**TRADE SECRET**

***Study Title***

H-24768: Influence on Growth and Growth Rate of the Green Alga *Selenastrum capricornutum*

**TEST GUIDELINES:** OECD Guideline for the Testing of Chemicals
Section 2: Effects on Biotic Systems, Number 201 (1984)

Commission Directive 92/69/EEC
EEC Method C3 (1992)

**AUTHOR:** Terry Lee Sloman, B.S.

**STUDY COMPLETED ON:** October 31, 2003

**PERFORMING LABORATORY:** E.I. du Pont de Nemours and Company
Haskell Laboratory for Health and Environmental Sciences
Elkton Road, P.O. Box 50
Newark, Delaware 19714-0050

**LABORATORY PROJECT ID:** DuPont-11954

[redacted]

## GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are consistent with the OECD Principles of Good Laboratory Practice (as revised in 1997) published in ENV/MC/CHEM(98)17 and MAFF Japan Good Laboratory Practice Standards (59 NohSan Number 3850).

The test substance was characterized by the sponsor prior to the initiation of this study. Although the characterization was not performed under Good Laboratory Practice Standards, the characterization was done by an ISO 9000 certified laboratory, and the accuracy of the data is considered sufficient for the purposes of this study.

Applicant / Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Director: Terry Lee Sloman 31-OCT-2003
Terry Lee Sloman, B.S. Date
Associate Scientist

Applicant / Sponsor: _____ _____
DuPont Representative Date

Company Sanitized. Does not contain TSCA CBI

## QUALITY ASSURANCE STATEMENT

Haskell Sample Number(s):

24768

Dates of Inspections:

Protocol: July 10, 2003

Conduct: July 23, 2003

Records, Reports: October 8, 9, 2003

Dates Findings Reported to:

Study Director: October 13, 2003

Management: October 13, 30, 2003

Reported by: Matthew Beardsley for JCH 31-Oct-2003

Joseph C. Hamill
Staff Quality Assurance Auditor

Date

## CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

**Analytical Evaluation by:** Boyk Sz 30-OCT-2003

Bogdan Szostek, Ph.D.
Senior Research Chemist
Date

**Statistical Analysis Reported by:** John Green 30 Oct 2003

John W. Green, Ph.D., Ph.D.
Principal Research Statistician
Date

**Issued by Study Director:** Terry Lee Sloman 31-OCT-2003

Terry Lee Sloman, B.S.
Associate Scientist
Date

## TABLE OF CONTENTS

## LIST OF TABLES



## LIST OF FIGURES



## LIST OF APPENDICES

## STUDY INFORMATION

9th Collective Nomenclature: [redacted]

Synonyms/Codes: [redacted]

- H-24768

Haskell Number: 24768

CAS Registry Number: [redacted]

Composition: [redacted]

Purity: [redacted]

Known Impurities: [redacted]

Physical Characteristics: Tan to light brown paste

Stability: The test substance stability was evaluated based on analytical measurements conducted during the study.

Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Initiated/Completed: July 3, 2003 / (see report cover page)

Experimental Initiated/Completed: July 22, 2003 / July 25, 2003

## STUDY PERSONNEL

Study Director: Terry Lee Sloman, B.S.
Management: Robert A. Hoke, Ph.D.

Analytical Chemist: Bogdan Szostek, Ph.D.
Management: S. Mark Kennedy, Ph.D.

Statistical Analysis: John W. Green, Ph.D., Ph.D.
Management: Janice L. Connell, M.S., B.A., C.I.H.

Ecotoxicology Report Preparation: Wanda F. Dinbokowitz
Management: Nancy S. Selzer, M.S.

## SUMMARY

A study was conducted to determine the effect of H-24768 on the growth and growth rate of the green alga *Selenastrum capricornutum*.[a] The algae were exposed to nominal concentrations of 6.25, 12.5, 25, 50, and 100 mg H-24768/liter of nutrient medium (ppm).

For the definitive (dose-response) test, the organisms were exposed for 72 hours (3 days) without test medium renewal. The effect was expressed as percent inhibition in growth based on healthy cell count (cell density), area under the growth curve, and growth rate relative to the blank (normal culture medium) control for the 72-hour (day 3) interval of the test.

The 72-hour results,[b] based on nominal concentrations are as follows:

**Healthy Cell Count**

| | |
|---|---|
| $EC_{50}$ | > 100 mg H-24768/L |
| NOEC[c] | 25 mg H-24768/L |

**Area Under the Growth Curve**

| | |
|---|---|
| $EC_{50}$ | > 100 mg H-24768/L |
| NOEC[c] | 25 mg H-24768/L |

**Growth Rate**

| | |
|---|---|
| $EC_{50}$ | > 100 mg H-24768/L |
| NOEC[c] | 25 mg H-24768/L |

None of the definitive test concentrations exhibited a 50% or greater growth inhibition based on healthy cell count relative to the blank control. Therefore, a recovery test was not performed.

a Also known as *Pseudokirchneriella subcapitata*.

b The $EC_{50}$ is defined as the "effective concentration" producing a 50% inhibition of growth relative to the control. The NOEC is defined as the highest concentration of test substance that has no significant effect on the measured parameter relative to the control.

c As determined by the Jonckheere trend test.

## INTRODUCTION

This study was conducted to determine the effect of H-24768 on the growth and growth rate of the green alga *Selenastrum capricornutum.*[a]

## MATERIALS AND METHODS

### A. Test Guidelines

The study design complies with the following test guidelines:

- Organisation for Economic Co-Operation and Development (OECD) (1984). 201 Effects on Biotic Systems. *Guideline for the Testing of Chemicals.*
- European Economic Communities (EEC) (1992). Directive 92/69/EEC Annex V, Part C.3, Algal Inhibition Test. *Methods for the Determination of Toxicity.*

### B. Test Substance

The test substance, H-24768, was supplied by the sponsor as a tan to light brown paste.

### C. Test System

*Selenastrum capricornutum*, a freshwater, unicellular, non-motile, green alga, was used in this study.

#### 1. Original Culture Source

The original culture source was the Department of Botany - Culture Collection of Algae - The University of Texas at Austin - Austin, Texas 78713-7640.

#### 2. Culture Maintenance

The culture method for *Selenastrum capricornutum* was based on published literature.[1-2] Prior to the study, *Selenastrum capricornutum* cultures were maintained under the same environmental conditions used in the study. The organisms were cultured in sterilized 250 mL Erlenmeyer flasks containing approximately 50 mL of filtered (= filter-sterilized) AAP nutrient medium and were aseptically transferred to fresh medium every 3 to 7 days. The flasks were fitted with foam stoppers to permit gas exchange.

---

a Also known as *Pseudokirchneriella subcapitata.*

## D. Test Design

The test design used for the *Selenastrum capricornutum* definitive and recovery tests is described below:

| Organism | Nutrient Medium | Flask Volume (mL) | Solution Volume (mL) | Volume Ratio |
|---|---|---|---|---|
| *Selenastrum capricornutum* | AAP | 250 | 50 | 5:1 |

## E. Test Preparation
(Appendix B)

### 1. AAP Nutrient Medium Preparation for *Selenastrum capricornutum*

AAP nutrient medium[1] was prepared by adding 1 mL of each of the 6 macronutrient stock solutions and 1 mL of the micronutrient stock solution to approximately 800 mL of Milli-Q® (deionized) water, with mixing after each addition. The volume of the medium was brought to 1 liter with additional Milli-Q® water. Appropriate proportions (1 mL of each stock solution for each 1 liter of medium) were used to prepare the larger volumes of the medium required for the definitive and recovery tests.

The medium pH was adjusted to 7.52 with 0.1N sodium hydroxide. For both the definitive and recovery tests, the medium was filter-sterilized using Corning® pre-sterilized filtration systems each with a 0.22 µm cellulose acetate filter. The containers with the resulting filter-sterilized AAP nutrient medium for use in the definitive and recovery tests were stored in the refrigerator in the dark at approximately 4°C and acclimated to room temperature prior to use. Any remaining unused medium was properly stored.

### 2. Test Solution Preparation

Prior to the initiation of the definitive test, a range-finding test was conducted to determine the appropriate concentrations to be used in the definitive test.

A working stock solution was prepared by dissolving the test substance in 1000 mL filter-sterilized AAP nutrient medium for a nominal concentration of 100 mg H-24768/L (= 100 ppm). Aliquots of the filter-sterilized AAP nutrient medium were used for the blank (normal culture medium) control solution. Test solutions were prepared using aliquots of the nominal 100 mg/L working stock solution and diluting with filter-sterilized AAP nutrient medium to make nominal concentrations of 6.25, 12.5, 25, and 50 mg H-24768/L. Aliquots of the nominal 100 mg/L working stock solution were used for the 100 mg H-24768/L test concentration solution and the abiotic (stability) control solution.

### 3. Test Culture Preparation

For each definitive control and test solution, 3 aliquots (50 mL each) were placed in separate sterilized 250 mL Erlenmeyer flasks fitted with sterilized foam stoppers. Each flask, excluding the abiotic control, was randomly assigned a number to eliminate bias while counting. To achieve the desired nominal concentration of approximately 10,000 *Selenastrum capricornutum* cells/mL at test initiation, an approximate 0.340 mL (= 340 µL) aliquot of algal inoculum from a logarithmically growing stock culture was aseptically transferred to each flask, except the abiotic control.

## F. Experimental Design

### 1. Definitive Test

The organisms were exposed for 72 hours (3 days), without test medium renewal. Test flasks were arranged on a lighted shaker table in a chamber in a non-systematic design and were re-positioned each working day. Each blank control, test concentration, and abiotic control was tested as 3 replicates. Cell counts were made approximately 24, 48, and 72 hours after the definitive test initiation (0-hour or day 0).

### 2. Recovery Test

Since none of the definitive test concentration exhibited a 50% or greater growth inhibition based on healthy cell count relative to the blank control, a recovery test was not necessary.

## G. Test Solutions

Solutions used in the definitive test were as follows:

| | |
|---|---|
| Blank Control | AAP nutrient medium containing no H-24768 |
| Treatment | nominal 6.25, 12.5, 25, 50, and 100 mg H-24768/liter nutrient medium |
| Abiotic Control | nominal 100 mg H-24768/liter nutrient medium; (no *Selenastrum capricornutum)* |

## H. Test Conditions - Definitive and Recovery Tests

The control and test flasks were placed in a chamber (Hotpack model 06084) and air temperature in the chamber was recorded continuously with a continuous temperature recorder (Dickson Model SL445C7). The algae were incubated for 72 hours without test medium renewal for the definitive test. Illumination was supplied by cool-white fluorescent tubes. The **target** environmental parameters are described in the following table.

| Test | Initial Population | Illumination (lumens/m² = lux) | Photo-Period (hours) | Shaking Speed (rpms) | Temperature (°C) |
|---|---|---|---|---|---|
| Definitive | 10,000 cells/mL | 6000 to 10,000 | 24 | 100 | 24 ± 2 |
| Recovery | variable; based on definitive test, termination-day counts from selected test concentrations | 6000 to 10,000 | 24 | 100 | 24 ± 2 |

## I. *Selenastrum capricornutum* Growth Measurement

### 1. Definitive Test

*Selenastrum capricornutum* growth measurement was determined by visually counting the number of cells taken from an approximate 0.2 mL sample from each flask at approximately 24, 48, and 72 hours after the definitive test initiation. The counts were conducted using a hemacytometer and a compound microscope. An aliquot of each sample was loaded into the 2 grid areas of the hemacytometer and 8 squares from each grid area (16 total squares) were selected for counting. All cells located in the 16 squares were counted and recorded as healthy or unhealthy. The total number of cells counted was multiplied by 10,000 to determine the number of cells per milliliter. Cells outside these 16 squares were not counted nor included in the total number. Observations and counts of healthy and unhealthy cells (e.g., deformed, scenescent, stunted) were recorded in the study records. Most counts were made at approximately the same time each day. Statistical calculations of the $EC_{50}$ and NOEC were based on mean healthy cell counts and nominal concentrations.

## J. Statistical Analyses

The mean healthy cell count, area under the growth curve, and growth rate at the 72-hour interval for each test concentration were expressed relative to the blank control. All calculations were based on the nominal concentrations.

The area under the growth curves for each interval were calculated according to the following formula:

$$A = [0.5 \times t_1 \times (N_1 - N_0)] + [0.5 \times (t_2 - t_1) \times (N_2 + N_1 - 2N_0)] \\ +\ldots+ [0.5 \times (t_n - t_{n-1}) \times (N_n + N_{n-1} - 2N_0)]$$

where $t_1$, $t_2$, $t_{n-1}$, and $t_n$ are times of observation measured from the initiation of the test and $N_0$, $N_1$, $N_{n-1}$, and $N_n$ are the initial and subsequent healthy cell counts (cells/mL) corresponding to the observation times.

The growth rate (μ) for each test concentration and for the controls were calculated according to the following formula:

$$\mu = \frac{\ln (N_n / N_0)}{t_n}$$

where $t_n$ is the time of observation measured from the initiation of the test and $N_0$ and $N_n$ are the initial and subsequent healthy cell counts (cells/mL) corresponding to the observation time.

The percent growth inhibition, % I, was calculated according to the following formula:

$$\% I = \frac{C - T}{C} \times 100$$

where C is equal to the mean healthy cell count, area under the growth curve, or growth rate for the blank control at a selected sampling interval and T is the mean healthy cell count, area under the growth curve, or growth rate for each test concentration at a selected sampling interval. Negative values of inhibition indicate stimulation of growth.

The means and standard errors of the healthy cell counts, areas under the growth curve, and growth rate for each test concentration and the blank control were calculated using standard procedures.[3]

The healthy cell counts, areas under the growth curve, and growth rates were used to calculate the appropriate $EC_{50}$ values. This "effective concentration" was defined as the concentration producing a 50% inhibition of growth relative to the blank control. The appropriate $EC_{50}$ values and associated 95% confidence limits were determined by weighted least-squares non-linear regression of the log of the test concentration against the measured parameter. The NOEC, defined as the highest concentration of test substance that had no significant effect on the measured parameter relative to the blank control, was determined from a trend test (Jonckheere-Terpstra) applied in a step-down manner. Such a procedure assumed a monotone dose response. All tests of significance were at $\alpha = 0.05$.[4]

## K. Analysis of Test Solutions - Definitive Test

### 1. Sample Collection

The concentrations of H-24768 in the working stock (nominal 100 mg H-24768/L), blank control, and test concentration (nominal 6.25, 12.5, 25, and 50 mg H-24768/L) solutions were verified at test initiation (day 0). An aliquot from each of the blank control and test concentration solutions was taken prior to the addition of *Selenastrum capricornutum*. The pH was determined for each of the working stock, blank control, and test concentration solutions.

