



Kirkpatrick Group Inc.
Dynagard BLUE Product Study
48 Hr Acute Definitive Test Report
Mysidopsis bahia
December 5, 2005

Approved by: *Chris Robason*
Chris Robason,
President

TABLE OF CONTENTS

TOXICITY TEST REPORT	3
SURVIVAL TEST SUMMARY	5
STATISTICAL & CHEMICAL ANALYSIS	Appendix A
REFERENCE TOXICANTS	Appendix B
LITERATURE REFERENCES	Appendix C

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

BIO-AQUATIC TESTING, INC.

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TOXICITY TEST REPORT - 48 Hr Acute

Client:	Kirkpatrick	Sample:	Dynagard BLUE (25000 ppm)
Product:	Dynagard BLUE	Laboratory Number:	33009
Permit No.	N/A	Date:	12/5/05

Result Summary: *Mysidopsis bahia* LC₅₀ = >25000ppm

INTRODUCTION: Bio-Aquatic Testing, Inc. was contracted by Kirkpatrick Group, Inc. to design a study to determine the toxicity of their product Dynagard BLUE to the saltwater organism *Mysidopsis bahia*.

SAMPLE PREPARATION: A sample of the product Dynagard BLUE was delivered to Bio-Aquatic Testing, Inc via the client. Samples were received on November 28, 2005. The sample was used to create a 25000 ppm stock solution, and then diluted with synthetic saltwater to the dilution series chosen during the study design. Preparation of the 25000 ppm stock solution began by adding 25 mLs of product to 1000 mLs of synthetic saltwater. The resulting mixture was exposed for 48 Hours before the water phase was collected and used for the dilution series.

TEST PROCEDURES:

Mysidopsis bahia

The 48 Hr Acute *Mysidopsis bahia* survival test was initiated at 1130 hours on December 5, 2005. Six concentrations of 100ppm, 500ppm, 1000ppm, 5000ppm, 15000ppm and 25000ppm were prepared utilizing laboratory synthetic saltwater as dilution water. A control of synthetic laboratory saltwater of approximately 24+/-2 ppt was also prepared, and ran concurrently with the test. Each replicate was set up with 300mL plastic cups containing 100 mL of test solution, and each effluent concentration included four replicate cups with five organisms per cup. Test organisms were five day old laboratory cultured juveniles. The test proceeded for 48 hours during which survival data were collected daily, and was not renewed. The test ended at 1130 hours on December 7, 2005. Survival data was statistically (p=0.05) analyzed according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC), the No Observable Effect Concentration (NOEC), and the LC₅₀.

SURVIVAL:*Mysidopsis bahia*

The *Mysidopsis bahia* survival data were not normally distributed at the alpha level of 0.01 (13.277) using the Shapiro-Wilk's test for normality. Survival data were shown not to be homogeneous using Bartlett's test at the alpha level of 0.01 (15.09) without data transformations. Steel's Many-One Rank test on survival data demonstrated no statistically significant differences between the control and and of the concentrations. The trimmed Spearman-Kärber method was used to calculate the LC₅₀ for this data set.

LOEC: Not Applicable/Calculable**NOEC: 25000ppm****LC₅₀: >25000ppm**

BIO-AQUATIC TESTING, INC.

TOXICITY TEST

48 Hr Acute *Mysidopsis*

Client: Kirkpatrick Group Inc. Product Test

Permit Number: N/A

Sample Type: Product

Receiving Water Name: N/A

Outfall Name: Dynagard Blue

Lab ID: 33009

Test Temperature (oC): 25 ± 1

Photo Period: 16 hours light
8 hours dark

Begin Date: 12/5/2005

End Date: 12/7/2005

SURVIVAL

Effluent Con. ppm	Number Of Alive Per Replicate															Avg% Surv.
	11/30					12/1					12/2					
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	
Synthetic Control	5	5	5	5		5	5	5	5		5	5	5	5		100.0%
100	5	5	5	5		5	5	5	5		5	5	5	5		100.0%
500	5	5	5	5		5	5	5	5		5	5	5	5		100.0%
1000	5	5	5	5		5	5	5	5		5	5	5	5		100.0%
5000	5	5	5	5		5	5	5	5		5	5	5	5		100.0%
15000	5	5	5	5		5	5	5	5		5	5	5	5		100.0%
25000	5	5	5	5		4	4	5	5		4	4	5	5		90.0%

* cup spilled

APPENDIX A

STATISTICS SUMMARY

Both the lethal and sublethal endpoints are statistically calculated according to their respective EPA guidelines. The chronic freshwater organisms are calculated according to EPA-600-4-91-002, July 1994 Third Edition. The chronic marine and estuarine organisms are calculated according to EPA-600-4-91-003, July 1994 Second Edition. The acute freshwater and marine organisms are calculated according to EPA-600-4-90-027F, August 1993 Fourth Edition. Listed below are the basic principles of these guidelines. If you would like a copy of the raw statistical calculations for your test, please contact us.

