FYI-0794-1164

Contains No CBI

ENVIRONMENTAL SAFETY TEST SUMMARY REVIEW

Test Substance: CAS RN= 124-28-7

Test Substance Identification Number: B0793.02

Contract Laboratory: Springborn Bionomics Report #: BW-87-4-2359

Type of Study: Marine Invertebrate Toxicity

Principal Investigator:

Date Report Written: 4-21-87 Date Rec'd by Operations Section: 4-27-8.

This report has been reviewed and found in agreement with the Protocol and there appear to be no inaccuracies in the numerical data or written portions with the following exceptions: See Sec page 9

_____ Date: 5/18/87

This report has been reviewed for scientific quality and is summarized, with comments (if any), as follows:

FYI-94-001164
INIT 07/14/94

84940000234

_____ Date: 6/1/87

Date: 6/3/87

_____ Date: 6-12-87

RECEIVED
OPPT CBIC
94 JUL 14 AM 9:13

Microfilming Completed by_____ Date:_____

TEST SUMMARY SHEET

B. Text: (Note - tabulations must be limited to 71 characters in width)

Test Type: Acute Marine Invertebrate Toxicity - Static

Test Protocol: Static Acute Marine Shrimp Toxicity Study of B0793.02

Species: Mysidopsis bahia

Age: ≤ 24 hrs old

Source: SBI culture unit

Dilution Water: filtered seawater

Salinity: 30°/oo

pH: 8.0

Test Temperature: 24-25 degrees C

Results

96 hr LC50 (95% C.I.): 74 (62 - 85) ppb

Control Mortality: 0 %

Behavioral Observations: All surviving mysids were lethargic at 400 µg/L at 24 hours. Several mysids were lethargic at 240 µg/L at 24 hours.

Reference Response (96 hr LC50): _____

Material Solubilization: Material added via [x] stock solution; [ ] direct weighing. Solvent used - [ ] water; [x] IPA.

Comments: All results are based on nominal concentrations of the active ingredient tested as 100 % active.

C. Project Status Sheet Code No. _____ _____ _____

D. Test Type: ACMN (KEYWORD) Acute Marine Invertebrate Toxicity - Static

E. Test Material: B0793.02

F. Title/Date: Acute Toxicity of B0793.02 to Mysid Shrimp (Mysidopsis bahia)/ 7/21/86

G. Test Location: SPRINGBORN BIONOMICS ... No.: 1011-0986-6185-520

I. Date: __/__/__ (YY MM DD)

K. Sponsor's Principal Invest.(sign) (print) _

M. TSIN: B0793.02 _____ _____

N. Date Work Done: 19 - 23 February 1987

bg:TSACMN

ACUTE TOXICITY OF B0793.02 TO MYSID SHRIMP (*Mysidopsis bahia*)

THE PROCTER AND GAMBLE COMPANY

CINCINNATI, OHIO

BIONOMICS REPORT #BW-87-4-2359

BIONOMICS STUDY #1011.0986.6185.510

COPY SENT TO:

RECEIVED BY
APR 27 1987

Springborn Bionomics, Inc.
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
17 April 1987

SUMMARY

96-Hour Static Acute Toxicity Test with *Mysidopsis bahia*

Springborn Bionomics, Inc.
790 Main Street
Wareham, Massachusetts 02571

SPONSOR: The Procter & Gamble Company

TEST PROTOCOL: Static Acute Marine Shrimp Toxicity Study of B0793.02; Principal Investigator 21 July 1986.

REPORT NUMBER AND DATE: #BW-87-4-2359, 17 April 1987

STUDY NUMBER: #1011.0986.6185.510

MATERIAL: B0793.02 **DATE RECEIVED:** 6 August 1986

DESCRIPTION: a clear, colorless liquid tested as 100% active ingredient

TEST DATE: 19 - 23 February 1987

SPECIES: *Mysidopsis bahia*

Age: ≤ 24 hours old

Source: Bionomics culture facility

DILUTION WATER: Natural filtered seawater from the Cape Cod Canal, Bourne, Massachusetts.

pH: 8.0

Salinity: 30°/oo

TEST TEMPERATURE: 24 - 25°C

METHOD OF TEST MATERIAL ADDITION: Stock solution: 4.0 mg B0793.02/mL of isopropyl alcohol.

NOMINAL TEST CONCENTRATIONS: 52, 88, 140, 240 and 400 ug/L

RESULTS: The 96-hour LC50 (95% confidence interval) was 74 (62-85) µg/L.

INTRODUCTION

The purpose of this study was to estimate the acute toxicity (LC50) of B0793.02 to mysid shrimp (Mysidopsis bahia) under static conditions. The LC50 is defined as the concentration of the test material in dilution water which causes mortality of 50% in the exposed test population after a fixed period of time. This value is often used as a relative indicator of potential acute hazards resulting from the release of the test material into the aquatic environment. A 96-hour definitive test was conducted from 19 - 23 February 1987, at the Aquatic Toxicology Laboratory of Springborn Bionomics, Inc., Wareham, Massachusetts. All raw data generated and the final report are stored at the above location.

MATERIALS AND METHODS

Test Material

A sample of B0793.02, a clear, colorless liquid, tested as 100% active ingredient, was received from The Procter & Gamble Company, Cincinnati, Ohio on 6 August 1986. Nominal test concentrations are reported as micrograms of B0793.02 per liter of solution (µg/L).

Protocol

Procedures used in this acute toxicity study followed those described in the protocol entitled "Static Acute Marine Shrimp

Toxicity Study of E0793.02"; Principal Investigator, [redacted], 21 July 1986. This protocol closely follows "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians", ASTM 1980.

Test Organisms

The mysid shrimp used in this toxicity test were obtained from laboratory cultures maintained at Springborn Bionomics, Inc., Wareham, Massachusetts. Juvenile mysids (≤ 24 hours old) were collected using a variation of the method described by Reitsema and Neff (1980). The culture water was prepared by filtering natural seawater collected from the Cape Cod Canal, Bourne, Massachusetts, which derives water from either Cape Cod Bay or Buzzards Bay depending upon tidal direction. The seawater was filtered through a 5-micrometer porosity polypropylene core filter and an activated carbon bed prior to use.

The mysid culture area received a regulated photoperiod of 16 hours of light and eight hours darkness. Light at an intensity of 70 - 140 footcandles at the culture solution surfaces was provided by a combination of Sylvania Growlux^{R} and Cool White^{R} fluorescent bulbs. Mysids were fed live brine shrimp nauplii twice daily and the soil nematode (Panagrellus redivivus) three times weekly. Commercial aquarium heaters were used to maintain the culture solution temperatures at 25 ± 1°C.

0 0 0 8

Dilution Water

The dilution water used was from the same source as the culture water and was characterized as having a salinity of 30°/oo and a pH of 8.0. This water was aerated prior to use to ensure the dissolved oxygen concentration was greater than 90% of saturation.

Test Procedure

The toxicity test was conducted in 1.6-liter (L) glass vessels. The definitive test was conducted at nominal B0793.02 test concentrations of 52, 88, 140, 240 and 400 µg/L.

A clear, colorless stock solution of 4.0 mg/mL was prepared by diluting 0.040 grams (g) of B0793.02 with warm (25 - 30°C) isopropyl alcohol (IPA) to volume in a 10-mL volumetric flask. The test solutions were prepared by adding the appropriate volumes of stock solution and dilution water to each test vessel to total 1000 mL. The test solutions were duplicated. Each solution was mixed with a glass rod. The test solution depth was 3.5 centimeters (cm) with a surface area of 280 cm^2. Two sets of duplicate control vessels containing the same dilution water and maintained under the same conditions as the exposure concentrations, but containing no B0793.02, were established. One set of control solutions was treated with the highest concentration (100 µL/L) of IPA present in any test solution and therefore termed the solvent control. A temperature controlled water bath maintained the test solution temperatures at 25

± 1°C. Test solutions were not aerated and were covered with plate glass to minimize evaporation. The test area received a light intensity of 40 - 100 footcandles at the surface of the solutions. The photoperiod and light source during the test were the same as in the culture area.

Twenty mysids, ≤ 24 hours old, were impartially distributed to each concentration (ten mysids per replicate) within 20 minutes after the test solutions had been prepared. Mysids were fed brine shrimp nauplii each day of exposure.

Test Monitoring

Biological observations and observations of the physical characteristics of each replicate test solution were made and recorded at 0, 24, 48, 72 and 96 hours of exposure. The number of dead mysid shrimp in each replicate test solution was recorded at 24, 48, 72 and 96 hours. Dead mysids were removed daily.

Water Quality Measurements

The pH, dissolved oxygen, temperature and salinity were measured at 0 hour and each subsequent 24-hour exposure interval in all replicate treatment and control solutions.

Salinity concentrations presented in this report were measured with an Argent refractometer; the pH's were measured with an

Instrumentation Laboratory Model #175 pH meter and combination electrode; the dissolved oxygen concentrations were measured with a YSI Model #57 dissolved oxygen meter and probe and the temperatures were measured with a Brooklyn alcohol thermometer. Light intensity was measured with a General Electric type 214 light meter.

Statistics

The concentrations tested and the corresponding mortality data derived from the toxicity test were used to estimate the median lethal concentrations (LC50) and 95% confidence intervals for each 24-hour interval of the exposure period. The LC50 is defined as the concentration of the test material in dilution water lethal to 50% of the test organism population at the stated time interval. LC50 values were empirically estimated as being greater than the highest concentration tested when no test concentrations caused 50% or more mortalities. If at least one test concentration caused mortality of greater than or equal to 50% of the test population, then a computer program (Stephan, 1977, 1982) was used to calculate the LC50 values and 95% confidence intervals.

Three statistical methods were available in the computer program: moving average angle analysis, probit analysis, and nonlinear interpolation with 95% confidence intervals calculated by binomial probability. Moving average angle and probit analyses yield statistically sound results only if at least two concentrations produce a mortality of between 0 and 100% of the test organism

population. The selection of reported LC50 values and 95% confidence intervals was based upon an examination of the data base and the results of the computer analysis. Selection criteria included the establishment of a concentration-effect relationship (mortality), the number of concentrations causing partial responses, and the span of responses bracketing the LC50 value. If two or more statistical methods produced acceptable results, then the method which yielded the smallest 95% confidence interval was selected.

RESULTS

The concentrations tested, corresponding mortalities and observations made during the toxicity test are presented in Table 1. The 96-hour LC50 value and 95% confidence interval for mysid shrimp exposed to B0793.02 were calculated by probit analysis to be 74 (62 - 85) µg/L. Table 2 summarizes the LC50 values, and corresponding confidence intervals. The results of the dissolved oxygen and pH analyses are presented in Table 3. These parameters remained within acceptable limits throughout the exposure period for survival of mysid shrimp. The temperature and salinity ranges of the exposure solutions were 24 - 25°C and 30 - 32°/oo, respectively, throughout the study.

PROTOCOL DEVIATIONS

1. The protocol states that the test solution temperature will be 22 ± 1°C. During this study the test temperature range was 24 -25°C. The present culture system at SBI is maintained at 25 ± 1°C; therefore this temperature range was provided for the acute study.

2. The protocol states that the test organisms will be fed live brine shrimp nauplii on test days 0 and 2. During this study the test organisms were fed brine shrimp nauplii daily. The interval between feedings was shortened to minimize the chance of cannibalism.

It is our opinion that these deviations did not affect the results of this study.

James R. Hoberg 4/21/87
James R. Hoberg
Study Director

D C Surprenant 4/22/87
Donald C. Surprenant
Laboratory Director

LITERATURE CITED

American Society for Testing and Materials Committee E-35 on Pesticides, 1980. Standard Practice #729-80, Standard Practice for Conducting Basic Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians, Philadelphia, PA. 25 pp.

Reitsema, L. A. and J. M. Neff. 1980. A recirculating artificial seawater system for the laboratory culture of Mysidopsis bahia (Crustacea; Pericaridae). Estuaries 3: 321-323.

Stephan, C. E. 1977. Methods for calculating an LC50, Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, F. L. Mayer and J. L. Hamelink, Eds., American Society for Testing and Materials, pp. 65-84.

Stephan, C. E. 1982. U. S. EPA., Environmental Research Laboratory, Duluth, Minnesota. Personal communication to Dr. Lowell Bahner, Chairman ASTM Task Group on Calculating LC50's.

U.S. EPA. 1985. Standard Evaluation Procedures for Acute Toxicity Test for Estuarine and Marine Organisms. Hazard Evaluation Division, Office of Pesticide Programs. Draft. June 17, 1985.

Table 1. Concentrations tested, corresponding cumulative mortalities and observations made during the 96-hour static exposure of mysid shrimp (Mysidopsis bahia) to B0793.02.

Nominal Concentration (mg/L)	24-Hour			48-Hour			72-Hour			96-Hour		
	A	B	$\bar{x}$	A	B	$\bar{x}$	A	B	$\bar{x}$	A	B	$\bar{x}$
Control	0	0	0	0	0	0	0	0	0	0	0	0
Solvent Control	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0[a]	10	10	10	20	20	20	20	20	20
88	10	0	5	30	30	30[a]	30	70	50[a]	40	80	60
140	10	40	25	100	100	100	100	100	100	100	100	100
240	50	40	45[b]	100	100	100	100	100	100	100	100	100
400	60	60	60[c]	100	100	100	100	100	100	100	100	100

[a] One mysid was lethargic.

[b] Several of the surviving mysids were lethargic.

[c] All of the surviving mysids were lethargic.

Table 2. The LC50 values and 95% confidence intervals for mysid shrimp (Mysidopsis bahia) exposed to 80793.02 under static conditions.

	LC50 (µg/L)	Confidence Intervals	
		Lower (µg/L)	Upper (µg/L)
24-Hour[a]	290	220	430
48-Hour[a]	89	78	100
72-Hour[a]	77	65	90
96-Hour[a]	74	62	85

[a] LC50 value and 95% confidence interval calculated by probit analysis.

Table 3. The pH and dissolved oxygen concentrations measured during the 96-hour static exposure of mysid shrimp (Mysidopsis bahia) to B0793.02.

Nominal Concentration (µg/L)	0-hour A	0-hour B	24-hour A	24-hour B	48-hour A	48-hour B	72-hour A	72-hour B	96-hour A	96-hour B
					pH					
Control	8.0	8.0	8.0	8.0	7.9	7.9	8.0	8.0	8.0	8.0
Solvent Control	8.0	8.0	7.9	8.0	8.0	7.9	7.9	8.0	7.8	7.9
52	8.0	8.0	8.0	8.0	7.9	7.9	7.9	7.9	7.9	7.9
88	8.0	8.0	8.0	8.0	7.8	7.9	7.9	8.0	7.9	7.9
140	8.0	8.0	7.9	7.9	8.0	7.9	---[b]	---	---	---
240	8.0	8.0	8.0	8.0	7.9	7.9	---	---	---	---
400	8.0	8.0	7.9	8.0	7.9	7.9	---	---	---	---
					Dissolved Oxygen[a] (mg/L)					
Control	7.4	7.3	6.0	6.3	5.9	6.0	5.6	5.1	5.7	5.5
Solvent Control	7.5	7.0	5.9	6.4	6.1	5.9	5.3	5.4	5.0	4.5
52	7.4	7.5	6.0	6.1	5.9	5.8	5.6	5.4	5.4	5.6
88	7.4	7.3	6.3	6.2	6.1	6.1	5.4	5.1	5.0	5.3
140	7.5	7.4	6.0	5.7	6.0	5.9	---	---	---	---
240	7.4	7.5	6.3	6.0	5.9	5.8	---	---	---	---
400	7.2	7.4	6.1	6.2	5.9	6.2	---	---	---	---

[a] Minimum dissolved oxygen concentration measured was 65% of saturation (4.5 mg/L @ 25°C and 32°/oo) and maximum dissolved oxygen concentration measured was 103% of saturation (7.5 mg/L @ 24°C and 30°/oo).

[b] Water quality measurements not required after 100% mortality occurred at the previous 24-hour interval.

The data and report prepared for this study were produced and compiled in accordance with all pertinent EPA Good Laboratory Practice regulations except in the case of the characterization and verification of the test substance identity. Maintenance of these records is the responsibility of the test sponsor.

James R. Hoberg 4/12/87

James R. Hoberg
Study Director

Date

The data contained in this report were audited by the Quality Assurance Unit to assure compliance with the protocols, standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations on the following dates: 3 and 16 April 1987. If discrepancies were found, reports were made immediately to the Study Director and management. It is the opinion of this Unit that these data accurately reflect the raw data generated during this study.

Jane Palmer Mayo 4/21/87
Jane P. Mayo
Quality Assurance

SUBMITTED BY:

Springborn Bionomics, Inc.
790 Main Street
Wareham, Massachusetts
17 April 1987

STUDY DIRECTOR: James R. Hoberg

James R. Hoberg 4/21/87
Aquatic Toxicologist

APPROVED BY: Donald C. Surprenant

D C Surprenant 4/22/87
Director, Aquatic Toxicology

AUDITED BY: Jane P. Mayo

Jane Palmer Mayo 4/21,
Quality Assurance

R-3

Laboratory Study No. 1011:0986.6105.510

PROTOCOL

SPONSOR: The Procter & Gamble Company; Cincinnati, Ohio

LABORATORY: Springborn Bionomics, Inc.
790 Main St.
Wareham, MA 02571

TITLE: Static Acute Marine Shrimp Toxicity Study of B0793.02

OBJECTIVE: To determine the 96-hr LC50 of the test material to a marine shrimp species.

JUSTIFICATION OF TEST SYSTEM: The Mysid shrimp (Mysidopsis bahia) is a readily available marine shrimp species, on which a large amount of toxicity data exists, and is recommended in ASTM E729-80[1], as a standard test organism.

TEST MATERIAL:

Sample Code B0793.02 Color CLEAR Form LIQUID

% Active 100 Density — Solubility LOW IN WATER

Expiration Date 11-86 Other —

Storage Conditions - ROOM TEMPERATURE

Safe Handling Precautions - AVOID SKIN OR EYE CONTACT. IF CONTACT OCCURS, FLUSH WITH WATER

The Sponsor accepts full responsibility for appropriate characterization and stability verification of this test material.

TEST MATERIAL ADDITION/PREPARATION: All calculations and measurements are to be based on the active ingredient. The maximum concentration to be tested is 1000 mg/L active ingredient. Dissolve in warm (~40°C) IPA.

TEST ORGANISM:

Species - Mysid shrimp (Mysidopsis bahia)
Source - Bionomics Marine Research Laboratory culture system.
Age - 1-4 days old.

A group of shrimp will not be used if individuals appear to be diseased or otherwise stressed. Shrimp will be fed brine shrimp nauplii during holding and during the test on days 0 and 2.

TEST CONTAINERS: Glass 1.6L bowls containing 1L of test solution or control water.

[1]ASTM Standard E729-80, Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

DILUTION WATER: Natural, filtered (5-micrometers) seawater, salinity 15-35 parts per thousand (°/oo) and pH 8.0 ± 0.5.

TEST CONDITIONS:

1) Temperature - 22 ± 1°C.
2) The dissolved oxygen concentration in seawater in each test container will be >90% of saturation on Day 0, before addition of the test material.
3) A control and at least five concentrations of the test material are to be used. Except for the control, the concentration of test material in each treatment must be ≥60% of the next higher one.

OPERATION: A minimum of 10 shrimp for each test concentration and the control will be randomly added to the test containers following laboratory's standard operating procedures. Shrimp will be added within 1 hour after the addition of the test material. A maximum of 10 shrimp will be added to each test container. One treatment should kill or affect more than 65% of the shrimp, and one treatment (not the control) should kill or affect less than 35% of the exposed shrimp. The test will be conducted for 96 hours, commencing when the shrimp are first exposed to the test material.

