



**Kirkpatrick Group, Inc.
Dynablue – 05JL**

48 Hr Acute Product Report

57086, 57141, and 57231

Mysidopsis bahia

June 6, 2014

Approved by: *Chris Robason*
Chris Robason,
President

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***HAND-WRITTEN RAW DATA TABLES ARE AVAILABLE UPON REQUEST**

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Toxicity Test Summary

Client: Kirkpatrick Group Inc.

Product: Dynaguard-05JL

Date: June 6, 2014

Overview:

A sample of Dynaguard -05JL from Kirkpatrick Group, Inc. was used for a product testing study to determine the toxicity of a part per million dosage of product to the *Mysidopsis bahia* (saltwater invertebrate).

Data Analysis:

The following is a list of bio-monitoring tests performed.

Bio-Monitoring Tests:

1. 48 Hour Acute Range Finder
Mysidopsis bahia

2. 48 Hour Definitive Test
Mysidopsis bahia

Range Finder Results:

LC₅₀ and 95% confidence limits in ppm of the 48 hour range finder tests

Organism	Lab ID	Time Initiated	Time Ended	LC50	Lower CL	Upper CL
<i>M. bahia</i>	57086	16:30	16:00	>50,000	NR	NR
<i>M. bahia</i>	57141	16:25	13:55	<60,000	NR	NR

Definitive Results:

LC₅₀ and 95% confidence limits in ppm of 48 hour definitive test

Organism	Lab ID	Time Initiated	Time Ended	LC50	Lower CL	Upper CL
<i>M. bahia</i>	57231	16:00	15:15	56,108.72	52,410.50	60,067.91

48 HOUR RANGE FINDER TEST REPORT

Test Procedures:

Mysidopsis bahia

EPA METHOD: 2007

The 48 hour *Mysidopsis bahia* range finder test was initiated at 16:30 hours on May 8, 2014. A stock solution of 50,000 ppm was prepared 48 hours prior to the test by adding 100 g of product to 2 L of synthetic seawater. From this stock solution, six product concentrations of 1,000, 5,000, 10,000, 25,000, 35,000 and 50,000 ppm (100% stock solution) were prepared utilizing synthetic water. The test was set up with 266 ml plastic cups containing 200 ml of test solution. Each concentration or control consisted of 5 replicate cups with 8 organisms each, giving a total of 40 per treatment. The control was conducted concurrently with the test. Test organisms were one to five days old laboratory cultured juveniles. Juveniles were randomly introduced into the test. The number of surviving organisms was recorded after each 24 hour period. Surviving organisms in each test chamber were fed freshly hatched brine shrimp two times per day. The test ended at 16:00 hours on May 10, 2014. Survival data was statistically ($p=0.05$) analyzed according to EPA procedures to determine the LC_{50} and 95% confidence limits.

Survival:

Mysidopsis bahia

The 48 hour LC_{50} (concentration at which 50% mortality is expected to occur) for Dynaguard-05JL on *Mysidopsis bahia* survival data was calculated by the Spearman-Kärber program as > 50,000 ppm.

LC_{50} : >50,000 ppm

95% Lower Confidence Limit: Not Reliable*

95% Upper Confidence Limit: Not Reliable*

*A confidence limit of “not reliable” occurs when two or more concentrations have no partial mortality. The LC_{50} is still considered valid.

48 HOUR RANGE FINDER TEST REPORT

Test Procedures:

Mysidopsis bahia

EPA METHOD: 2007

A second 48 hour *Mysiopsis bahia* range finder test was initiated at 16:25 hours on May 16, 2014. A stock solution of 1,000,000 ppm was prepared 48 hours prior to the test by adding 2000 g of product to 2 L of synthetic seawater. From this stock solution, five product concentrations of 60,000, 125,000, 250,000, 500,000, and 1,000,000 ppm (100% stock solution) were prepared utilizing synthetic water. The test was set up with 266 ml plastic cups containing 200 ml of test solution. Each concentration or control consisted of 5 replicate cups with 8 organisms each, giving a total of 40 per treatment. The control was conducted concurrently with the test. Test organisms were one to five days old laboratory cultured juveniles. Juveniles were randomly introduced into the test. The number of surviving organisms was recorded after each 24 hour period. Surviving organisms in each test chamber were fed freshly hatched brine shrimp two times per day. The test ended at 13:55 hours on May 18, 2014. Survival data was statistically ($p=0.05$) analyzed according to EPA procedures to determine the LC_{50} and 95% confidence limits.