The concentrations of H-24768 in the blank control, test concentration (nominal 6.25, 12.5, 25, 50, and 100 mg H-24768/L), and abiotic control (nominal 100 mg H-24768/L) solutions were verified at test termination (day 3). These samples were prepared by pooling all 3 replicates for

each of the control and test concentration solutions and taking an aliquot from each of the pooled samples. The pH was determined for each of these day 3 samples.

The concentrations of H-24768 in each of the test solutions were determined by high performance liquid chromatography/mass spectrometry (HPLC/MS).

### 2. Sample Treatment

An aliquot from the blank control, working stock, and test concentrations were sampled at the study start (day 0) and submitted for analysis. An aliquot from the pooled replicates of the blank control, test concentrations, and abiotic control were sampled on day 3 and submitted for analysis. Back-up solutions also were provided for each sample. All samples were analyzed on the day of receipt. The day 3 exposure samples were centrifuged in the IEC clinical centrifuge for 30 minutes in order to remove algae. Aliquots of all test solutions were transferred to autosampler vials for analysis by LC/MS. All samples were diluted 1:1 with acetonitrile (5 mL of sample added to 5 mL acetonitrile) at the time of sampling. Additionally, the following samples were diluted with 50/50% acetonitrile/AAP nutrient medium before analysis: 6.25 mg/L (diluted 1:6.25), 12.5 mg/L (diluted 1: 12.5), 25 mg/L (diluted 1:25), 50 mg/L (diluted 1:50), 100 mg/L (diluted 1:100).

## 3. Instrumentation and Conditions

HPLC Instrument: Model 2795, Waters
MS Instrument: ZQ, Micromass

*LC Parameters:*
Column: Luna 3u C18 (2), 20 mm x 2mm
Mobile Phase:
A - 25 mM ammonium acetate in water
B - acetonitrile
C - 25 mM ammonium acetate in acetonitrile
Gradient:

| Time (min) | % A | %B | %C | Flow (mL/min) |
|---|---|---|---|---|
| 0 | 40 | 0 | 60 | 0.3 |
| 5 | 0 | 0 | 100 | 0.3 |
| 6.5 | 0 | 0 | 100 | 0.3 |
| 6.51 | 0 | 100 | 0 | 0.3 |
| 8.5 | 0 | 100 | 0 | 0.3 |
| 8.51 | 40 | 0 | 60 | 0.3 |
| 12 | 40 | 0 | 60 | 0.3 |

Column Temperature: 30°C
Injection Volume: 35 µL
Column Switch: 1.25 and 7 min

*MS Parameters:*
Ionization mode: Electrospray, positive ions
Capillary voltage: 3.5 kV
Cone voltage: 30 V
Source Temperature: 120°C
Desolvation Temperature: 500°C
Data Acquisition Function: [redacted] SIR of 690.5, 734.5, 778.6, 822.6, 866.6 m/z; 0-7 min
[redacted] SIR of 790.5, 834.5, 878.6, 922.7, 966.7 m/z; 0-7 min
[redacted] SIR of 890.6, 934.6, 978.7, 1022.7, 1066.7 m/z; 0-7 min

## 4. Quantitation

H-25436 and H-24768 chemically represent [redacted] of the following structure: $CF_3$-$(CF_2)_n$-$CH_2$-$CH_2$-O-$(CH_2$-$CH_2$-$O)_x$-H, where [redacted] The major components of the mixture are derived for three [redacted] Five of the most intense individual [redacted] were selected for each of the [redacted] For each of the [redacted] considered [redacted] five of the most intense [redacted] represented [redacted] The [redacted] are

ionized in positive ion mode electrospray predominately as the ammonium ion adducts, when the mobile phase contains significant concentration of ammonium acetate. The quantitation was based on the total signal obtained for five selected [redacted] Separate calibration curves were made for each group of [redacted] The content of each of the selected [redacted] in the test substance was not known. The total concentration of the test substance (either H-25436 or H-24768) was assigned to each group of [redacted] that were monitored.

A primary stock solution of the reference compound, H-25436 [redacted] was made in 10 mL of acetonitrile. Calibration standards were prepared on each analysis day by dilution with 50/50 % acetonitrile/AAP nutrient medium at concentrations ranging from 0.103 to 1.03 mg/L. Duplicate injections of calibration solutions were made and peak heights were determined by electronic integration.

The calibration curves were generated by regression analysis of the peak height data obtained from chromatographic analysis of the calibration solutions. Data for test solutions was fitted to the calibration curve to determine the concentrations of H-24768. The limit of detection (LOD) and limit of quantitation (LOQ) were determined by calculating the average noise level in chromatograms of blank control samples and comparing them to the signal of a calibration standard of known concentration. Two chromatograms were examined for noise-related peaks near the retention time of the analyte. The LOD was calculated as 3 times the concentration equivalent of the mean noise level. The LOQ was calculated as 10 times the concentration equivalent of the mean noise level.

## RESULTS AND DISCUSSION

### A. Environmental Conditions - Definitive and Recovery Tests
(Tables 1-2)

- For the definitive test, the pH measurements of the test solutions ranged from 7.49 to 7.68 at the 0-hour and from 7.23 to 7.59 at the 72-hour interval.
- For the definitive test, light intensity for the areas used in the chamber ranged from 6250 to 7440 lumens/m² (= lux). The mean light intensity was 6800 lumens/m².
- For the definitive test, the shaking speed was 98 revolutions per min (rpm).
- For the definitive test, the temperature from the digital readout on the temperature recorder ranged from 24.7 to 24.8°C for the duration of the study.

### B. Test Substance Stability and Analytical Verification
(Table 3, Figures 1-4)

#### 1. Chromatographic Results

[redacted] eluted as a well-resolved peak with a retention time of approximately 2.0 minutes. [redacted] eluted as a well-resolved peak with a retention time of approximately 3.6 minutes. [redacted] eluted as a well-resolved peak with a retention time of approximately 5.0 minutes. Figure 1 shows representative calibration curves obtained for [redacted] Chromatograms of typical standards are shown in Figure 2. Figures 3 and 4 show chromatograms of a blank control solution and test concentration solution, respectively.

The LOD and LOQ for [redacted] were determined to be 0.005 mg/L and 0.016 mg/L of H-25436, respectively. The LOD and LOQ for [redacted] were determined to be 0.002 mg/L and 0.007 mg/L of H-25436, respectively. The LOD and LOQ for [redacted] were determined to be 0.019 mg/L and 0.062 mg/L of H-25436, respectively.

#### 2. Test Solution Results

The mean, measured concentration of [redacted] in the day 0 working stock (= nominal 100 mg H-24768/L test concentration, = abiotic control) was 110 mg/L. This represents 110% recovery of the active ingredient. The mean, measured concentration of [redacted] in the day 0 working stock (=nominal 100 mg H-24768/L test concentration, = abiotic control) was 95 mg/L. This represents 95% recovery of the active ingredient. The mean, measured concentration of [redacted] in the day 0 working stock (= nominal

100 mg H-24768/L test concentration, = abiotic control) was 99 mg/L. This represents 99% recovery of the active ingredient.

The mean, measured concentrations of [redacted] in the day 0 nominal 6.25, 12.5, 25, and 50 mg/L test concentration solutions were 6.3, 12, 26, and 52 mg/L, respectively. This represents 101, 96, 104, and 104% recovery of the active ingredient, respectively. The mean, measured concentrations of [redacted] in the day 0 nominal 6.25, 12.5, 25, and 50 mg/L test concentration solutions were 5.4, 11, 23, and 47 mg/L, respectively. This represents 86, 88, 92, and 94% recovery of the active ingredient, respectively. The mean, measured concentrations of [redacted] in the day 0 nominal 6.25, 12.5, 25, and 50 mg/L test concentration solutions were 5.3, 11, 24, and 49 mg/L, respectively. This represents 85, 88, 96, and 98% recovery of the active ingredient, respectively. These data indicated the stock and test concentration solutions were prepared at the desired nominal concentrations. After 3 days, the mean, measured concentrations of [redacted] in the nominal 6.25, 12.5, 25, 50, and 100 mg/L test concentrations, and abiotic control solutions were 4.3, 8.9, 18, 35, and 73 mg/L and 75 mg/L, respectively. This represents 69, 71, 72, 70, 73, and 75% recovery of the active ingredient, respectively. The blank control solutions contained no detectable concentrations of [redacted] on both day 0 and day 3. After 3 days, the mean, measured concentrations of [redacted] in the nominal 6.25, 12.5, 25, 50, and 100 mg/L test concentrations, and abiotic control solutions were 3.2, 7.1, 16, 32, and 68 mg/L and 69 mg/L, respectively. This represents 51, 57, 64, 64, 68, and 69% recovery of the active ingredient, respectively. The blank control solutions contained no detectable concentrations of [redacted] on both day 0 and day 3. After 3 days, the mean, measured concentrations of [redacted] in the nominal 6.25, 12.5, 25, 50, and 100 mg/L test concentrations, and abiotic control solutions were 2.9, 7.1, 16, 32, and 72 mg/L and 74 mg/L, respectively. This represents 46, 57, 64, 64, 72, and 74% recovery of the active ingredient, respectively. The blank control solutions contained no detectable concentrations of [redacted] on both day 0 and day 3.

The stability of H-24768 is difficult to assess since it is composed [redacted] Recovery of the [redacted] in the abiotic control on day 3 based on measured concentration were 68, 73, and 75% of day 0 measured concentrations. These results suggest marginal stability of these [redacted] over the 3 day study period using 70% of day 0 measured concentrations as the benchmark for stability on day 3.[5] Recoveries of the [redacted] on day 3 in test solutions containing *Selenastrum capricornutum* ranged from 65-74% of day 0 measured values with the exception of the day 3 recoveries for the [redacted] in the nominal 6.25 mg/L H-24768 test concentration.

## C. *Selenastrum capricornutum* Growth

(Tables 4-8, Figures 5-7, Appendices C-E)

### 1. Definitive Test

The organisms were exposed for 72 hours without test medium renewal. The effects were expressed in terms of percent inhibition in growth based on healthy cell count, area under the

growth curve, and growth rate relative to the blank control for the 72-hour interval of the test based on nominal test concentrations. The 72-hour results are as follows:

**Healthy Cell Count**

| | |
|---|---|
| $EC_{50}$ | > 100 mg H-24768/L |
| NOEC | 25 mg H-24768/L |

**Area Under the Growth Curve**

| | |
|---|---|
| $EC_{50}$ | > 100 mg H-24768/L |
| NOEC | 25 mg H-24768/L |

**Growth Rate**

| | |
|---|---|
| $EC_{50}$ | > 100 mg H-24768/L |
| NOEC | 25 mg H-24768/L |

The reductions in healthy cell count, area under the growth curve, and growth rate indicate a dose-dependent response with increasing concentrations of the test substance.

### 2. Recovery Test

None of the definitive test concentrations exhibited a 50% or greater growth inhibition based on healthy cell count relative to the blank control. Therefore, a recovery test was not performed.

## CONCLUSIONS

The reductions in healthy cell count, area under the growth curve, and growth for *Selenastrum capricornutum* at 72 hours (3 days) indicate a dose-dependent response for increasing concentrations of the test substance, H-24768. However, the $EC_{50}$ is greater than 100 mg/L based on nominal test concentrations.

## RECORDS AND SAMPLE STORAGE

Laboratory-specific or site-specific raw data, such as personnel files and equipment records will be retained by the facility where the work was done.

Specimens (if applicable), raw data, and the final report will be retained at Haskell Laboratory, Newark, Delaware, the DuPont Crop Protection Archives, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware. Characterization data (percent purity, composition, and known impurities) will be stored at Regional Analytical Services (RAS), Jackson Laboratories, Deepwater, New Jersey.

## REFERENCES

1. Miller, W.E.; Greene, J.C.; Shiroyama, T. The *Selenastrum capricornutum* Printz Algal Assay Bottle Test; U.S. Environmental Protection Agency; U.S. Government Printing Office: Washington, DC, 1978; EPA-600/9-78-018.

2. American Society for Testing and Materials (ASTM). (1990). "Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae" in *ASTM Annual Book of Standards*, E1218-90. Vol. 11.04, Philadelphia, PA.

3. Snedecor, G.W. and Cochran, W.G. (1967). *Statistical Methods*, 6th edition. The Iowa State University Press, Ames.

4. Tamhane, A.C., Dunnett, C.W., Green J.W., and Wetherington, J.D. (2001). Multiple test procedures for identifying maximum safe dose. *J Amer. Statist. Assoc.* **96**, 835-843.

5. U.S. Environmental Protection Agency. (1996). Ecological Effects Test Guidelines. OPPTS 850.1000. Special Considerations for Conducting Aquatic Laboratory Studies, Public Draft. EPA 712-C-96-113.

