Na2S2O3, 4° 7 - 4°
Horald Davis Pilaring Pride	ride	1532	) (Intact)				Matrix Codes:  DW - Drinking W	
Signature:		Send Results/Report to:	TAT:				WW - Wastewat GW - Groundwa SW - Sruface W	ter SD - Sediment
Hall Dais	<del>-</del>			(			ST – Storm Water W – Water	
	Time M O C	Sample Container Matrix (Size/Mat'l)	Preserv.	Field by Millered	-		Lab I.D.	Remarks
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Signature: Lahl Oc	Time: 12130	Signature: Comanda Bid	le Bidlad	l	Time: 101Š		AECOM Toxicology Lab	
Relinquished by: (Print Name)/(Affiliation)	Date:	Received by: (Print N	Name)/(Affiliation)		Date:		Fort Collins, CO 80521	
Signature:	Time:	Signature:			Time:		(970) 490-2963 (FAX)	(x) campic)
Relinquished by: (Print Name)/(Affiliation)	Date:	Received by: (Print Name)/(Affliation)	Vame)/(Affiliation)		Date:	Sample Shipped Via:	Via:	Temp blank
Signature:	Time:	Signature:		٠	Time:	UPS (FedEx	Courier Other	Yes
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Serial No. 050682

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# **CHAIN OF CUSTODY RECORD**

Lage T or T	Container Type Preservation Plastic 1 - HCl, 4° A Amber Glass 2 - H2SO4, 4° G - Char Glass 3 - Landon	V – VOA Vial 4 – NaOH, 4° O – Other 5 – NaOH/ZhAc, E – Encore 4°	6 – Na2S2O3, 4° 7 – 4°		WW – Wastewater SL – Sludge GW – Groundwater SD − Sediment SW – Surface Water SO – Solid	ST – Storm Water A – Air W – Water – — — — CLiquid P – Product	Lab I.D.	24479 Ph= 7.26	Temp = 21.4 cc	10.6.= 4.34		·					ory (Destination):	AECOM Toxicology, Lab	Fort Collins, CO 80521 NO 1CC.	(970) 490-2963 (FAX) TRESENT		uner Other
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Serial No. 050683

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# CHAIN OF CUSTODY RECORD

Page of	Type Preservation 1 + HCl. 4° 1 - HCl. 4°		6 - Na2S2O3, 4° 7 - 4°		undwater SD - Sediment ace Water SO - Solid n Water A - Air	Remarks	Ph-7.21	4.12-00	<b>-</b>	Do-5.56					0.00 (0-34)	logy Lab NO TCE	80521 present		Temp blank	
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5))[	Flight Project Name:	BOD-9721200	Sampler (Print Name)/(Affiliation):	Harold Davis Pilgrims Pride		-		11 15-12-1	,						Relinquished by: (Print Name) Kaffillation) Hazald Davis Pol grims Pride	Signature: Howly Dan	Relinquished by: (Print Name)/(Affiliation)	Signature:	Relinquished by: (Print Name)/(Affiliation)	Signature:

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**APPENDIX B** 

**Test Data** 

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#### **TOXICITY DATA PACKAGE COVER SHEET**

an: m202/02/11

Test Type:	Chronic		Project Number: 60147216-408-180
Test Substance:	Effluent		Species: <u>Ceriodaphnia dubia</u>
Dilution Water Type:	Mod Hard		Organism Lot of Ratch Number: 01911
Concurrent Control Water Typ	oe:	NA	Age: < 24 hr) Supplier: FCETL
Date and Time Test Began:	01/20/11	@ 1500	Date and Time Test Ended: 1/26 11 @ 1515
Protocol Number:	CD4FL,WE	R408.005	Investigator(s): BRAWL 86 CO
Background Information		-	pH control? Yes
Type of Test:	Static-Rene	wal	If yes, give % CO <sub>2</sub> : N/A
Test Temperature	25 ± 1 °C		Env. Chmbr/Bath #: 21 Test Chmbrs: 30-ml cups
Test Solution Vol.:	15 ml		Number of Replicates per Treatment:10
Length of Test:	3 broods		Number of Organisms per Replicate: 1
Type of Food and Quantity pe	r Chamber:	0.2 ml YTC/AL	G Feeding Frequency: 1 x Daily
Test Substance Characterizat Hardness: <u>Sx Receipt</u> pH: <u>Daily</u>	Alkalinity:	Sx Receipt	•
Test Concentrations (Volume	:Volume):	MH, 6.25, 12.5	, 25, 50 and 100% effluent
Agency Summary Sheet(s)?:	FDEP		<del>-</del>
Reference Toxicant Data:	Test Dates:	V3 II	to 1911 1C25: 472 mg L c1
Hist. 95% Control Limits:Լ	19	to <u>606</u>	Method for Determining Ref. Tox. Value: Linear Interpolation
Special Procedures and Co	nsiderations		
*Conductivity measured in dil	ution water a	nd 100% effluer	nt at test termination
D.O. maintained ≥ 4.0 mg/L			
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Study Director Initials: $ ho$	37	Date: 1/2	20/11