If any additive (solvent or carrier other than seawater or deionized water) is used to solubilize the test material, a solvent control containing the greatest volume of additive present in any other container will also be maintained. A test will not be valid if mortality in either the seawater or solvent control is >10%.

WATER CHEMISTRY: Dissolved oxygen (DO) concentrations will be determined in all replicates for all treatments in which there are living shrimp at a minimum of 0 and 96 hours of exposure. The pH will be measured in Replicate A only of all treatments in which there are living shrimp at 0 and 96 hours of exposure. When 100% mortality is observed in a test concentration, pH and DO measurements will be conducted then, but further measurements for that concentration will be discontinued. Temperature will be measured in Replicate A of the seawater control daily. Salinity will be measured on day 0 in Replicate A of the seawater control.

OBSERVATIONS: Observations of mortality, morbidity, and behavior will be made and recorded for each 24 hour period for all test concentrations and control(s). Dead shrimp will be removed during these observations.

RANGE-FINDING TEST: Will be conducted at the discretion of the testing laboratory unless otherwise specified.

CALCULATIONS: Test results are used to calculate the 96 hr LC50 following laboratory's standard operating procedures. The 24, 48, and 72 hr LC50's are calculated when possible. The LC50 is defined as the calculated concentration of the test material which causes 50% mortality in populations of test shrimp at the specified time of exposure. Results are to be calculated and reported on the basis of added (nominal) test concentrations or from concentrations confirmed by actual analysis, if requested.

0 0 2 2

RECORDS TO BE MAINTAINED: All records necessary to reconstruct the study and demonstrate adherence to the Protocol.

PROTOCOL CHANGES: If a change in the approved protocol becomes necessary, verbal agreement should be made between the Study Director and Sponsor's Principal Investigator. As soon as practical thereafter, this change and the reasons for it should be put in writing, approved by both persons, and attached to the protocol as an addendum.

REPORTING: The report is to be a typed document in triplicate, describing the results of the study and is to be signed and dated by the Study Director, Quality Assurance Officer and Laboratory Manager. It is to include, but is not limited to, the following:

1) Identification of test material by sample code, percent active, color, form, and date received.
2) Procedures followed for test material preparation and addition.
3) Reference to laboratory notebook or other file containing raw data.
4) Date definitive test was conducted.
5) Species tested, source, and age.
6) Percentage mortality in all test containers, including the control.
7) The calculated LC50 values, 95% confidence intervals, and a reference to the method used to calculate these values.
8) Description of dilution water used, including the source, measured pH, and salinity.
9) Description of holding conditions and acclimation procedures.
10) All temperature, pH and DO determinations and all visual observations.
11) Laboratory Study Number.
12) Reference to the Protocol (title, author, and date) and addenda if made, and any analytical procedu s used.
13) Any Protocol deviations and their implications.
14) Description of the quality assurance methods used to i ···e the quality of the data.

ALTERNATE PRINCIPAL INVESTIGATOR _____ PHONE:

8/1/86 PHONE:
Date

A : 8/21/86 PHONE:
ate

TO BE COMPLETED BY STUDY DIRECTOR:

Study No. 1011·0986·6185·510

Estimated Starting Date 6 October 1986

Estimated Reporting Date 2 January 1987

Date Test Material Received 6 August 1986

Approved James R. Hoberg 9/12/86 PHONE: 617-295-2550
Study Director Date

0 0 2 3

DILUTION WATER: Natural, filtered (5-micrometers) seawater, salinity 15-35 parts per thousand (‰) and pH 8.0 ± 0.5.

TEST CONDITIONS:

1) Temperature - 22 ± 1°C.
2) The dissolved oxygen concentration in seawater in each test container will be >90% of saturation on Day 0, before addition of the test material.
3) A control and at least five concentrations of the test material are to be used. Except for the control, the concentration of test material in each treatment must be ≥60% of the next higher one.

OPERATION: A minimum of 10 shrimp for each test concentration and the control will be randomly added to the test containers following laboratory's standard operating procedures. Shrimp will be added within 1 hour after the addition of the test material. A maximum of 10 shrimp will be added to each test container. One treatment should kill or affect more than 65% of the shrimp, and one treatment (not the control) should kill or affect less than 35% of the exposed shrimp. The test will be conducted for 96 hours, commencing when the shrimp are first exposed to the test material.

If any additive (solvent or carrier other than seawater or deionized water) is used to solubilize the test material, a solvent control containing the greatest volume of additive present in any other container will also be maintained. A test will not be valid if mortality in either the seawater or solvent control is >10%.

WATER CHEMISTRY: Dissolved oxygen (DO) concentrations will be determined in all replicates for all treatments in which there are living shrimp at a minimum of 0 and 96 hours of exposure. The pH will be measured in Replicate A only of all treatments in which there are living shrimp at 0 and 96 hours of exposure. When 100% mortality is observed in a test concentration, pH and DO measurements will be conducted then, but further measurements for that concentration will be discontinued. Temperature will be measured in Replicate A of the seawater control daily. Salinity will be measured on day 0 in Replicate A of the seawater control.

OBSERVATIONS: Observations of mortality, morbidity, and behavior will be made and recorded for each 24 hour period for all test concentrations and control(s). Dead shrimp will be removed during these observations.

RANGE-FINDING TEST: Will be conducted at the discretion of the testing laboratory unless otherwise specified.

CALCULATIONS: Test results are used to calculate the 96 hr LC50 following laboratory's standard operating procedures. The 24, 48, and 72 hr LC50's are calculated when possible. The LC50 is defined as the calculated concentration of the test material which causes 50% mortality in populations of test shrimp at the specified time of exposure. Results are to be calculated and reported on the basis of added (nominal) test concentrations or from concentrations confirmed by actual analysis, if requested.

RECORDS TO BE MAINTAINED: All records necessary to reconstruct the study and demonstrate adherence to the Protocol.

PROTOCOL CHANGES: If a change in the approved protocol becomes necessary, verbal agreement should be made between the Study Director and Sponsor's Principal Investigator. As soon as practical thereafter, this change and the reasons for it should be put in writing, approved by both persons, and attached to the protocol as an addendum.

REPORTING: The report is to be a typed document in triplicate, describing the results of the study and is to be signed and dated by the Study Director, Quality Assurance Officer and Laboratory Manager. It is to include, but is not limited to, the following:

1) Identification of test material by sample code, percent active, color, form, and date received.
2) Procedures followed for test material preparation and addition.
3) Reference to laboratory notebook or other file containing raw data.
4) Date definitive test was conducted.
5) Species tested, source, and age.
6) Percentage mortality in all test containers, including the control.
7) The calculated LC50 values, 95% confidence intervals, and a reference to the method used to calculate these values.
8) Description of dilution water used, including the source, measured pH, and salinity.
9) Description of holding conditions and acclimation procedures.
10) All temperature, pH and DO determinations and all visual observations.
11) Laboratory Study Number.
12) Reference to the Protocol (title, author, and date) and addenda if made, and any analytical procedures used.
13) Any Protocol deviations and their implications.
14) Description of the quality assurance methods used to insure the quality of the data.

ALTERNATE PRINCIPAL INVESTIGATOR _____ PHONE: _____

NOTE _____ Date _____ PHONE

APPROVED: _____ Principal Investigator _____ Date _____ PHONE

TO BE COMPLETED BY STUDY DIRECTOR:

Study No. 1011-0986-6185-510

Estimated Starting Date 6 October 1986

Estimated Reporting Date 2 January 1987

Date Test Material Received 6 August 1986

Approved James R. Holsen 9/12/86 PHONE: 617-275-2550
Study Director Date

0 0 0 2 5

167

A. MATERIAL

Formula or nature of product: (Exact Structure/Nominal Composition; Analytical Information)

Octadecyl-dimethylamine

Active Molecular Weight 297

SPECIAL INFORMATION: (additional information of value not included in above, reference to testing done on similar formulations or chemicals, etc.).

Date: 2/3/86

B. ENVIRONMENTAL TESTS:

Lab

Algae Toxicity (Microcystis and Selenastrum) Stillwater Pacific

Marine Fish Toxicity (Sheepshead minnow) Bionomics

Marine Invertebrate Toxicity (mysid shrimp) "

SAMPLE IDENTIFICATION

B0793-02

Est. Cost $8,500

Est. Reporting Date 9/10/86

Date: 7/18/86

RHH392/dh
7/18/86

0 0 2 6

TEST SUBSTANCE CHARACTERIZATION REPORT (TSCR)

Test Substance Identification Number (TSIN): B0793-02
Safety Test Request Number:
Principal Investigator:

Physical Description: Clear Liquid solubility: NK
Recommended Storage Conditions: Room Temperature Expiration Date: 11-86
Hazards (i.e. flammability, toxic gases): Alkaline Corrosive
Dept. of Transportation Hazard Classification: Nos. NA1719 CAS No.(a): 124-28-7

Formulated Composition (b)

Component (c)	Mol. Wt.	Nominal Level (% by Wt.)	Acceptable Range	Stock Code No.	Supplier	Lot Number (NB-Ref.)
(Alkyl Dimethylamine)	297	100%	NA	NK	Ethyl Corp	ADMA 8-0

(a) Include CAS number(s) for the three most major components of a formulation or for single chemical products. Footnote to the material with which the respective number is associated.

(b) If information requested is not known, then the symbol NK will be entered.

(c) Chemical names which are inconveniently long may be abbreviated in tables but should be listed in full in referenced footnotes. Non-chemical names, such as Tergitol 15-S-0 or Yellow Dye #10, may not be acceptable but should be previewed with the responsible toxicologist. Nondefinitive identification (e.g. Arquad, BC-base) is not acceptable.

The above information provided by: (Signature) 10/23/85 (Date)

The above information reviewed and accepted by:

Principal Investigator: 7/4/86 (Date)

TEST SUBSTANCE CHARACTERIZATION REPORT (TSCR)

Test Substance Identification Number (TSIN): B0793-02

Analyzed Composition

Date Submitted	Submitter Code	Analysis Code/Analysis	Estimated Value	Measured Value	Testing Laboratory

Analytical Information Verified By: _____ (Signature) Date:

Date: 2/4/8

This test substance is suitable for human (clinical) safety testing.

Principle Investigator: _____ (Signature) Date:

0028

ENVIRONMENTAL SAFETY TEST SUMMARY REVIEW

Test Material: CAS RN 124-28-7

Test Substance Identification Number: B0793.02

Contract Laboratory: Malcolm Pirnie Report #: 165-14-1100-1 & -2

Type of Study: Algae Toxicity (Microcystis & Selenastrum) DRD #:

Name of Originator:

Date Report Written: 10-22-86 Date Rec'd by Operations Section: 11-4-86

This report has been reviewed and found in agreement with the Protocol and there appear to be no inaccuracies in the numerical data or written portions with the following exceptions:

Date: 11/26/86

This report has been reviewed for scientific quality and is summarized, with comments (if any), as follows:

Divisional Toxicologist _____ Date: 11/18/86

Date: 11/26/86

_____ Date: 12-2-86

_____ Date: 1-16-87

Microfilming Completed by_ _____ Date: 6-1-87

KEY SUMMARY SHEET

A

B. Text: (Note - tabulations must be limited to 71 characters in width.)

Test Type: Algal Toxicity

Test Protocol: Toxicity of B0793.02 to Algae (Algistatic Determination)

Species: Selenastrum capricornutum and Microcystis aeruginosa

Test Media: AAP

Light Level: 400 ft.c ±15% for S. capricornutum; 200 ft.c ±15% for M. aeruginosa

Test Temperature: 24 ± 2°C

Material Solubilization: N,N-dimethylformamide

Results (mg/l):	5-Day AC	95% Conf. Intervals	Algicidal Concentration
S. capricornutum	0.029	0.015-0.064	> 0.032
M. aeruginosa	0.11	0.04-0.27	0.16

Comments: All results based on nominal concentrations of the active ingredient tested as 100% active. All calculations were made from cell counts.

C. Project Status Sheet Code No. _____

D. Test Type: ALTX (KEYWORD) Algal Toxicity

E. Test Material: B0793.02

F. Title/Date: _____

G. Test Location: Malcolm Pirnie H. Lab Project No.: 0105-14-1100

I. Date: __/__/__ (YY MM DD)

K. Sponsor's Principal Invest.(sign

M. TSIN: B0793.02

N. Date Work Done: 9/12/86 - 9/26/86 and 9/19/86 - 10/3/86

bg:TSALTX

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Aquatic Toxicology
MPI-QA-SIGN-1

Malcolm Pirnie, Inc.
2 Corporate Park Drive
White Plains, New York 10602

Sponsor: The Procter and Gamble Company
Cincinnati, Ohio 45217

Title of Report: The Toxicity of B0793.02 to Selenastrum capricornutum and Microcystis aeruginosa

Date of Issue: October 31, 1986

Dates of Testing: Range-Finding: 8/15/86-8/20/86; 9/5/86-9/10/86; 9/12/86-9/17/86
Definitive: 9/12/86-9/26/86; 9/19/86-10/3/86

MPI Project No.: 165-14-1100-1 and -2

Protocol No.: Prepared by Sponsor, dated 7/21/86

MPI Notebook No.(s): 43

Archive Reference No.: PG/B0793.02/ALGST, SEL, MIC/0165-14-1100

Report prepared by:
Jane S. Hughes Date: 10/23/86
Jane S. Hughes
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RECEIVED BY
NOV 4 1986
OPERATIONS SECTION

Report approved by:
Richard J. Califano Date: 10/31/86
Richard J. Califano, Ph.D.
Project Manager

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I. SUMMARY

Algal toxicity tests were conducted for the Procter and Gamble Company, Cincinnati, Ohio by Malcolm Pirnie, Inc., White Plains, NY during the periods September 12 to September 26, 1986 and September 19 to October 3, 1986. The objective of the study was to determine the algistatic concentration of the test material B0793.02 to the freshwater green alga, Selenastrum capricornutum and the freshwater blue-green alga, Microcystis aeruginosa. Algal cultures were exposed to a series of nominal concentrations (0.001 to 0.032 mg/L for S. capricornutum and 0.01 to 0.32 mg/L for M. aeruginosa) of the test material for five days. At the end of five days, algal cells from cultures with populations less than or similar to the initial inoculum level were transferred to test material-free medium. Transferred cultures were then incubated for a nine-day recovery period. Biomass was estimated during the assays by cell counts using an electronic particle counter for both species and also by chlorophyll a relative fluorescence for S. capricornutum.

The algistatic concentration of B0793.02 to Selenastrum capricornutum was determined to be 0.029 mg/L (95% confidence limits: 0.015-0.064 mg/L). The algicidal concentration for this species was greater than 0.032 mg/L. For Microcystis aeruginosa, the algistatic concentration was determined to be 0.11 mg/L (95% confidence limits: 0.04-0.27 mg/L). The algicidal concentration for this species was 0.16 mg/L.

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II. INTRODUCTION

Payne and Hall (1979) developed an algal toxicity test specifically for use in the environmental safety assessment of new chemicals. The procedure, based upon the Algal Assay Procedure Bottle Test (EPA, 1971; Miller et al., 1978), employs a five-day exposure of algal cultures to a range of test material concentrations followed by a nine-day recovery period in the absence of test material. Biomass estimates are made by cell counts, in vivo fluorescence, or both. The primary response sought in this method is the algistatic concentration of the test material, defined as the concentration that causes no net increase in cell number after the 5-day exposure period but permits regrowth when the cells are resuspended in test material-free medium. The algicidal concentration is the lowest concentration tested which causes no net increase in cell number during either the exposure or recovery period; i.e. cells do not recover when transferred to test material-free medium.

The objective of the study was to determine the algistatic concentration of the test material B0793.02 to Selenastrum capricornutum and Microcystis aeruginosa. These representative freshwater species, a green alga and a blue-green alga, respectively, are readily available, easily cultured and have ecological importance.

The study was conducted by Malcolm Pirnie, Inc. during the periods September 12 to September 26, 1986 (for M. aeruginosa) and September 19 to October 3, 1986 (for S. capricornutum). The study was conducted for the Procter and Gamble Company, Cincinnati, Ohio under MPI Project Nos. 0165-14-1100-1 and 0165-14-1100-2. Methods were according to the protocol prepared by of the Procter and Gamble Co., dated July 21, 1986, as supplemented by Malcolm Pirnie's Standard Operating Procedures.

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III. METHODS AND MATERIALS

All testing was conducted at the laboratory of Malcolm Pirnie, Inc. in White Plains, New York.

Test Organisms

Selenastrum capricornutum used in this test came from laboratory stock cultures. The original culture was obtained from the National Eutrophication Research Program, USEPA, Corvallis, Oregon. Stock cultures are maintained in algal assay procedure medium (AAP medium) (Appendix A) in Erlenmeyer flasks under constant illumination of 4304 ± 650 lumens/m^2 and temperature of 24 ± 2°C. Flasks are continuously shaken at 100 ± 10 oscillations/minute. Transfers into fresh medium are conducted weekly according to Standard Operating Procedures.

Microcystis aeruginosa used in this test came from laboratory stock cultures. The original culture was obtained from the University of Texas Culture Collection, Austin, TX (UTEX L2061). Stock cultures are maintained in algal assay procedure medium (AAP medium) (Appendix A) in Erlenmeyer flasks under constant illumination of 2152 ± 323 lumens/m^2 and temperature of 24 ± 2°C. Flasks are continuously shaken at 100 ± 10 oscillations/minute. Transfers into fresh medium are conducted weekly according to Standard Operating Procedures.

Preparation of Glassware - All glassware used in testing was thoroughly scrubbed with non-phosphate detergent and rinsed with tap water. This was followed by a rinse with acetone, further rinses with tap water, a rinse in 10 percent reagent grade hydrochloric acid, and thorough rinsing in tap water and distilled deionized water. Since the Coulter Counter was used, the final rinse was in distilled deionized water passed through a 0.22 micron pore size membrane-filter. Glassware was dried in an oven at 50-70°C. Foam plugs were inserted and

the glassware was autoclaved for 20 minutes at 1.1 Kg/cm^2 and 121°C.

Preparation of Medium - AAP medium was prepared by adding 1 mL of each of the macronutrient stock solutions and 1 mL of the micronutrient stock solution, in the order listed in Appendix A, to 900 mL of distilled deionized water, with mixing after each addition. The volume was brought to 1 liter and the pH was adjusted to 7.5 ± 0.1 with 0.1N sodium hydroxide or hydrochloric acid. The medium was immediately filtered through a 0.22 micron porosity membrane filter into a sterile container. Medium was stored in the dark at 4°C and brought to room temperature prior to use.

Preparation of Test Material

The test material was received on August 8, 1986. The test material is characterized by the sponsor as B0793.02 and, at room temperature, is a clear liquid containing white solids. The test material is 100% active ingredient and has a low water solubility. It was stored at room temperature.

A range-finding test with each species, conducted using test material concentrations from 0.001 to 10 mg/L, had indicated that the appropriate test concentrations for the definitive test would be 0.02 to 0.64 mg/L for S. capricornutum and 0.1 to 3.2 mg/L for M. aeruginosa. However, poor growth was obtained at all test concentrations for both species in the first attempted definitive test, and also in a second attempted definitive test with S. capricornutum. Successful definitive tests were conducted using concentrations of 0.001 to 0.032 mg/L for S. capricornutum and 0.01 to 0.32 mg/L for M. aeruginosa.

All calculations and measurements are based on the percent active ingredient (a.i.). To begin the definitive test

with S. capricornutum, 25 mL of a stock solution of 2 mg a.i./mL was prepared in N, N-dimethylformamide (DMF). The stock solution was heated in a water bath to approximately 40°C to dissolve all of the test material. Stock solutions of 0.2, 0.02 and 0.002 mg/mL in DMF were prepared by serial dilution. To begin the definitive test with M. aeruginosa, 25 mL of a stock solution of 4 mg a.i./mL was prepared in DMF. The stock solution was heated, and stock solutions of 0.4 and 0.04 mg/L in DMF were prepared by serial dilution. For both assays, the test treatments were prepared by adding calculated amounts of the appropriate stock solution to AAP medium in labelled 250 mL or 500 mL volumetric flasks. The volume was then brought up in each flask with AAP medium. The test concentrations were 0.001, 0.002, 0.004, 0.008, 0.016 and 0.032 mg/L for the assay with S. capricornutum and 0.01, 0.02, 0.04, 0.08, 0.16, and 0.32 mg/L for the assay with M. aeruginosa.