# TABLES

## TABLE 1

## pH MEASUREMENTS OF TEST SOLUTIONS

| Nominal H-24768 Concentration | pH | |
|---|---|---|
| | Exposure Initiated: 22-Jul-2003 0-Hour (Day 0) | Exposure Ended: 25-Jul-2003 72-Hour (Day 3) |
| Blank Control | 7.49 | 7.49 |
| 6.25 mg/L | 7.51 | 7.56 |
| 12.5 mg/L | 7.68 | 7.59 |
| 25 mg/L | 7.60 | 7.59 |
| 50 mg/L | 7.54 | 7.58 |
| 100 mg/L | 7.59 | 7.52 |
| Abiotic Control (100 mg/L) | 7.59 | 7.23 |

## TABLE 2

## CHAMBER LIGHT INTENSITY, SHAKING SPEED, AND TEMPERATURE RANGE

| Test | Mean Light Intensity at Test Initiation (lumens/$m^2$ = lux) | Oscillations (rpms) | Temperature (°C) |
|---|---|---|---|
| Definitive | 6800 | 98 | 24.7 to 24.8 |

## TABLE 3

## MEASURED CONCENTRATIONS OF H-24768 IN TEST SOLUTIONS

| Sample Description[a] | Nominal [redacted] Concentration[b] (mg/L) | Measured [redacted] Concentration (mg/L) | | Mean, Measured Concentration (mg/L)[c] | % Recovery[d] |
|---|---|---|---|---|---|
| | | Day 0 | Day 3 | | |
| Blank Control | 0 | ND[e] | ND | - | - |
| Working Stock (100 mg/L) | 100 | 110 | NS[f] | - | - |
| 6.25 mg/L | 6.25 | 6.3 | 4.3 | 5.3 | 85 |
| 12.5 mg/L | 12.5 | 12 | 8.9 | 10.5 | 84 |
| 25 mg/L | 25 | 26 | 18 | 22.0 | 88 |
| 50 mg/L | 50 | 52 | 35 | 43.5 | 87 |
| 100 mg/L | 100 | 110[g] | 73 | 91.5 | 92 |
| Abiotic Control (100 mg/L) | 100 | 110[g] | 75 | 92.5 | 93 |

a Defined in terms of H-24768 mg/liter nutrient medium.
b H-24768 was [redacted] pure by analysis.
c Mean, measured concentration calculated as (day 0 + day 3)/2.
d Calculated as nominal/mean, measured concentration.
e ND stands for not detected. The limit of detection was calculated as 0.005 mg/L.
f NS stands for not submitted.
g No separate analysis was conducted since the working stock, the day 0 nominal 100 mg/L test concentration, and the day 0 abiotic control were aliquots of the same solution. Therefore, the measured concentration obtained for the working stock can be used for the 100 mg/L test concentration and the abiotic control.

**TABLE 3 (Continued)**

**MEASURED CONCENTRATIONS OF H-24768 IN TEST SOLUTIONS**

| Sample Description[a] | Nominal [redacted] Concentration[b] (mg/L) | Measured [redacted] Concentration (mg/L) | | Mean, Measured Concentration (mg/L)[c] | % Recovery[d] |
|---|---|---|---|---|---|
| | | Day 0 | Day 3 | | |
| Blank Control | 0 | ND[e] | ND | - | - |
| Working Stock (100 mg/L) | 100 | 95 | NS[f] | - | - |
| 6.25 mg/L | 6.25 | 5.4 | 3.2 | 4.3 | 69 |
| 12.5 mg/L | 12.5 | 11 | 7.1 | 9.1 | 73 |
| 25 mg/L | 25 | 23 | 16 | 19.5 | 78 |
| 50 mg/L | 50 | 47 | 32 | 39.5 | 79 |
| 100 mg/L | 100 | 95[g] | 68 | 81.5 | 82 |
| Abiotic Control (100 mg/L) | 100 | 95[g] | 69 | 82 | 82 |

a Defined in terms of H-24768 mg/liter nutrient medium.
b H-24768 was [redacted]ure by analysis.
c Mean, measured concentration calculated as (day 0 + day 3)/2.
d Calculated as nominal/mean, measured concentration.
e ND stands for not detected. The limit of detection was calculated as 0.002 mg/L.
f NS stands for not submitted.
g No separate analysis was conducted since the working stock, the day 0 nominal 100 mg/L test concentration, and the day 0 abiotic control were aliquots of the same solution. Therefore, the measured concentration obtained for the working stock can be used for the 100 mg/L test concentration and the abiotic control.

**TABLE 3 (Continued)**

**MEASURED CONCENTRATIONS OF H-24768 IN TEST SOLUTIONS**

| Sample Description[a] | Nominal [redacted] Concentration[b] (mg/L) | Measured [redacted] Concentration (mg/L) | | Mean, Measured Concentration (mg/L)[c] | % Recovery[d] |
|---|---|---|---|---|---|
| | | Day 0 | Day 3 | | |
| Blank Control | 0 | ND[e] | ND | - | - |
| Working Stock (100 mg/L) | 100 | 99 | NS[f] | - | - |
| 6.25 mg/L | 6.25 | 5.3 | 2.9 | 4.1 | 66 |
| 12.5 mg/L | 12.5 | 11 | 7.1 | 9.1 | 73 |
| 25 mg/L | 25 | 24 | 16 | 20 | 80 |
| 50 mg/L | 50 | 49 | 32 | 40.5 | 81 |
| 100 mg/L | 100 | 99[e] | 72 | 85.5 | 86 |
| Abiotic Control (100 mg/L) | 100 | 99[e] | 74 | 86.5 | 87 |

a Defined in terms of H-24768 mg/liter nutrient medium.
b H-24768 was [redacted]ure by analysis.
c Mean, measured concentration calculated as (day 0 + day 3)/2.
d Calculated as nominal/mean, measured concentration.
e ND stands for not detected. The limit of detection was calculated as 0.019 mg/L.
f NS stands for not submitted.
g No separate analysis was conducted since the working stock, the day 0 nominal 100 mg/L test concentration, and the day 0 abiotic control were aliquots of the same solution. Therefore, the measured concentration obtained for the working stock can be used for the 100 mg/L test concentration and the abiotic control.

# TABLE 4

## HEALTHY CELL COUNT DATA SUMMARY - DEFINITIVE TEST

| Nominal H-24768 Concentration | Rep. | Exposure Initiated: Day 0: 22 July 2003 | | Exposure Ended: Day 3: 25 July 2003 | |
|---|---|---|---|---|---|
| | | Healthy Cell Count (cells/mL) by Exposure Period | | | |
| | | 0-Hour | 24-Hour | 48-Hour | 72-Hour |
| Blank Control | 1 | 10,000 | 50,000 | 290,000 | 2,360,000 |
| | 2 | 10,000 | 30,000 | 270,000 | 2,590,000 |
| | 3 | 10,000 | 140,000 | 600,000 | 2,160,000 |
| Mean | | 10,000 | 73,333 | 386,667 | 2,370,000 |
| Standard Error | | 0.00 | 33,829.64 | 106,822.80 | 124,230.97 |
| Std. Dev. | | 0.00 | 58,594.65 | 185,022.52 | 215,174.35 |
| Variance | | 0.00E+00 | 3.43E+09 | 3.42E+10 | 4.63E+10 |
| 6.25 mg/L | 1 | 10,000 | 100,000 | 630,000 | 2,520,000 |
| | 2 | 10,000 | 60,000 | 340,000 | 2,030,000 |
| | 3 | 10,000 | 20,000 | 470,000 | 2,180,000 |
| Mean | | 10,000 | 60,000 | 480,000 | 2,243,333 |
| Standard Error | | 0.00 | 23,094.01 | 83,864.97 | 144,952.10 |
| Std. Dev. | | 0.00 | 40,000.00 | 145,258.39 | 251,064.40 |
| Variance | | 0.00E+00 | 1.60E+09 | 2.11E+10 | 6.30E+10 |
| % Inhibition | | 0.00 | 18.18 | -24.14 | 5.34 |
| 12.5 mg/L | 1 | 10,000 | 120,000 | 500,000 | 2,940,000 |
| | 2 | 10,000 | 20,000 | 340,000 | 2,310,000 |
| | 3 | 10,000 | 70,000 | 150,000 | 2,860,000 |
| Mean | | 10,000 | 70,000 | 330,000 | 2,703,333 |
| Standard Error | | 0.00 | 28,867.51 | 101,159.94 | 198,017.96 |
| Std. Dev. | | 0.00 | 50,000.00 | 175,214.15 | 342,977.16 |
| Variance | | 0.00E+00 | 2.50E+09 | 3.07E+10 | 1.18E+11 |
| % Inhibition | | 0.00 | 4.55 | 14.66 | -14.06 |
| 25 mg/L | 1 | 10,000 | 120,000 | 310,000 | 1,870,000 |
| | 2 | 10,000 | 50,000 | 330,000 | 1,630,000 |
| | 3 | 10,000 | 40,000 | 470,000 | 1,490,000 |
| Mean | | 10,000 | 70,000 | 370,000 | 1,663,333 |
| Standard Error | | 0.00 | 25,166.11 | 50,332.23 | 110,955.45 |
| Std. Dev. | | 0.00 | 43,588.99 | 87,177.98 | 192,180.47 |
| Variance | | 0.00E+00 | 1.90E+09 | 7.60E+09 | 3.69E+10 |
| % Inhibition | | 0.00 | 4.55 | 4.31 | 29.82 |
| 50 mg/L | 1 | 10,000 | 50,000 | 340,000 | 1,630,000 |
| | 2 | 10,000 | 20,000 | 110,000 | 1,640,000 |
| | 3 | 10,000 | 80,000 | 470,000 | 1,520,000 |
| Mean | | 10,000 | 50,000 | 306,667 | 1,596,667 |
| Standard Error | | 0.00 | 17,320.51 | 105,251.02 | 38,441.88 |
| Std. Dev. | | 0.00 | 30,000.00 | 182,300.12 | 66,583.28 |
| Variance | | 0.00E+00 | 9.00E+08 | 3.32E+10 | 4.43E+09 |
| % Inhibition | | 0.00 | 31.82 | 20.69 | 32.63 |
| 100 mg/L | 1 | 10,000 | 10,000 | 320,000 | 1,410,000 |
| | 2 | 10,000 | 40,000 | 150,000 | 1,400,000 |
| | 3 | 10,000 | 80,000 | 170,000 | 1,370,000 |
| Mean | | 10,000 | 43,333 | 213,333 | 1,393,333 |
| Standard Error | | 0.00 | 20,275.88 | 53,644.92 | 12,018.50 |
| Std. Dev. | | 0.00 | 35,118.85 | 92,915.73 | 20,816.66 |
| Variance | | 0.00E+00 | 1.23E+09 | 8.63E+09 | 4.33E+08 |
| % Inhibition | | 0.00 | 40.91 | 44.83 | 41.21 |

# TABLE 5

## AREA UNDER THE GROWTH CURVE DATA SUMMARY - DEFINITIVE TEST

| Nominal H-24768 Concentration | Rep. | Exposure Initiated: Day 0: 22 July 2003 — Area Under the Growth Curve Based on Healthy Cell Count (cells/mL) by Exposure Period: 0-24 Hour | 0-48 Hour | Exposure Ended: Day 3: 25 July 2003 — 0-72 Hour |
|---|---|---|---|---|
| Blank Control | 1 | 480,000 | 4,320,000 | 35,880,000 |
| | 2 | 240,000 | 3,600,000 | 37,680,000 |
| | 3 | 1,560,000 | 10,200,000 | 43,080,000 |
| Mean | | 760,000 | 6,040,000 | 38,880,000 |
| Standard Error | | 405,955.66 | 2,090,358.82 | 2,163,330.77 |
| Std. Dev. | | 703,135.83 | 3,620,607.68 | 3,746,998.80 |
| Variance | | 4.94E+11 | 1.31E+13 | 1.40E+13 |
| 6.25 mg/L | 1 | 1,080,000 | 9,600,000 | 47,160,000 |
| | 2 | 600,000 | 5,160,000 | 33,360,000 |
| | 3 | 120,000 | 5,760,000 | 37,320,000 |
| Mean | | 600,000 | 6,840,000 | 39,280,000 |
| Standard Error | | 277,128.13 | 1,390,827.09 | 4,102,487.05 |
| Std. Dev. | | 480,000.00 | 2,408,983.19 | 7,105,716.01 |
| Variance | | 2.30E+11 | 5.80E+12 | 5.05E+13 |
| % Inhibition | | 21.05 | -13.25 | -1.03 |
| 12.5 mg/L | 1 | 1,320,000 | 8,520,000 | 49,560,000 |
| | 2 | 120,000 | 4,200,000 | 35,760,000 |
| | 3 | 720,000 | 3,120,000 | 39,000,000 |
| Mean | | 720,000 | 5,280,000 | 41,440,000 |
| Standard Error | | 346,410.16 | 1,649,727.25 | 4,166,341.32 |
| Std. Dev. | | 600,000.00 | 2,857,411.42 | 7,216,314.85 |
| Variance | | 3.60E+11 | 8.16E+12 | 5.21E+13 |
| % Inhibition | | 5.26 | 12.58 | -6.58 |
| 25 mg/L | 1 | 1,320,000 | 6,240,000 | 32,160,000 |
| | 2 | 480,000 | 4,800,000 | 28,080,000 |
| | 3 | 360,000 | 6,240,000 | 29,520,000 |
| Mean | | 720,000 | 5,760,000 | 29,920,000 |
| Standard Error | | 301,993.38 | 480,000.00 | 1,194,654.76 |
| Std. Dev. | | 523,067.87 | 831,384.39 | 2,069,202.75 |
| Variance | | 2.74E+11 | 6.91E+11 | 4.28E+12 |
| % Inhibition | | 5.26 | 4.64 | 23.05 |
| 50 mg/L | 1 | 480,000 | 4,920,000 | 28,320,000 |
| | 2 | 120,000 | 1,440,000 | 22,200,000 |
| | 3 | 840,000 | 7,200,000 | 30,840,000 |
| Mean | | 480,000 | 4,520,000 | 27,120,000 |
| Standard Error | | 207,846.10 | 1,674,753.71 | 2,565,307.00 |
| Std. Dev. | | 360,000.00 | 2,900,758.52 | 4,443,242.06 |
| Variance | | 1.30E+11 | 8.41E+12 | 1.97E+13 |
| % Inhibition | | 36.84 | 25.17 | 30.25 |
| 100 mg/L | 1 | 0 | 3,720,000 | 24,240,000 |
| | 2 | 360,000 | 2,400,000 | 20,760,000 |
| | 3 | 840,000 | 3,600,000 | 21,840,000 |
| Mean | | 400,000 | 3,240,000 | 22,280,000 |
| Standard Error | | 243,310.50 | 421,426.15 | 1,028,396.81 |
| Std. Dev. | | 421,426.15 | 729,931.50 | 1,781,235.53 |
| Variance | | 1.78E+11 | 5.33E+11 | 3.17E+12 |
| % Inhibition | | 47.37 | 46.36 | 42.70 |

