



**Biomonitoring Report**  
*Pimephales promelas*

prepared for  
**MATRIX Construction Products**

Services Requested By: John Berry  
**BIG-FOOT™ 5lbs / 1000 gallons.**  
**EE USA Project No.: Q-158I-14**

Non-protocol.


***P. promelas***

SURVIVAL NOEC / LOEC = 10.0% / >10.0% LPS  
LPC %CV = 0.0

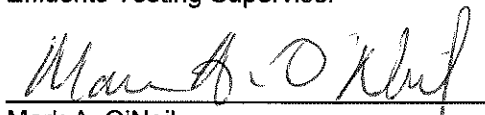
Report Date: March 24, 2014

by  
**ENVIRONMENTAL ENTERPRISES USA, INC.**  
58485 PEARL ACRES ROAD, SUITE D  
SLIDELL, LOUISIANA 70461  
(985) 646-2787

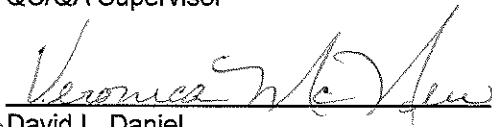
*This report contains three pages plus four appendices, A - D. This report must not be reproduced in part, only in whole. The results and conclusions presented in this report apply only to the sample(s) tested. All results included in this report are from a valid test.*

  
Veronica McNew  
Effluents Testing Supervisor

03/24/14  
DATE

  
Mark A. O'Neil  
QC/QA Supervisor

3/25/14  
DATE

  
David L. Daniel  
Laboratory Director

03/25/14  
DATE

BIG-FOOT™ 5lbs/1000 gallons

Q-158I-14

Non-protocol. Sample not for permit compliance.

**Pimephales promelas ACUTE STATIC 48-HOUR DEFINITIVE TEST,**  
**EPA-821-R-02-012: SECTION 9, Method 2000.**

**TEST OVERVIEW**

A 48-hour static toxicity test was conducted by **EE USA** to determine toxicity of a laboratory prepared sample (LPS) BIG-FOOT™ 5lbs/1000 gallons to *Pimephales promelas* larvae. Test organisms were cultured at **EE USA** and two days old when this test was initiated. Moderately hard synthetic freshwater was used as the diluent and a laboratory performance control was evaluated. Five replicates of the laboratory control and five LPS concentrations were prepared initially. LPS concentrations tested were 1.3, 2.16, 3.6, 6.0, and 10.0%. This test was initiated March 10, 2014, at 1710 and completed March 12, 2014, at 1615.

**MATERIALS AND METHODS**

Materials and methods for the work performed are stated in EPA-821-R-02-012: Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Actual materials and methods are detailed below. This test was performed with strict adherence to the requirements of EPA-821-R-02-012, Section 9, Method 2000 with the following exception(s):

- 1) Water quality parameters were not measured during this test.

Additionally, the recommendations and suggestions made elsewhere in EPA-821-R-02-012 were incorporated whenever applicable to optimize the experimental design.

*P. promelas* was cultured and maintained in moderately hard synthetic freshwater at 25±1°C. Several clutches from different females comprised the embryo pool from which the test organism population hatched. Test organisms were fed *Artemia* nauplii less than 24-hours prior to test initiation.

Sensitivity of test organisms to a known toxicant was determined by performing an acute Standard Reference Toxicant (SRT) test, PP1402-48, with potassium chloride (GFS Chemical, Lot C363173). The SRT test was initiated on February 26, 2014, with 1-day-old *P. promelas* larvae. Appendix D contains *P. promelas* SRT control charts.

48-hr LC50:	811 mg/l
Upper & Lower 95% Confidence Interval:	760 – 864 mg/l

The product used in this test was delivered to **EE USA** on February 18, 2014 (Appendix C). The product received was used to prepare the BIG-FOOT™ 5 lbs/1000 gallon sample. This laboratory prepared sample was used to prepare the initial dilutions. Test chambers were labeled with replicate identification, and test chamber boards with **EE USA's** project number. Six treatments, five LPS concentrations and a laboratory performance control were prepared daily (Appendix A, page 1).