A solvent control was prepared to contain an amount of DMF equivalent to the greatest amount used in any test treatment. The concentration of DMF in the solvent control was 0.5 mL/L for both assays. The control contained AAP medium with no additions.

The pH of the highest test treatment (in each assay) fell within the prescribed range of 7.5 ±1; therefore, the pH of the test treatments did not require adjustment.

For each assay, the test concentrations, control and solvent control treatments were thoroughly mixed and 50 mL of each was added to each of three replicate test vessels (250 mL Erlenmeyer flasks).

Inoculation

The inoculum of Selenastrum capricornutum came from a 7-day old stock culture which had previously been maintained in AAP medium for at least three transfers. A portion of the

stock culture was diluted by about 75% with AAP medium to be used as the inoculum. Population density in the inoculum was determined with a Model ZBI Coulter Counter equipped with a C-1000 Channelyzer and MER Computer. The inoculum contained 1,830,000 cells/mL. A 0.546 mL volume of this inoculum was aseptically added to 50 mL medium in each flask, yielding a nominal initial concentration of 20,000 cells/mL.

The inoculum of Microcystis aeruginosa came from a 7-day old stock culture which had previously been maintained in AAP medium for at least three transfers. Population density in the stock culture was determined with the Coulter Counter as described above. The stock culture contained 5,070,000 cells/mL. A 0.493 mL volume of this culture was aseptically added to 50 mL medium in each flask, yielding a nominal initial concentration of 50,000 cells/mL.

Incubation

Test flasks were kept in a Psycrotherm Controlled Environment Incubator Shaker, Model G-27, at a temperature of 24 ± 2°C. Temperature was recorded daily. Flasks were continuously shaken at 100 ± 10 oscillations/minute. Continuous illumination at 4304 ± 650 lumens/m^2 for S. capricornutum and 2152 ± 323 lumens/m^2 for M. aeruginosa was provided by overhead cool-white fluorescent lights. Flasks were randomly repositioned each working day to minimize spatial differences in the incubator.

Biomass Estimation

Biomass measurements during the assay were estimated by cell counts using an electronic particle counter for both species and also by chlorophyll a relative fluorescence for S. capricornutum. These measurements were made on days 3 and 5 of the exposure period and days 2, 6, and 9 of the recovery period.

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Cell counts were made using a Model ZBI Coulter Counter with C-1000 Channelyzer and MHR Computer. The Coulter Counter operates on the principle that cells are poor electrical conductors. The algal cells, suspended in an electrolyte, can be sized and counted by passing them through an aperture with a specific path of current flow. As cells pass through the aperture and displace a volume of electrolyte equal to their volume, the resistance changes, causing current and voltage changes which are translated into number and size of cells. On each counting day, a sample was collected aseptically from each flask using an automatic micropipette with a sterile tip. Flasks were swirled prior to pipetting to ensure representative sampling. The samples were placed in individual particle-free disposable containers and diluted with the electrolyte Isoton II (Coulter Electronics, Inc.). Three counts per replicate flask were made. All counts were multiplied by the appropriate conversion factors (for sample dilution and volume counted) to yield cells/mL. The Coulter Counter was calibrated, using an organic calibration material, as per Standard Operating Procedures. The instrument settings used are given in Appendix B.

For S. capricornutum, in vivo chlorophyll a relative fluorescence was measured with a Turner Model 111 Fluorometer equipped (as recommended by A.P.H.A. et al., 1980) with a F4T.5 blue lamp and a R-136 red-sensitive photomultiplier tube. A CS5-60 filter was used as the primary filter while a CS2-64 filter was used as the secondary filter. All results are expressed in terms of a range setting of 1 x. Clean test medium was used as a "blank" to zero the fluorometer. A sample was collected aseptically from each flask using an automatic micropipette with a sterile tip. Flasks were swirled prior to pipetting to ensure representative sampling.

Samples were placed in individual sterile disposable glass cuvettes. Three fluorescence readings were made per sample. When fluorescence was low, 3.5 to 4.0 mL samples were taken from the flasks and returned after measurement, so as not to significantly reduce the volume in the test flasks. When fluorescence was higher, the samples had to be diluted with a measured amount of AAP medium in order to obtain fluorescence readings. Diluted samples were not returned to the test flasks.

Recovery Phase

After five days exposure of the algal cultures to the test material, the recovery phase was initiated. Algal cells from cultures with day 5 cell concentrations similar to or less than the initial inoculum level were washed by centrifugation and resuspended in test material-free medium for a nine-day recovery phase. For S. capricornutum, the test concentrations that were submitted to the recovery phase were 0.016 and 0.032 mg/L. For M. aeruginosa, the test concentrations that were submitted to the recovery phase were 0.16 and 0.32 mg/L. Algal cells were also transferred from control and solvent control treatments for both assays.

To conduct the recovery phase, the contents of the replicate flasks were pooled for each treatment, yielding approximately 120-150 mL. (An exception to this procedure occured for the 0.016 mg/L test concentration in the assay with S. capricornutum. Growth in replicate 0.016C was noticeably greater than in the other two replicates, so it was not combined with the others.) The pooled volumes were divided into aliquots for centrifuging in centrifuge tubes of approximately 40 mL capacity. Cells were centrifuged for 10 minutes at approximately 1000 x g. The supernatant was removed and the cells resuspended in test material-free medium. Again the

cells were centrifuged, the supernatant removed and the cells resuspended. The divided aliquots were then re-combined.

An insufficient number of algal cells was available to begin the recovery phase with the original inoculum level of 20,000 cells/mL for S. capricornutum and 50,000 cells/mL for M. aeruginosa. For S. capricornutum, the recovery phase was begun with 5,000 cells/mL from each transferred test concentration, the control and solvent control. For M. aeruginosa, the recovery phase was begun with 2,300 cells/mL for each transferred test concentration, the control and solvent control.

Cell density in the washed suspensions was determined using the Coulter Counter, as previously described. The computed volumes were aseptically added to each of three replicate flasks per test concentration containing test material-free medium. The flasks were incubated for nine days under the same conditions previously described for the exposure phase.

IV. RESULTS - DISCUSSION

Data Analysis

The algistatic concentration with its associated 95% confidence intervals is determined by linear regression analysis of $\log_{10}$ of the ratio of the cell numbers (at the end of the exposure period) to the initial inoculum level regressed against the $\log_{10}$ of the concentration of material tested, using a linear model. The algistatic concentration is then selected by "inverse estimation" as that concentration of test material that corresponds to a day 5 ratio of one. Algistatic concentrations and 95% confidence intervals were calculated using a computer program developed by Procter and Gamble.

Discussion

Cell counts during the assay are presented in Tables 1 and 3 and are depicted graphically in Figures 1 and 3 for S. capricornutum and M. aeruginosa, respectively. Relative fluorescence readings for S. capricornutum are given in Table 2 and are plotted in Figure 2.

For S. capricornutum, exposure to increasing test concentrations of B0793.02 caused proportionately reduced growth, relative to the solvent control. In one replicate of the 0.016 mg/L test concentration (0.016C), growth on exposure days 3 and 5 was noticeably greater than in the other two replicates. The cause of this is not known, but the data for this replicate were determined to be "outlier" values according to Dixon's criteria (Dixon, 1953), as indicated on Tables 1 and 2. The data given for the recovery phase for this test concentration represents transferred cells from the 0.016A and B replicates only. In the assay with S. capricornutum, population density on day 5 of the exposure period was similar to the initial inoculum level of 20,000 cells/mL in the two highest test concentrations (0.016 and

0.032 mg/L). Algal cells exposed to these test concentrations were transferred to clean medium for the recovery phase. The transferred cultures were able to recover, although at the end of the recovery period, cell concentrations were less than that in the solvent control.

The algistatic concentration of B0793.02 for S. capricornutum is 0.029 mg/L (95% confidence limits: 0.015-0.064 mg/L). This value was determined by inverse estimation linear regression using the mean day 5 cell counts for the four highest test concentrations. (See Appendix C.) The algicidal concentration is greater than 0.032 mg/L.

Exposure to concentrations of 0.01, 0.02, 0.04 and 0.08 mg/L of B0793.02 resulted in similar or slightly increased growth of M. aeruginosa, relative to the solvent control. No growth occured in the two highest test concentrations. Algal cells exposed to these test concentrations (0.16 and 0.32 mg/L) were transferred to clean medium for the recovery phase. Recovery did not occur in these test concentrations. Although the cell concentration used to begin the recovery phase (2300 cells/mL) was quite low, substantial growth occurred by day 9 in the control and solvent control.

The algistatic concentration of B0793.02 for M. aeruginosa is 0.11 mg/L (95% confidence limits: 0.04-0.27 mg/L). This value was determined by inverse estimation linear regression using the mean day 5 cell counts for the four highest test concentrations. (See Appendix C.) The algicidal concentration is 0.16 mg/L.

Deviations from Protocol

There were no deviations from protocol.

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American Public Health Association, American Water Works Association, Water Pollution Control Federation, 1980, Standard Methods for the Examination of Water and Wastewater, 15th ed., Washington, DC.

Dixon, W.J., 1953, "Processing data for outliers," Biometrics, 9:74-89.

Miller, W.E., J.C. Greene and T. Shiroyama, 1978, The Selenastrum capricornutum Printz Algal Assay Bottle Test, EPA-600/78-018, 126 pp.

Payne, A.G. and R.H. Hall, 1979, "A method for measuring algal toxicity and its application to the safety assessment of new chemicals," Aquatic Toxicology, ASTM STP 667, L.L. Marking and R.A. Kimerle, eds., American Society for Testing and Materials, 1979, pp. 171-180.

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The Toxicity of 80793.02 to S. capricornutum and M. aeruginosa

Table 1. Cell counts (cells/mL) during assay for S. capricornutum

	Sample	Exposure Day 3 9-22-86	Exposure Day 5 9-24-86	Recovery Day 2 9-26-86	Recovery Day 6 9-30-86	Recovery Day 9 10-03-86
0	A	1,470,000	5,640,000	72,000	4,080,000	6,880,000
	B	1,000,000	3,600,000	85,000	3,980,000	6,680,000
	C	1,320,000	5,120,000	75,000	4,260,000	7,240,000
	Mean	1,260,000	4,790,000	77,000	4,110,000	6,930,000
	SD[1]	2.4E5	1.0EE6	6.8E3	1.4E5	2.8E5
	Var[2]	5.76E10	1.12E12	4.63E7	2.01E10	8.05E10
Sol[3]	A	848,000	2,920,000	91,000	4,620,000	7,320,000
	B	720,000	2,500,000	83,000	4,420,000	6,720,000
	C	608,000	3,800,000	85,000	4,660,000	7,640,000
	Mean	725,000	3,070,000	86,000	4,570,000	7,230,000
	SD	1.2E5	6.6E5	4.2E3	1.3E5	4.7E5
	Var	1.44E10	4.40E11	1.73E7	1.65E10	2.18E11
0.001	A	620,000	2,860,000	NT	NT	NT
	B	584,000	2,360,000	NT	NT	NT
	C	692,000	2,380,000	NT	NT	NT
	Mean	632,000	2,530,000	--	--	--
	SD	5.5E4	2.8E5	--	--	--
	Var	3.02E9	8.01E10	--	--	--
0.002	A	640,000	2,400,000	NT	NT	NT
	B	584,000	2,220,000	NT	NT	NT
	C	568,000	2,440,000	NT	NT	NT
	Mean	597,000	2,350,000	--	--	--
	SD	3.8E4	1.2E5	--	--	--
	Var	1.43E9	1.37E10	--	--	--
0.004	A	448,000	1,970,000	NT	NT	NT
	B	604,000	2,580,000	NT	NT	NT
	C	632,000	2,590,000	NT	NT	NT
	Mean	561,000	2,380,000	--	--	--
	SD	9.9E4	3.6E5	--	--	--
	Var	9.83E9	1.26E11	--	--	--

(Continued)

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The Toxicity of [illegible] to *S. marcescens* and *M. aeruginosa*

Table 1. Cell counts (cells/mL) during assay for *S. marcescens*

Sample		Exposure Day 3 9-22-86	Exposure Day 5 9-24-86	Recovery Day 2 9-26-86	Recovery Day 6 9-30-86	Recovery Day 9 10-03-86
0.008	A	212,000	1,200,000	NT	NT	NT
	B	173,000	880,000	NT	NT	NT
	C	258,000	1,280,000	NT	NT	NT
	Mean	214,000	1,110,000	--	--	--
	SD	4.2E4	2.2E5	--	--	--
	Var	1.8E9	4.9E10	--	--	--
0.016	A	33,000	31,000	25,000	1,030,000	3,940,000
	B	33,000	39,000	19,000	1,250,000	4,540,000
	C	(78,000)*	(254,000)*	20,000	1,360,000	5,260,000
	Mean	33,000	35,000	21,000	1,213,000	4,580,000
	SD	0	6.0E3	3.0E3	1.7E5	6.6E5
	Var	0	3.2E7	1.0E7	2.9E10	4.3TE11
0.032	A	18,000	18,000	9,000	242,000	2,270,000
	B	29,000	28,000	8,000	202,000	2,030,000
	C	23,000	24,000	10,000	231,000	2,610,000
	Mean	23,000	23,000	9,000	225,000	2,303,000
	SD	6.0E3	5.0E3	1.0E3	2.1E4	2.9E5
	Var	3.0E7	2.5E7	1.0E6	4.2TE8	8.46E10

[1] SD = Standard deviation
[2] Var = Variance
[3] Sol = Solvent control
[4] NT = Not transferred
*Value considered an outlier, not included in mean.

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The Toxicity of 80793.02 to S. capricornutum and M. aeruginosa

Table 2. Relative fluorescence readings during assay for S. capricornutum

Sample		Exposure Day 3 9-22-86	Exposure Day 5 9-24-86	Recovery Day 2 9-26-86	Recovery Day 6 9-30-86	Recovery Day 9 10-03-86
0	A	215	840	22	660	1420
	B	150	610	22.5	680	1080
	C	185	840	30.5	700	1480
	Mean	183.5	763.5	25	680	1326.5
	SD[1]	32.5	132.5	4.8	20	215.7
	Var[2]	1058	17633	22.8	400	46533
Sol[3]	A	156.5	520	30.5	820	1660
	B	141.5	440	28.5	760	1640
	C	141.5	660	22	800	1240
	Mean	146.5	540	27.0	793.5	1680
	SD	8.7	111.4	4.3	30.5	52.9
	Var	75.0	12400	19.8	933	2800
0.001	A	141.5	520	NT[4]	NT	NT
	B	141.5	400	NT	NT	NT
	C	165	480	NT	NT	NT
	Mean	149.5	466.5	--	--	--
	SD	13.6	61.1	--	--	--
	Var	184.1	3733	--	--	--
0.002	A	140	390	NT	NT	NT
	B	150	380	NT	NT	NT
	C	130	400	NT	NT	NT
	Mean	140	383.5	--	--	--
	SD	10	20.8	--	--	--
	Var	100	433	--	--	--
0.004	A	128.5	340	NT	NT	NT
	B	121.5	405	NT	NT	NT
	C	130	420	NT	NT	NT
	Mean	126.5	388.5	--	--	--
	SD	4.5	42.5	--	--	--
	Var	20.6	1808	--	--	--

(Continued)

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The Toxicity of 80798.02 to S. capricornutum and M. aeruginosa

Table 2. Relative fluorescence readings during assay for S. capricornutum

Sample		Exposure Day 3 9-22-86	Exposure Day 5 9-24-86	Recovery Day 2 9-26-86	Recovery Day 6 9-30-86	Recovery Day 9 10-03-86
0.008	A	38.5	195	NT	NT	NT
	B	41.5	135	NT	NT	NT
	C	60	195	NT	NT	NT
	Mean	53.5	175	--	--	--
	SD	10.3	34.6	--	--	--
	Var	106	1200	--	--	--
0.016	A	2.6	1.5	4.5	190	750
	B	4.2	3.0	4.5	225	800
	C	16.5	(50)*	4.0	245	930
	Mean	7.8	2.2	4.3	220	827
	SD	7.6	1.1	0.3	27.8	92.9
	Var	57.8	1.1	0.01	775.0	8633.3
0.032	A	3.0	1.5	0.5	39.5	385
	B	3.5	1.1	0.5	35	350
	C	4.0	1.5	0.5	39	445
	Mean	3.5	1.4	0.5	38	393
	SD	0.5	0.2	0	2.5	48.0
	Var	0.25	0.05	0	6.1	2308.3

[1] SD = Standard deviation
[2] Var = Variance
[3] Sol = Solvent control
[4] NT = Not transferred
*Value considered an outlier, not included in mean

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The Toxicity of 80793.02 to S. capricornutum and M. aeruginosa

Table 3. Cell counts (cells/mL)* during assay for M. aeruginosa

	Sample	Exposure Day 3 9-15-86	Exposure Day 5 9-17-86	Recovery Day 2 9-19-86	Recovery Day 6 9-23-86	Recovery Day 9 9-26-86
0	A	148,000	326,000	8,000	67,000	390,000
	B	130,000	260,000	8,000	67,000	326,000
	C	152,000	456,000	7,000	66,000	384,000
	Mean	143,000	347,000	8,000	67,000	367,000
	SD[1]	1.2E4	1.0E5	1.0E3	1.0E3	3.5E4
	Var[2]	1.37E8	9.94E9	3.33E5	3.33E5	1.25E9
Sol[3]	A	145,000	404,000	6,000	69,000	444,000
	B	141,000	360,000	6,000	73,000	442,000
	C	146,000	392,000	6,000	76,000	448,000
	Mean	144,000	385,000	6,000	73,000	411,000
	SD	2.6E3	2.3E4	0	3.5E3	5.8E4
	Var	7.00E6	5.17E8	0	1.23E7	3.41E9
0.01	A	146,000	430,000	NT[4]	NT	NT
	B	153,000	414,000	NT	NT	NT
	C	150,000	408,000	NT	NT	NT
	Mean	150,000	417,000	--	--	--
	SD	3.5E3	1.1E4	--	--	--
	Var	1.23E9	1.29E8	--	--	--
0.02	A	149,000	466,000	NT	NT	NT
	B	170,000	538,000	NT	NT	NT
	C	161,000	482,000	NT	NT	NT
	Mean	160,000	502,000	--	--	--
	SD	1.0E4	4.9E4	--	--	--
	Var	1.11E8	2.42E9	--	--	--
0.04	A	173,000	448,000	NT	NT	NT
	B	157,000	424,000	NT	NT	NT
	C	146,000	400,000	NT	NT	NT
	Mean	159,000	424,000	--	--	--
	SD	1.4E4	2.4E4	--	--	--
	Var	1.8eE8	5.76E8	--	--	--

(Continued)

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The Toxicity of 80799.02 to *S. capricornutum* and *M. serpentinae*