Survival:

Daphnia pulex

The 48 hour LC_{50} (concentration at which 50% mortality is expected to occur) for Dynaguard-05JL on *Mysidopsis bahia* survival data was calculated by the Spearman-Kärber program as < 60,000 ppm.

LC_{50} : <60,000 ppm

95% Lower Confidence Limit: Not Reliable*

95% Upper Confidence Limit: Not Reliable*

*A confidence limit of “not reliable” occurs when two or more concentrations have no partial mortality. The LC_{50} is still considered valid.

48 HOUR DEFINITIVE TEST REPORT

Test Procedures:

Mysidopsis bahia

EPA METHOD: 2007

The 48 hour *Mysidopsis bahia* definitive test was initiated at 16:00 hours on June 6, 2014. A stock solution of 167,000 ppm was prepared 48 hours prior to the test by adding 501 g of product to 3 L of synthetic seawater. From this stock solution, six product concentrations of 10,000, 25,000, 50,000, 75,000, 100,000 and 167,000 ppm (100% stock solution) were prepared utilizing synthetic water. The test was set up with 266 ml plastic cups containing 200 ml of test solution. Each concentration or control consisted of 5 replicate cups with 8 organisms each, giving a total of 40 per treatment. The control was conducted concurrently with the test. Test organisms were one to five days old laboratory cultured juveniles. Juveniles were randomly introduced into the test. The number of surviving organisms was recorded after each 24 hour period. Surviving organisms in each test chamber were fed freshly hatched brine shrimp two times per day. The test ended at 15:15 hours on June 8, 2014. Survival data was statistically ($p=0.05$) analyzed according to EPA procedures to determine the LC_{50} and 95% confidence limits.

Survival:

Mysidopsis bahia

The 48 hour LC_{50} (concentration at which 50% mortality is expected to occur) for Dynaguard-05JL on *Mysidopsis bahia* survival data was calculated by the Spearman-Kärber program as 56,108.72 ppm.

LC_{50} : 56,108.72 ppm

95% Lower Confidence Limit: 52,410.50 ppm

95% Upper Confidence Limit: 60,067.91 ppm

BIO-AQUATIC TESTING, INC.

TOXICITY TEST

48 Hr Acute *Mysidopsis bahia*

Client: Kirkpatrick Group Inc. Product Test

Permit Number: N/A

Sample Type: Product

Receiving Water Name: N/A

Outfall Name: Dynablue-05JL

Lab ID: 57086

Test Temperature (oC): 25 ± 1

Photo Period: 16 hours light
8 hours dark

Begin Date: 5/8/2014

End Date: 5/10/2014

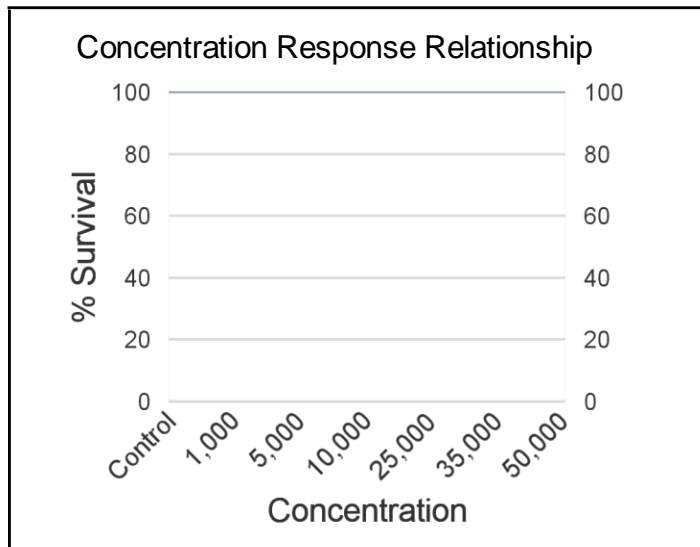
Test Start Time:

Test End Time:

SURVIVAL

Effluent Con. ppm	Number Of Alive Per Replicate															Avg% Surv.
	5/8					5/9					5/10					
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	
Control	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
1,000	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
5,000	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
10,000	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
25,000	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
35,000	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
50,000	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%

*spilled cup



BIO-AQUATIC TESTING, INC.

TOXICITY TEST

48 Hr Acute *Mysidopsis bahia*

Client: Kirkpatrick Group Inc. Product Test

Permit Number: N/A

Sample Type: Product

Receiving Water Name: N/A

Outfall Name: Dynablue-05JL

Lab ID: 57231

Test Temperature (oC): 25 ± 1

Photo Period: 16 hours light
8 hours dark

Begin Date: 6/6/2014

End Date: 6/8/2014

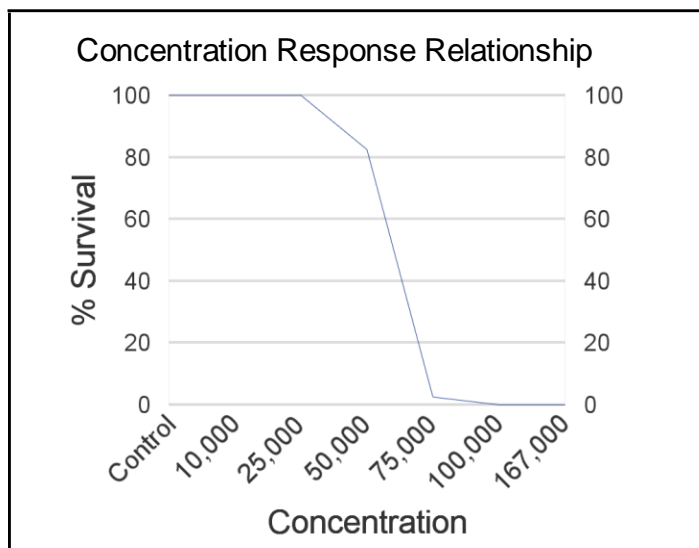
Test Start Time:

Test End Time:

SURVIVAL

Effluent Con. ppm	Number Of Alive Per Replicate															Avg% Surv.
	6/6					6/7					6/8					
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	
Control	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
10,000	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
25,000	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
50,000	8	8	8	8	8	8	8	8	8	8	5	7	6	7	8	82.5%
75,000	8	8	8	8	8	3	4	3	3	4	0	0	0	0	1	2.5%
100,000	8	8	8	8	8	0	0	0	0	0	0	0	0	0	0	0.0%
167,000	8	8	8	8	8	0	0	0	0	0	0	0	0	0	0	0.0%

*spilled cup



BIO-AQUATIC TESTING, INC.