Each treatment was poured directly into test chambers and placed in an environmental chamber to warm up to test temperature. Alkalinity, hardness, and conductivity were measured in the control March 10, 2014 (Appendix A, page 1).

On Day 0, the treatments were poured into their respective test chambers, ten *P. promelas* larvae were distributed randomly to each, and then this test was placed in the environmental chamber. Every 24 hours, survival was recorded (Appendix A, page 2). After 48 hours, the final survival data were recorded and this test was terminated.

### Summary of Experimental Conditions

Test Organisms: 2-days old  
Dilution Water: Moderately-hard synthetic freshwater  
Temperature: 25±1°C  
Photoperiod: 16 hours light; 8 hours dark  
Test Chambers: Rectangular Pyrex dish, 21cm x 11cm x 7cm Total volume = 1.45 liters  
Test Solution Volume: 200 ml  
Aeration: No  
Test Solution Renewal: Yes

### RESULTS AND CONCLUSION

The response used in statistical analysis of survival data was the proportion of surviving test organisms per replicate. These proportions were transformed by the Arc Sine Square Root Transformation and then tested for normal distribution and homogeneity of variance using Shapiro-Wilk's and Bartlett's tests, respectively. Survival data were not normally distributed and further evaluated by the nonparametric alternative, Steel's Many-One Rank Test. The NOEC for survival was 10.0% LPS. The LOEC was >10.0% LPS. Dunnett's Test was used to determine the MSDp between survival in the control and survival at any LPS concentration tested. For this *P. promelas* survival data set, the MSDp was 3.0% (Appendix B, page 1).

Survival of *P. promelas* larvae exposed to BIG-FOOT™ 5lbs/1000 gallons was not reduced significantly at any concentration tested ( LOEC >10.0% LPS). Survival data summary statistics are presented in Appendix B. Survival in the concurrent laboratory performance control was 100.0%.

### REFERENCES

EE USA. January 2014. Quality Assurance Plan. EE USA, Slidell, LA 70461.

EE USA. December 2013. Standard Operating Procedures. EE USA, Slidell, LA 70461.

NELAC Institute. TNI Standard, Environmental Laboratory Sector, adopted September 8, 2009. Management and Technical Requirements for Laboratories Performing Environmental Analysis. Volume 1. EL-V1-2009-ISO. Weatherford, TX 76086.

Tidepool Scientific Software. 2007. ToxCalc™ Toxicity Data Analysis Software. Version 5.0.32. McKinleyville, CA.

U.S. Environmental Protection Agency. July 2000. Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR Part 136). EPA 821-B-00-004. Office of Water (4303). Washington, DC 20460.

U.S. Environmental Protection Agency. October 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA-821-R-02-012. 5<sup>th</sup> Edition. Office of Water (4303T). Washington, DC 20460.

**Environmental Enterprises USA, Inc.**

**APPENDIX A**

**Matrix Construction Products**  
**5 lbs/ 1000 gallons BIG-FOOT™**  
 3075 Book Road, Suite 103, Naperville, IL 60567  
 John Berry (877) 591-3137

**Test Concentrations, % Laboratory Prepared Sample (LPS)**

	<i>Pimephales promelas</i>	Total Volume/ Concentration, ml	Color Code	ml LPS	ml DH2O
10.00		1000.00	Black	100.00	990.00
6.00		"	Red	60.00	940.00
3.60		"	Yellow	36.00	964.00
2.16		"	Green	21.60	978.40
1.30		"	Blue	13.00	987.00
0.00	LPC	"	White	0.00	1000.00