Table 3. Cell counts (cells/mL)* during assay for *M. serpentinae*

Sample		Exposure Day 5 9-15-86	Exposure Day 5 9-17-86	Recovery Day 2 9-19-86	Recovery Day 6 9-23-86	Recovery Day 9 9-26-86
0.08	A	134,000	402,000	NT	NT	NT
	B	129,000	370,000	NT	NT	NT
	C	127,000	362,000	NT	NT	NT
	Mean	130,000	378,000	--	--	--
	SD	3.6E3	2.1E4	--	--	--
	Var	1.3E7	4.4E8	--	--	--
0.16	A	3,000	5,000	3,000	4,000	9,000
	B	5,000	5,000	2,000	2,000	6,000
	C	4,000	5,000	2,000	4,000	6,000
	Mean	4,000	5,000	2,000	3,000	7,000
	SD	1.0E3	0	1.0E3	1.2E3	1.7E3
	Var	1.0E6	0	3.3E5	1.3E6	3.0E6
0.32	A	3,000	4,000	2,000	4,000	3,000
	B	5,000	4,000	2,000	5,000	3,000
	C	6,000	4,000	1,000	4,000	4,000
	Mean	5,000	4,000	2,000	4,000	3,000
	SD	1.5E3	0	1.0E3	1.0E3	1.0E3
	Var	2.3E6	0	3.3E5	3.3E5	3.3E5

[1] SD = Standard deviation
[2] Var = Variance
[3] Sol = Solvent control
[4] NT = Not transferred

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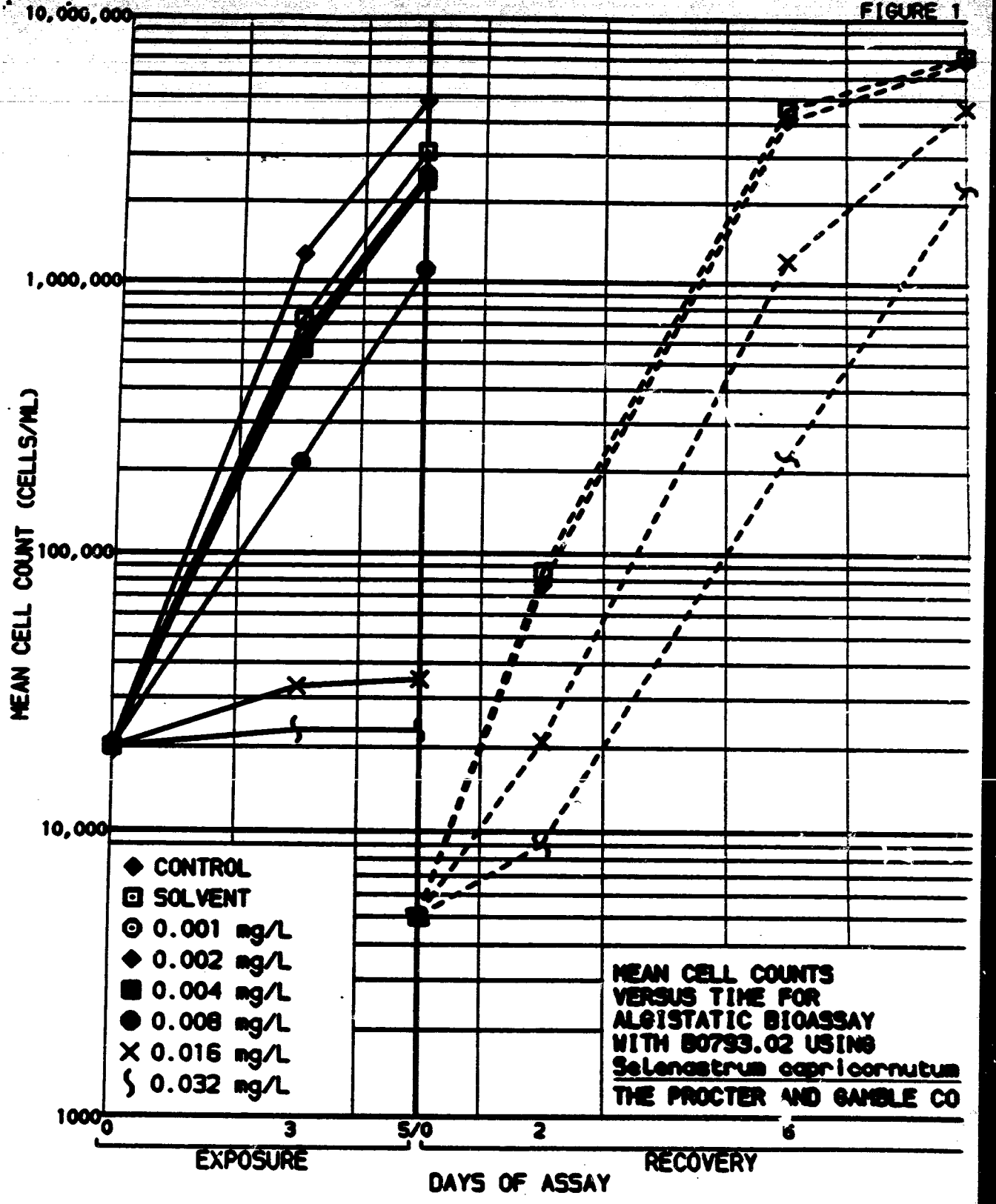
FIGURE 1



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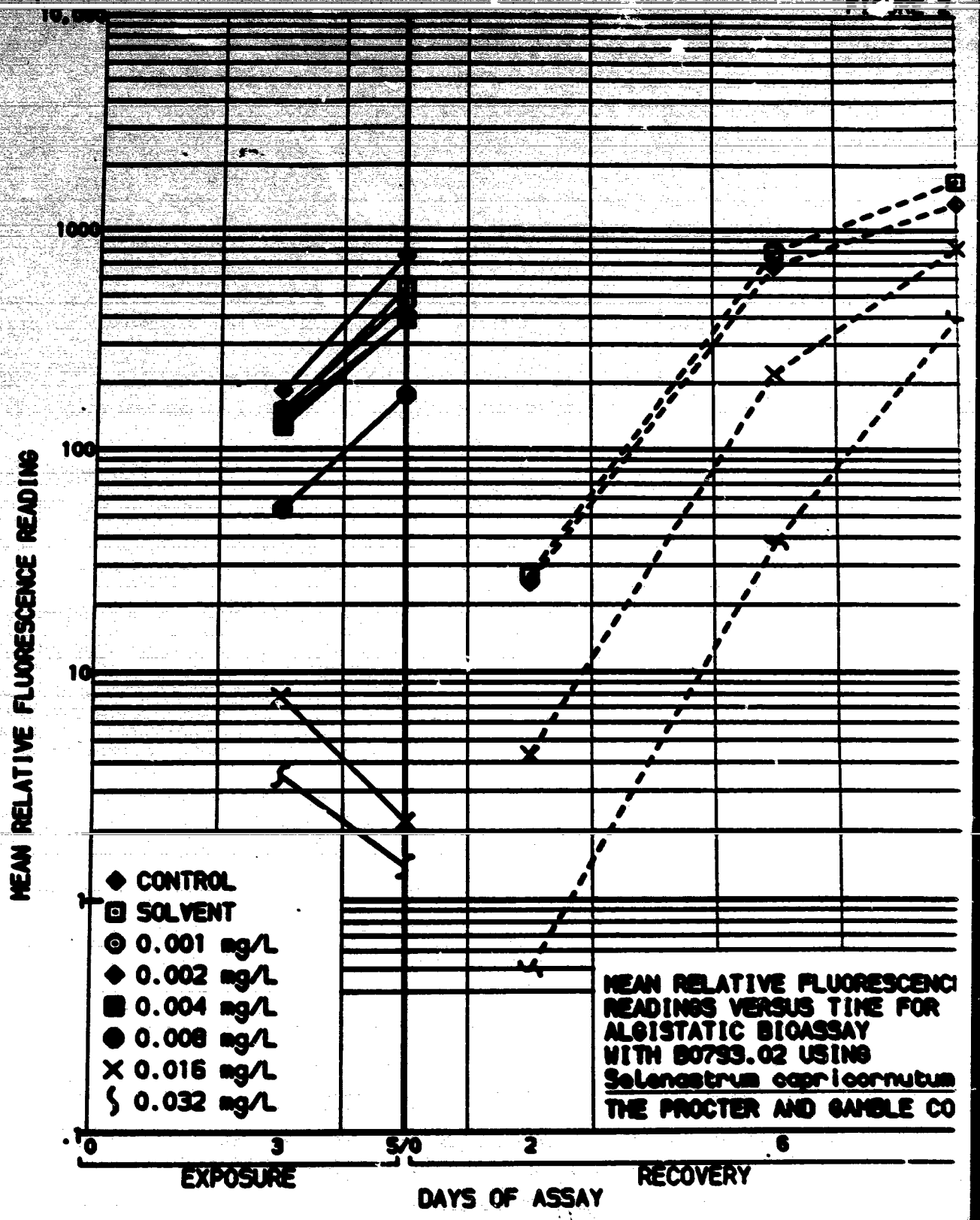
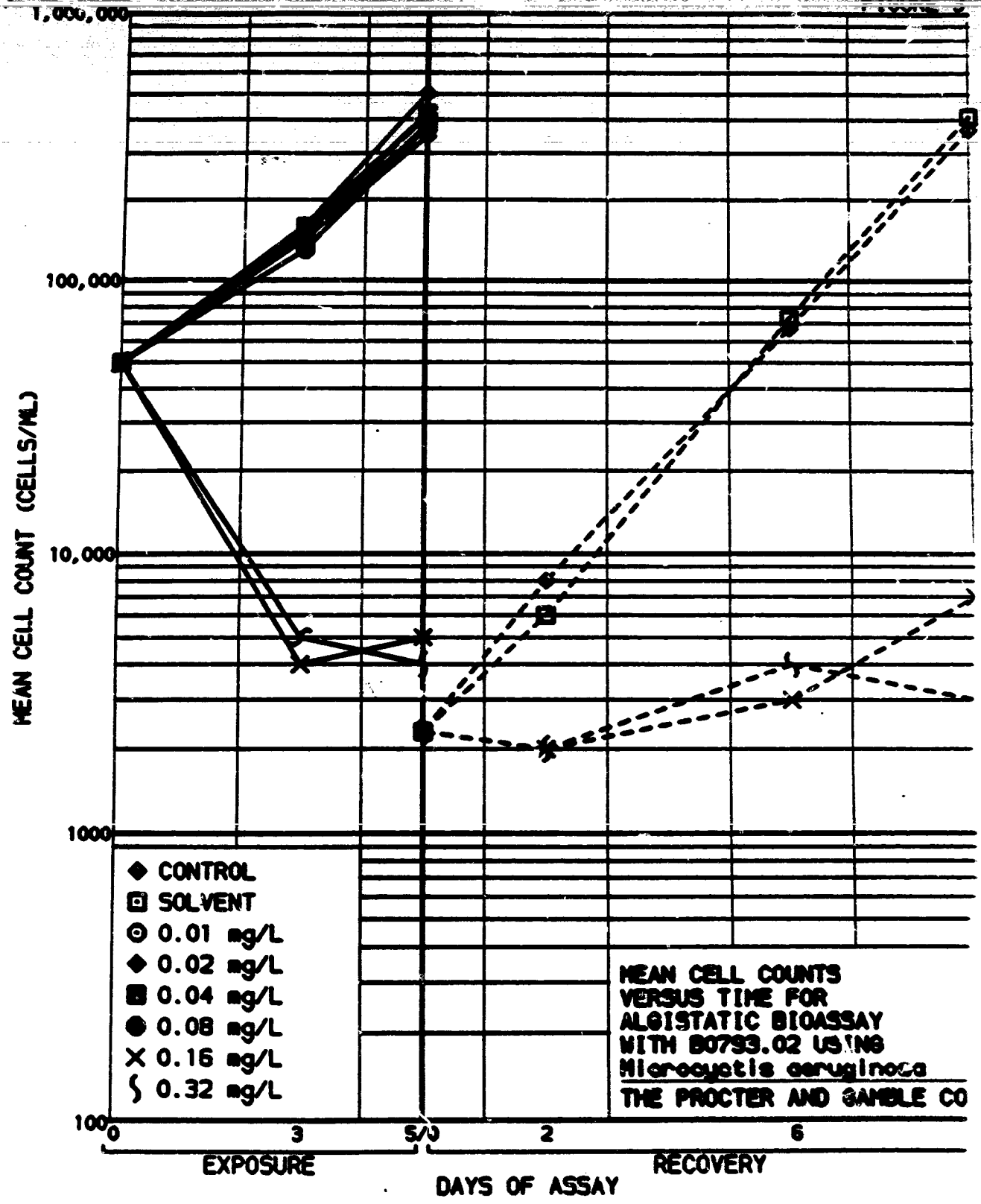

MEAN RELATIVE FLUORESCENCE READING
1000
100
10
1
◆ CONTROL
⊡ SOLVENT
⊙ 0.001 mg/L
◆ 0.002 mg/L
■ 0.004 mg/L
● 0.008 mg/L
× 0.016 mg/L
ʃ 0.032 mg/L
MEAN RELATIVE FLUORESCENCE READINGS VERSUS TIME FOR ALGISTATIC BIOASSAY WITH B0793.02 USING Selenastrum capricornutum
THE PROCTER AND GAMBLE CO
0
3
5/0
2
6
EXPOSURE
RECOVERY
DAYS OF ASSAY

0 0 5 8

FIGURE 3



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APPENDIX A. COMPOSITION OF ALGAL ASSAY PROCEDURE (AAP) NUTRIENT MEDIUM (AFTER MILLER ET AL, 1978)

a. Macronutrients

Stock solution		Nutrient composition of prepared medium	
Compound	Concentration, g/L	Element	Nominal Concentration, mg/L
$NaNO_3$	25.500	N	4.200
$NaHCO_3$	15.000	Na	11.001
		C	2.143
K_2HPO_4	1.044	K	0.469
		P	0.186
$MgSO_4.7H_2O$	14.700	S	1.911
$MgCl_2.6H_2O$	12.164	Mg	2.904
$CaCl_2.2H_2O$	4.410	Ca	1.202

b. Micronutrients

Stock solution		Nutrient composition of prepared medium	
Compound	~~Concentration~~ mg/L	Element	Nominal ~~Concentration~~, ug/L
H_3BO_3	185.520	B	32.460
$MnCl_2.4H_2O$	415.610	Mn	115.374
$ZnCl_2$	3.271	Zn	1.570
$CoCl_2.6H_2O$	1.428	Co	0.354
$CuCl_2.2H_2O$	0.012	Cu	0.004
$Na_2MoO_4.2H_2O$	7.260	Mo	2.878
$FeCl_3.6H_2O$	160.000	Fe	33.051
$Na_2EDTA.2H_2O$	300.000	...	...

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0 0 5 4

THE PROCTER & GAMBLE COMPANY

The Toxicity of B0793.02 to S. capricornutum and M. aeruginosa

APPENDIX B. OPERATING PARAMETERS FOR THE COULTER COUNTER

For counting algae using the Model ZBI Coulter Counter with C-1000 Channelyzer and MIR Computer, the following parameters were used:

Aperture tube	100 um
Volume	500 uL
Separate/Locked switch	Separate
1/aperture	1/2
1/amplification	1/2
Matching switch	20K
Gain trim	3
Lower threshold	8
Upper threshold	100
MIR threshold	8
Base channel threshold	5
Window width	100
Edit switch	On
Mean Threshold factor	1.231 (n = 3)
Range of cell volumes included in count	10-140 um^3

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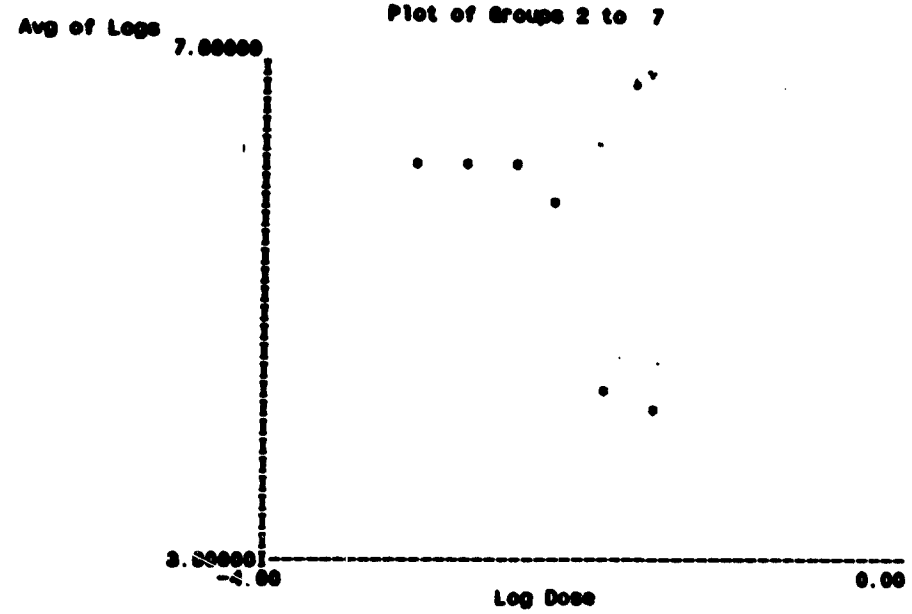
APPENDIX C

DETERMINATION OF ALGISTATIC CONCENTRATIONS

ALGISTATIC CONCENTRATION OF 80793.02 TO Selenastrum capricornutum



4 4 5 6 7

Conc used in Regression

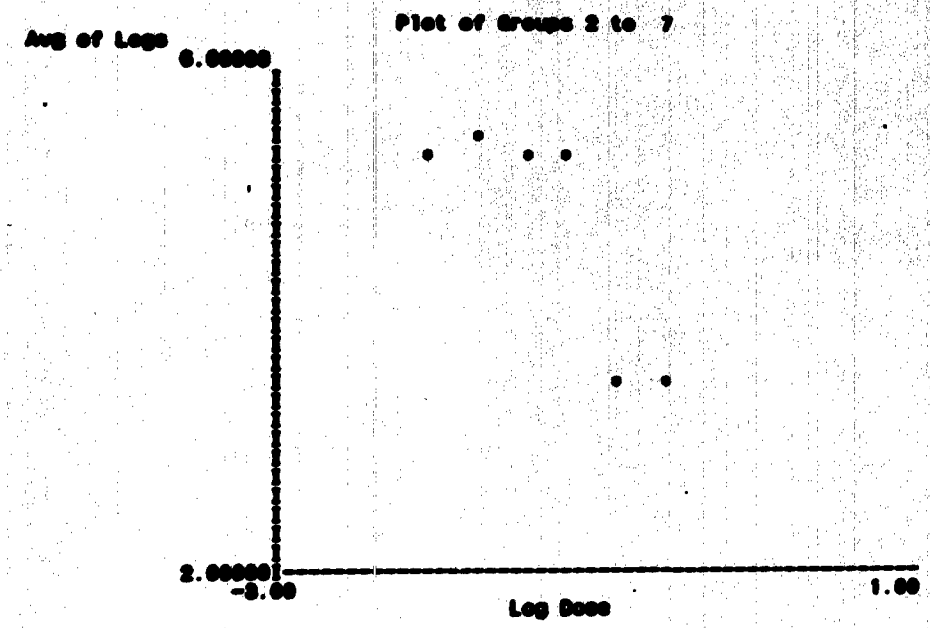
Group	Conc	Log Conc
4	0.004	-2.3979
5	0.008	-2.0969
6	0.016	-1.7959
7	0.032	-1.4949

Random Error =	0.02521
Mean of conc =	-1.9464
Mean of counts =	1.0281
Slope =	-2.50303
Intercept =	-3.04380
Standard Error =	0.31492
Ratio =	1.00000
Algistatic Conc	0.02913

95% Confidence Limits for this Conc

Low Conc =	0.01483
Hi Conc =	0.06425

ALGISTATIC CONCENTRATION OF [illegible]0768.02 TO Microcystis aeruginosa



4 4 5 6 7

Conc used in Regression

Group	Conc	Log Conc
4	0.04	-1.3979
5	0.06	-1.0969
6	0.10	-0.7959
7	0.32	-0.4949

Random Error =	0.00120
Mean of conc =	-0.9464
Mean of counts =	-0.0727
Slope =	-2.04100
Intercept =	-2.57201
Standard Error =	0.48251
Ratio =	1.00000
Algistatic Conc	0.10019

95% Confidence Limits for this Conc

Low Conc =	0.03100
Hi Conc =	0.27100

THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

P.O. BOX 39175
CINCINNATI, OHIO 45247

August 4, 1986

Mr. S. K. Krishnaswami
Malcolm Pirnie, Inc.
2 Corporate Park Drive
White Plains, NY 10602

Dear Mr. Krishnaswami:

This is to authorize you to carry out the following study according to the attached protocol, and in conformance with the stipulations of our current Laboratory Services Agreement.