TOXICITY TEST

48 Hr Acute *Mysidopsis bahia*

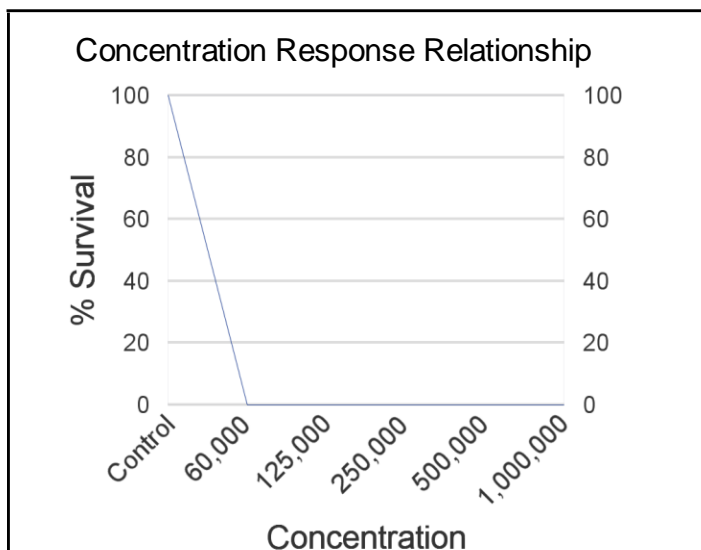
Client: Kirkpatrick Group Inc.	<u>Product Test</u>	Lab ID: 57141
Permit Number: N/A		Test Temperature (oC): 25 ± 1
Sample Type: Product	Outfall Name: Dynablue-05JL	Photo Period: 16 hours light 8 hours dark
Receiving Water Name: N/A		Begin Date: 5/16/2014
		End Date: 5/18/2014

Test Start Time: Test End Time:

SURVIVAL

Effluent Con. mg/L	Number Of Alive Per Replicate															Avg% Surv.
	5/16					5/17					5/18					
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	
Control	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
60,000	8	8	8	8	8	8	8	8	8	8	0	0	0	0	0	0.0%
125,000	8	8	8	8	8	4	5	4	3	4	0	0	0	0	0	0.0%
250,000	8	8	8	8	8	2	3	2	0	2	0	0	0	0	0	0.0%
500,000	8	8	8	8	8	0	0	0	0	0	0	0	0	0	0	0.0%
1,000,000	8	8	8	8	8	0	0	0	0	0	0	0	0	0	0	0.0%

*spilled cup



APPENDIX A

STATISTICS SUMMARY

Both the lethal and sub-lethal endpoints were statistically calculated according to their respective EPA guidelines. The Chronic Freshwater organisms were calculated according to EPA-821-R-02-013, October 2002 Fourth Edition. The Chronic Marine and Estuarine organisms were calculated according to EPA-821-R-02-014, October 2002 Third Edition. The Acute Freshwater and Marine organisms were calculated according to EPA-821-R-02-012, October 2002 Fifth Edition. The fertilization organisms were calculated according to EPA-600-R-95-136 or EPA-600-R-12-022, dependent upon the species. Listed below are the basic principles of these guidelines. If you would like a copy of the raw statistical calculations for your test then please contact us.

The chronic and acute *Pimephales promelas* and *Menidia beryllina* survival data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts (parametric). If the data fails Shapiro Wilks Test or Bartlett's Test then Steels Many One Test (non-parametric) is used. The chronic *Pimephales promelas* and *Menidia beryllina* growth data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Shipiro Wilks Test and Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The chronic *Mysidopsis bahia* survival data is analyzed using Chi-square test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test or Bartlett's Test then Steels Many One Test is used. *Mysidopsis bahia* growth data is analyzed using Chi-square test and Bartlett's Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test and Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The acute *Mysidopsis bahia* survival data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shipiro Wilks Test or Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The chronic *Ceriodaphnia dubia* survival data are analyzed using the Fisher's Exact Test. The chronic *Ceriodaphnia dubia* reproduction and are analyzed using the Chi-square test and Bartlett Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test and Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The acute *Daphnia pulex* and *Ceriodaphnia dubia* survival data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shapiro Wilks Test or Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The fertilization data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shapiro Wilks Test or Bartlett's Test then Steels Many One Test is used. Point estimation may or TST methodology may also be used.

TOXICANT : dynaguard blue
SPECIES: Mysidopsis bahia

RAW DATA:	Concentration	Number	Mortalities
--- ----	(ppm)	Exposed	
	.00	40	0
	1000.00	40	0
	*****	40	0
	*****	40	0
	*****	40	0
	*****	40	0
	*****	40	40
	*****	40	40
	*****	40	40
	*****	40	40
	*****	40	40

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 54772.26
95% CONFIDENCE LIMITS
ARE NOT RELIABLE.