5 lbs/1000 gallons LPS: 0.59 g BIG- FOOT™ / 1000 ml

Weight: 0.5901 g Scale ID: B2 Initials: VB Date & Time: 03/10/14 1501

Data Pages & Calculations by: Vanessa McNeel QA/QC Check by: Michelle Miller

*P. promelas* = 5 Reps x 200 ml  
 = 1000 ml

DH<sub>2</sub>O = Dilution Water = **Moderately Hard Synthetic Freshwater**

	LPC	M #
Date	03/10	
Alkalinity	68	//
Conductivity	322	1B
Hardness	100	//
pH	8.1	1B3
TRC	///	//
	<u>VB</u>	

LPC: Laboratory Performance Control, synthetic moderately hard water  
 Alkalinity: mg/l as CaCO<sub>3</sub> Conductivity: μS/cm Hardness: mg/l as CaCO<sub>3</sub>  
 pH: su TRC: mg/l

Prep Date	03/10
DH <sub>2</sub> O Lot #	FW- 004 -14
Sample #	1
Ph	—
M#	—
Initial	<u>ME</u>

Comments: \_\_\_\_\_

**Fathead Minnow, *Pimephales promelas***

Acute Static 48 – Hour Definitive Test  
EPA-821-R-02-012: Section 9 Method 2000

**Matrix Construction Products – BIG-FOOT™**

Test Organisms Age: 2 Days Old      Test Organisms Source: EE  
 Test Initiation At: 1710 on 03/10/14  
 Counted by: Calvin M. Mays      QC/QA by: Colleen Beard  
 Loaded by: Colleen Beard      Organism Lot # pp-070-14

Exposure Chamber: 16 oz. plastic cups.      Feeding: None

**Survival Data**

*% wrong data 03/21/14*

Treatment g/L PR													
Time	REP	LPC White	REP	1.30 Blue	REP	2.16 Green	REP	3.60 Yellow	REP	6.00 Red	REP	10.00 Black	Initials
0 HR	1	10	6	10	11	10	16	10	21	10	26	10	03/10/14
	2	10	7	10	12	10	17	10	22	10	27	10	
	3	10	8	10	13	10	18	10	23	10	28	10	
	4	10	9	10	14	10	19	10	24	10	29	10	
	5	10	10	10	15	10	20	10	25	10	30	10	
24 HR	1	10	6	10	11	10	16	10	21	10	26	10	03/11/14
	2	10	7	10	12	10	17	10	22	10	27	10	
	3	10	8	10	13	10	18	10	23	10	28	10	
	4	10	9	10	14	10	19	10	24	10	29	10	
	5	10	10	10	15	10	20	10	25	10	30	10	
48 HR	1	10	6	10	11	9	16	10	21	10	26	10	03/12/14
	2	10	7	10	12	10	17	9	22	10	27	10	
	3	10	8	10	13	10	18	10	23	9	28	10	
	4	10	9	10	14	10	19	10	24	10	29	10	
	5	10	10	10	15	10	20	10	25	10	30	10	
% Sur	100		100		98		98		98		100		

Data Entry by: Veronica McNew  
 Double Data Entry: Veronica McNew or  
 QA/QC Officer: Maria-O King

**P. promelas Water Quality Data**

All Treatments: Temp., 19.5 to 21.4°C. Initial & Final Dissolved Oxygen (DO): 4.0 to 9.5 mg/l.  
 LPC: Initial Conductivity, 250 to 365 uS/cm. I: initial water quality. F: final water quality.

0 HR	Treatment <sup>1.0</sup> g/L PR						
03/10/14	LPC	1.30	2.16	3.60	6.00	10.00	Meter #
DO	I						
Temp	I						
Conductivity	I						
Tech Initials:				Time:			

Comments  
 water quality not measured because polymer sample would likely turn probes of meters  
 03/10/14

24 HR	Treatment <sup>1.0</sup> g/L PR						
03/11/14	LPC	1.30	2.16	3.60	6.00	10.00	Meter #
DO	F						
Temp	F						
pH	F						
Tech Initials:				Time:			

Comments  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

48 HR	Treatment <sup>1.0</sup> g/L PR						
03/12/14	LPC	1.30	2.16	3.60	6.00	10.00	Meter #
DO	F						
Temp	F						
pH	F						
Tech Initials:				Time:			

Comments  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

Ⓐ wrong data 03/25/14 VL.