The chronic and acute *Pimephales promelas* and *Menidia beryllina* survival and growth data are analyzed using the Shipiro Wilkes Test and Bartlett's Test. If the data pass both tests, then the data are analyzed using an ANOVA and Dunnett's (parametric) Test. If the data fail the Shipiro Wilkes Test or Bartlett's Test, then the Steel's Many One Test (non-parametric) is used.

The chronic *Mysidopsis bahia* survival and growth, as well as the acute growth data are analyzed using the Chi-square test and Bartlett's Test. If the data pass both tests, then the data are analyzed using an ANOVA and Dunnett's Test. If the data fail the Chi-square test or Bartlett's Test, then the Steel's Many One Test is used. The acute *Mysidopsis bahia* survival data are analyzed using the Shipiro Wilkes Test and Bartlett's Test. If the data pass both tests, then the data are analyzed using an ANOVA and Dunnett's Test. If the data fail the Shipiro Wilkes Test or Bartlett's test, then the Steel's Many One Test is used.

The chronic *Ceriodaphnia dubia* survival data are analyzed using the Fisher Exact Test. The chronic *Ceriodaphnia dubia* growth and reproduction data are analyzed using the Chi-square test and Bartlett's Test. If the data pass one of these tests, then the data are analyzed using an ANOVA and Dunnett's Test. If the data fail the Chi-square test, then Steel's Many One Test is used.

The acute *Daphnia pulex* and *Ceriodaphnia dubia* survival data are analyzed using the Shipiro Wilkes Test and Bartlett's Test. If the data pass both tests, then the data are analyzed using an ANOVA and Dunnett's Test. If the data fail the Shipiro Wilkes Test or Bartlett's Test, then the Steel's Many One Test is used.

Bio-Aquatic Testing, Inc.

33009

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SALT WATER TEST SETUP FORM

Client: Kirkpatrick Group Inc.

Permit _____

Facility: Product Test

Lab Number 33009

Outfall Name: Dynagard Blue

Number of samples 1

Dilution Water: Synthetic Lab

Sx #	Rcvd Date	Rcvd Time	Begin Sampling		End Sampling	
			Date	Time	Date	Time
1	11/28/05	12:00				

Receiving Water Name: N/A

Dechlorinate Sample: No

Type of Test(s)

<u>Mysidopsis bahia</u>	<u>48 Hr Acute</u>

Start Sx # 1 Date: 12/5/2005

Renew Sx # _____ Date: _____

Renew Sx # _____ Date: _____

Renew Sx # _____ Date: _____

Renew Sx # _____ Date: _____

Renew Sx # _____ Date: _____

Renew Sx # _____ Date: _____

Test Start Date: _____ Test End Date: _____

12/5/2005 12/7/2005

Controls: Synthetic Lab

pH Match: _____

Hardness Match: _____

Mysidopsis bahia Test Set Up: 4 Reps & 5 Organisms per Rep

Test Set Up: _____

Concentrations: 100 500 1000 5000 15000 25000 ppm LF %

Test Chemistry on these dilutions: 100 500 1000 5000 15000 25000

Samples received by:

<input type="radio"/> Greyhound	<input type="radio"/> UPS Next Day	<input type="radio"/> Delta Dash	<input type="radio"/> Delta
<input type="radio"/> Pony Express	<input checked="" type="radio"/> Client Delivered	<input type="radio"/> Southwest Airlines	
<input type="radio"/> Federal Express	<input type="radio"/> American Airlines	<input type="radio"/> Bio Pick Up	

Other: _____

BIO-AQUATIC TESTING, INC.

Hardness, Alkalinity, Residual Chlorine, Specific Conductivity, and Salinity Analysis Data

Client: Kirkpatrick Group Inc.