Mysid Survival
 File: 57231.mys Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 6.000

W = 0.647

Critical W (P = 0.05) (n = 35) = 0.934

Critical W (P = 0.01) (n = 35) = 0.910

Data FAIL normality test. Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normal data and should not be performed.

Mysid Survival
 File: 57231.mys Transform: NO TRANSFORMATION

Hartley's test for homogeneity of variance
 Bartlett's test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
 Additional transformations are useless.

Mysid Survival
 File: 57231.mys Transform: NO TRANSFORMATION

STEEL'S MANY-ONE RANK TEST - Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRI T. VALUE	df	SIG
1	Con	8.000				
2	10,000	8.000	27.50	16.00	5.00	
3	25,000	8.000	27.50	16.00	5.00	
4	50,000	6.600	17.50	16.00	5.00	
5	75,000	0.200	15.00	16.00	5.00	*
6	100,000	0.000	15.00	16.00	5.00	*
7	167,000	0.000	15.00	16.00	5.00	*

Critical values use k = 6, are 1 tailed, and alpha = 0.05

DATE: 6/6/14 TEST NUMBER: 57231 DURATION: 48 g
 TOXICANT : Dynaguard Blue
 SPECIES: Mysidopsis bahia

RAW DATA:	Concentration (ppm)	Number Exposed	Mortalities
---	.00	40	0
----	*****	40	0
	*****	40	0
	*****	40	7
	*****	40	39
	*****	40	40
	*****	40	40

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 56108.72
 95% LOWER CONFIDENCE: 52410.50
 95% UPPER CONFIDENCE: 60067.91

Appendix B

Americamysis bahia

BIO-AQUATIC TESTING, INC.

Carrollton, TX

REFERENCE TOXICANTS

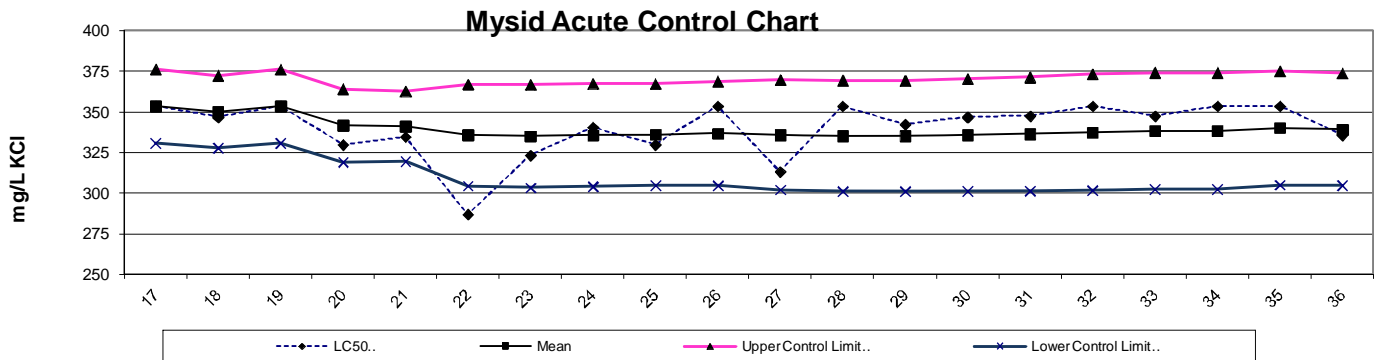
Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals are used of the following choices: sodium laurel sulfate (SLS), copper sulfate, copper chloride, potassium chloride, and sodium chloride. Organism responses are tracked with control charts for each reference toxicant/organism combination. The data are examined for sensitivity trends and to determine if results are within EPA described limits.