DO: mg/l pH: su Conductivity: uS/cm Temp: °C

**Environmental Enterprises USA, Inc.**

**APPENDIX B**



**Acute Toxicity Test-48 Hr Survival**

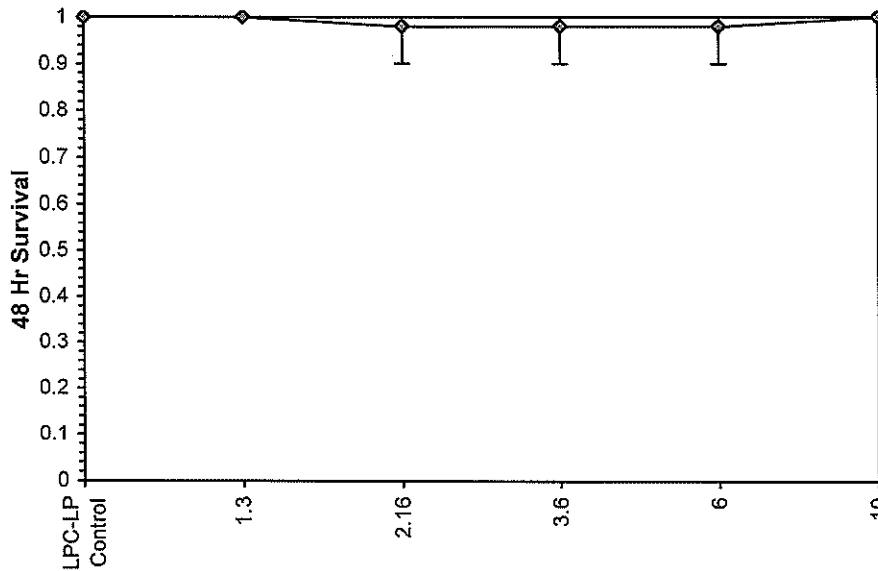
Start Date: 3/10/2014	Test ID: pp158114	Sample ID: LAB-Lab Sample
End Date: 3/12/2014	Lab ID: EE-Environmental Enterprise	Sample Type: PRD-Product
Sample Date:	Protocol: EPAF 02-EPA Freshwater	Test Species: PP-Pimephales promelas
Comments:		

Conc-%	1	2	3	4	5
PC-LP Control	1.0000	1.0000	1.0000	1.0000	1.0000
1.3	1.0000	1.0000	1.0000	1.0000	1.0000
2.16	0.9000	1.0000	1.0000	1.0000	1.0000
3.6	1.0000	0.9000	1.0000	1.0000	1.0000
6	1.0000	1.0000	0.9000	1.0000	1.0000
10	1.0000	1.0000	1.0000	1.0000	1.0000

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root				Rank Sum	1-Tailed Critical
			Mean	Min	Max	CV%		
PC-LP Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5	
1.3	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5	27.50
2.16	0.9800	0.9800	1.3794	1.2490	1.4120	5.284	5	25.00
3.6	0.9800	0.9800	1.3794	1.2490	1.4120	5.284	5	25.00
6	0.9800	0.9800	1.3794	1.2490	1.4120	5.284	5	25.00
10	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5	27.50

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.59678	0.927	-2.2346	4.3922
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	10	>10		10
Treatments vs LPC-LP Control				

**Dose-Response Plot**



Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnnett's Test	10	>10		10	0.02953	0.03029	0.00159	0.00266	0.70034	5, 24
Treatments vs LPC-LP Control										

**Environmental Enterprises USA, Inc.**

**APPENDIX C**



**ENVIRONMENTAL ENTERPRISES USA, INC.**

58485 Pearl Acres Rd., Suite D  
Slidell, Louisiana 70461  
(985) 646-2787

Kit No.