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#### **TEST SUBSTANCE USAGE LOG**

QA: ARO2/02/11

Project Number:

60147216-408-180

	A Semiples (Semiples Company)	Sample/2	A Par Semple 3	a sasShimple/Assassa
เอาเลย (Proprietal Number	24466	24479	24481	
	From: 1/18/11	From: 1/2,0/11	From: 1/23/11	From:
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Preparation of Test Solutions

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25%	50	150	200						
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### CERIODAPHNIA DUBIA CHRONIC BIOLOGICAL DATA

RIA: SPIRIDE/11

Project Number: 60147216-408-180

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	2	0	0	Ö	0	0	0	0	0	0	0	Ó						
	3	0	0	6/1	41	5/1	5/1	%X	91	0	0	31						
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	5	14/2	18/2	12/2	13/2	10/2	10/2		15/2	13/2	12/2	N7						
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	Time:			1325		nao	1515	ļ	ļ		M = Male							
	Initials:	KIN	চন্দ্	A8	BP	(W	CN	<u> </u>	<u></u>	<u></u>								

### CERIODAPHNIA DUBIA CHRONIC BIOLOGICAL DATA

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PB: Opher 1/28/11

Project Number: 60147216-408-180

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Conc.	Day	Α	В	С	D	E	F	G	Н	ı	J	Total	Mean	Remarks
25%	1	σ	Q	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	$e_{l}$	6/1	5/1	6/1	6/1	0	7/1	0	50		,
	4	٥	٥	0	0	0	٥	٥	6/1	0	6/	12		
	5	10/2	13/2	12/2	14/2	12/2	12/2	14/2	13/2	12/2	1/2	123		
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	6	17/3	18 3	21/3	20/3	16/3	17/3	20/3	18/3	17/3	<u> </u>	164		
	7			-										
	8					····							<u> </u>	1
Tota		37	भो	38	37	37	34	39	37	34	TE	334	37.1	ก=ๆ
100%	1	0	0	0	0	O	0	0	0	0	0	O		100%
	2	0	0	0	0		0	$\bigcirc$	0	0	0	<u> </u>		
·	3	2/1	21	4/1	5/1	6/1	0	21	0	7/1	0	22		
	4		2/1	0	1/2*	0	0	3/1	4/1	0	6/1	17		
	5	10/2	9/2	5/2			0 3/7	8/2	10/2	11/2	8/2	66		*under-neos-n
	6	11/3	9/3	11/3	o*	12/3	0*	0	9/3	11/3	0	63.	<u> </u>	*under.neos-NC
	7													
***-	8				,			:		-		11.5		]
Tota		23	17	2C	(g	25	0	11	23	29	14	168	16.8	101
	Day:	<u>1</u> الإيا	2 100/4	3	4 0\/24/11	5 11 1	6	7	8					ot counted
·		(1/10)	'bəlib				126111		<u> </u>	<u></u>		ginal orgar	nism died.	<u> </u>
	Time:		1120	1325	1030 869	100 W	1515	<u> </u>			M = Ma	ale		
	Initials:	MIC	150	80	(D)	I en	ധ	<u></u>	<u> </u>				<del></del> -	

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#### **CHRONIC CHEMICAL DATA (INITIAL)**