# TABLE 6

## GROWTH RATE DATA SUMMARY - DEFINITIVE TEST

| Nominal H-24768 Concentration | Rep. | Exposure Initiated: Day 0: 22 July 2003 — Growth Rate Based on Healthy Cell Count (cells/mL) by Exposure Period: 0-24 Hour | 0-48 Hour | Exposure Ended: Day 3: 25 July 2003 — 0-72 Hour |
|---|---|---|---|---|
| Blank Control | 1 | 0.0671 | 0.0702 | 0.0759 |
| | 2 | 0.0458 | 0.0687 | 0.0772 |
| | 3 | 0.1100 | 0.0853 | 0.0747 |
| Mean | | 0.0743 | 0.0747 | 0.0759 |
| Standard Error | | 0.0189 | 0.0053 | 0.0007 |
| Std. Dev. | | 0.0327 | 0.0092 | 0.0013 |
| Variance | | 1.07E-03 | 8.43E-05 | 1.56E-06 |
| 6.25 mg/L | 1 | 0.0959 | 0.0863 | 0.0768 |
| | 2 | 0.0747 | 0.0735 | 0.0738 |
| | 3 | 0.0289 | 0.0802 | 0.0748 |
| Mean | | 0.0665 | 0.0800 | 0.0751 |
| Standard Error | | 0.0198 | 0.0037 | 0.0009 |
| Std. Dev. | | 0.0342 | 0.0064 | 0.0015 |
| Variance | | 1.17E-03 | 4.10E-05 | 2.33E-06 |
| % Inhibition | | 10.50 | -7.05 | 1.05 |
| 12.5 mg/L | 1 | 0.1035 | 0.0815 | 0.0789 |
| | 2 | 0.0289 | 0.0735 | 0.0756 |
| | 3 | 0.0811 | 0.0564 | 0.0786 |
| Mean | | 0.0712 | 0.0705 | 0.0777 |
| Standard Error | | 0.0221 | 0.0074 | 0.0011 |
| Std. Dev. | | 0.0383 | 0.0128 | 0.0018 |
| Variance | | 1.47E-03 | 1.64E-04 | 3.33E-06 |
| % Inhibition | | 4.22 | 5.71 | -2.33 |
| 25 mg/L | 1 | 0.1035 | 0.0715 | 0.0727 |
| | 2 | 0.0671 | 0.0728 | 0.0707 |
| | 3 | 0.0578 | 0.0802 | 0.0695 |
| Mean | | 0.0761 | 0.0748 | 0.0710 |
| Standard Error | | 0.0139 | 0.0027 | 0.0009 |
| Std. Dev. | | 0.0242 | 0.0047 | 0.0016 |
| Variance | | 5.83E-04 | 2.20E-05 | 2.61E-06 |
| % Inhibition | | -2.47 | -0.13 | 6.54 |
| 50 mg/L | 1 | 0.0671 | 0.0735 | 0.0707 |
| | 2 | 0.0289 | 0.0500 | 0.0708 |
| | 3 | 0.0866 | 0.0802 | 0.0698 |
| Mean | | 0.0609 | 0.0679 | 0.0704 |
| Standard Error | | 0.0169 | 0.0092 | 0.0003 |
| Std. Dev. | | 0.0294 | 0.0159 | 0.0006 |
| Variance | | 8.61E-04 | 2.52E-04 | 3.03E-07 |
| % Inhibition | | 18.08 | 9.14 | 7.24 |
| 100 mg/L | 1 | 0.0000 | 0.0722 | 0.0687 |
| | 2 | 0.0578 | 0.0564 | 0.0686 |
| | 3 | 0.0866 | 0.0590 | 0.0683 |
| Mean | | 0.0481 | 0.0625 | 0.0685 |
| Standard Error | | 0.0255 | 0.0049 | 0.0001 |
| Std. Dev. | | 0.0441 | 0.0085 | 0.0002 |
| Variance | | 1.94E-03 | 7.18E-05 | 4.33E-08 |
| % Inhibition | | 35.22 | 16.32 | 9.75 |

## TABLE 7

## 72-HOUR (DAY 3) STATISTICAL ANALYSES SUMMARY - DEFINITIVE TEST

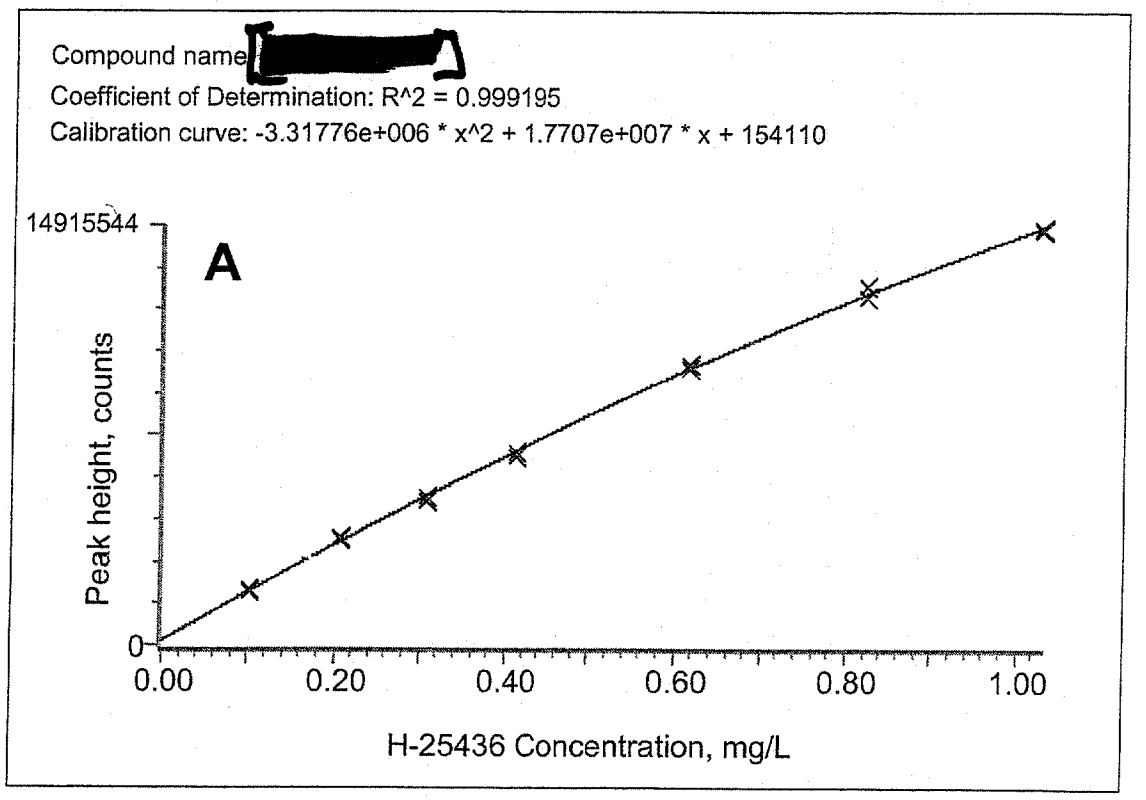
| | Nominal H-24768 Concentration | Mean Response | Standard Error | N | Test Concentration Response (mg/L) Level | Value |
|---|---|---|---|---|---|---|
| **Healthy Cell Count** | Blank Control | 2,370,000 | 124,231 | 3 | | |
| | 6.25 mg/L | 2,243,333 | 144,952 | 3 | $EC_{50}$ | > 100 |
| | 12.5 mg/L | 2,703,333 | 198,018 | 3 | | |
| | 25 mg/L | 1,663,333 | 110,955 | 3 | NOEC | 25 |
| | 50 mg/L | 1,596,667 | 38,442 | 3 | | |
| | 100 mg/L | 1,393,333 | 12,019 | 3 | | |
| **Area Under the Growth Curve** | Blank Control | 38,880,000 | 2,163,331 | 3 | | |
| | 6.25 mg/L | 39,280,000 | 4,102,487 | 3 | $EC_{50}$ | > 100 |
| | 12.5 mg/L | 41,440,000 | 4,166,341 | 3 | | |
| | 25 mg/L | 29,920,000 | 1,194,655 | 3 | NOEC | 25 |
| | 50 mg/L | 27,120,000 | 2,565,307 | 3 | | |
| | 100 mg/L | 22,280,000 | 1,028,397 | 3 | | |
| **Growth Rate** | Blank Control | 0.0759 | 0.0007 | 3 | | |
| | 6.25 mg/L | 0.0751 | 0.0009 | 3 | $EC_{50}$ | > 100 |
| | 12.5 mg/L | 0.0777 | 0.0011 | 3 | | |
| | 25 mg/L | 0.0710 | 0.0009 | 3 | NOEC | 25 |
| | 50 mg/L | 0.0704 | 0.0003 | 3 | | |
| | 100 mg/L | 0.0685 | 0.0001 | 3 | | |