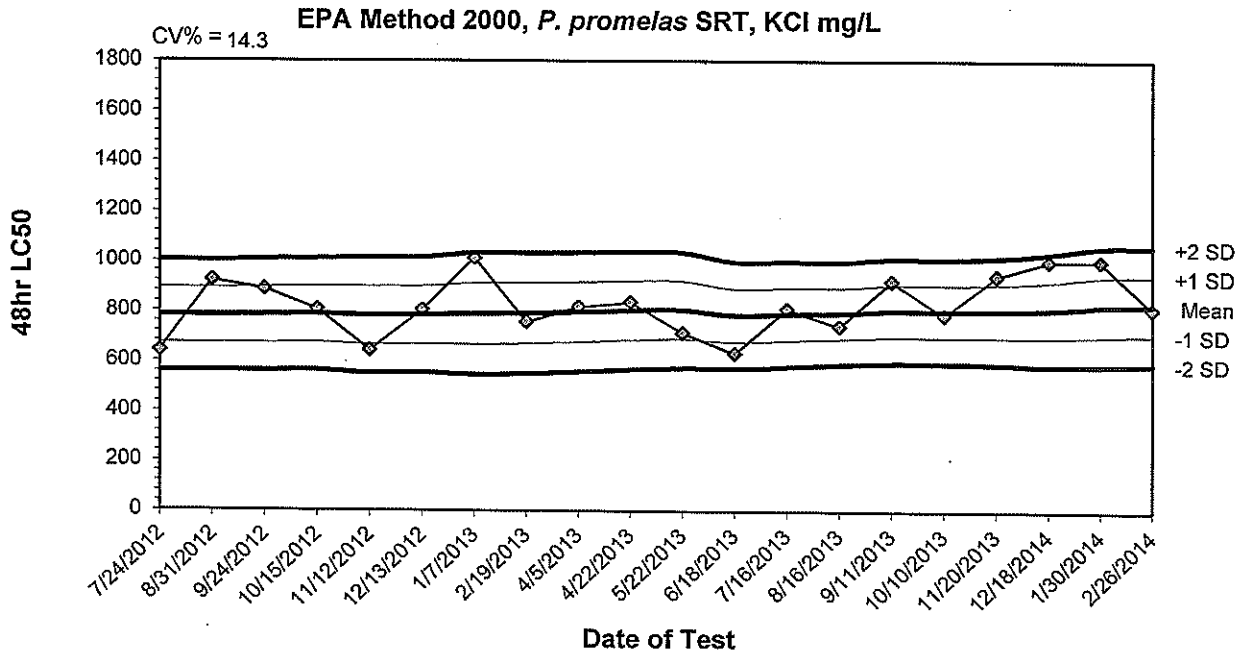
**CHAIN OF CUSTODY RECORD**

Client: Matrix Construction Contact Person: John Berry Special Handling  
Address: Products Phon#: 630-791-0893 Request  
P.O. # \_\_\_\_\_ ( ) RUSH  
FAX #: \_\_\_\_\_ ( ) VERBAL  
Project: \_\_\_\_\_ ( ) OTHER

Lab Sample Description	Date Collected	Time Collected	No. of Containers	Analysis Request	S/R No.	Lab No.
Big-Foot™			1	48-hr P. promises		Q-158I-14
Collected By:	Date	Time		Relinquished By:	Date	Time
Received By: <u>Marcus McNew</u>	Date <u>02/18/14</u>	Time <u>1101</u>		Relinquished By:	Date	Time
Received By:	Date	Time		Relinquished By:	Date	Time
Received By:	Date	Time		Relinquished By:	Date	Time
Received By:	Date	Time		Relinquished By:	Date	Time