Protocol: Toxicity of B0793.02 to Algae; (Algistatic Determination)
Date: 7/21/86
Sponsor's Principal Investigator:

Notice: The stipulations of this protocol are to be implemented in conformance with EPA Good Laboratory Practice Regulations (40 CFR, Part 792).

Please have the Study Director approve and complete both copies of the attached protocol by adding your study or project number, estimated starting and reporting dates, and date the test material was received. Retain one copy for your files and return the other to me.

Please return all unused portions of the test material to the Sponsor's Principal Investigator at the following address:

We understand the estimated cost for this study is $3,000. Invoices are also to be sent to the Principal Investigator.

Three copies of the final report are to be sent to my attention at the letterhead address.

Matters involving the scientific aspects of the work can be handled directly with the Sponsor's Principal Investigator. Feel free to contact me at if you have any other questions or concerns.

Sincerely,

THE PROCTER & GAMBLE COMPANY

Attachments
c

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THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

P. O. BOX 39175
CINCINNATI, OHIO 45247

August 11, 1986

Ms. Jane S. Hughes
Malcolm Pirnie, Inc.
2 Corporate Park Drive
White Plains, NY 10602

Dear Jane:

In my authorization letters dated August 4, 1986, for the toxicity to algae (algistatic determination) of test substances B0792.02 and B0793.02, I listed the estimated cost incorrectly. There are two species to be conducted for each test substance. The estimated cost for each test substance, therefore, is $6,000 ($3,000 per species).

If you have any questions, please telephone.

Sincerely yours,

THE PROCTER & GAMBLE COMPANY
Research & Development Department

mh

0 0 6 0

MALCOLM PIRNIE

MALCOLM PIRNIE, INC.
ENVIRONMENTAL ENGINEERS, SCIENTISTS & PLANNERS

November 13, 1986

The Procter and Gamble Co.
Miami Valley Laboratories
P. O. Box 39175
Cincinnati, Ohio 45247

As we discussed in our recent telephone conversation, I have enclosed the completed test summary sheet for the study with B0793.02 (which I neglected to include with the report) and a copy of a telephone confirmation record documenting the use of DMF as a solvent rather than isopropanol as per protocol. (This telephone confirmation will substitute for a formal "addendum to protocol".)

Also enclosed is a reprint describing the results of some work I conducted several years ago on the use of DMF as a solvent for S. capricornutum. This work obviously needs to be expanded upon, but I'm afraid we have neither the time nor the money at present!

Yours very truly,

MALCOLM PIRNIE INC.

Jane S. Hughes

Jane S. Hughes
Study Director, Aquatic Toxicology

JSH/kg
Enclosure

cc: T. M. Kennel

CORPORATE PARK DR BOX 751 WHITE PLAINS NY 10602 914 694 2100

0 0 6 1

MALCOLM PIRNIE

TELEPHONE CALL CONFIRMATION

Local _____ Long Distance _____ Date 8/8/86

To/From _____ Time _____

Procter and Gamble Project _____

MPI Name Jane Hughes Proj. No. 0165-13-1100 and 0165-14-1100

Subject:

Characteristics of Test Materials

1. **B0793.02**

   Protocol identifies this as a clear liquid. We can see some white suspension in it. said this is normal. If it is heated up about 5° above room temperature, it will become clear. We are to do this prior to making a stock solution. This compound may be quite toxic to algae.

2. **B0792.02** - not expected to be as toxic

3. **Solvent**

   knows the compounds will disolve in isopropanol. However, we can use any other solvent we have experience with (such as DMF), if it will dissolve the compounds.

Route to: Meryl Alexander

File: 0165-13-1100
0165-14-1100

0 0 6 2

Laboratory Study No. 0165-14-1000

PROTOCOL

SPONSOR: Procter & Gamble Company; Cincinnati, Ohio

LABORATORY: Malcolm Pirnie, Inc. 2 Corporate Park Drive White Plains, NY 10602

TITLE: Toxicity of B0793.02 to Algae; (Algistatic Determination)

OBJECTIVE: To determine the algistatic concentration of the test material to algae.

TEST SPECIES AND JUSTIFICATION: (check 1 or more) [✓] Selenastrum capricornutum [✓] Microcystis aeruginosa [ ] Navicula pelliculosa [ ] Dunaliella tertiolecta. All four species are readily available, easily cultured and are representative of common classes of algae.

TEST MATERIAL:

Sample Code B0793.02 Color CLEAR Form LIQUID

% Active 100 Density — Solubility LOW IN WATER

Expiration Date 11-86 Other —

Storage Conditions - ROOM TEMPERATURE

Safe Handling Precautions - AVOID SKIN OR EYE CONTACT - IF CONTACT OCCURS, FLUSH WITH WATER

The Sponsor accepts full responsibility for appropriate characterization and stability verification of the test material.

TEST MEDIA: For Selenastrum, Microcystis, and Navicula, use synthetic algal nutrient media containing the levels of nutrients specified on pages 18 and 19, Sections 5.71 and 5.72 of reference 1. (For Navicula only, 20 mg Si/L is included.) For Dunaliella, nutrient levels are to be as specified on pages 8 and 9, Section 6.1 of reference 2, with a salinity level of 20 °/oo. Media are prepared following laboratory's Standard Operating Procedure (SOP).

TEST MATERIAL PREPARATION/ADDITION: All calculations and measurements are based on the active ingredient. The maximum concentration tested is 1,000 mg active/L.

Prepare a stock solution in the test medium at concentrations ≥ the highest test concentration. Dilutions of subsequent stock solutions to achieve lower test concentrations are also made in the test medium.

(Note: Check here [✓] and include special instructions, ~~as attachment~~ BELOW 1, for compounds of low solubility in water.) DISSOLVE IN WARM (40°C) ISOPROPANOL

Determine the pH of the highest test concentration. If the pH change is greater than one full unit from the test medium control, adjust to within this range using either NaOH or HCl. Follow this same procedure for the next lower concentration until no adjustment is required.

Test concentrations - A control and at least five concentrations of the test material are used. Except for the control, the test material concentration in each treatment must be at least 50% of the next higher one.

Based on a range-finding study, the test concentration range is selected to demonstrate inhibitory responses above and below the algistatic level (i.e. the original inoculum level).

0 0 6 3

TEST PROCEDURE: Algal cultures are exposed to the test material under [illegible] for five days. At the end of this five-day period, algal cells from cultures that have populations less than or similar to the initial inoculum level are washed and transferred into test material free medium. Cells from the control cultures are also washed and transferred. The transferred cultures are then incubated for a recovery period of nine days under the same conditions as the exposure period.

Biomass measurements - Algal growth is determined by cell counts following laboratory's SOP on days 3 and 5 of the exposure period and days 2, 6 and 9 of the recovery period. Chlorophyll a relative fluorescence measurements are also made on each of these days following laboratory's SOP. (Note: Fluorescence measurements are not required for Microcystis)

Cell washing procedure -

1) pool replicates from cultures to be transferred;
2) centrifuge at ≤1,000 X g to form a pellet;
3) decant the supernatant;
4) resuspend cells in test material free medium;
5) repeat steps 2, 3, and 4.

Washed cells are added at the original inoculum level to clean Erlenmeyer flasks containing test material free medium. If a sufficient number of cells is not available, the entire quantity is divided equally into the replicate flasks. Under these circumstances, washed cells from the control culture are added at both the original inoculum level and the lowest level included in the recovery period.

Test conditions are as follows:

1) Test containers and solution volume - 125 ml or 250 ml Erlenmeyer flasks, containing solution in a sample-to-volume ratio of twenty to fifty percent. Test containers and solution volumes should be consistent for all test concentrations and the controls.

2) Number of replicates - minimum of three.

3) Inoculum source and age - laboratory stock cultures 7 to 14 days old and previously maintained in the respective test medium for a minimum of three transfers.

4) Inoculum level - 2×10^4 cells/ml for Selenastrum, Navicula, and Dunaliella
- 5×10^4 cells/ml for Microcystis .

5) ~~Temperature - as per laboratory's SOP.~~

6) Light level - 400 footcandles ± 15% for Selenastrum, Navicula, and Dunaliella
- 200 footcandles ± 15% for Microcystis.

7) Shaker speed - as per laboratory's SOP's.

8) Glassware cleaning - as per laboratory's SOP.

CALCULATIONS: The algistatic concentration, with its associated 95% confidence intervals, is determined by linear regression analysis of $\log_{10}$ of the ratio of the cell numbers (at the end of the exposure period, that is, Day 5) to the initial inoculum level regressed against the $\log_{10}$ of the concentration of material tested, using a linear model. The algistatic concentration is then selected by "inverse estimation" as that concentration of test material that

corresponds to a Day 5 ratio of one (i.e., where the $\log_{10}$ of the ratio is zero). When test concentrations causing 100% die-off are used in the calculation, an arbitrary value representing one half of the minimum count possible (as per laboratory's SOP) is used in place of zero.

The algicidal concentration is reported as the lowest concentration tested demonstrating inhibitory effects (equal to or below the original inoculum level) and from which cells do not recover when transferred into test material free medium.

RECORDS TO BE MAINTAINED: All records necessary to reconstruct the study and demonstrate adherence to the Protocol.

PROTOCOL CHANGES: If a change in the approved protocol becomes necessary, verbal agreement should be made between the Study Director and Sponsor's Principal Investigator. As soon as practical thereafter, this change and the reasons for it should be put in writing, approved by both persons, and attached to the protocol as an addendum.

REFERENCES:

1. USEPA 1978. The Selenastrum capricornutum Printz Algal Assay Bottle Test, Environmental Research Laboratory, Corvallis, OR, 126 pp.

2. USEPA 1974. Marine Algal Assay Procedure: Bottle Test, Pacific Northwest Environmental Research Laboratory, Corvallis, OR, 43 pp.

REPORTING: The report is to be a typed document in triplicate, describing the results of the study and is to be signed and dated by the Study Director, Quality Assurance Officer and Laboratory Manager. It is to include, but is not limited to, the following:

1. Identification of test material by sample code, percent active, color, form and date received.
2. Procedures followed for test material preparation and addition.
3. Reference to laboratory notebook or other file containing raw data.
4. Laboratory Study Number.
5. Date definitive test was conducted.
6. Species tested, including the source and age of the inoculum.
7. Calculated algistatic concentrations with associated 95% confidence intervals and the algicidal concentration.
8. Plots and tables of biomass measurements across the 14-day testing period.
9. Description of the test conditions including test media, temperature, shaker speed, and light intensity.
10. Reference to the Protocol (title, author and date), and any standard test methods or any analytical procedures used.
11. Any Protocol deviations and their implications.
12. Description of the quality assurance methods used to insure the quality of the data.

Study No. 0405-M-1104

Estimated Starting Date 9/5/86 Defined As: definitive test

Estimated Reporting Date 10/3/86

Date Test Material Received 8/8/86

Approved Jerry S. Hughes 8/8/86 PHONE: 914-694-2100
Study Director Date

A. MATERIAL Name:
Formula or nature of product: (Exact Structure/Nominal Composition; Analytical Information)

Octadecyl-dimethylamine

Active Molecular Weight 297

For

SPECIAL INFORMATION: (additional information of value not included in above, reference to testing done on similar formulations or chemicals, etc.).

Or

Date: 2/3/86

B. ENVIRONMENTAL TESTS:	Lab	SAMPLE IDENTIFICATION
Algae Toxicity (Microcystis and Selenastrum)	[illegible]	B0793-02
Marine Fish Toxicity (Sheepshead minnow)	[illegible]	
Marine Invertebrate Toxicity (mysid shrimp)	"	

Est. Cost $8,500

Est. Reporting Date 9/10/86

Date: 7/18/86

RHH392/dh
7/18/86

TEST SUBSTANCE CHARACTERIZATION REPORT (TSCR)

ORIG. SECT. NO.

Test Substance Identification Number (Serial): 80793-02
Safety Test Request Number:
Principal Investigator:

Product or Ingredient:
Physical Description: Clear Liquid Solubility: NK
Recommended Storage Conditions: Room Temperature Expiration Date: 11-86
Hazards (i.e. flammability, toxic gases): Alkaline Corrosive
Dept. of Transportation Hazard Classification: Non. HAI719 CAS No.(a): 124-28-7

Formulated Composition (b)

Component (c)	Mol. Wt.	Nominal Level (% by Wt.)	Acceptable Range	Stock Code No.	Supplier	Lot Number (MB-Ref.)
(Alkyl Dimethylamine)	297	100%		NK	Ethyl Corp	ADMA 8-0

(a) Include CAS number(s) for the three most major components of a formulation or for single chemical products. Footnote to the material with which the respective number is associated.

(b) If information requested is not known, then the symbol NK will be entered.

(c) Chemical names which are inconveniently long may be abbreviated in tables but should be listed in full in referenced footnotes. Non-chemical names, such as Tergitol 15-S-0 or Yellow Dye #10, may not be acceptable but should be previewed with the responsible toxicologist. Nondefinitive identification (e.g. Arquad, DC-base) is not ---------.

The above information provided by: 10/23/85 (Date)

The above information reviewed and accepted by:

Principal Investigator: 7/4/86 (Date)

0 0 6 9

TEST SUBSTANCE CHARACTERIZATION REPORT (TSCR)

Test Substance Identification Number (TSIN): 20793-02

Analyzed Composition

Date Submitted	Submitter Code	Analysis Code/Analysis	Estimated Value	Measured Value	Testing Laboratory

nalytical Information Verified By: _____ Date:
(Signature)

hi:

rir Date: 2/4/82

his test substance is suitable for human (clinical) safety testing.

rinciple Investigator: _____ Date:
(Signature)

0 0 6 9

MALCOLM PIRNIE

MALCOLM PIRNIE, INC.
ENVIRONMENTAL ENGINEERS, SCIENTISTS & PLANNERS

December 18, 1986

The Procter and Gamble Company
PO Box 39175
Cincinnati, Ohio 45247

In reviewing past files, it was found that the Compliance Statements were omitted from two Algal Assay reports submitted recently to Procter and Gamble. The reports are entitled "The Toxicity of B0792.02 to Selenastrum capricornutum and Microcystis aeruginosa", dated October 1986, and "The Toxicity of B0793.02 to Selenastrum capricornutum and Microcystis aeruginosa", also dated October 1986. Enclosed are the completed Compliance Statements for these reports, as well as forms MPI-QA-RAE-1, Completed Study Report Addenda/Errata Notification. Please sign one copy of each of the Addenda Notification forms, and return the signed copies to me.

I would like to apologize for any inconvenience this may have caused, and I assure you, that in the future, a completed Compliance Statement will be sent with each report submitted to you.

Very truly yours,

MALCOLM PIRNIE, INC.

Teresa M. Kennel

Teresa M. Kennel
Quality Assurance Inspector

TMK/kg
Enclosure

2 CORPORATE PARK DR BOX 751 WHITE PLAINS, NY 10602 914-694-2100 TELEX 137364

0 0 7 0

Completed Study Report Addenda/Errata Notification

A quality assurance inspection has been conducted of the following final report:

MPI Archive Reference No.: PG/BO792.02/ALGST, SEL, MIC/0165-13-1100

Title of Report: The Toxicity of BO792.02 to Selenastrum capricornutum and Microcystis aeruginosa

Sponsor: The Procter and Gamble Company

MPI Protocol No.: Prepared by Sponsor, dated 7/12/86

Notebook No.(s): 42 Issue Date: 10/2/86

The following changes or clarifications are necessary:

Page	Line(s)	Comments/Corrections
____	_____	Add form MPI-QA-CS-2, Quality Assurance Compliance
____	_____	Statement to end of report.
____	_____	
____	_____	
____	_____	
____	_____	
____	_____	
____	_____	
____	_____	
____	_____	
____	_____	

(X) The above changes do not affect the results of the study.

( ) The above changes affect the results of the study.
Explanation attached.

Report reviewed by: Teresa M. Kennedy Date: _____

Copy of MPI-QA-RAB-1 sent to Jane S. Hughes (facility director) Date: 12/19/86

Copy of MPI-QA-RAB-1 sent to _____ (sponsor representative) Date: 1/6/87

Copy of MPI-QA-RAB-1 archived with report by: _____

Date: _____

Aquatic Toxicology
MPI-QA-CS-2

Quality Assurance Compliance Statement

The study entitled The Toxicity of B0792.02 to Selenastrum capricornutum and Microcystis aeruginosa

conducted and reported by Malcolm Pirnie, Inc., White Plains, NY for The Proctor and Gamble Company

is in compliance with EPA Good Laboratory Practice Standards under the Federal Insecticide, Fungicide and Rodenticide Act and the Toxic Substances Control Act (Fed. Reg. Vol. 48, No. 230, 11/29/83) except as follows: study was conducted in compliance with EPA Good Laboratory Practice Standards

Inspections were conducted during the course of this study for compliance verification

I) Instrumentation Inspection

Date of Inspection	Inspector	Date Findings Reported	Date Corrective Action Taken	Comments
8/22/86	T. Kennel	8/22/86	N.A.	None
8/28/86	T. Kennel	8/28/86	N.A.	None

II) Study Inspection (Test System and Raw Data)

Date of Inspection	Inspector	Date Findings Reported	Date Corrective Action Taken	Comments
9/4/86	T. Kennel	9/4/86	N.A.	None

Quality Assurance Inspector: Teresa M. Kennel
Date: 12-16-86

0 0 7 3

ENVIRONMENTAL SAFETY TEST SUMMARY REVIEW

Test Material Name: CAS RN 134-28-7

Test Substance Identification Number: B0793.01 project # suffix): B0793.01-03

Type of Study: Acute Daphnia Toxicity in River Water Report or Project #: BS-85-6-1782

Name of Originator: Division: DRD #:

Laboratory Involved: Springborn Bionomics, Inc.

Date Report Written: 6-27-85 Date Received by Operations Section: 7-11-85

This report has been received and found in agreement with the Protocol and there appear to be no inaccuracies in the numerical data or written portions with the following exceptions:

None

Logistics Reviewer Date 10/24/85

This report has been reviewed for scientific quality and is summarized with the following comments:

Principal Investigator Date 10/25/85

System:

C Date 10/29/85

Date 11/3/85

Microfilming Completed: Date _____ By _____

Copy returned to: _____ Date _____

0 0 3 9

TEST SUMMARY SHEET

B. Text: (Note - tabulations must be limited to 71 characters in width)

Test Type: Acute Freshwater Invertebrate Toxicity

Species: Daphnia magna
Age: < 24 hours
Source: Bionomics culture

Dilution Water: Town River Water
Total Hardness: 35 mg/l as CaCO3
pH: 6.5
Test Temperature: 20-21 degrees C
Total Suspended Solids: 15 mg/l

Results
48 hr LC50: 0.35 mg/l
95% Conf. Intervals: 0.29-0.42 mg/l

Observations: Abnormal behavior noted at all test concentrations.

Reference Response (48 hr LC50): 5.3(3.2-9.0) mg/l sodium lauryl sulfate
Analytical Method: None
Material Solubilization: Material added via [✓] stock solution; [ ] direct weighing. Solvent used - [ ] water; [✓] IPA.
Undissolved test material noted at 1.8 mg/l and higher.

Comments: All results are based on nominal concentrations of the active ingredient tested as 100 % active.