OA: AROZ 02/11

Project Number:	60147216-408-180			
Test Species:	Ceriodaphnia dubia			

%	Day	Day	Day	Day	Day	Day	Day	Day	Meter#	Remarks
	0	1	2	3 -	4	5	6	7		· · · · · · · · · · · · · · · · · · ·
Conc.: MH									All Conc.	
pH	8,2	8,3	6.9	<i>B,</i> a	8.2	8.2			16	
D.O. (mg/L)	6.6	6.8	6.8	69	7.0	6.9			5	
Temp. (°C)	25	25	25	25	25	25			D53	
Cond. (µS/cm)	304	301	321	326	813	308			15	
Hard. (mg/L)	90		376			90			Tite	
Alk. (mg/L)	63		241			43			Titr	
TRC (mg/L) /10/11	<0.02		<del>(0.02</del> 0						<b>a</b> 0	
NH <sub>3</sub> (mg/L)	410								HA#1	
Conc.: 6.25%										
рН	8.2	8.3	8.2	B.2	8.2	8.2				
D.O. (mg/L)	6.9	6.8	6.8	6.9	7.0	6.9				
Temp. (°C)	*	*	K	*	*	*				
Cond. (µS/cm)	347	348	361	354	360	35 <i>3</i>				
Hard. (mg/L)								·		
Alk. (mg/L)							-			
TRC (mg/L)					-					
NH <sub>3</sub> (mg/L)										
Conc.: 12.5%										*
рН	8.2	8.2	8.2	8.2	8.2	8.2				
D.O. (mg/L)	6.9	6.8	6.8	69	4.0	6.8				
Temp. (°C)	*	*	R	K	*	*				
Cond. (µS/cm)	405	408	417	406	418	402				
Conc.: 25%										
рН	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)	*	*	X	À	*	*				
Cond. (µS/cm)	502	503	537	520	520	510				
Date:	alzolu	1/21/11	1/22/11	12311	0/24/10	1/25/11				
Time:	1445	1185	1100	1255	1005	1050				
Initials	BP	Am	ઉદ	A8	BP	డు	·			

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

<sup>\*</sup>Dillution/control water and effluent were brought to 25°C prior to making the dillution series. The temperature of resulting effluent dilution is assumed to also be 25°C.

#### **CHRONIC CHEMICAL DATA (INITIAL)**

aA: AROZ/02/11

Project Number:	60147216-408-180		
Test Species:	Ceriodaphnia dubia		

%		Day	Day	Day 2	Day 3	Day	Day 5	Day	Day	Meter#	Remarks
		0	1	2	3	4	5	6	7	1	·
Conc.:	50%									All Conc.	
рH		8.1	8.1	8.1	8.1	8.1	8-1				
D.O. (mg/L)		69	6.7	8.1	<i>(0.</i> 7	6.9	6.8				
Temp. (°C)		*	<b>X</b> .	A .	A I	*	*			-	
Cond. (µS/cm)		709	709	745	742	743	722				
Conc.:											
рН											
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm)											
Conc.:											
рН											
D.O. (mģ/L)											
Temp. (°C)											
Cond. (µS/cm)											•
Conc.:											
рН											
D.O. (mg/L)											_
Temp. (°C)											
Cond. (µS/cm)		-									
Conc.:	100%										
рН		8.0	8.0	8.	8.1	8	8.				
D.O. (mg/L)		6.9	20 10 10 10	6.	6.6	6.9	6.7				
Temp. (°C)		26	S	လ	25	25	25				
Cond. (µS/cm)		1120	1129	1201	1196	1197	1135				
Hard. (mg/L)		308		376			370				
Alk. (mg/L)		263		241			203				
TRC (mg/L)		0,02	,	~0.0Z			<0.02	-			
NH <sub>3</sub> (mg/L)		21.0		41.0			0.ا				
	Date:	culsolu	1/21/11	1/00/11	1/03/11	01/24/11	125 11				
	Time:	1445	1135	1100	1255	1005					
	Initials:	BP	Am	08	AS	108	లు				

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

<sup>\*</sup>Dillution/control water and effluent were brought to 25°C prior to making the dillution series. The temperature of resulting effluent dilution is assumed to also be 25°C.

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CHRONIC CHEMICAL DATA (FINAL)

QA	: AR02/0	2/11
----	----------	------

Project Number:	60147216-408-180	***************************************	
Test Species:	Ceriodaphnia dubia		

%	Day	Day	Day	Day	Day	Day	Day	Day	Meter#	Remarks
	1	2	3	4	5	6 **	7	8		
Conc.: MH	1	n 0	0 -	_		* 324				* conductivity
pH		8,3	8.3		8.2	8.2			16	
D.O. (mg/L)	<u>65</u>	6.6	(0'J	6.9	6.8	6.5		<del></del>	S	
Temp (°C)	25	25	26	26	26	26			046	
Conc. 6.25%										****
рН	8.3	•	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)	20	26	26	25	26	25				
Conc.: 12.5%		·								
рН	8.4	8,4	8.5	8.3	8,4	8.4				
D.O. (mg/L.)	65	6.6	6.6	6.7	6.7	6.5				,
Temp (°C)	26	24	24	26	26	26				
Conc.: 25%										
pН	8,5	8.5	8.5	8.4	8.5	8,5				
D.O. (mg/L)	64	6.3	18:165	6.7	6.5	6.5				
Temp (°C)	as	25	26	26	26	26				
Conc.: 50%							· • • · · · · · · · · · · · · · · · · ·			
pH	8.6	8.6	8.6	8.6	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
pН	8.7	8.7	8.7	8.6	8,7	8.4				
D.O. (mg/L)	6.5		6.0	6.7	67	6,4				
Temp (°C)	26	25	26	26	26	26				
Conc.:			·							
pН								<u> </u>		
D.O. (mg/L)										
Temp (°C)								<u> </u>	<b></b>	
	1/0/11	1120/11	1/83/11	01/24/11	12511	Ilaulii				
Time	II!	1105	1325		1125	1525				
Initials	TII 🛕 T		18	137	ري دي	(0)				, , , , , , , , , , , , , , , , , , ,
		<u> </u>		. , ,	!	<del></del> ,	<u> </u>	<del></del>	٠	