**Environmental Enterprises USA, Inc.**

**APPENDIX D**



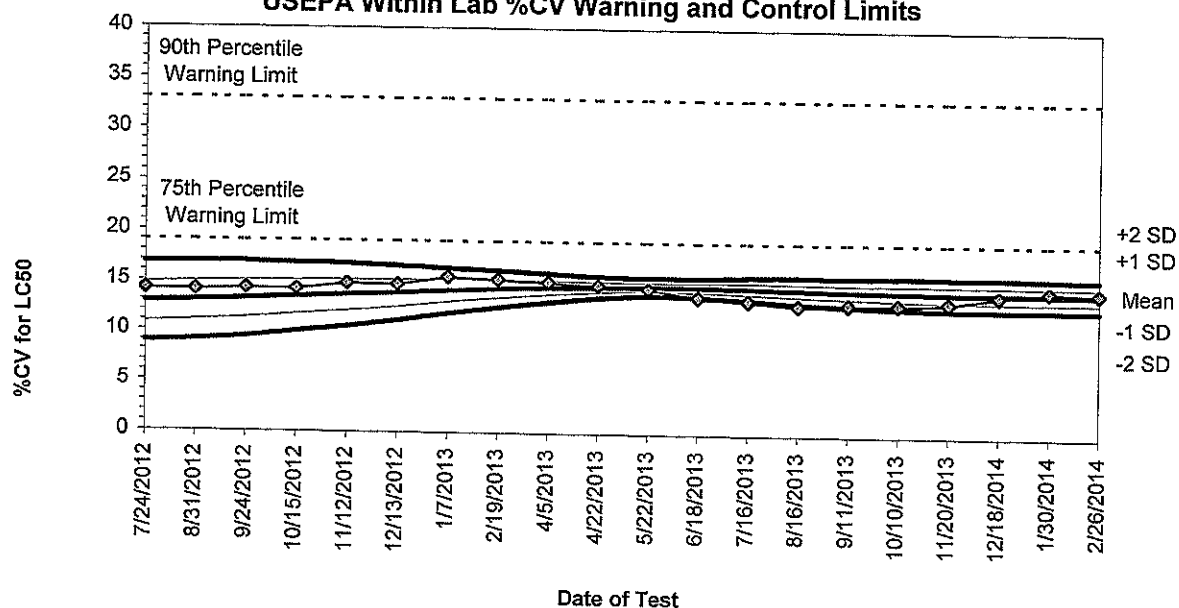
Dilution Series = 288, 412, 588, 840, & 1200 mg/L KCl; Dilution Factor = 0.70

Test #	Test Date	48-hr LC50 mg/L KCl	Cumulative Mean LC50	-1 SD	-2 SD	+1 SD	+2 SD	Control Survival	Toxicant Lot #
PP1208-48	7/24/2012	641	783	672	562	894	1005	97.5	081M0170V
PP1209-48	8/31/2012	922	782	673	563	892	1002	100.0	081M0170V
PP1210-48	9/24/2012	887	785	673	562	896	1008	97.5	081M0170V
PP1211-48	10/15/2012	806	787	675	564	899	1010	100.0	081M0170V
PP1212-48	11/12/2012	644	783	667	552	898	1014	100.0	081M0170V
PP1213-48	12/13/2012	804	785	670	554	900	1016	100.0	081M0170V
PP1301-48	1/7/2013	1010	789	667	545	911	1033	100.0	081M0170V
PP1302-48	2/19/2013	756	792	671	550	913	1033	100.0	SLBD2389V
PP1304-48	4/5/2013	817	797	677	558	916	1036	100.0	SLBD2389V
PP1305-48	4/22/2013	836	803	685	568	921	1039	100.0	SLBD2389V
PP1306-48	5/22/2013	714	806	690	574	921	1037	97.5	SLBD2389V
PP1307-48	6/18/2013	633	785	678	572	891	998	100.0	SLBC2414V
PP1308-48	7/16/2013	810	790	685	581	895	1000	100.0	SLBC2414V
PP1309-48	8/16/2013	741	793	692	590	895	997	97.5	SLBC2414V
PP1310-48	9/11/2013	921	803	699	595	908	1012	100.0	SLBC2414V
PP1311-48	10/10/2013	785	802	698	593	906	1010	97.5	C256341
PP1312-48	11/20/2013	942	804	697	591	910	1016	92.5	C256341
PP1313-48	12/18/2014	1000	808	695	583	920	1032	100.0	SLBH1238V
PP1401-48	1/30/2014	1000	821	703	584	940	1058	100.0	C363173
PP1402-48	2/26/2014	811	824	707	589	941	1059	100.0	C363173