Test Protocol: Static Acute Freshwater Invertebrate Toxicity Study of B0793.01 in River Water; 5/85

C. Project Status Sheet Code No. B0793.01.04 [illegible] 11-6 85

D. Test Type: ACIN KEYWORD Acute Freshwater Invertebrate Toxicity - Static

E. Test Material: Di-Methyl Stearyl Amine

F. Title/Date/ESD File No. Acute Toxicity of B0793.01 in River Water to the Daphnia magna / 6-17-85

G. Test Location: Bionomics H. Lab Project No.:

I. Date: 85/10/22 (YY MM DD)

K. Prepared by:

M. TSIN: B0793.01

N. Date Work Done: 4/8/85 - 4/10/85

ACUTE TOXICITY OF B0793.01 IN RIVER WATER TO *Daphnia magna*

TOXICITY TEST REPORT

SUBMITTED TO

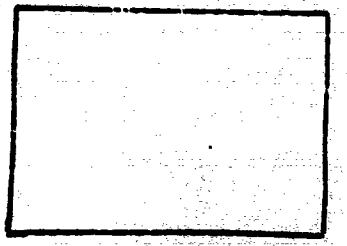
THE PROCTER & GAMBLE COMPANY

CINCINNATI, OHIO

BIONOMICS REPORT #BW-85-6-1782

BIONOMICS STUDY #1011-0385-6134-110

Springborn Bionomics, Inc.
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
June, 1985



SUMMARY

48-Hour Static Toxicity Test: Freshwater Invertebrate

Springborn Bionomics, Inc.
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts

CLIENT: The Procter & Gamble Company

PROTOCOL CITED: Static Acute Freshwater Invertebrate Toxicity Study of B0793.01 in River Water, Principal Investigator, , 5 March 1985

REPORT NUMBER AND DATE: #BW-85-6-1782, June, 1985

STUDY NUMBER: 1011-0385-6134-110

MATERIAL: B0793.01

DESCRIPTION: a cloudy, white-colored waxy gel tested as 100% active ingredient

DATE MATERIAL RECEIVED: 25 January 1985

TEST DATE: 8-10 April 1985

SPECIES: Daphnia magna

Age: ≤24 hours old

Source: Bionomics culture facility

DILUTION WATER: Town River Water

Total hardness as $CaCO_3$: 35 mg/L

Total alkalinity as $CaCO_3$: 11 mg/L

pH: 6.5

0 0 7 7

Specific conductivity: 190 umhos/cm

Total suspended solids: 15 mg/L

TEST TEMPERATURE: 20-21°C

METHOD OF TEST MATERIAL ADDITION: stock solution; 6.0 mg of B0793.01/mL isopropyl alcohol

TEST CONCENTRATIONS: 3.0, 1.8, 1.1, 0.66, 0.39, 0.23 and 0.14 mg of B0793.01/L

RESULTS: Results are reported as nominal concentrations of B0793.01 based on 100% active ingredient. The 48-hour LC50 value and 95% confidence interval were calculated by moving average angle analysis to be 0.35(0.29-0.42) mg/L.

INTRODUCTION

The purpose of this study was to estimate the acute toxicity of B0793.01 in river water to daphnids (Daphnia magna) under static conditions. A 48-hour definitive test was conducted from 8-10 April 1985, at the Aquatic Toxicology Laboratory of Springborn Bionomics, Inc., Wareham, Massachusetts. All raw data generated are stored at the above location.

MATERIALS AND METHODS

A sample of B0793.01, a cloudy white-colored waxy gel and tested as 100% active ingredient, was received from The Procter & Gamble Company, Cincinnati, Ohio, on 25 January 1985. Nominal test concentrations are reported as milligrams of B0793.01 active ingredient per liter of solution (mg/L).

Procedures used in this acute toxicity study followed those described in "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA, 1975) and the protocol entitled "Static Acute Freshwater Invertebrate Toxicity Study of B0793.01 in River Water" Principal Investigator, 5 March 1985).

The daphnids used in this toxicity test were obtained from laboratory stocks cultured at Springborn Bionomics,

Inc., Wareham, Massachusetts. The culture water was prepared by fortifying well water based on the formula for hard water (U.S. EPA, 1975) and filtering it through a carbon filter and an Amberlite XAD-7 resin column to remove any potential organic contaminants. This water had total hardness and alkalinity ranges as calcium carbonate ($CaCO_3$) of 160-180 mg/L and 110-130 mg/L, respectively, a pH range of 7.9-8.3, a temperature of 20 ± 1°C, a dissolved oxygen concentration of greater than 60% of saturation and a specific conductance range of 400-600 micromhos per centimeter (umhos/cm).

The daphnid culture area received a regulated photoperiod of 16 hours of light and eight hours darkness. Light at an intensity of 5-10 hectolux at the culture solution surfaces was provided by a combination of Sylvania Growlux® and Cool White® fluorescent bulbs. Daphnids were fed a solution of green algae (Ankistrodesmus sp. or Selenastrum sp.) and yeast suspension once daily. The ambient air temperature in the culture area was controlled in order to maintain the culture solution temperatures at 20 ± 1°C.

A sodium lauryl sulfate reference test was conducted with the daphnid test population from 19-21 March 1985. The 48-hour LC50 and 95% confidence interval was 5.3 (3.2-9.0) mg/L (Reference Test Log).

0 0 9 0

The toxicity test was conducted in 250-milliliter (mL) glass beakers. The dilution water used was river water collected from the Town River in West Bridgewater, Massachusetts, at the point where the river intersects Route 28. The river water was characterized as having a total hardness and alkalinity of 35 and 11 mg/L as CaC_3, respectively, a pH of 6.5, a specific conductivity of 190 umhos/cm and total suspended solids concentration of 15 mg/L.

A clear, colorless stock solution of 6.0 mg/mL was formulated by diluting 0.60 grams of B0793.01 with isopropyl alcohol (IPA) to volume in a 100-mL volumetric flask. The stock solution was then mixed for ~30 seconds using an ultrasonicator. Test solutions were formulated by adding the appropriate volumes of the stock solution to dilution water to total 1000 mL. Each solution was mixed on a magnetic stirrer for one minute and then divided into three beakers to provide replicate exposure treatments, each containing 200 mL. The excess 400 mL of test solution was discarded. The test solution depth was 6.2 centimeters (cm) with a surface area of 33 cm^2. Two sets of three control beakers containing the same dilution water and maintained under the same conditions as the exposure concentrations, but containing no B0793.01, were established. One set of control beakers was treated with the maximum quantity of IPA (0.5 mL/L) present in any test solution and therefore termed the solvent control. The air temperature in the laboratory was

controlled in order to maintain test solution temperatures at 21 ± 1°C. Test solutions were not aerated. The test area was illuminated with Sylvania Growlux® and Cool White® fluorescent lights at an intensity of 5-8 hectolux at the solution surfaces. The photoperiod during the test was the same as in the culture area.

Fifteen daphnids, ≤24 hours old, were impartially distributed to each concentration (five daphnids per replicate) within 25 minutes after the test solutions had been prepared. Daphnids were not fed during the exposure. Mortalities in replicate test solutions were recorded at 24 and 48 hours of exposure. Biological observations and observations of the physical characteristics of each replicate test solution were also made and recorded at 0, 24, and 48 hours. The pH's and dissolved oxygen concentrations were measured at 0 and 48 hours in one replicate vessel of each test concentration and the controls. Temperature was measured in one replicate vessel of the control at 0, 24 and 48 hours of exposure.

Total hardness concentrations presented in this report were measured by the EDTA titrimetric method and total alkalinity concentrations were determined by potentiometric titration to an endpoint of pH 4.5 (APHA et al., 1980). Specific conductivities were measured with a Yellow Springs Instrument Company (YSI) Model #33 salinity-conductivity-

temperature meter and probe; the pH's were measured with an Instrumentation Laboratory Model #175 pH meter and combination electrode; the dissolved oxygen concentrations were measured with a YSI Model #57 dissolved oxygen meter and probe and the temperatures were measured with a Brooklyn alcohol thermometer. Light intensity was measured with a General Electric type 213 light meter.

Statistics

The concentrations tested and the corresponding mortality data derived from the toxicity test were used to estimate 24- and 48-hour median lethal concentrations (LC50) and 95% confidence intervals. The LC50 is defined as the concentration of the test material in dilution water which caused mortality of 50% of the test animal population at the stated exposure interval. If sufficient toxicant-related mortality occurred during the test (e.g., presence of at least one test concentration causing mortality of $\geq$50% of the animals in the test population), then a computer program (Stephan, 1982, personal communication) was used to calculate the LC50 values. The computer program scanned the data base, identified the most appropriate statistical methods and performed the analyses. Three statistical methods, in the following order of preference, were available in the computer program: moving average angle analysis, probit analysis, binomial probability (Stephan, 1977). The binomial

probability method calculates only the 95% confidence interval. In such a case, a point estimate of the LC50 is obtained by nonlinear interpolation (i.e., logarithm transformation of the concentration and the angle transformation of the percent dead) (Stephan, 1982). The method selected was determined by the above order of preference and by the characteristics of the data base (e.g., presence or absence of several test concentrations causing mortality of a partial number of animals in the respective test population). LC50 values were empirically estimated when insufficient toxicant-related mortality occurred.

RESULTS

The concentrations tested, corresponding mortalities and observations made during the toxicity test are presented in Table 1. The 48-hour LC50 value and 95% confidence interval for D.magna exposed to B0793.01 in river water was calculated by moving average angle analysis to be 0.35(0.29-0.42) mg/L. Table 2 summarizes the 24-hour and 48-hour LC50 values. The results of the water quality analyses are presented in Table 3.

0 0 8 4

LITERATURE CITED

APHA, AWWA, WPCF. 1980. Standard methods for the examination of water and wastewater. 15th Edition, Washington, D.C., 1134 pp.

Stephan, C. E. 1977. Methods for calculating an LC50, Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, F. L. Mayer and J. L. Hamelink, Eds., American Society for Testing and Materials, pp. 65-84.

Stephan, C. E. 1982. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal communication to Dr. Lowell Bahner, Chairman ASTM Task Group on Calculating LC50's.

U.S. EPA. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecological Research Series (EPA-660/3-75-009), 61 pp.

Table 1. Concentrations tested, corresponding mortalities and observations made during the 48-hour exposure of daphnids (Daphnia magna) to B0793.01 in river water.

Nominal[a] concentration (mg/l)	Cumulative Mortality (%)							
	24-hour				48-hour			
	A	B	C	$\bar{x}$	A	B	C	$\bar{x}$
3.0[b]	100	100	100	100	100	100	100	100
1.8[b]	100	100	100	100	100	100	100	100
1.1	100	100	100	100	100	100	100	100
0.66	0	40	40	27	100	100	100	100
0.39	0	0	0	0	60	40	40	47[cd]
0.23	0	0	0	0	20	0	20	13[e]
0.14	0	0	0	0	0	0	0	0[e]
Solvent Control	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0

[a]Throughout the exposure period, all test and control solutions were yellow in color due to the coloration of the dilution water.

[b]Small white colored flakes of undissolved test material were on the test solution surface at 0-hour.

[c]Several of the surviving daphnids were lethargic.

[d]Several daphnids were swimming erratically.

[e]Several of the surviving daphnids were on the bottom of the test vessel.

Table 3. The pH's, dissolved oxygen concentration and temperatures measured during the 48-hour exposure of Daphnia magna to B0793.01 in river water.

	Nominal concentration (mg/ℓ)	0-hour	24-hour	48-hour
pH	3.0	6.6	—[b]	6.8
	1.8	6.5	—	6.8
	1.1	6.5	—	6.8
	0.66	6.5	—	6.8
	0.39	6.5	—	6.8
	0.23	6.5	—	6.8
	0.14	6.5	—	7.0
	Solvent Control	6.5	—	7.2
	Control	6.5	—	7.4
Dissolved oxygen (mg/ℓ)	3.0	8.9 (97)[a]	—	8.5 (92)
	1.8	8.9 (97)	—	8.4 (91)
	1.1	9.2 (100)	—	8.5 (93)
	0.66	8.6 (93)	—	8.6 (93)
	0.39	9.1 (99)	—	8.7 (94)
	0.23	9.2 (100)	—	8.8 (96)
	0.14	9.2 (100)	—	8.7 (94)
	Solvent control	9.1 (99)	—	8.9 (97)
	Control	9.2 (100)	—	8.6 (93)
Temperature (°C)	Control	20	21	20

[a] Percent of saturation.

[b] Measurement not required at stated time interval.

0 0 8 7

Table 2. The estimated 24- and 48-hour LC50 values for daphnids (*Daphnia magna*) exposed to B0793.01 in river water.

LC50 (mg/L)	
24-hour	48-hour
0.75[a] (0.39-1.1)	0.35[b] (0.29-0.42)

[a]The LC50 value was estimated by non-linear interpolation with 95% confidence interval estimated by binomial probability.

[b]LC50 value and 95% confidence interval calculated by moving average angle analysis.

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The data contained in this report were audited by the Quality Assurance Unit to assure compliance with the protocols, standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations on the following dates. 15 May and 25 June, 1985. If discrepancies were found, reports were made immediately to the Study Director and management. It is the opinion of this Unit that these data accurately reflect the raw data generated during this study.

Robert E. Bentley
Director, Quality Assurance and
Special Projects

SUBMITTED BY:

Springborn Bionomics, Inc.
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
June, 1985

STUDY DIRECTOR: James R. Hoberg

James R. Hoberg 6/17/85

Aquatic Toxicologist

APPROVED BY: Donald C. Surprenant

D.C. Surprenant 6/24/85

Director, Aquatic Toxicology

DATA AUDITED BY: Robert E. Bentley

R.E. Bentley 25 Jun 85

Director, Quality Assurance Unit

5 Composition; Analytical Information;

2. DQ Base (monotallow dimethyl amine;

Active Molecular Weight (2) 279

SPECIAL INFORMATION: (additional information of value not included in above, reference to testing done on similar formulations or chemicals, etc.)

Date: 2/12/85

B. ENVIRONMENTAL TESTS:

SAMPLE IDENTIFICATION

(1) Acute Daphnia Tox. River Water on and B0793.01 — $1200.00
(2) Acute Fish Tox. in River Water and recon. water on and B0793.01 — $3200.00
(3) Microbial Toxicity on and B0793.01 — $1000.00
(4) Batch Anaerobic Digester Inhibition on and B0793.01 — $5000.00

B0793.01

TOTAL: $10,200.00

Est. Cost $10,200.00

Est. Reporting Date April 1985

Date: 2/12/85

0 0 9 1

[illegible]

Test Substance Identification Number (TSTM): [illegible]
Safety Test Request Number:
Principle Investigator:

Product or Ingredient:
Physical Description: White Solid/Semi Solid Solubility: NK pH: NK
Recommended Storage Conditions: Room Temperature Expiration Date: > 1 year
Hazards (i.e. flammability, toxic gases): Non-Hazardous
Dept. of Transportation Hazard Classification: Non-Hazardous CAS No.(a): 124-28-7

Formulated Composition (b)

Component (c)	Mol. Wt.	Nominal Level (% by Wt.)	Acceptable Range	Stock Code No.	Supplier	Lot Number (NB-Ref.)
Di-Methyl Stearyl Amine (	297	100.00		NK	Sherex	4069-S-666334

(a) Include CAS number(s) for the three most major components of a formulation or for single chemical products. Footnote to the material with which the respective number is associated.

(b) If information requested is not known, then the symbol NK will be entered.

(c) Chemical names which are inconveniently long may be abbreviated in tables but should be listed in full in referenced footnotes. Non-chemical names, such as Tergitol 15-S-0 or Yellow Dye #10, may not be acceptable but should be previewed with the responsible toxicologist. Nondefinitive identification (e.g. Arquad, DC-base) is not acceptable.

The above information provided by: (Name) (Signature) 12/18/84 (Date)

The above information reviewed and accepted by:

Principle Investigator: (Name) (Signature) 12/18/84 (Date)

LMS009/lad

0092

Test Substance Identification Number (TSIN):

Analyzed Composition

Date Submitted	Submitter Code	Analysis Code/Analysis	Estimated Value	Measured Value	Testing Laboratory

Analytical Information Verified By: _____ Date:
(Signature)

Date: 3/5/86

This test substance is suitable for human (clinical) safety testing.

Principle Investigator: _____ Date:
(Signature)

THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

P. O. BOX 39175
CINCINNATI, OHIO 45247

March 13, 1985

Mr. Robert Bentley
Springborn Bionomics, Inc.
790 Main St.
Wareham, MA 02571

Dear Mr. Bentley:

This is to authorize you to carry out the following study according to the attached protocol, and in conformance with the stipulations of our current Laboratory Services Agreement.

Protocol: Static Acute Freshwater Invertebrate Toxicity Study of B0793.01 in River Water
Date: 3/5/85
Sponsor's Principal Investigator:

Notice: The stipulations of this protocol are to be implemented in conformance with EPA Good Laboratory Practice Regulations (40 CFR, Part 792).

Please have the Study Director approve and complete both copies of the attached protocol by adding your study or project number, estimated starting and reporting dates, and date the test material was received. Retain one copy for your files and return the other to me.

Please return all unused portions of the test material to the Sponsor's Principal Investigator at the following address:

The Procter & Gamble Company
Sharon Woods Technical Center
11520 Reed Hartman Hwy.
Cincinnati, OH 45241

Please use the same test materials from previous acute studies with Daphnia submitted 1/24/85. Verbal results are needed by April 5, 1985.

We understand the estimated cost for this study is $850. Invoices are also to be sent to the Principal Investigator.

Matters involving the scientific aspects of the work can be handled directly with the Sponsor's Principal Investigator. Feel free to contact me at ... if you have any other questions or concerns.

VY

0 0 9 9

Laboratory Study No. 1011.0365.6134.110

PROTOCOL

SPONSOR: The Procter & Gamble Company; Cincinnati, Ohio
Springborn Bionomics, Inc.
LABORATORY: 790 Main St.
Wareham, MA 02571

TITLE: Static Acute Freshwater Invertebrate Toxicity Study of B0793.01 in River Water

OBJECTIVE: To determine the 48 hr LC_{50} of the test material to a freshwater invertebrate species.

JUSTIFICATION FOR TEST SYSTEM: Daphnia magna is a readily available freshwater invertebrate species, on which a large amount of toxicity data exists, and is recommended by the USEPA[1] as a standard test organism.

TEST MATERIAL:

Sample Code B0793.01 Color Opaque Form Gel/liquid

% Active 100 Density -- Solubility in IPA to 5%

Expiration Date in progress Other

Storage Conditions - room temperature

Safe Handling Precautions - Caution: Eye and skin irritant. If exposed rinse with plenty of water.

The Sponsor accepts full responsibility for appropriate characterization and stability verification of this test material.

TEST MATERIAL ADDITION/PREPARATION: All calculations and measurements are to be based on the active ingredient. The maximum concentration to be tested is 1000 mg/L active ingredient. Stock solution to be prepared in IPA. Maximum solvent in test as per laboratory SOP.

TEST ORGANISM:

Species - Daphnia magna

Handling and Preparation - On the day preceding test initiation, reproductively mature adults are to be isolated from any young to assure only adult sizes are present in the stock cultures. The young produced by these isolated adults are to be removed from the stock cultures and used for testing. The maximum time to initiate a test following isolation is 36 hours.

TEST CONTAINERS: 250 ml glass beakers containing 200 ml of test solution. Beakers are to be covered with glass or clear plastic during the test period to minimize evaporation.

[1]USEPA, Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. EPA-3-75-009.

DILUTION WATER: Town River water collected above the sewage treatment plant.

TEST CONDITIONS:

(1) Maintain temperature at 21 ± 1°C.

(2) The dissolved oxygen concentration in each test container must be at least 90% of saturation on Day 0.

(3) A control and at least five concentrations of the test material are to be used. Except for the control, the concentration of test material in each treatment must be at least 56% of the next higher one.