# FIGURES

## FIGURE 1

## REPRESENTATIVE ANALYTICAL CALIBRATION STANDARD CURVES FOR H-25436

A
B
C

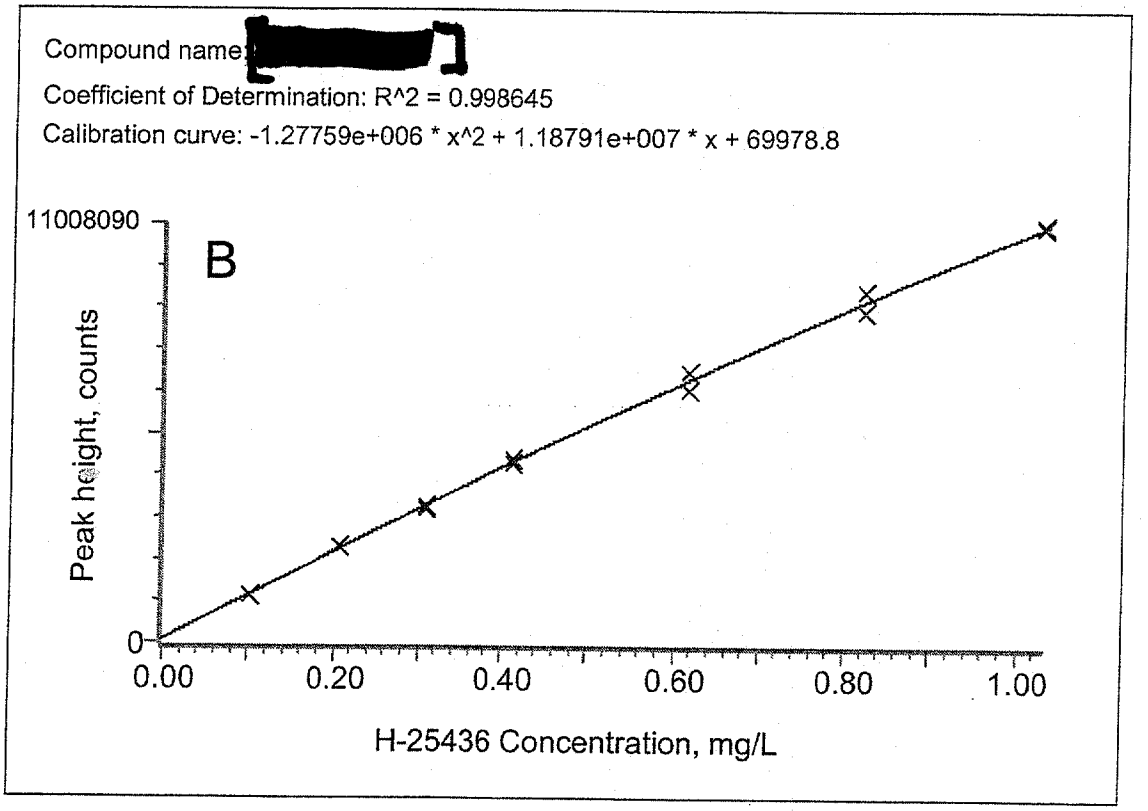



Company Sanitized. Does not contain TSCA CBI

**FIGURE 1 (Continued)**

**REPRESENTATIVE ANALYTICAL CALIBRATION STANDARD CURVES FOR H-25436**

A [redacted]
B
C

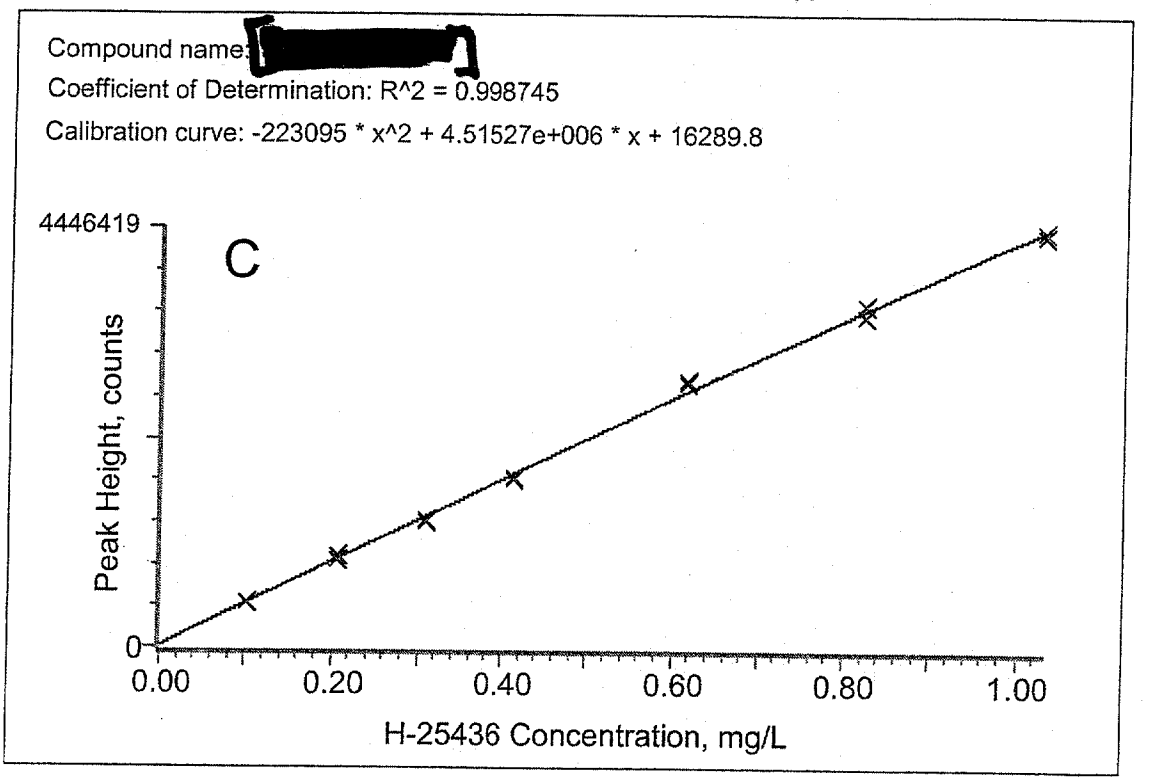



Company Sanitized. Does not contain TSCA CBI

**FIGURE 1 (Continued)**

**REPRESENTATIVE ANALYTICAL CALIBRATION STANDARD CURVES FOR H-25436**

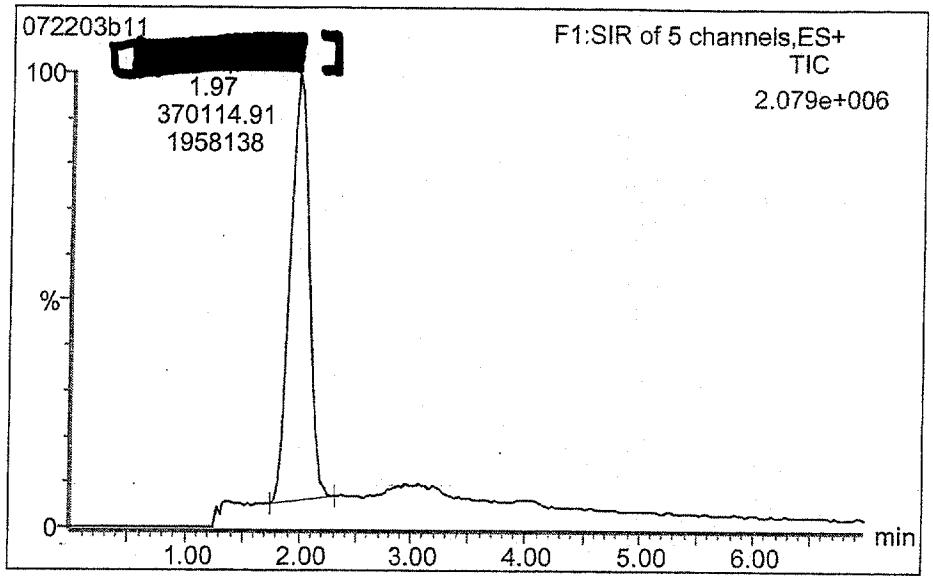
A
B
C



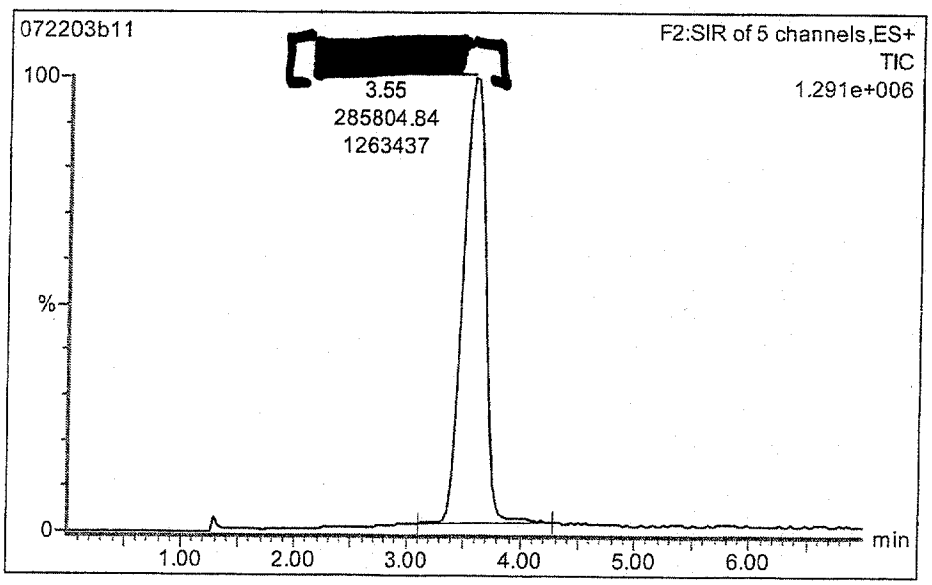

Company Sanitized. Does not contain TSCA CBI

## FIGURE 2

## REPRESENTATIVE CHROMATOGRAM OF A CALIBRATION STANDARD SOLUTION



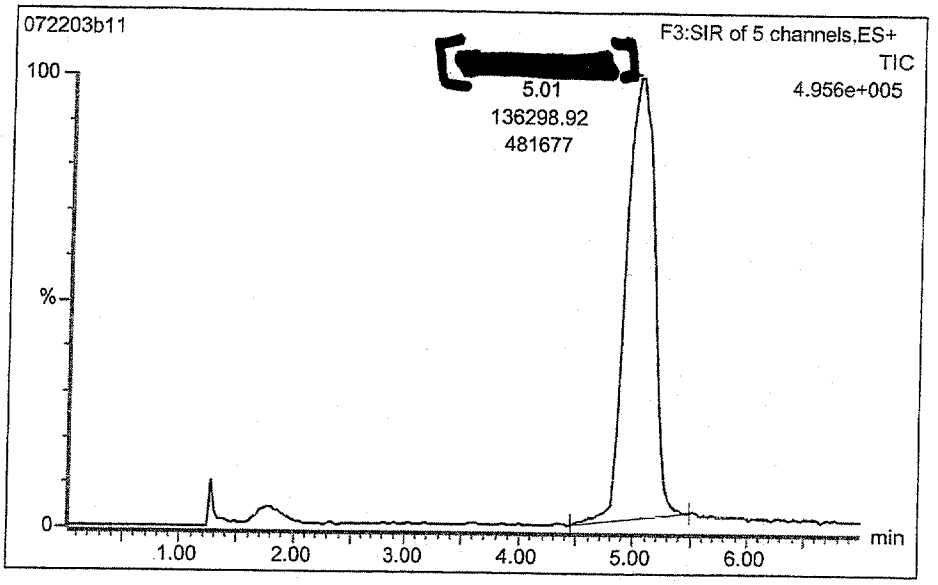

[redacted] elute at a retention time of approximately 2.0 minutes. Calibration standard solution contains H-25436 at a concentration of 0.103 mg/L.




[redacted] elute at a retention time of approximately 3.6 minutes. Calibration standard solution contains H-25436 at a concentration of 0.103 mg/L

**FIGURE 2 (Continued)**

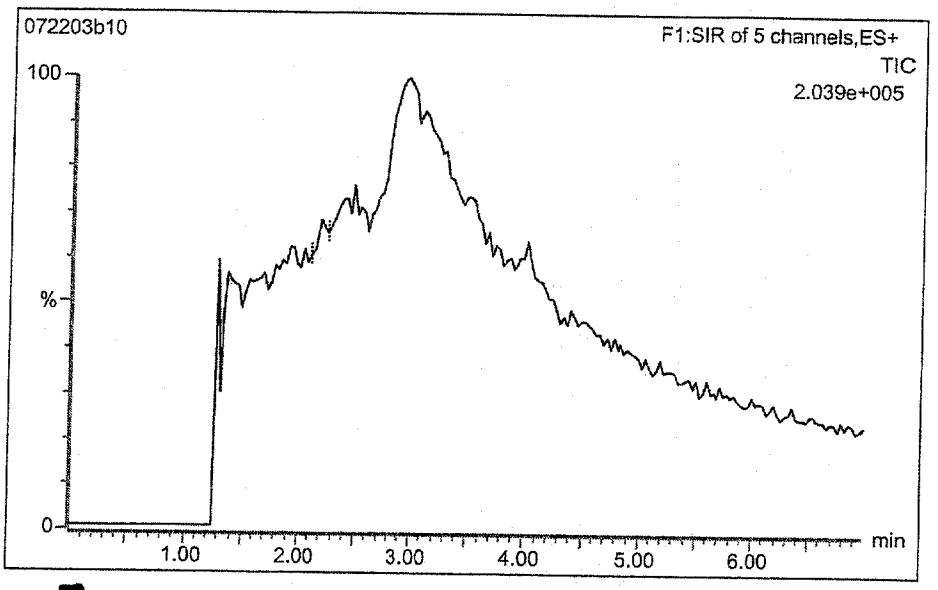
**REPRESENTATIVE CHROMATOGRAM OF A CALIBRATION STANDARD SOLUTION**



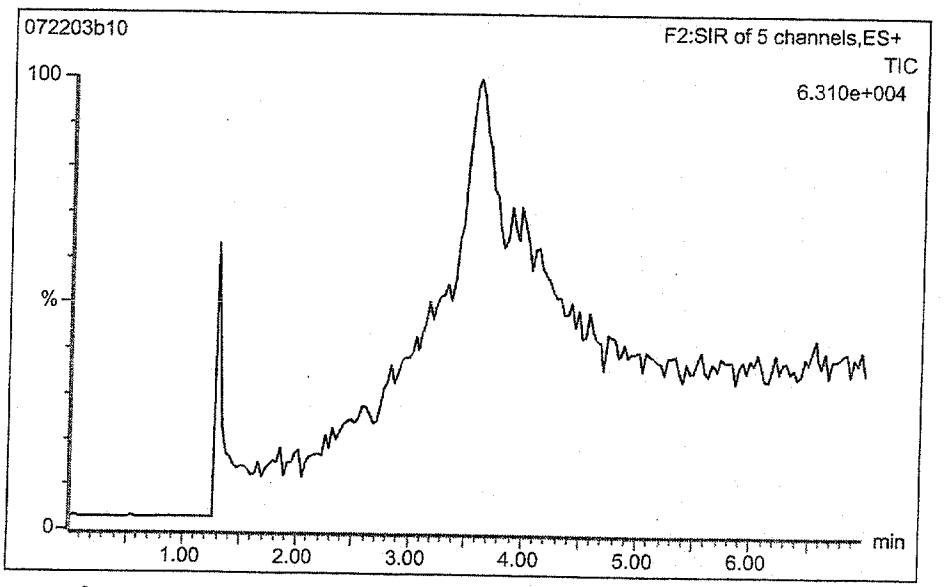

[redacted] elute at a retention time of approximately 5.0 minutes. Calibration standard solution contains H-25436 at a concentration of 0.103 mg/L.

## FIGURE 3

## REPRESENTATIVE CHROMATOGRAM OF THE BLANK CONTROL SOLUTON



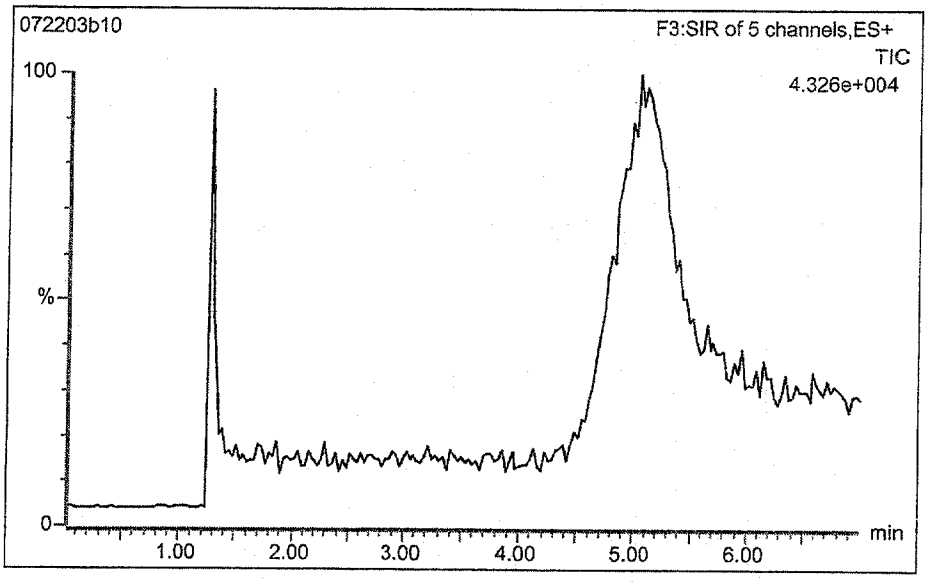

[redacted] would elute at a retention time of approximately 2.0 minutes.




[redacted] would elute at a retention time of approximately 3.6 minutes.