PP1303-48 - LC50 >1200 mg/l KCl. Obvious mixing error seen by conductivity measurements. Two tests will be completed in April.

QAQC by: HWB 3/10/14

**EPA Method 2000, *P. promelas* SRT KCI, Survival LC50,  
USEPA Within Lab %CV Warning and Control Limits**



8%CV = 10th percentile, 10%CV = 25th percentile, 16%CV = 50th percentile

Test #	Test Date	%CV for LC50	Mean %CV	-1 SD	-2 SD	+1 SD	+2 SD	75th Warning Limit	90th Warning Limit	Toxicant Lot #
PP1208-48	7/24/2012	14.1	12.8	10.8	8.8	14.8	16.8	19.0	33.0	081M0170V
PP1209-48	8/31/2012	14.0	13.0	11.0	9.1	14.9	16.9	19.0	33.0	081M0170V
PP1210-48	9/24/2012	14.2	13.2	11.3	9.4	15.0	16.9	19.0	33.0	081M0170V
PP1211-48	10/15/2012	14.2	13.4	11.7	10.0	15.1	16.8	19.0	33.0	081M0170V
PP1212-48	11/12/2012	14.8	13.6	12.0	10.4	15.2	16.7	19.0	33.0	081M0170V
PP1213-48	12/13/2012	14.7	13.8	12.5	11.1	15.2	16.6	19.0	33.0	081M0170V
PP1301-48	1/7/2013	15.5	14.1	13.0	11.8	15.2	16.4	19.0	33.0	081M0170V
PP1302-48	2/19/2013	15.2	14.3	13.4	12.4	15.2	16.1	19.0	33.0	SLBD2389V
PP1304-48	4/5/2013	15.0	14.4	13.7	13.0	15.2	15.9	19.0	33.0	SLBD2389V
PP1305-48	4/22/2013	14.7	14.6	14.0	13.5	15.1	15.7	19.0	33.0	SLBD2389V
PP1306-48	5/22/2013	14.4	14.6	14.2	13.7	15.1	15.5	19.0	33.0	SLBD2389V
PP1307-48	6/18/2013	13.6	14.6	14.1	13.6	15.1	15.6	19.0	33.0	SLBC2414V
PP1308-48	7/16/2013	13.3	14.5	14.0	13.4	15.1	15.7	19.0	33.0	SLBC2414V
PP1309-48	8/16/2013	12.8	14.4	13.7	13.1	15.1	15.8	19.0	33.0	SLBC2414V
PP1310-48	9/11/2013	13.0	14.3	13.6	12.9	15.0	15.7	19.0	33.0	SLBC2414V
PP1311-48	10/10/2013	13.0	14.2	13.5	12.7	15.0	15.8	19.0	33.0	C256341
PP1312-48	11/20/2013	13.2	14.2	13.4	12.6	15.0	15.8	19.0	33.0	C256341
PP1313-48	12/18/2014	13.9	14.2	13.4	12.6	14.9	15.7	19.0	33.0	SLBH1238V
PP1401-48	1/30/2014	14.4	14.1	13.4	12.6	14.9	15.7	19.0	33.0	C363173
PP1402-48	2/26/2014	14.3	14.1	13.3	12.6	14.9	15.6	19.0	33.0	C363173

QAQC by: MAO 3/11/14