OPERATION:

Five Daphnia are randomly assigned to each test container, following the laboratory's standard operating procedure, within 30 minutes after the addition of the test material. A minimum of three test containers are to be used for each test concentration and the control. One treatment must have killed or affected more than 65% of the Daphnia, and one treatment (not the control) must have killed or affected less than 35% of the Daphnia. The test is conducted for 48 hours, commencing when the test Daphnia are first exposed to the test material. Daphnia are not to be fed during the test period.

If any additive (e.g. solvent) is used to solubilize the test material, another control containing the greatest amount of solvent present in any other container is also required. A test is not valid if there is a greater than 10% mortality in either control.

WATER CHEMISTRY: Dissolved oxygen (DO) and pH are measured at 0 and 48 hours in at least one replicate for all test concentrations, including the controls. The 0 hour measurements are taken prior to the addition of the test organisms. If 100% mortality is observed in a test concentration at 24 hours, determinations are to be made at that time, but further determinations for that concentration are not required.

SPECIAL INSTRUCTIONS (If any, for operation, sampling, analyses, or monitoring): Analysis of suspended solids in the Town River water sample should be included in the final report.

RANGE-FINDING TEST: To be conducted at the discretion of the laboratory unless otherwise specified.

OBSERVATIONS: Mortality observations are to be made and recorded at 24 and 48 hours for all test containers, including the control. The criteria for death is the lack of reaction to gentle prodding.

CALCULATIONS: Test results are used to calculate the 48 hour LC_{50}. The 24 hour LC_{50} is calculated when possible. The LC_{50} is defined as the calculated concentration of the test material which causes 50% mortality in populations of test organisms at the specified time of exposure. Results are to be calculated and reported on the basis of added (nominal) test concentrations or from concentrations confirmed by actual analysis, if requested.

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RECORDS TO BE MAINTAINED: All records necessary to reconstruct the study and demonstrate adherence to the Protocol.

PROTOCOL CHANGES: If a change in the approved protocol becomes necessary, verbal agreement should be made between the Study Director and Sponsor's Principal Investigator. As soon as practical thereafter, this change and the reasons for it should be put in writing, approved by both persons, and attached to the protocol as an addendum.

REPORTING: The report is to be a typed document in triplicate, describing the results of the study and is to be signed and dated by the Study Director, Quality Assurance Officer and Laboratory Manager. It is to include, but is not limited to, the following:

1) Identification of test material by sample code, percent active, color, form, and date received.
2) Procedures followed for test material preparation and addition.
3) Reference to laboratory notebook or other file containing raw data.
4) Date definitive test was conducted.
5) Species tested, including the source and age.
6) Percentage mortality in all test treatments, including the control.
7) The calculated LC_{50} values, 95% confidence intervals, and a reference to the method used to calculate these values.
8) Description of dilution water used, including a range of the measured pH, hardness, alkalinity, and conductivity.
9) All temperature, pH, and DO determinations and all visual observations.
10) Laboratory Study Number.
11) Reference to the Protocol (title, author, and date) and addenda if made, and any analytical procedures used.
12) Any Protocol deviations and their implications.
13) Results of reference toxicant and date conducted.
14) Description of the quality assurance methods used to insure the quality of the data.

ALTERNATE PRINCIPAL INVESTIGATOR ____ PHONE:

____ PHONE:

____ PHONE:

TO BE COMPLETED BY STUDY DIRECTOR:

Study No. 1011.0385.6134.110

Estimated Starting Date 21 March 1985

Estimated Reporting Date 13 May 1985 (5 April 1985-verbal)

Date Test Material Received 25 January 1985

Approved James R. Hobbs (Study Director) 3/21/85 (Date) PHONE: 617-295-2550

/kd

0 0 9 7

Date: 5/22/85

LOGISTICS MONITORING REPORT

Monitor(s):

Date of Visit: 5/16 & 5/17/85

TSIN: #60793.01

Test: Bluegill acute in 160 river waters; Daphnia acute in river water

~~**Project No.:**~~

Laboratory Project No.: 1011.0385.6134 thru 6139

Date Started: 3/26/85

Date Ended: 4/13/85

Principal Investigator:

Location: Springborn Bionomics

Study Director: J. Sousa for fish tests; J. Hoberg for Daphnia tests.

Discussed With: D. Surprenant (aquatic lab manager); R. Bentley (QAU)

Phases Audited/Inspected:

1) Protocol
2) Test material tracking and usage
3) Study records
4) Final reports

Findings are discussed in Attachment I.

(signature) (date) 5/22/85

CIRCULATION (Do not copy. Please initial and date):

Contains No CBI

ENVIRONMENTAL SAFETY TEST SUMMARY REVIEW

Test Material Name: CAS RN 124-28-7

Test Substance Identification Number: 80793.01 project # suffix): (.06)

Type of Study: Bluegill Static Acute Report or ~~Project~~ #: BW-85-5-1778

Name of Originator: Division:

Laboratory Involved: Springborn Bionomics; Wareham, MA

Date Report Written: 5/13/85 Date Received by Operations Section: 6/11/85

This report has been received and found in agreement with the Protocol and there appear to be no inaccuracies in the numerical data or written portions with the following exceptions: None

Logistics Reviewer ___ Date 6/27/85

This report has been reviewed for scientific quality and is summarized with the following comments:

Principal Investigator Date 7/2/85

This report is recommended for entry into ESD Safety Data System:

___ Date 7/17/85

File approved for entry:

___ Date 7-25-85

(Return Operations Section)

ce _____ By _____

Microfilming Completed: Date _____ By _____

Copy returned to: _____ Date _____

0 0 9 9

TEST SUMMARY SHEET

B. Text: (Note - tabulations must be limited to 71 characters in width)

Test Type: Acute Freshwater Fish Toxicity - Static

Species: Bluegill sunfish, Lepomis macrochirus

Dilution Water: Soft Reconstituted

Length: 37(34-41) mm

Total Hardness: 42 mg/l as CaCO3

Weight: 0.52(0.39-0.72) gm

pH: 7.6

Source: Lot # 84B15B

Test Temperature: 22 degrees C

Results:

96 Hour LC50: 0.30 mg/l

95% Conf. Intervals: 0.23-0.39 mg/l

Observations: Abnormal behavior was noted at 0.23 mg/l and higher.

Reference Response (Bluegill-96 hr LC50): 7.8(5.8-10) mg/l sodium lauryl sulfate

Analytical Method: None

Material Solubilization: Material added via [✓] stock solution; [ ] direct weighing. Solvent used - [ ] water; [✓] DMF.

Comments: All results are based on nominal concentrations of the active ingredient tested as 100% active.

Test Protocol: Static Acute Freshwater Fish Toxicity Study of B0793.01 1/7/85

C. Project Status Sheet Code No. B0793.01.06

D. Test Type: ACFT (KEYWORD) ~~Acute Freshwater~~ Fish Toxicity - Static

E. Test Material: D-Methyl Stearyl Amine

F. Title/Date/ESD File No. Acute Toxicity of B0793.01 to Bluegill (Lepomis macrochirus) / 5-13-85

G. Test Location: Springborn Bionomics H. Lab Project No.: 101-0385-6138-100

I. Date: 86/06/27 (YY MM DD)

K. Prepared by:

M. TSIN: B0793.01 V

N. Date Work Done: 3/20/85 - 3/30/85

ACUTE TOXICITY OF B0793.01 TO BLUEGILL (*Lepomis macrochirus*)

TOXICITY TEST REPORT

SUBMITTED TO

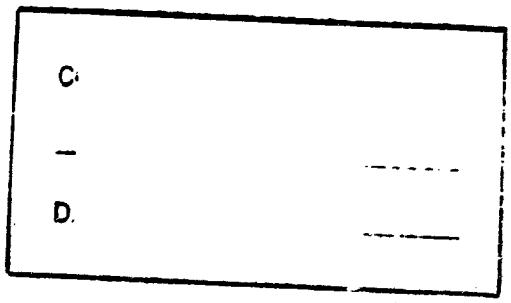
THE PROCTER & GAMBLE COMPANY

CINCINNATI, OHIO

BIONOMICS REPORT #BW-85-5-1778

BIONOMICS STUDY # 2011-0385-6138-100

Springborn Bionomics, Inc.
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
May, 1985



0 1 0 1

SUMMARY

96-Hour Static Toxicity Test: Freshwater Fish

Springborn Bionomics, Inc.
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts

CLIENT: THE PROCTER & GAMBLE COMPANY

PROTOCOL CITED: Static Acute Freshwater Fish Toxicity Study of B0793.01; Principal Investigator, 3/5/85

REPORT NUMBER AND DATE: BW-85-5-1778, May, 1985

STUDY NUMBER: 1011-0385-6138-100

MATERIAL: B0793.01

DESCRIPTION: a cloudy white-colored waxy gel, 100% active ingredient

DATE MATERIAL RECEIVED: 25 January 1985

TEST DATE: 26-30 March 1985

SPECIES: Lepomis macrochirus

Total length: Mean (range, N=30), 37(34-41) mm

Wet weight: Mean (range, N=30), 0.52(0.39-0.70) g

Source: Commercial fish supplier in Connecticut

DILUTION WATER: Deionized, reconstituted well water

Total hardness as $CaCO_3$: 49 mg/L

Total alkalinity as $CaCO_3$: 32 mg/L

pH: 7.6

Specific conductivity: 140 umhos/cm

TEST TEMPERATURE: 22°C

METHOD OF TEST MATERIAL ADDITION: Stock solution; 6.0 mg of B0793.01/mL of isopropanol

TEST CONCENTRATIONS: 3.0, 1.8, 1.1, 0.64, 0.39, 0.23 and 0.14 mg/L of B0793.01.

RESULTS: Results are reported as nominal concentrations of B0793.01 based on 100% active ingredient. The 96-hour LC50 was estimated by nonlinear interpolation to be 0.30 mg/L with a 95% confidence limit calculated by binomial probability to be between 0.23 and 0.39 mg/L.

INTRODUCTION

The purpose of this study was to estimate the acute toxicity of B0793.01 to bluegill (Lepomis macrochirus) under static conditions. A 96-hour definitive test was conducted from 26-30 March 1985, at the Aquatic Toxicology Laboratory of Springborn Bionomics, Inc., Wareham, Massachusetts. All raw data generated are stored at the above location.

MATERIALS AND METHODS

The B0793.01, a cloudy white-colored waxy gel, 100% active ingredient, was received from The Procter & Gamble Company, Cincinnati, Ohio, on 25 January 1985. Nominal test concentrations are expressed as milligrams of B0793.01 active ingredient per liter of test solution (mg/L).

Procedures used in this acute toxicity test followed those described in "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA, 1975) and the protocol entitled "Static Acute Freshwater Fish Toxicity Study of B0793.01" (Principal Investigator, 5 March 1985) issued to Springborn Bionomics, Inc., by The Procter & Gamble Company, Cincinnati, Ohio.

The bluegill (Bionomics lot #84B15B) were obtained from a commercial fish supplier in Connecticut and held in a

1700-L expoxy-coated tank under a photoperiod of 16 hours light and 8 hours darkness. The well water which flowed into this tank was characterized as having total hardness and alkalinity ranges as calcium carbonate ($CaCO_3$) of 25-34 mg/L and 22-26 mg/L, respectively, and a specific conductance range of 100-190 micromhos per centimeter (umnos/cm) (Weekly Gravity Feed Tank Water Quality Analysis Logbook). Other parameters monitored in the holding tank were a pH range of 6.6-7.1, a dissolved oxygen concentration range of 73-96% of saturation and a flow rate of 6-7 tank volume replacements/day (Weekly Record of Fish Holding Water Characteristics). Test fish were maintained under these conditions for a minimum of 14 days. The temperature range in the holding tank was 21-22°C during this 14-day period. All fish were fed a dry commercial pelleted food, ad libitum, daily except during the 48 hours prior to testing. There was mortality of <1.0% of the test fish population during this two-day period (Daily Record of Fish Holding Conditions). The mean (range, n=30) wet weight and total length of the test fish population was 0.52 (0.39-0.70) grams and 37 (34-41) millimeters (Fish Weight and Lengths Log).

A sodium lauryl sulfate reference test was conducted with the test fish population from 27-31 March 1985. The resulting 96-hour LC50 and 95% confidence interval was 7.8 (4.8-10) mg/L (Reference Test Log).

The toxicity test was conducted in 19.6-L glass jars which contained 15 L of test solution. The test solution depth was 27.5 cm with a surface area of 545 cm^2. The dilution water used was soft water reconstituted from deionized water according to recommended procedures (U.S. EPA, 1975). This water had a total hardness and alkalinity as $CaCO_3$ of 49 mg/L and 32 mg/L, respectively, a pH of 7.6 and a specific conductance of 140 umhos/cm (Reconstituted Water, Water Quality Analysis Log).

A clear colorless stock solution of 6.0 mg/milliliter (mg/mL) was prepared by diluting 0.60 grams of B0793.01 with isopropanol to volume in a 100-mL volumetric flask. The appropriate amount of the stock solution was then added to each test jar and mixed by stirring with a glass rod.

Two control jars containing the same dilution water and maintained under the same conditions as the exposure jars, but containing no B0793.01, were established. One control jar contained the greatest amount of isopropanol present in any test jar (0.50 mL/L). All test solution temperatures were controlled by a system designed to maintain temperatures at 22 $\pm$ 1°C. Test solutions were not aerated. The photoperiod during testing was the same as that provided in the fish culture area.

Ten bluegill were impartially distributed to each test jar within 15 minutes after the test solutions had been

prepared. The resulting test organism loading concentration was 0.35 grams of biomass per liter of test solution. Fish were not fed during exposure.

Mortalities were recorded and removed from each test jar every 24 hours during exposure. Biological observations of the fish and observations of the physical characteristics of the test solutions were also made at each 24-hour interval. The pH's and dissolved oxygen concentrations were measured at 0, 24, 48, 72 and 96 hours in the control and all test concentrations. If mortality of 100% of the fish occurred in a test concentration, measurements were made at that time interval, but further measurements were discontinued. The temperature was measured in the control jar at the above-mentioned time intervals.

Total hardness concentrations presented in this report were measured by the EDTA titrimetric method and total alkalinity concentrations were determined by potentiometric titration to an endpoint of pH 4.5 (APHA et al., 1980). Specific conductivities were measured with a Yellow Springs Instrument Company (YSI) Model #33 salinity-conductivity-temperature meter and probe; the pH's were measured with an Instrumentation Laboratory Model #175 pH meter and combination electrode; the dissolved oxygen concentrations were measured with a YSI Model #57 dissolved oxygen meter and probe and the temperatures were measured with a Brooklyn

alcohol thermometer.

The concentrations tested and the corresponding mortality data derived from the toxicity test were used to estimate 24-, 48-, 72- and 96-hour median lethal concentrations (LC50) and 95% confidence intervals. The LC50 is defined as the concentration of the test material in dilution water which caused mortality of 50% of the test animal population at the stated exposure interval. If sufficient toxicant-related mortality occurred during the test (e.g., presence of at least one test concentration causing mortality of >50% of the animals in the test population), then a computer program (Stephan, 1982, personal communication) was used to calculate LC50 values. The computer program scanned the data base, identified the most appropriate statistical method and performed the analyses. Three statistical methods, in the following order of preference, were available in the computer program: moving average angle analysis, probit analysis, and binomial probability (Stephan, 1977). Binomial probability method calculates only the 95% confidence interval. In such a case, a point estimate of the LC50 is obtained by nonlinear interpolation (i.e., logarithm transformation of the concentration and the angle transformation of the percent dead; Stephan, 1982). The method selected was determined in the above order of preference and by the characteristics of the data base (e.g., presence or absence of several test

concentrations causing mortality of a partial number of animals in the respective test population). The LC50 values were empirically estimated when insufficient toxicant-related mortalities occurred.

RESULTS

The nominal test concentrations, the corresponding cumulative mortalities and the observations made during the toxicity test are presented in Table 1. The 96-hour LC50 for bluegill exposed to B0793.01 was estimated by nonlinear interpolation to be 0.30 mg/L with a 95% confidence interval calculated by binomial probability to be between 0.23 and 0.39 mg/L. Table 2 summarizes the 24-, 48-, 72- and 96-hour LC50's. The pH's, dissolved oxygen concentrations and temperatures measured during the toxicity test are presented in Table 3.

LITERATURE CITED

APHA, AWWA, WPCF. 1980. Standard methods for the examination of water and wastewater. 15th Edition, Washington, D.C., 1134 pp.

Stephan, C. E. 1977. Methods for calculating an LC50, Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, F. L. Mayer and J. L. Hamelink, Eds., American Society for Testing and Materials, pp. 65-84.

Stephan, C. E. 1982. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal communication to Dr. Lowell Bahner, Chairman ASTM Task Group on Calculating LC50's.

U.S. EPA. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecological Research Series (EPA-660/3-75-009), 61 pp.

Table 1. Concentrations tested and corresponding cumulative mortalities of bluegill (Lepomis macrochirus) exposed to B0793.01 for 24, 48, 72 and 96 hours.

Nominal concentration (mg/L)	Cumulative Mortality (%)			
	24-hour	48-hour	72-hour	96-hour
3.0[a]	100[b]	100[b]	100[e]	100[e]
1.8[a]	100[b]	100[b]	100	100
1.1[a]	100[b]	100[b]	100	100
0.64[a]	60[bcd]	100[b]	100	100
0.39[a]	0[bc]	60[bcd]	80[cd]	100
0.23	0	0[c]	0[c]	0[c]
0.14	0	0	0	0
solvent control	0	0	0	0
control	0	0	0	0

[a]A film was present on the surface of the test solution at 0 hour.

[b]A film was present on the surface of the test solution.

[c]All fish were respiring rapidly.

[d]All fish were lethargic.

[e]The test solution was cloudy.

Table 2. The 24-, 48-, 72- and 96-hour LC50 values and 95% confidence intervals for bluegill (Lepomis macrochirus) exposed to B0793.01.

LC50 (mg/L)			
24-hour	48-hour	72-hour	96-hour
0.60[a] (0.39-1.1)[b]	0.36[a] (0.23-0.64)[b]	0.33[a] (0.23-0.64)[b]	0.30[a] (0.23-0.39)[b]

[a]LC50 value estimated by nonlinear interpolation.

[b]95% confidence limits calculated by binomial probability.

Table 3. The pH's, dissolved oxygen concentrations and temperatures measured during the 96-hour exposure of bluegill (Lepomis macrochirus) to B0793.01.

	Nominal concentration (mg/L)	0-hour	24-hour	48-hour	72-hour	96-hour
pH	3.0	7.7	7.2	—[b]	—	—
	1.8	7.7	7.2	—	—	—
	1.1	7.6	7.2	—	—	—
	0.64	7.6	7.1	7.0	—	—
	0.39	7.5	7.0	7.0	6.8	6.8
	0.23	7.5	7.0	6.9	6.9	6.8
	0.14	7.5	7.0	6.9	6.9	6.6
	solvent control	7.5	6.9	6.7	6.9	6.7
	control	7.4	6.8	6.6	6.8	6.7
Dissolved oxygen (mg/L)	3.0	9.3(100)[a]	8.2(93)	—	—	—
	1.8	9.4(107)	8.1(92)	—	—	—
	1.1	9.5(108)	8.2(93)	—	—	—
	0.64	9.6(109)	8.1(92)	6.3(72)	—	—
	0.39	9.3(106)	8.3(94)	6.3(72)	3.3(38)	2.6(30)
	0.23	9.3(106)	8.4(95)	6.7(76)	5.2(59)	4.0(45)
	0.14	9.4(107)	8.4(95)	6.7(76)	5.1(58)	4.2(48)
	solvent control	9.4(107)	7.2(82)	6.2(70)	4.5(51)	4.0(45)
	control	9.5(108)	7.0(80)	6.2(70)	4.9(56)	4.5(51)
Temperature (°C)	control	22	22	22	22	22

[a] % of saturation.