**FIGURE 3 (Continued)**

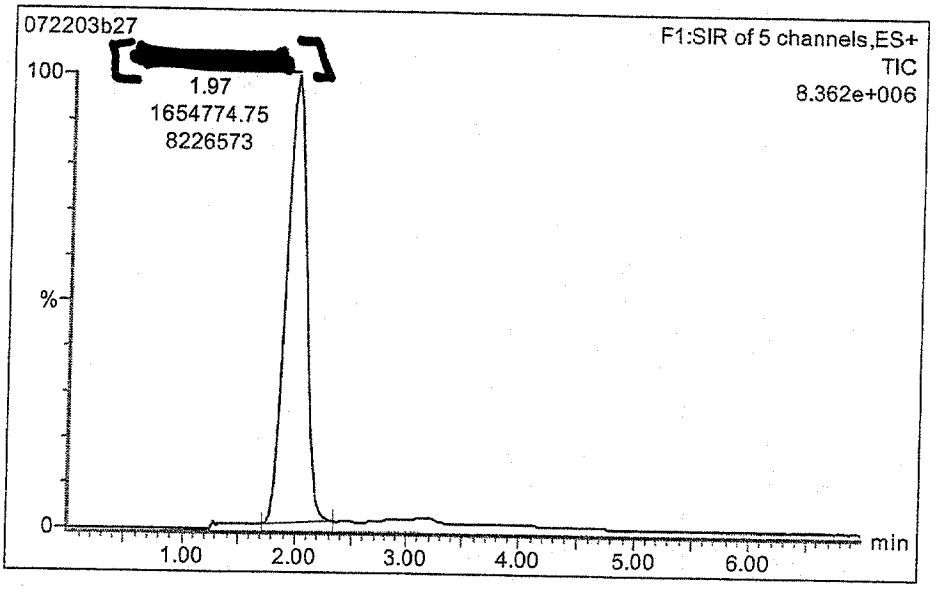
**REPRESENTATIVE CHROMATOGRAM OF THE BLANK CONTROL SOLUTON**



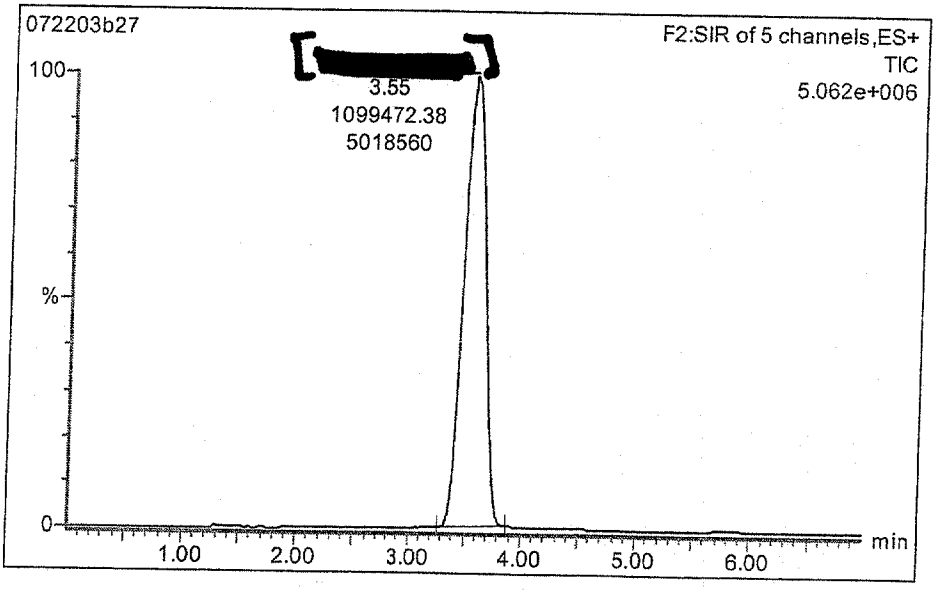

[redacted] would elute at a retention time of approximately 5.0 minutes.

## FIGURE 4

## REPRESENTATIVE CHROMATOGRAM OF A TEST CONCENTRATION SOLUTION



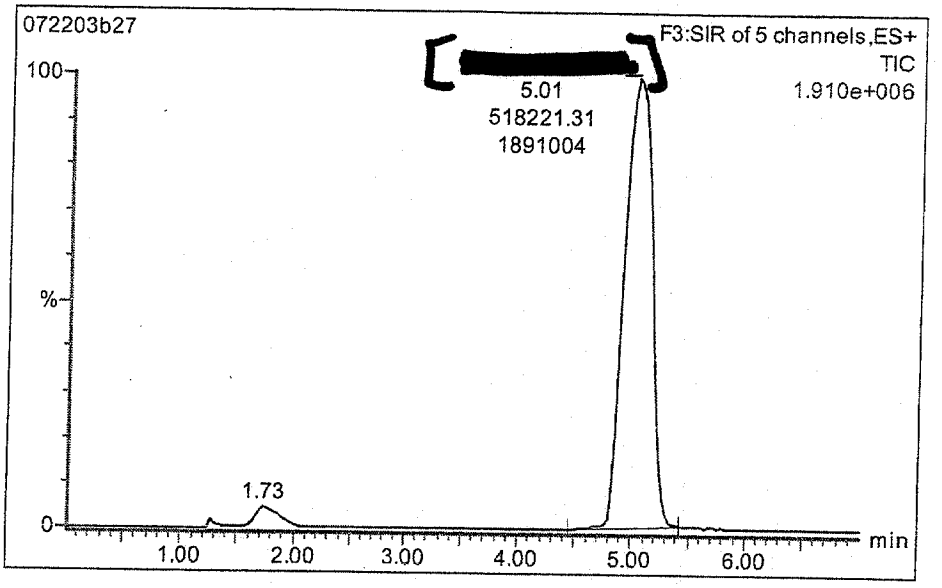

[redacted]elute at a retention time of approximately 2.0 minutes. Test solution sample contains H-24768 at a nominal concentration of 6.25 mg/L (diluted 1:12.5 before analysis).




[redacted]elute at a retention time of approximately 3.6 minutes. Test solution sample contains H-24768 at a nominal concentration of 6.25 mg/L (diluted 1:12.5 before analysis).

**FIGURE 4 (Continued)**

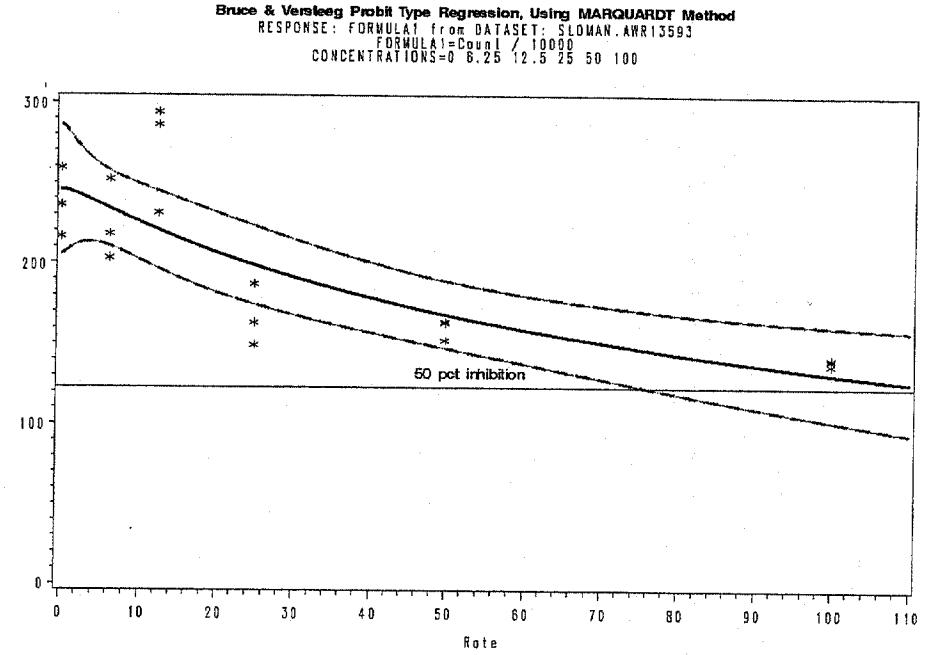
**REPRESENTATIVE CHROMATOGRAM OF A TEST CONCENTRATION SOLUTION**

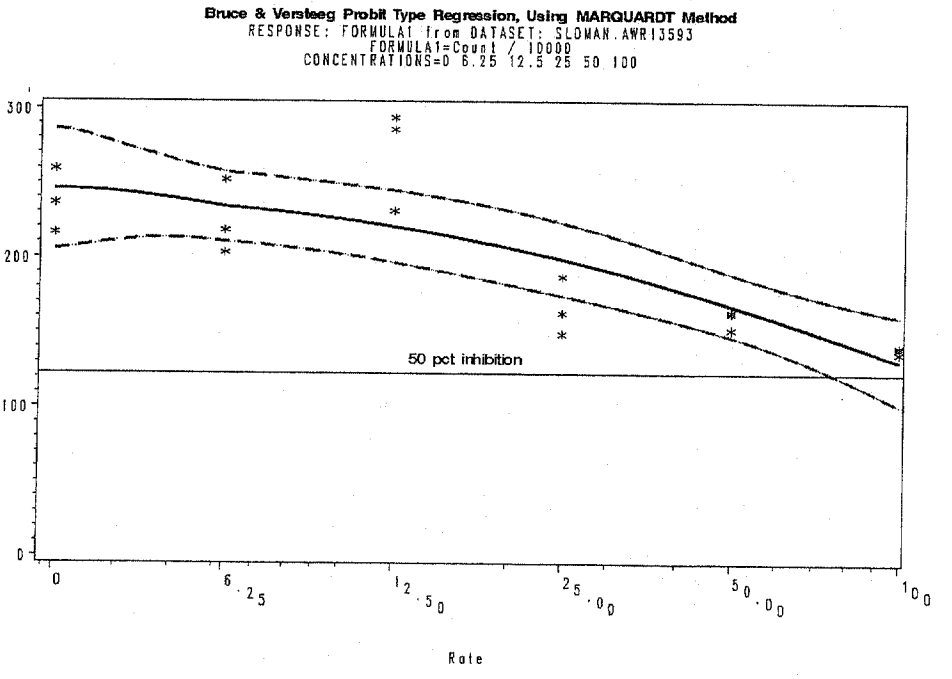



[redacted] elute at a retention time of approximately 5.0 minutes. Test solution sample contains H-24768 at a nominal concentration of 6.25 mg/L (diluted 1:12.5 before analysis).