ACUTE REFERENCE TOXICANT TEST RESULTS

DILUTION WATER:	Standard Synthetic Saltwater						
CHEMICAL:	Potassium Chloride						
DURATION:	48 Hour Acute						
TEST NUMBER:	36						
PROJECT NUMBER:	57185						
START DATE:	5/28/2014						
START TIME:	10:50						
TOTAL NUMBER EXPOSED:	40 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	25	50	125	250	500	1000
NUMBER DEAD PER CONCENTRATION:	0	0	0	0	3	40	40
TEST METHODS:	As listed in EPA-821-R-02-012						
STATISTICAL METHODS:	SURVIVAL: Trimmed Spearman-Karber						
LC50:	335.64	mg/L					
95% LOWER CONFIDENCE LIMITS:	316.81	mg/L					
95% UPPER CONFIDENCE LIMITS:	355.59	mg/L					

**REFERENCE TOXICANT STATISTICAL RESULTS: LC₅₀ AND CONTROL LIMITS
Americamysis bahia EXPOSED TO POTASSIUM CHLORIDE, 48 HOUR STATIC RENEWAL**

Test Number	Date	Project Number	Toxicant Lot Number	Statistical Method	LC ₅₀ mg/L	Mean	Twice Standard Deviation 2S	Upper Control Limit Mean+2S, mg/L	Lower Control Limit Mean-2S, mg/L
17	12/4/2012	52633	217304	Trimmed Spearman-Kärber	353.6	353.6	22.7	376.2	330.9
18	12/11/2012	53193	217304	Trimmed Spearman-Kärber	346.8	350.2	22.3	372.5	327.9
19	12/28/2012	53572	217304	Trimmed Spearman-Kärber	353.6	353.6	22.6	376.1	331.0
20	1/30/2013	53768	217304	Trimmed Spearman-Kärber	329.9	341.7	22.5	364.2	319.2
21	3/1/2013	53834	217304	Trimmed Spearman-Kärber	334.7	341.1	21.6	362.7	319.5
22	3/26/2013	53913	217304	Trimmed Spearman-Kärber	287.0	335.8	31.3	367.0	304.5
23	5/3/2013	54044	217304	Trimmed Spearman-Kärber	323.3	335.2	31.8	366.9	303.4
24	5/28/2013	54103	217304	Trimmed Spearman-Kärber	340.6	335.8	31.7	367.5	304.1
25	6/28/2013	54183	217304	Trimmed Spearman-Kärber	329.9	336.1	31.2	367.3	304.9
26	8/7/2013	54289	217304	Trimmed Spearman-Kärber	353.6	336.8	32.1	368.9	304.7
27	8/27/2013	54341	314803	Trimmed Spearman-Kärber	313.2	335.9	33.7	369.6	302.2
28	10/11/2013	55798	314803	Trimmed Spearman-Kärber	353.6	335.3	34.2	369.5	301.1
29	10/31/2013	56242	314803	Trimmed Spearman-Kärber	342.3	335.2	34.1	369.3	301.1
30	11/26/2013	56336	314803	Trimmed Spearman-Kärber	346.8	335.8	34.5	370.3	301.3
31	12/26/2013	56532	314803	Trimmed Spearman-Kärber	347.5	336.5	34.9	371.4	301.5
32	1/31/2014	56669	314803	Trimmed Spearman-Kärber	353.6	337.4	35.8	373.2	301.7
33	2/27/2014	56885	314803	Trimmed Spearman-Kärber	347.5	338.3	35.9	374.2	302.5
34	3/28/2014	56961	314803	Trimmed Spearman-Kärber	353.6	338.3	35.9	374.2	302.5
35	4/30/2014	57061	314803	Trimmed Spearman-Kärber	353.6	340.2	35.1	375.3	305.2
36	5/28/2014	57185	314803	Trimmed Spearman-Kärber	335.6	339.3	34.5	373.8	304.8



APPENDIX C

LITERATURE REFERENCES

- U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms (Fifth Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-012.
- U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents and Receiving Water To Marine And Estuarine Organisms (Third Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-014.
- U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms (Fourth Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-013.
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- U.S.E.P.A., 1995. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To West Coast Marine and Estuarine Organisms (First Edition) U.S. Environmental Protection Agency, EPA-600-R-95-136.
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