

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

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***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 E (25000 ppm) |
| Product: | Dynagard E | Laboratory Number: | 33008 |
| Permit No. | N/A | Date: | 12/5/05 |

Result Summary: *Mysidopsis bahia* LC₅₀ = 21,085.82 ppm

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

SAMPLE PREPARATION: A sample of the product Dynagard E 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. Anova and Dunnett's test on survival data demonstrated a statistically significant difference between the control and the 25000ppm concentration only. The trimmed Spearman-Kärber method was used to calculate the LC₅₀ for this data set.

LOEC: 25000 ppm**NOEC: 15000 ppm****LC₅₀: 21085.82 ppm**

BIO-AQUATIC TESTING, INC.

TOXICITY TEST

48 Hr Acute *Mysidopsis*

Client: Kirkpatrick Group Inc. Product Test

Permit Number: N/A

Sample Type: Product

Outfall Name: Dynagard E

Receiving Water Name: N?A

Lab ID: 33008

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 | | 2 | 1 | 3 | 2 | | 0 | 0 | 3 | 2 | | 25.0% |
| | | | | | | | | | | | | | | | | |

* 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.

33008

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SALT WATER TEST SETUP FORM

Client: Kirkpatrick Group Inc.

Permit _____

Facility: Product Test

Lab Number 33008

Outfall Name: Dynagard E

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: 33008

Facility: Product Test

Dilution Water(s): Synthetic Lab

Outfall: Dynagard E

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 |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |

DATE: 12/5/05 TEST NUMBER: 33008 DURATION: 48 h
TOXICANT : Dynagard E
SPECIES: Mysidopsis bahia

| RAW DATA: | Concentration | Number | Mortalities |
|-----------|----------------|---------|-------------|
| ---- | (ppm) | Exposed | |
| | .00 | 20 | 0 |
| | 100.00 | 20 | 0 |
| | 500.00 | 20 | 0 |
| | 1000.00 | 20 | 0 |
| | 5000.00 | 20 | 0 |
| | ***** (15,000) | 20 | 0 |
| | ***** (25,000) | 20 | 15 |

SPEARMAN-KARBER TRIM: 25.00%

SPEARMAN-KARBER ESTIMATES: LC50: 21085.82
95% CONFIDENCE LIMITS
ARE NOT RELIABLE.

Dynagard E Survival
File: 33008.mys Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 6.750

W = 0.533

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 E Survival
File: 33008.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 E Survival
File: 33008.mys Transform: NO TRANSFORMATION

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|----------------|----|--------|-------|--------|
| Between | 6 | 48.214 | 8.036 | 25.000 |
| Within (Error) | 21 | 6.750 | 0.321 | |
| Total | 27 | 54.964 | | |

Critical F value = 2.57 (0.05,6,21)

Since $F > \text{Critical } F$ REJECT H_0 : All equal

Dynagard E Survival
 File: 33008.mys

Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|-------|----------------|------------------|-----------------------------------|--------|-----|
| 1 | Con | 5.000 | 5.000 | | |
| 2 | 100 | 5.000 | 5.000 | 0.000 | |
| 3 | 500 | 5.000 | 5.000 | 0.000 | |
| 4 | 1000 | 5.000 | 5.000 | 0.000 | |
| 5 | 5000 | 5.000 | 5.000 | 0.000 | |
| 6 | 15000 | 5.000 | 5.000 | 0.000 | |
| 7 | 25000 | 1.250 | 1.250 | 9.354 | * |

Dunnnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

Dynagard E Survival
 File: 33008.mys

Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|-------|----------------|-------------|-----------------------------------|--------------|-------------------------|
| 1 | Con | 4 | | | |
| 2 | 100 | 4 | 0.986 | 19.7 | 0.000 |
| 3 | 500 | 4 | 0.986 | 19.7 | 0.000 |
| 4 | 1000 | 4 | 0.986 | 19.7 | 0.000 |
| 5 | 5000 | 4 | 0.986 | 19.7 | 0.000 |
| 6 | 15000 | 4 | 0.986 | 19.7 | 0.000 |
| 7 | 25000 | 4 | 0.986 | 19.7 | 3.750 |

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.

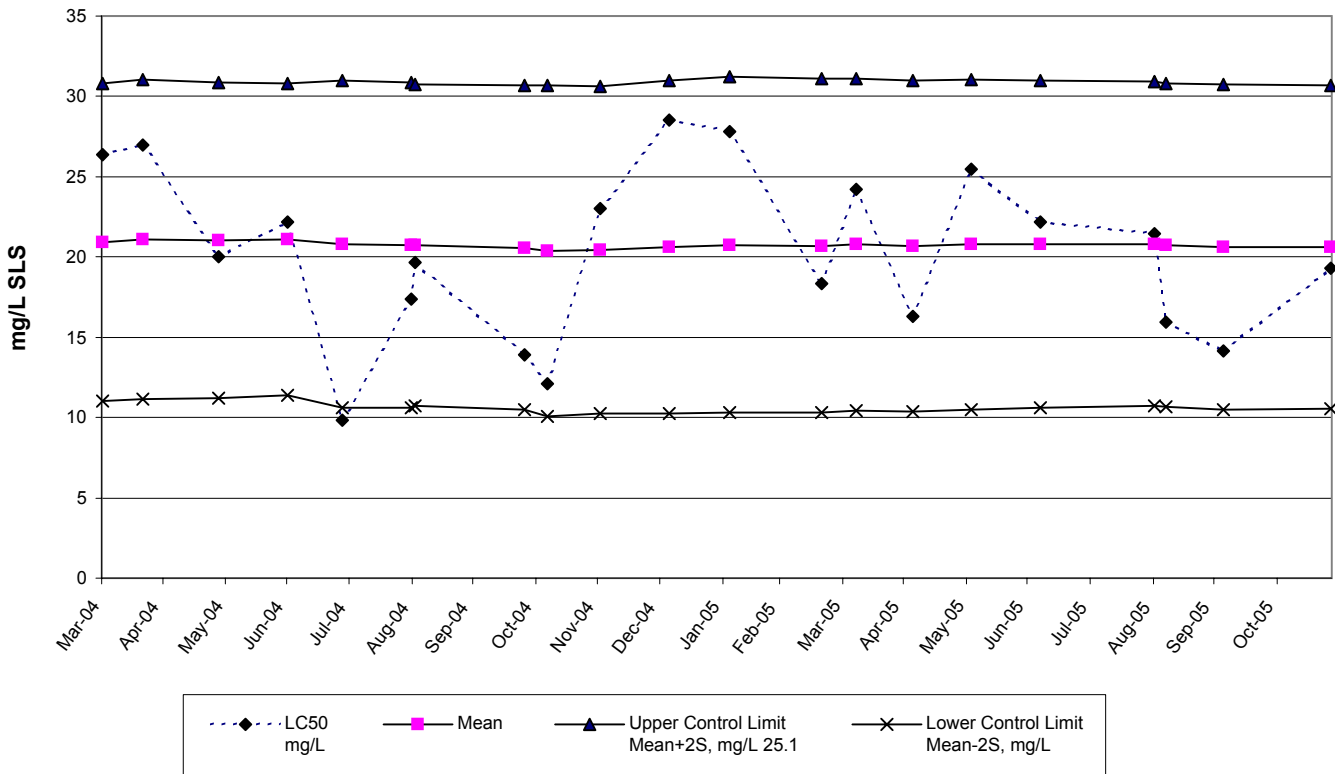
ACUTE REFERENCE TOXICANT TEST RESULTS

| | | | | | | | |
|-------------------------------|---|------|-----|---|----|----|----|
| 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.
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- 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.



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