# FIGURE 5

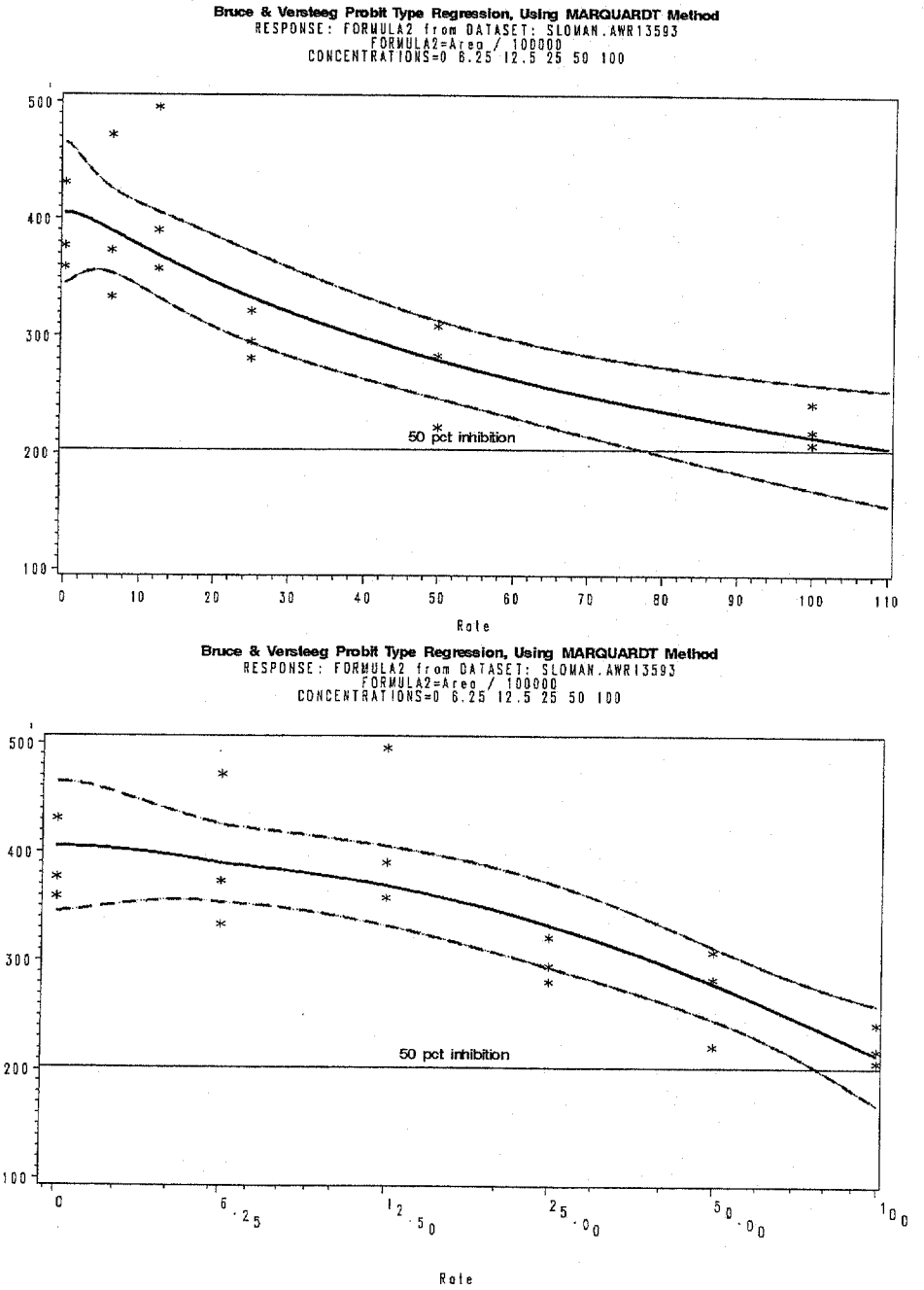
## 72-HOUR HEALTHY CELL COUNT DOSE-RESPONSE CURVE FOR THE DEFINITIVE TEST



# FIGURE 6

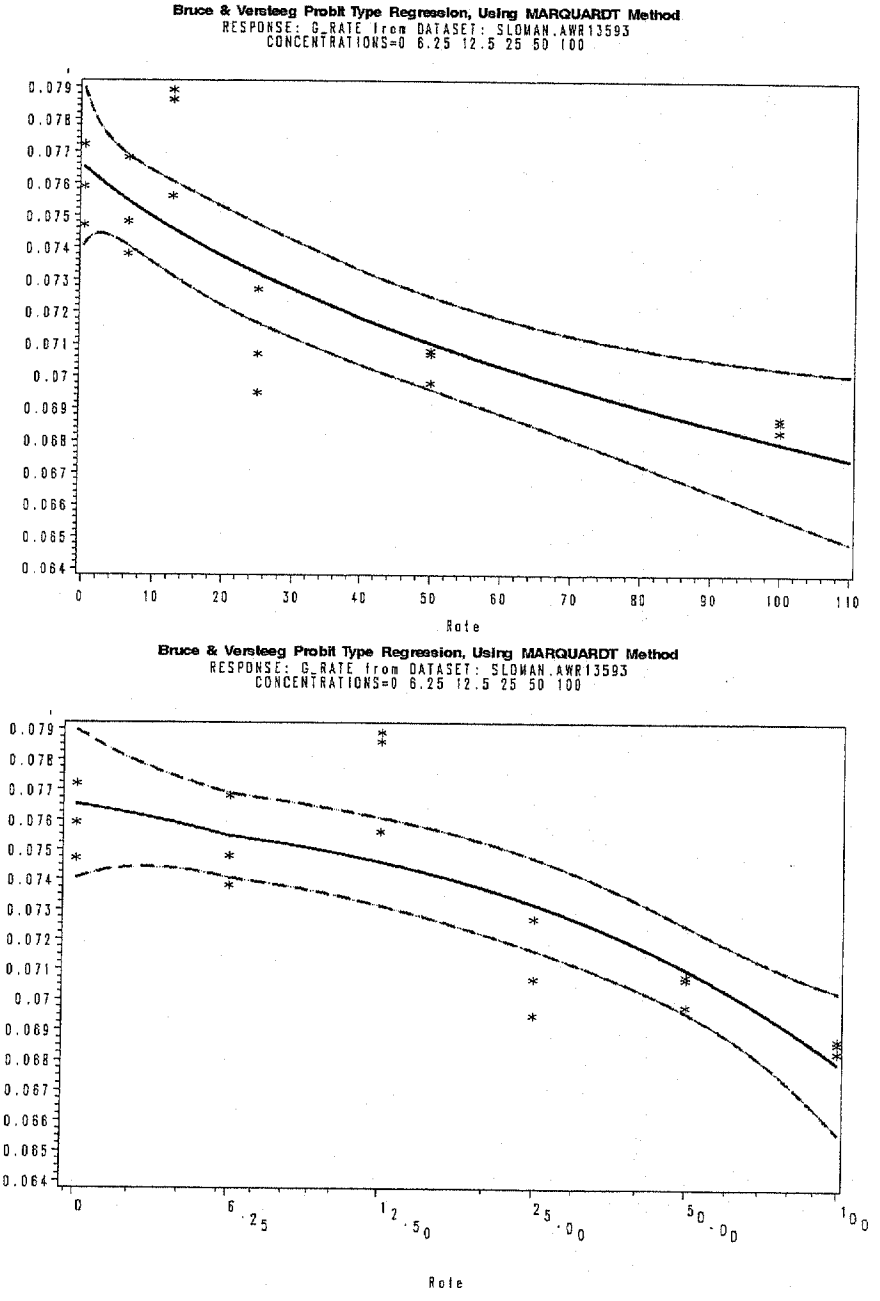
## 72-HOUR AREA UNDER THE GROWTH CURVE DOSE-RESPONSE CURVE FOR THE DEFINITIVE TEST




Company Sanitized. Does not contain TSCA CBI

# FIGURE 7

## 72-HOUR GROWTH RATE DOSE-RESPONSE CURVE FOR THE DEFINITIVE TEST




Company Sanitized. Does not contain TRADE SECRET

# APPENDICES

# APPENDIX A

## AAP Nutrient Medium Constituents

## AAP NUTRIENT MEDIUM CONSTITUENTS

| Stock Solutions[a] | | Final Prepared Medium | |
|---|---|---|---|
| **Component** | **Concentration (g/L)** | **Component** | **Concentration (mg/L)** |
| Macronutrient Stock #1 | | | |
| $NaNO_3$ | 25.500 | Na | 11.001 |
| | | N | 4.200 |
| Macronutrient Stock #2 | | | |
| $NaHCO_3$ | 15.000 | C | 2.143 |
| | | K | 0.469 |
| Macronutrient Stock #3 | | | |
| $K_2HPO_4$ | 1.044 | P | 0.186 |
| | | Mg | 2.904[b] |
| Macronutrient Stock #4 | | | |
| $MgSO_4 \cdot 7H_2O$ | 14.700 | Ca | 1.202 |
| | | S | 1.911 |
| Macronutrient Stock #5 | | | |
| $MgCl_2 \cdot 6H_2O$ | 12.164 | | |
| Macronutrient Stock #6 | | | |
| $CaCl_2 \cdot 2H_2O$ | 4.410 | | |

| Stock Solutions[a] | | Final Prepared Medium | |
|---|---|---|---|
| **Component** | **Concentration (mg/L)** | **Component** | **Concentration (µg/L)** |
| Micronutrient Stock | | | |
| $H_3BO_3$ | 185.520 | B | 32.460 |
| $MnCl_2 \cdot 4\ H_2O$ | 415.610 | Mn | 115.374 |
| $ZnCl_2$ | 3.271 | Zn | 1.570 |
| $CoCl_2 \cdot 6\ H_2O$ | 1.428 | Co | 0.354 |
| $CuCl_2 \cdot 2H_2O$ | 0.012 | Cu | 0.004 |
| $Na_2MoO_4 \cdot 2\ H_2O$ | 7.260 | Mo | 2.878 |
| $FeCl_3 \cdot 6\ H_2O$ | 160.000 | Fe | 33.051 |
| $Na_2EDTA \cdot 2\ H_2O$ | 300.000 | | |

a As reported in Reference 1.
b Includes magnesium from both magnesium sulfate and magnesium chloride.

# APPENDIX B

## 72-Hour Healthy Cell Count Statistics for the Definitive Test

## 72-HOUR HEALTHY CELL COUNT STATISTICS FOR THE DEFINITIVE TEST

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 17:19 THURSDAY 31JUL03
ECOTOX MEASUREMENTS FROM DATASET [redacted]
GROUP STATISTICS FOR Count BY DOSE

| | dose | doseval mg/L | COUNT | MEAN | MEDIAN | STD_DEV | STD_ERR |
|---|---|---|---|---|---|---|---|
| 1 | | 0 | 3 | 2370000.00 | 2360000 | 215174.35 | 124230.97 |
| 2 | | 6.25 | 3 | 2243333.33 | 2180000 | 251064.40 | 144952.10 |
| 3 | | 12.5 | 3 | 2703333.33 | 2860000 | 342977.16 | 198017.96 |
| 4 | | 25 | 3 | 1663333.33 | 1630000 | 192180.47 | 110955.45 |
| 5 | | 50 | 3 | 1596666.67 | 1630000 | 66583.28 | 38441.88 |
| 6 | | 100 | 3 | 1393333.33 | 1400000 | 20816.66 | 12018.50 |

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 17:19 THURSDAY 31JUL03
SHAPIRO-WILK TEST OF NORMALITY OF Count
AQUATIC SELENASTRUM: FULL DATA

| STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|---|---|---|---|---|---|
| 177819.03 | -0.32849 | -0.034462 | 0.95919 | 0.58599 | |

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 17:19 THURSDAY 31JUL03
LEVENE TEST FOR Count - FULL Model
ANALYSIS OF VARIANCE ON FULL DATA SET

| Effect | DF | LEVENE | P_VALUE | SIGNIF |
|---|---|---|---|---|
| DOSE | 5 | 0.67167 | 0.65269 | |

NOTE

The data was found to be normally distributed
with equal variances.
An analysis of variance will be performed.

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 17:19 THURSDAY 31JUL03
ANALYSIS OF SELENASTRUM - FULL DATA
DOSES 0, 6.25, 12.5, 25, 50, 100 mg/L

| Obs | Class | Levels | Values |
|---|---|---|---|
| 1 | dose | 6 | 1 2 3 4 5 6 |

OVERALL F-TESTS FOR ANOVA
Count FROM FULL DATA Using Weight=NO WEIGHT and Variance Ratio=0.33

| Obs | Effect | Num DF | Den DF | FValue | ProbF |
|---|---|---|---|---|---|
| 1 | dose | 5 | 12 | 17.88 | <.0001 |

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 17:19 THURSDAY 31JUL03
ANALYSIS OF SELENASTRUM - FULL DATA
LSMEANS - DOSE MEANS ADJUSTED FOR THE EFFECTS OF SELENASTRUM
Count FROM FULL DATA Using Weight=NO WEIGHT and Variance Ratio=0.33

| Obs | LEVEL | LSMEAN | SE | DDF |
|---|---|---|---|---|
| 1 | 0 | 2370000.00 | 122194.44 | 12 |
| 2 | 6.25 | 2243333.33 | 122194.44 | 12 |
| 3 | 12.5 | 2703333.33 | 122194.44 | 12 |
| 4 | 25 | 1663333.33 | 122194.44 | 12 |
| 5 | 50 | 1596666.67 | 122194.44 | 12 |
| 6 | 100 | 1393333.33 | 122194.44 | 12 |

ESTIMATED DOSE EFFECTS & DUNNETT FOR Decreasing ALTERNATIVE
USING ALPHA=.05 FOR COMPARISONS TO CONTROL
Count FROM FULL DATA Using Weight=NO WEIGHT and Variance Ratio=0.33

| Estimate | SIGNIF | Dunnett 1-sided p-value | Test Group Mean | N |
|---|---|---|---|---|
| DOSE TREND | ** | 0.00001 | . | . |
| DOSE QUAD | * | 0.04616 | . | . |
| DOSE 2-1 | | 0.91420 | 2243333.33 | 3 |
| DOSE 3-1 | | 0.25156 | 2703333.33 | 3 |
| DOSE 4-1 | ** | 0.00613 | 1663333.33 | 3 |
| DOSE 5-1 | ** | 0.00315 | 1596666.67 | 3 |
| DOSE 6-1 | ** | 0.00046 | 1393333.33 | 3 |

ESTIMATED DOSE EFFECTS & DUNNETT FOR Decreasing ALTERNATIVE
USING ALPHA=.05 FOR COMPARISONS TO CONTROL
Count FROM FULL DATA Using Weight=NO WEIGHT and Variance Ratio=0.33

Check for Number of Reps in count
Maximum Number of Reps in Any Treatment Group or Control
Maximum Number of Reps per Group is 3
Exact Permutation Methods Recommended

Analysis of [redacted] Data
Test for TREND IN EITHER DIRECTION in Response=count
ANALYSIS USING ALPHA=0.05
FULL DATA

KEY
_JT_ IS JONCKHEERE STATISTIC
Z_JT IS STANDARDIZED JONCKHEERE STATISTIC
XPR_JT IS P-VALUE FOR UPWARD TREND
XPL_JT IS P-VALUE FOR DOWNWARD TREND
P-VALUES ARE FOR TIE-CORRECTED TEST WITH CONTINUITY CORRECTION FACTOR
SIGNIF RESULTS ARE FOR D ALTERNATIVE HYPOTHESIS

Jonckheere Trend Test on Dose 0 + Lowest 5 Doses thru 100 MG/L

| JT | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|---|---|---|---|---|---|
| 31 | -3.466368 | 0.0001346 | . | ** | 6 |

Jonckheere Trend Test on Dose 0 + Lowest 4 Doses thru 50 MG/L

| JT | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|---|---|---|---|---|---|
| 29.5 | -2.281172 | 0.0114348 | . | ** | 5 |

Jonckheere Trend Test on Dose 0 + Lowest 3 Doses thru 25 MG/L

| JT | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|---|---|---|---|---|---|
| 24 | -1.279204 | 0.1166342 | . | | 4 |

NOTE
Jonckheere test results are included in the summary table.
Group means should be examined to check for lack-of-fit
to a linear trend before trend test results are accepted.

**EC50 for Cell Count** (Note: Formula1=Cell Count/100000)

OBSERVED & PREDICTED PERCENT CHANGE FROM CONTROL MEAN
RESPONSE: FORMULA1 from DATASET:[redacted]

| Obs | CONC mg/L | N | MEAN | PRED | PCTCHNG | PREDPCTCHNG |
|---|---|---|---|---|---|---|
| 1 | 0.00 | 3 | 237.000 | 245.544 | 0 | 0 |
| 2 | 6.25 | 3 | 224.333 | 233.638 | -5 | -5 |
| 3 | 12.50 | 3 | 270.333 | 220.312 | 14 | -10 |
| 4 | 25.00 | 3 | 166.333 | 198.514 | -30 | -19 |
| 5 | 50.00 | 3 | 159.667 | 167.920 | -33 | -32 |
| 6 | 100.00 | 3 | 139.333 | 131.071 | -41 | -47 |

EC50 using Method=MARQUARDT, Bruce & Versteeg Weighted Probit
Covariance Matrix and Standard Error of Parameter Estimates

| _NAME_ | logecx | gamma | y0 | SE |
|---|---|---|---|---|
| logecx | 0.08794 | 0.05019 | -2.085 | 0.2965 |
| gamma | 0.05019 | 0.40994 | 7.924 | 0.6403 |
| y0 | -2.08530 | 7.92425 | 355.372 | 18.8513 |

Model Parameter Estimates, EC50 and 95% Confidence Bounds

| logecx | gamma | y0 | LB | UB | EC50 |
|---|---|---|---|---|---|
| 4.75450 | 1.76051 | 245.544 | 64.9268 | 207.625 | 116.105 |

Model Evaluation Measures

| SSREG | SSERR | SSTOTC | SSTOTU | RSQUARE |
|---|---|---|---|---|
| 3591 | 78.6661 | 237.796 | 3669.67 | 0.66919 |

note
Graph descriptions include the following:
formula1: EC50 BVP FIT BY MARQUARDT,

# APPENDIX C

## 72-Hour Area Under the Growth Curve Statistics for the Definitive Test

## 72-HOUR AREA UNDER THE GROWTH CURVE STATISTICS FOR THE DEFINITIVE TEST

STATISTICAL ANALYSIS LIST FILE
ECOTOX MEASUREMENTS FROM DATASET [redacted]
GROUP STATISTICS FOR Area BY DOSE

| dose | doseval mg/L | COUNT | MEAN | MEDIAN | STD_DEV | STD_ERR |
|---|---|---|---|---|---|---|
| 1 | 0 | 3 | 38880000 | 37680000 | 3746998.80 | 2163330.77 |
| 2 | 6.25 | 3 | 39280000 | 37320000 | 7105716.01 | 4102487.05 |
| 3 | 12.5 | 3 | 41440000 | 39000000 | 7216314.85 | 4166341.32 |
| 4 | 25 | 3 | 29920000 | 29520000 | 2069202.75 | 1194654.76 |
| 5 | 50 | 3 | 27120000 | 28320000 | 4443242.06 | 2565307.00 |
| 6 | 100 | 3 | 22280000 | 21840000 | 1781235.53 | 1028396.81 |

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 17:19 THURSDAY 31JUL03
SHAPIRO-WILK TEST OF NORMALITY OF Area

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|---|---|---|---|---|---|---|
| 18 | 4113152.51 | 0.57085 | -0.17048 | 0.94614 | 0.36764 | |

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 17:19 THURSDAY 31JUL03
LEVENE TEST FOR Area - FULL Model
ANALYSIS OF VARIANCE ON FULL DATA SET

| Effect | DF | LEVENE | P_VALUE | SIGNIF |
|---|---|---|---|---|
| DOSE | 5 | 0.55480 | 0.73255 | |

NOTE
The data was found to be normally distributed with equal variances.
An analysis of variance will be performed.