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FCETL QA Form No. 055
Revision 2
Effective 1/94
OU 1/26/11
AA: AROZ/02/11

#### **DAILY TOXICITY TEST LOG**

Project Number: 60147216-408-180
Test Species: Ceriodaphnia dubia

General	Neonates obtained from 01(1	II НТА+НТВ cerio monocultu	re board	Feeding	Initials/Date
Comments	( GREEN marked cups)			0.2 ml YTC/ALG	
	Roundom Chart: Shen'd	len		Daily	
Test Day 0	Test Solution Mixed at: ነላዛር			Fed @	BP
	Test Organisms Added at: (5	000		1450	01/20/11
Test Day 1	ct= <i>2</i> 4.⊋ °c	Range = 22,6-25,0	°C	Fed @/130	1/01/11
Test Day 2	CT= 24,2, °C	Range = 22.6-25.0	°C	Fed @ (\00	0-8- 1/00/11
Test Day 3	CT=24,0°C	Range = 22 . 6-24 . 6	°C	Fed @ 1315	1/23/11
Test Day 4	CT= 24.0 °C	Range = 22.6 - 2년.6	°C	Fed @ 1005	BP 01/24/11
Test Day 5	CT=24.0 °C	Range = 22.6 - 25.0	°C	Fed @ 1055	cu 1/25/11
Test Day 6	СТ= ач.о °C	Range = 22.6/24.6	°C	Fed @	en Haulii
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 CU 1/27/11 GVAT MC 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	МН	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

Toxstat version 3.5, Study # 60147216-408-180 Pilgrim's Pride

Page 10 of 12 CU 1|27|11

Ceriodaphnia dubia Chronic

Determination of NOEC, LOEC for Reproduction, per Original female

ODE GARIBEL

File:

408180RO.dat

Transform:

NO TRANSFORMATION

#### Chi-Square Test for Normality

_	_	_	
Actual	and	Evnected	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

				<b></b>			_
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 )

Toxstat version 3.5, Study # 60147216-408-180 Pilarim's Pride Ceriodaphnia dubia Chronic Determination of PMSD

Page 11 of 12 en Hazin QA1 9ME1/28/11

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

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	МН	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 Page 12 of 12 CN 1127111 QA: ME 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: Ceriodaphia dubia Test Duration: 6 days

DATA FILE: 408180R.icp OUTPUT FILE: 408180R.i25

Conc. ID Means	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

AECOM Environment 60147216-408-180

#### **APPENDIX C**

**Reference Toxicant Summary and Control Chart** 

11-nsL-5b ◆ ◆ 01-Deb-10 \$1-voN-40 01-00-10 01-ge2-40 AECOM / FCETL Ceriodaphnia dubia Chronic Ref Tox at 25C IC25 In-House Organisms July 2009 through January 2011 01-ģuA-10 ◆ 01-lub-10 ◆ ՕԼ-սոՐ-ֆԼ ◀ 0լ-unr-լ0 01-ysM-80 ◆ 01-1qA-10 **Test Dates** 02-Mar-1D O1-Feb-10 ♦ 01-ngL-∂0 01-Ded-09 ♦ ◆ 60-þon-e0 01-bct-09 80-qə2-10 60-guA-8¢ 60-Int-10 700 9 200 200 100 400 300 mg/L chloride

## FILE IS CERIO CHRONIC 60147216-804-097 REFERENCE TOXICANT DATA FOR IN-HOUSE CERIODAPHNIA CHRONICS SODIUM CHLORIDE - EXPRESSED AS MG/L CLAECOM 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. <del>9</del>	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		344	373.75	110.98	151.80	595.70	29.69	18.1	4.96
01-Aug-10		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