Lab ID: 33009

Facility: Product Test

Dilution Water(s): Synthetic Lab

Outfall: Dynagard Blue

Test Date: December 5, 2005

Sample #	Received		Residual Cl ₂	DeChlor (ml/L)	Analyst Initials	Initial Salinity	Adjusted Salinity	Temp. Received
	Date	Time						
1	11/28/2005	12:00	N/A	N/A	CH	N/A	N/A	N/A

Dynagard Blue Survival
File: 33009.mys Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.000

W = 0.000

Critical W (P = 0.05) (n = 28) = 0.924

Critical W (P = 0.01) (n = 28) = 0.896

Data FAIL normality test. Try another transformation.

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

Dynagard Blue Survival
File: 33009.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.

Dynagard Blue Survival

File: 33009.mys

Transform: NO TRANSFORMATION

STEEL'S MANY-ONE RANK TEST

-

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	Con	5.000				
2	100	5.000	18.00	10.00	4.00	
3	500	5.000	18.00	10.00	4.00	
4	1000	5.000	18.00	10.00	4.00	
5	5000	5.000	18.00	10.00	4.00	
6	15000	5.000	18.00	10.00	4.00	
7	25000	5.000	18.00	10.00	4.00	

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

Mysidopsis 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, cadmium 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.

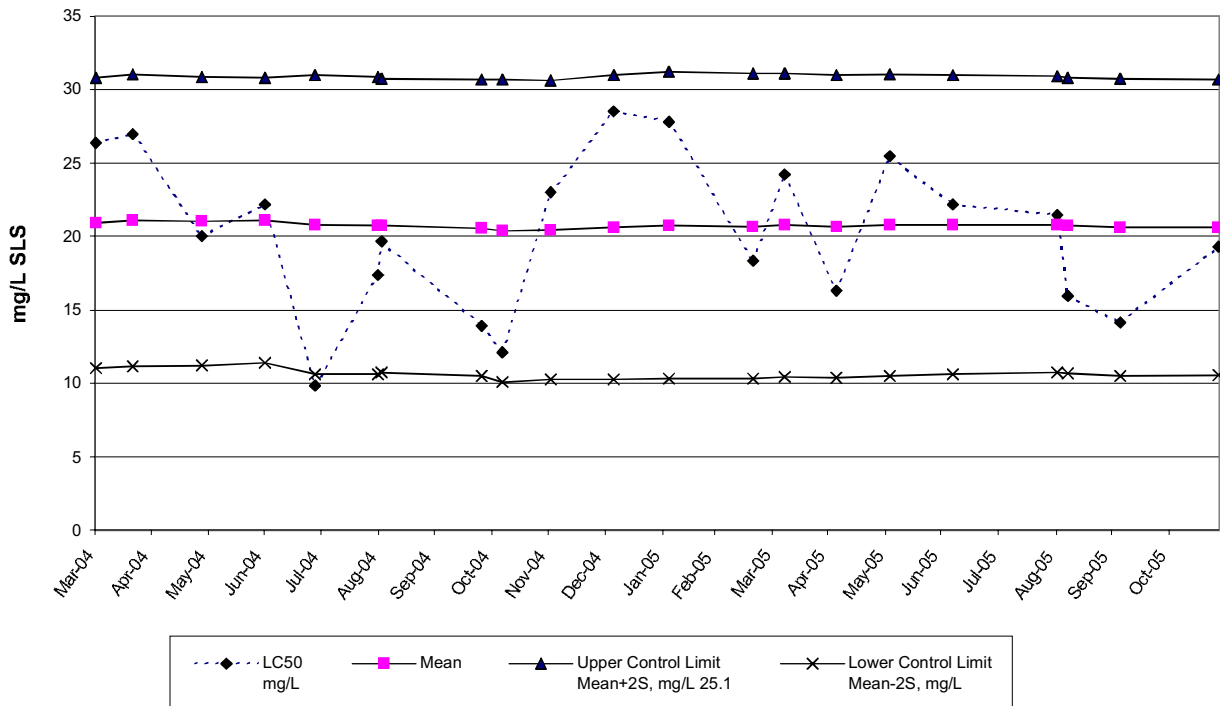
ACUTE REFERENCE TOXICANT TEST RESULTS

DILUTION WATER:	Synthetic Seawater
CHEMICAL:	Sodium Laurel Sulfate
SOURCE:	Fisher Scientific
DURATION:	48 Hours
TEST NUMBER:	141
PROJECT NUMBER:	32903
TEST DATE:	11/26/2005
START TIME:	13:05
TOTAL NUMBER EXPOSED:	40 organisms per concentration
CONCENTRATIONS:	CON 1.25 2.5 5 10 20 40
NUMER DEAD PER CONCENTRATION:	0 0 0 0 1 21 40
STATISTICAL METHOD:	Trimmed Spearman-Karber Method
CHEMICAL METHODS:	Standard Wet Chemistry listed in EPA-821-R-02-012
LC50:	19.32 mg/l
95% LOWER CONFIDENCE LIMITS:	17.23 mg/l
95% UPPER CONFIDENCE LIMITS:	21.67 mg/l

REFERENCE TOXICANT STATISTICAL RESULTS: LC₅₀ AND CONTROL LIMITS
Mysidopsis bahia EXPOSED TO SODIUM LAUREL SULFATE, 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
120	02/25/2004	29792	176710-02.5	Trimmed Spearman-Kärber	21.4	20.8	9.9	30.6	10.9
121	03/31/2004	29938	176710-02.5	Trimmed Spearman-Kärber	26.4	20.9	9.9	30.8	11.0
122	04/20/2004	30071	176710-02.5	Trimmed Spearman-Kärber	27.0	21.1	9.9	31.0	11.1