REPORT CREATED ON 17:19 THURSDAY 31JUL03
ANALYSIS OF SELENASTRUM - FULL DATA
DOSES 0, 6.25, 12.5, 25, 50, 100 mg/L
ECOTOX OF AQUATIC SELENASTRUM

| Obs | Class | Levels | Values |
|---|---|---|---|
| 1 | dose | 6 | 1 2 3 4 5 6 |

OVERALL F-TESTS FOR ANOVA
Area FROM FULL DATA Using Weight=NO WEIGHT and Variance Ratio=0.33

| Obs | Effect | Num DF | Den DF | FValue | ProbF |
|---|---|---|---|---|---|
| 1 | dose | 5 | 12 | 7.61 | 0.0020 |

LSMEANS - DOSE MEANS ADJUSTED FOR THE EFFECTS OF SELENASTRUM
Area FROM FULL DATA Using Weight=NO WEIGHT and Variance Ratio=0.33

| Obs | LEVEL | LSMEAN | SE | DDF |
|---|---|---|---|---|
| 1 | 0 | 38880000 | 2826493.71 | 12 |
| 2 | 6.25 | 39280000 | 2826493.71 | 12 |
| 3 | 12.5 | 41440000 | 2826493.71 | 12 |
| 4 | 25 | 29920000 | 2826493.71 | 12 |
| 5 | 50 | 27120000 | 2826493.71 | 12 |
| 6 | 100 | 22280000 | 2826493.71 | 12 |

ESTIMATED DOSE EFFECTS & DUNNETT FOR Decreasing ALTERNATIVE
USING ALPHA=.05 FOR COMPARISONS TO CONTROL
Area FROM FULL DATA Using Weight=NO WEIGHT and Variance Ratio=0.33

| Estimate | SIGNIF | Dunnett 1-sided p-value | Test Group Mean | N |
|---|---|---|---|---|
| DOSE TREND | ** | 0.00013 | . | . |
| DOSE QUAD | | 0.10086 | . | . |
| DOSE 2-1 | | 0.99999 | 39280000 | 3 |
| DOSE 3-1 | | 0.94736 | 41440000 | 3 |
| DOSE 4-1 | | 0.15377 | 29920000 | 3 |
| DOSE 5-1 | * | 0.04655 | 27120000 | 3 |
| DOSE 6-1 | ** | 0.00549 | 22280000 | 3 |

ESTIMATED DOSE EFFECTS & DUNNETT FOR Decreasing ALTERNATIVE
USING ALPHA=.05 FOR COMPARISONS TO CONTROL
Area FROM FULL DATA Using Weight=NO WEIGHT and Variance Ratio=0.33

KEY
_JT_ IS JONCKHEERE STATISTIC
Z_JT IS STANDARDIZED JONCKHEERE STATISTIC
XPR_JT IS P-VALUE FOR UPWARD TREND
XPL_JT IS P-VALUE FOR DOWNWARD TREND
P-VALUES ARE FOR TIE-CORRECTED TEST WITH CONTINUITY CORRECTION FACTOR
SIGNIF RESULTS ARE FOR Decreasing ALTERNATIVE HYPOTHESIS

Jonckheere Trend Test on Dose 0 + Lowest 5 Doses thru 100 MG/L

| _____JT_____ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|---|---|---|---|---|---|
| 28 | -3.733532 | 0.0000343 | . | ** | 6 |

Jonckheere Trend Test on Dose 0 + Lowest 4 Doses thru 50 MG/L

| _____JT_____ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|---|---|---|---|---|---|
| 25.5 | -2.734396 | 0.0027539 | . | ** | 5 |

Jonckheere Trend Test on Dose 0 + Lowest 3 Doses thru 25 MG/L

| _____JT_____ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|---|---|---|---|---|---|
| 21 | -1.705606 | 0.0514529 | . | | 4 |

NOTE
Jonckheere test results are included in the summary table.
Group means should be examined to check for lack-of-fit
to a linear trend before trend test results are accepted.

## EC50 for AREA (UC)

OBSERVED & PREDICTED PERCENT CHANGE FROM CONTROL MEAN
RESPONSE: FORMULA2 from DATASET: [redacted]

| Obs | CONC mg/L | N | MEAN | PRED | PCTCHNG | PREDPCTCHNG |
|---|---|---|---|---|---|---|
| 1 | 0.00 | 3 | 388.8 | 404.834 | 0 | 0 |
| 2 | 6.25 | 3 | 392.8 | 389.304 | 1 | -4 |
| 3 | 12.50 | 3 | 414.4 | 368.726 | 7 | -9 |
| 4 | 25.00 | 3 | 299.2 | 332.613 | -23 | -18 |
| 5 | 50.00 | 3 | 271.2 | 279.532 | -30 | -31 |
| 6 | 100.00 | 3 | 222.8 | 214.191 | -43 | -47 |

EC50 using Method=MARQUARDT, Bruce & Versteeg Weighted Probit
Covariance Matrix and Standard Error of Parameter Estimates

| _NAME_ | logecx | gamma | y0 | SE |
|---|---|---|---|---|
| logecx | 0.06314 | 0.0352 | -2.563 | 0.2513 |
| gamma | 0.03519 | 0.3039 | 10.175 | 0.5513 |
| y0 | -2.56309 | 10.1754 | 781.952 | 27.9634 |

Model Parameter Estimates, EC50 and 95% Confidence Bounds

| logecx | gamma | y0 | LB | UB | EC50 |
|---|---|---|---|---|---|
| 4.72438 | 1.63377 | 404.834 | 68.8452 | 184.362 | 112.661 |

Model Evaluation Measures

| SSREG | SSERR | SSTOTC | SSTOTU | RSQUARE |
|---|---|---|---|---|
| 5967.60 | 111.608 | 390.035 | 6079.21 | 0.71385 |

note
Graph descriptions include the following:
formula2: EC50 BVP FIT BY MARQUARDT,

# APPENDIX D

## 72-Hour Growth Rate Statistics for the Definitive Test

## 72-HOUR GROWTH RATE STATISTICS FOR THE DEFINITIVE TEST

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 17:19 THURSDAY 31JUL03
ECOTOX MEASUREMENTS FROM DATASET [redacted]
GROUP STATISTICS FOR G_Rate BY DOSE

| dose | doseval mg/L | COUNT | MEAN | MEDIAN | STD_DEV | STD_ERR |
|---|---|---|---|---|---|---|
| 1 | 0 | 3 | 0.075933 | 0.0759 | .001250333 | .000721880 |
| 2 | 6.25 | 3 | 0.075133 | 0.0748 | .001527525 | .000881917 |
| 3 | 12.5 | 3 | 0.077700 | 0.0786 | .001824829 | .001053565 |
| 4 | 25 | 3 | 0.070967 | 0.0707 | .001616581 | .000933333 |
| 5 | 50 | 3 | 0.070433 | 0.0707 | .000550757 | .000317980 |
| 6 | 100 | 3 | 0.068533 | 0.0686 | .000208167 | .000120185 |

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 17:19 THURSDAY 31JUL03
SHAPIRO-WILK TEST OF NORMALITY OF G_Rate
AQUATIC SELENASTRUM: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|---|---|---|---|---|---|---|
| 18 | .001094729 | -0.13041 | -0.58919 | 0.96847 | 0.76851 | |

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 17:19 THURSDAY 31JUL03
LEVENE TEST FOR G_Rate - FULL Model
ANALYSIS OF VARIANCE ON FULL DATA SET

| Effect | DF | LEVENE | P_VALUE | SIGNIF |
|---|---|---|---|---|
| DOSE | 5 | 0.55036 | 0.73563 | |

NOTE
The data was found to be normally distributed with equal variances.
An analysis of variance will be performed.

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 17:19 THURSDAY 31JUL03
ANALYSIS OF SELENASTRUM - FULL DATA
DOSES 0, 6.25, 12.5, 25, 50, 100 mg/L
ECOTOX OF AQUATIC SELENASTRUM

| Obs | Class | Levels | Values |
|---|---|---|---|
| 1 | dose | 6 | 1 2 3 4 5 6 |

OVERALL F-TESTS FOR ANOVA
G_Rate FROM FULL DATA Using Weight=NO WEIGHT and Variance Ratio=0.33

| Obs | Effect | Num DF | Den DF | FValue | ProbF |
|---|---|---|---|---|---|
| 1 | dose | 5 | 12 | 23.27 | <.0001 |

Company Sanitized. Does not contain TSCA CBI

LSMEANS - DOSE MEANS ADJUSTED FOR THE EFFECTS OF SELENASTRUM
G_Rate FROM FULL DATA Using Weight=NO WEIGHT and Variance Ratio=0.33

| Obs | LEVEL | LSMEAN | SE | DDF |
|---|---|---|---|---|
| 1 | 0 | 0.075933 | .000752280 | 12 |
| 2 | 6.25 | 0.075133 | .000752280 | 12 |
| 3 | 12.5 | 0.077700 | .000752280 | 12 |
| 4 | 25 | 0.070967 | .000752280 | 12 |
| 5 | 50 | 0.070433 | .000752280 | 12 |
| 6 | 100 | 0.068533 | .000752280 | 12 |

ESTIMATED DOSE EFFECTS & DUNNETT FOR Decreasing ALTERNATIVE
USING ALPHA=.05 FOR COMPARISONS TO CONTROL
G_Rate FROM FULL DATA Using Weight=NO WEIGHT and Variance Ratio=0.33

| Estimate | SIGNIF | Dunnett 1-sided p-value | Test Group Mean | N |
|---|---|---|---|---|
| DOSE TREND | ** | 0.00000 | . | . |
| DOSE QUAD | * | 0.02339 | . | . |
| DOSE 2-1 | | 0.90629 | 0.075133 | 3 |
| DOSE 3-1 | | 0.37087 | 0.077700 | 3 |
| DOSE 4-1 | ** | 0.00227 | 0.070967 | 3 |
| DOSE 5-1 | ** | 0.00099 | 0.070433 | 3 |
| DOSE 6-1 | ** | 0.00007 | 0.068533 | 3 |

ESTIMATED DOSE EFFECTS & DUNNETT FOR Decreasing ALTERNATIVE
USING ALPHA=.05 FOR COMPARISONS TO CONTROL

KEY _JT_ IS JONCKHEERE STATISTIC
Z_JT IS STANDARDIZED JONCKHEERE STATISTIC
XPR_JT IS P-VALUE FOR UPWARD TREND
XPL_JT IS P-VALUE FOR DOWNWARD TREND
P-VALUES ARE FOR TIE-CORRECTED TEST WITH CONTINUITY CORRECTION
SIGNIF RESULTS ARE FOR D ALTERNATIVE HYPOTHESIS

Jonckheere Trend Test on Dose 0 + Lowest 5 Doses thru 100 MG/L

| _JT_ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|---|---|---|---|---|---|
| 31 | -3.466368 | 0.0001346 | . | ** | 6 |

Jonckheere Trend Test on Dose 0 + Lowest 4 Doses thru 50 MG/L

| _JT_ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|---|---|---|---|---|---|
| 29.5 | -2.281172 | 0.0114348 | . | ** | 5 |

Jonckheere Trend Test on Dose 0 + Lowest 3 Doses thru 25 MG/L

| _JT_ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|---|---|---|---|---|---|
| 24 | -1.279204 | 0.1166342 | . | | 4 |

NOTE
Jonckheere test results are included in the summary table.
Group means should be examined to check for lack-of-fit
to a linear trend before trend test results are accepted.

## EC50 for Growth Rate

OBSERVED & PREDICTED PERCENT CHANGE FROM CONTROL MEAN
RESPONSE: G_RATE from DATASET: [redacted]

| Obs | CONC mg/L | N | MEAN | PRED | PCTCHNG | PREDPCTCHNG |
|---|---|---|---|---|---|---|
| 1 | 0.00 | 3 | 0.07593333 | 0.076499 | 0 | 0 |
| 2 | 6.25 | 3 | 0.07513333 | 0.075457 | -1 | -1 |
| 3 | 12.50 | 3 | 0.0777 | 0.074588 | 2 | -2 |
| 4 | 25.00 | 3 | 0.07096667 | 0.073179 | -7 | -4 |
| 5 | 50.00 | 3 | 0.07043333 | 0.071030 | -7 | -7 |
| 6 | 100.00 | 3 | 0.06853333 | 0.067947 | -10 | -11 |

EC50 using Method=MARQUARDT, Bruce & Versteeg Weighted Probit
Covariance Matrix and Standard Error of Parameter Estimates

| _NAME_ | logecx | gamma | y0 | SE |
|---|---|---|---|---|
| logecx | 1.39520 | 1.11491 | .000743038 | 1.18119 |
| gamma | 1.11491 | 0.93955 | .000774690 | 0.96930 |
| y0 | 0.00074 | 0.00077 | .000001339 | 0.00116 |

Model Parameter Estimates, EC50 and 95% Confidence Bounds

| logecx | gamma | y0 | LB | UB | EC50 |
|---|---|---|---|---|---|
| 8.01017 | 2.79779 | 0.076499 | 297.391 | 30494.21 | 3011.43 |

Model Evaluation Measures

| SSREG | SSERR | SSTOTC | SSTOTU | RSQUARE |
|---|---|---|---|---|
| 1.3161 | .000910377 | .003008721 | 1.31701 | 0.69742 |

95% FIELLER CONFIDENCE BOUNDS ON TARGETED PERCENT EFFECT CONC

note
Graph descriptions include the following:
G_Rate: EC50 BVP FIT BY MARQUARDT,