123	05/27/2004	30169	35229	Trimmed Spearman-Kärber	20.0	21.1	9.8	30.9	11.2
124	06/30/2004	30314	35229	Trimmed Spearman-Kärber	22.2	21.1	9.7	30.8	11.4
125	07/27/2004	30490	35229	Trimmed Spearman-Kärber	9.8	20.8	10.2	31.0	10.6
126	08/30/2004	30710	35229	Trimmed Spearman-Kärber	17.4	20.7	10.1	30.9	10.6
127	09/01/2004	30616	35229	Trimmed Spearman-Kärber	19.7	20.7	10.0	30.7	10.7
128	10/25/2004	31021	35229	Trimmed Spearman-Kärber	13.9	20.6	10.1	30.7	10.5
129	11/05/2004	30909	35229	Trimmed Spearman-Kärber	12.1	20.4	10.3	30.7	10.1
130	12/01/2004	31033	35229	Trimmed Spearman-Kärber	23.0	20.4	10.2	30.7	10.2
131	01/04/2005	31211	35229	Trimmed Spearman-Kärber	28.5	20.6	10.4	31.0	10.2
132	02/03/2005	31483	35229	Trimmed Spearman-Kärber	27.8	20.8	10.5	31.2	10.3
133	03/20/2005	31744	35229	Trimmed Spearman-Kärber	18.3	20.7	10.4	31.1	10.3
134	04/06/2005	31918	35229	Trimmed Spearman-Kärber	24.2	20.8	10.3	31.1	10.5
135	05/04/2005	32092	35229	Trimmed Spearman-Kärber	16.3	20.7	10.3	31.0	10.4
136	06/02/2005	32263	35229	Trimmed Spearman-Kärber	25.5	20.8	10.3	31.1	10.5
137	07/06/2005	32416	35229	Trimmed Spearman-Kärber	22.2	20.8	10.2	31.0	10.6
138	08/31/2005	32610	35229	Trimmed Spearman-Kärber	21.4	20.8	10.1	30.9	10.7
139	09/06/2005	32628	35229	Trimmed Spearman-Kärber	16.0	20.7	10.1	30.8	10.6
140	10/04/2005	32771	35229	Trimmed Spearman-Kärber	14.1	20.6	10.1	30.8	10.5
141	11/26/2005	32903	35229	Trimmed Spearman-Kärber	19.32	20.6	10.1	30.7	10.5

Mysidopsis Acute Control Chart



APPENDIX C

LITERATURE REFERENCES

- U.S.E.P.A., 1994. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Marine and Estuarine Organisms (Second Edition) U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.
- U.S.E.P.A., 1994. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms (Third Edition) U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.
- U.S.E.P.A., 1991. Technical Support Document for Water Quality-Based Toxic Control, U.S. Environmental Protection Agency, EPA/505/2-90-001.
- Zarr, Jerrold, H., 1984. Biostatistical Analysis, (Second Edition). Prentice-Hall, Inc., Englewood Cliffs, New Jersey.
- Weber, C.I., (ed.) 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (Fourth Edition). U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio. EPA-600/4-90-027.