[b] Measurement of parameter not required at stated time interval.

The data contained in this report were audited by the Quality Assurance Unit to assure compliance with the protocols, standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations on the following date: 15 May 1985. If discrepancies were found, reports were made immediately to the Study Director and management. It is the opinion of this Unit that these data accurately reflect the raw data generated during this study.

R. E. Bentley

Robert E. Bentley
Director, Quality Assurance and
Special Projects

SUBMITTED BY:

Springborn Bionomics, Inc.
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
May, 1985

STUDY DIRECTOR: Joseph V. Sousa

Joseph V. Sousa

Aquatic Toxicologist

APPROVED BY: Donald C. Surprenant

Donald C. Surprenant 5/13/8

Director, Aquatic Toxicology

DATA AUDITED BY: Robert E. Bentley

R. E. Bentley

Director, Quality Assurance Unit

THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

P.O. BOX 39175
CINCINNATI, OHIO 45247

March 13, 1985

Mr. Robert Bentley
Springborn Bionomics, Inc.
790 Main St.
Wareham, MA 02571

Dear Mr. Bentley:

This is to authorize you to carry out the following study according to the attached protocol, and in conformance with the stipulations of our current Laboratory Services Agreement.

Protocol: Static Acute Freshwater Fish Toxicity Study of B0793.01
Date: 3/5/85
Sponsor's Principal Investigator:

Notice: The stipulations of this protocol are to be implemented in conformance with EPA Good Laboratory Practice Regulations (40 CFR, Part 792).

Please have the Study Director approve and complete both copies of the attached protocol by adding your study or project number, estimated starting and reporting dates, and date the test material was received. Retain one copy for your files and return the other to me.

Please return all unused portions of the test material to the Sponsor's Principal Investigator at the following address:

The Procter & Gamble Company
Sharon Woods Technical Center
11520 Reed Hartman Hwy.
Cincinnati, OH 45241

Please use the same test materials from previous acute studies with Daphnia submitted 1/24/85. Verbal results are needed by April 5, 1985.

We understand the estimated cost for this study is $800. Invoices are also to be sent to the Principal Investigator.

Matters involving the scientific aspects of the work can be handled directly with the Sponsor's Principal Investigator. Feel free to contact me at if you have any other questions or concerns.

Sincerely,

THE PROCTER & GAMBLE COMPANY

Approved:

0116

Laboratory Study No. 1011-0385-6138-NC

PROTOCOL

SPONSOR: The Procter & Gamble Company; Cincinnati, Ohio

LABORATORY: Springborn Bionomics, Inc.
790 Main St.
Wareham, MA 02571

TITLE: Static Acute Freshwater Fish Toxicity Study of B0793.01

OBJECTIVE: To determine the 96 hr LC_{50} of the test material to a freshwater fish species.

JUSTIFICATION FOR TEST SYSTEM: The bluegill (Lepomis macrochirus) is a readily available freshwater fish species, on which a large amount of toxicity data exists, and is recommended by the USEPA[1] as a standard test organism.

TEST MATERIAL:

Sample Code B0793.01 Color Opaque Form gel/liquid

%Active 100 Density -- Solubility 5% in IPA

Expiration Date in progress Other --

Storage Conditions - room temperature

Safe Handling Precautions - Caution: May cause irritation of skin and eyes. If exposed rinse with plenty of water. Strong fishy odor - use hood.

The Sponsor accepts full responsibility for appropriate characterization and stability verification of this test material.

TEST MATERIAL ADDITION/PREPARATION: All calculations and measurements are to be based on the active ingredient. The maximum concentration to be tested is 1000 mg/L active ingredient. Add test material from IPA stock solution. Use appropriate IPA control. Maximum IPA concentration as per laboratory SOP.

TEST ORGANISM:

Species - Bluegill (Lepomis macrochirus)
Age - All fish from the same year class.
Weight - Average is not to exceed 1.2 g.
Length - The largest fish is to be no more than twice the standard length of the shortest fish.

All fish must be in holding for a minimum of 14 days prior to starting the test. Fish are to be acclimated to the test temperature for a minimum of 48 hours in the "dilution water" or water of similar chemical composition to the "dilution water". Fish are not to be fed during this acclimation period or during the test period. A group of fish must not be used if individuals appear to be diseased or otherwise stressed or if more than 3% die during the 48 hours.

[1] USEPA, Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. EPA-660/3-75-009.

TEST CONTAINERS: Wide mouth glass jars (approximately 20 L capacity) containing 15 L of test solution.

DILUTION WATER: Soft reconstituted water prepared as described in USEPA[1], p. 14-2[illegible]. Do not use dechlorinated water.

TEST CONDITIONS:

(1) Maintain temperature at 22 ± 1°C.
(2) The dissolved oxygen concentration in each test container must be at least 90% of saturation on Day 0, and for the control, must remain greater than 40% of saturation throughout the test.
(3) The biomass to water ratio must not exceed 0.8 g/L.
(4) A control and at least five concentrations of the test material are to be used. Except for the control, the concentration of test material in each treatment must be at least 56% of the next higher one.

OPERATION:

Ten fish are randomly assigned to each test container, following the laboratory's standard operating procedures, within 30 minutes after the addition of the test material. One treatment must have killed or affected more than 65% of the fish, and one treatment (not the control) must have killed or affected less than 35% of the exposed fish. The test is conducted for 96 hours, commencing when the test fish are first exposed to the test material.

If any additive (e.g. solvent) is used to solubilize the test material, another control containing the greatest amount of solvent present in any other container is also required. A test is not valid if there is greater than 10% mortality in either control.

WATER CHEMISTRY: Dissolved oxygen (DO) and pH are determined at 0, 24, 48, 72, and 96 hours in all test concentrations, including the controls. When 100% mortality is observed in a test concentration, determinations are to be made, but further determinations for that concentration are discontinued.

SPECIAL INSTRUCTIONS (If any, for operation, sampling, analyses, or monitoring):

RANGE-FINDING TEST: To be conducted at the discretion of the laboratory unless otherwise specified.

OBSERVATIONS: Observations concerning mortality, morbidity, and behavior are to be recorded for all test concentrations. Of specific interest, but not exclusive to, are those discussed in USEPA[1], p. 38-39. Observations are made at 24, 48, 72, and 96 hours, at which time dead fish are removed.

CALCULATIONS: Test results are used to calculate the 96 hour LC_{50}. The 24, 48, and 72 hour LC_{50}'s are calculated when possible. The LC_{50} is defined as the calculated concentration of the test material which causes 50% mortality in populations of test fish at the specified time of exposure. Results are to be calculated and reported on the basis of added (nominal) test concentrations or from concentrations confirmed by actual analysis, if requested.

RECORDS TO BE MAINTAINED: All records necessary to reconstruct the study and demonstrate adherence to the Protocol.

PROTOCOL CHANGES: If a change in the approved protocol becomes necessary, verbal agreement should be made between the Study Director and Sponsor's Principal Investigator. As soon as practical thereafter, this change and the reasons for it should be put in writing, approved by both persons, and attached to the protocol as an addendum.

REPORTING: The report is to be a typed document in triplicate, describing the results of the study and is to be signed and dated by the Study Director, Quality Assurance Officer and Laboratory Manager. It is to include, but is not limited to, the following:

1) Identification of test material by sample code, percent active, color, form, and date received.
2) Procedures followed for test material preparation and addition.
3) Reference to laboratory notebook or other file containing raw data.
4) Date definitive test was conducted.
5) Species tested, source, mean and range of the length and weight.
6) Percentage mortality in all test containers, including the control.
7) Calculated LC_{50} values, 95% confidence intervals, and reference to the method used to calculate these values.
8) Description of dilution water used, including a range of the measured pH, hardness, alkalinity, and conductivity.
9) All temperature, pH, and DO determinations and all visual observations.
10) Laboratory Study Number.
11) Reference to Protocol (title, author, and date) and addenda if made, and any analytical procedures used.
12) Any Protocol deviations and their implications.
13) Results of reference toxicant and date conducted.
14) Description of the quality assurance methods used to insure the quality of the data.

ALTERNATE PRINCIPAL INVESTIGATOR _____ PHONE: _____

NOTED: _____ 3/26/85 PHONE: _____
Date

APPROVED: _____ 3/5/85 PHONE: _____
Principal Investigator Date

TO BE COMPLETED BY STUDY DIRECTOR:

Study No. 1011.0385.6138.100

Estimated Starting Date 21 March 1985

Estimated Reporting Date 13 May 1985 (Verbal - 5 April)

Date Test Material Received 25 January 1985

Approved _____ 2/21/85 PHONE: 617-295-2550
Study Director Date

/kd

CONFIDENTIAL
• ONLY SPRINGBORN COPY
• MUST BE STRICTLY CONTROLLED

Date: 5/22/85

LOGISTICS MONITORING REPORT

Monitor(s):

Date of Visit: 5/14 & 5/17/85

TSUM: #60793.01

Test: Bluegill acute in lab & river waters; Daphnia acute in river water

~~**EBD Project No.:**~~

Laboratory Project No.: 1011.0385.6134 thru 6139

Date Started: 3/26/85

Date Ended: 4/13/85

Principal Investigator: R.H. Hall

Location: Springborn Bionomics

Study Director: J. Sousa for fish tests; J. Hoberg for Daphnia tests.

Discussed With: D. Surprenant (aquatic lab manager); R Bentley (QAU)

Phases Audited/Inspected:

1) Protocol
2) Test material tracking and usage
3) Study records
4) Final reports

Findings are discussed in Attachment I.

(signature) _____ 5/22/85 (date)

CIRCULATION (Do not copy. Please initial and date):

0128

~~TEST SUBSTANCE CHARACTERIZATION REPORT (TSCR)~~

Test Substance Identification Number (TSIN): 80743.01
Safety Test Request Number:
Principle Investigator: ~~[redacted]~~

~~[redacted]~~

Product or Ingredient:
Physical Description: White Solid/Semi Solid Solubility: NK pH: NK
Recommended Storage Conditions: Room Temperature Expiration Date: > 1 year
Hazards (i.e. flammability, toxic gases): Non-Hazardous
Dept. of Transportation Hazard Classification: Non-Hazardous CAS No.(a): 124-28-7

Formulated Composition (b)

Component (c)	Mol. Wt.	Nominal Level (% by Wt.)	Acceptable Range	Stock Code No.	Supplier	Lot Number (MB-Ref.)
Di-Methyl Stearyl Amine	297	100.00		NK	Sherex	4069-S-666334

(a) Include CAS number(s) for the three most major components of a formulation or for single chemical products. Footnote to the material with which the respective number is associated.

(b) If information requested is not known, then the symbol NK will be entered.

(c) Chemical names which are inconveniently long may be abbreviated in tables but should be listed in full in referenced footnotes. Non-chemical names, such as Tergitol 15-S-0 or Yellow Dye #10, may not be acceptable but should be previewed with the responsible toxicologist. Nondefinitive identification (e.g. Arv is not acceptable.

The above information provided by: ~~[redacted]~~ (Name) ~~[redacted]~~ (Signature) 12/18/84 (Date)

The above information reviewed and accepted by

Principle Investigator 12/18/84 (Date)

LHS009/lad

TEST SUBSTANCE CHARACTERIZATION REPORT (TSCR)

Test Substance Identification Number (TSIN):

Analyzed Composition

Date Submitted	Submitter Code	Analysis Code/Analysis	Estimated Value	Measured Value	Testing Laboratory

Analytical Information Verified By: _____ (Signature) Date: _____

This test substance is suitable for animal

Principle Investigator: Date: 3/5/86

This test substance is suitable for human (clinical) safety testing.

Principle Investigator: _____ (Signature) Date:

Contains No CBI

(6)

ENVIRONMENTAL SAFETY TEST SUMMARY REVIEW

Test Material Name: CAS RN 124-28-7

Test Substance Identification Number: B0793.01 Suffix #: .05

Type of Study: Acute Fish Toxicity in River Water Report #: 85-10-1841

Name of Originator:

Laboratory Involved: Springborn Bionomics

Date Report Written: 11-12-85 Date Received by Operations Section: 12-10-85

This report has been received and found in agreement with the Protocol and there appear to be no inaccuracies in the numerical data or written portions with the following exceptions:

Total suspended solids were not reported for the river water. This information will be provided as an amendment.

Logistics Reviewer V. W. Williams Date 12/26/85

This report has been reviewed for scientific quality and is summarized with the following comments:

Principal Investigator R. E. Hall Date 1/28/86

This report is recommended for entry into ESD Safety Data System:

Corp. Date 1/29/86

File approved for entry into the

Date 1-30-86

(Return to L)

Entered into Safety Data System: Date 2-22-86 By

Microfilming Completed: Date _____ By _____

TEST SUPPART SHEET

A. _____

B. Text: (Note - tabulations must be limited to 71 characters in width)

Test Type: Acute Freshwater Fish Toxicity - Static

Test Protocol: Static Acute Freshwater Fish Toxicity Study of BO793.01 in River Water, 3/5/85

Species: Bluegill sunfish, Lepomis macrochirus

Dilution Water: Tama River Water

Length: 37(34-41) mm

Total Hardness: 35 mg/l as $CaCO_3$

Weight: 0.52(0.39-0.70) gm

pH: 6.5 TSS: 15 mg/l

Source: Lot # RMB158

Test Temperature: 22 degrees C

Results: 96 Hour LC50 (95% C.I.): 2.1 (1.7-2.8)mg/l

Control Mortality: 0 %

Behavioral Observations: Effects were noted at all concentrations.

Reference Response (96 hr LC50): 7.2(6.2-8.3) mg/l sodium lauryl sulfate

Material Solubilization: Material added via [✓] stock solution; [ ] direct weighing. Solvent used - [ ] water; [✓] IPA.

Comments: All results are based on nominal concentrations of the active ingredient tested as 100% active.

C. Project Status Sheet Code No. BO793.01.05 _____ _____

D. Test Type: ~~ACFT~~ KEYWO*** Acute Freshwater Fish Toxicity - Static

E. Test Material: _ test Substance

F. Title/Date: Acute Toxicity of BO793.01 to Bluegill (Lepomis Macrochirus)/in River Water/11-14-85

G. Test Location: Springborn Bionomics H. Lab Project No.: 101-0385-6136-100

I. Date: 86/01/28 YY MM DD

K. Sponsor's Principal Invest.(sign): _ (print): _____

M. TSIN: BO793.01 _____

N. Date Work Done: 9/9/85 - 9/13/85

bg:TSACFT

ACUTE TOXICITY OF B0793.01 to BLUEGILL (*Lepomis macrochirus*) IN RIVER WATER

TOXICITY TEST REPORT

SUBMITTED TO

THE PROCTER & GAMBLE COMPANY

CINCINNATI, OHIO

BIONOMICS REPORT #BW-85-10-1841

BIONOMICS STUDY #1011-0385-6136-100

Springborn Bionomics, Inc.
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts 02571
October, 1985

0 1 2 5

SUMMARY

96-Hour Static Toxicity Test: Freshwater Fish

Springborn Bionomics, Inc.
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts

CLIENT: THE PROCTER & GAMBLE COMPANY

PROTOCOL CITED: Static Acute Freshwater Fish Toxicity Study of B0793.01 in river water; Principal Investigator, 3/5/85.

REPORT NUMBER AND DATE: BW-85-10-1841, October, 1985

STUDY NUMBER: 1011-0385-6136-100

MATERIAL: B0793.01

DESCRIPTION: a cloudy white-colored gel, 100% active ingredient

DATE MATERIAL RECEIVED: 25 January 1985

TEST DATE: 9-13 April 1985

SPECIES: Lepomis macrochirus

Total length: Mean (range, N=30), 37(34-41) mm

Wet weight: Mean (range, N=30), 0.52(0.39-0.70) g

Source: Commercial fish supplier in Connecticut

DILUTION WATER: Town river water

Total hardness as $CaCO_3$: 35 mg/L

Total alkalinity as $CaCO_3$: 11 mg/L

pH: 6.5

Specific conductivity: 190 umhos/cm

TEST TEMPERATURE: 22°C

METHOD OF TEST MATERIAL ADDITION: Stock solution; 10 mg of B0793.01/mL of isopropyl alcohol

TEST CONCENTRATIONS: 5.0, 3.0, 1.8, 1.1, 0.65, 0.39, 0.23 and 0.14 of B0793.01 mg/L

RESULTS: Results are reported as nominal concentrations of B0793.01 based on 100% active ingredient. The 96-hour LC50 was estimated by nonlinear interpolation to be 2.1 mg/L with a 95% confidence limit calculated to be between 1.7 and 2.8 mg/L.

INTRODUCTION

The purpose of this study was to estimate the acute toxicity of B0793.01 in river water to bluegill (Lepomis macrochirus) under static conditions. A 96-hour definitive test was conducted from 9-13 April, 1985, at the Aquatic Toxicology Laboratory of Springborn bionomics, Inc., Wareham, Massachusetts. All raw data generated are stored at the above location.

MATERIALS AND METHODS

The B0793.01, a white-colored gel tested as 100% active ingredient, was received from The Procter & Gamble Company, Cincinnati, Ohio, on 25 January 1985. Nominal test concentrations are expressed as milligrams of B0793.01 active ingredient per liter of test solution (mg/L).

Procedures used in this acute toxicity test followed those described in "Methods for acute toxicity tests with fish, macroinvertebrates and amphibians" (U. S. EPA, 1975) and the protocol entitled "Static Acute Freshwater Fish Toxicity Study of B0793.01 in River Water" Principal Investigator, 5 March 1985) issued to Springborn Bionomics, Inc., by the Procter & Gamble Company, Cincinnati, Ohio.

The bluegill (Bionomics Lot #84B15B) were obtained from a commercial fish supplier in Connecticut and held in a 1700-L epoxy-coated tank under a photoperiod of 16 hours light

and 8 hours darkness. The well water which flowed into this tank was characterized as having total hardness and alkalinity ranges as calcium carbonate ($CaCO_3$) of 32-34 mg/L and 22-24 mg/L, respectively, and a specific conductance range of 110-130 micromhos per centimeter (umhos/cm) (Weekly Gravity Feed Tank Water Quality Analysis Logbook). Other parameters monitored in the holding tank were a pH range of 6.6-7.4, a dissolved oxygen concentration range of 73-94% of saturation and a flow rate of 3-7 tank volume replacements/day (Weekly Record of Fish Holding Water Characteristics). Test fish were maintained under these conditions for a minimum of 14 days. The temperature range in the holding tank was 21-22°C during this 14-day period. All fish were fed a dry commercial pelleted food, ad libitum, daily except during the 48 hours prior to testing. There was mortality of <1.0% of the test fish population during this two-day period (Daily Record of Fish Holding Conditions). The mean (range, n=30) wet weight and total length of the test fish population was 0.52 (0.39-0.70) grams and 37 (34-41) millimeters (Fish Weight and Lengths Log).

A sodium lauryl sulfate reference test was conducted with the test fish population from 27-30 March 1985. The resulting 96-hour LC50 and 95% confidence interval was 7.8 (4.8-10) mg/L (Reference Test Log).

The toxicity test was conducted in 19.6-L glass jars

which contained 15 L of test solution. The test solution depth was 27.5 cm with a surface area of 545 cm^2. The dilution water used was river water collected from the Town River in West Bridgewater, Massachusetts, at the point where the river intersects Route 28. This water had a total hardness and alkalinity as $CaCO_3$ of 35 mg/L and 11 mg/L, respectively, a pH of 6.5 and a specific conductance of 190 umhos/cm (Reconstituted Water, Water Quality Analysis Lot).

A clear colorless stock solution of 10 mg/milliliter (mg/mL) was prepared by diluting 0.50 grams of B0793.01 with isopropyl alcohol to volume in a 50-mL volumetric flask. The appropriate amount of the stock solution was then added to each test jar and mixed by stirring with a glass rod.

Two control jars containing the same dilution water and maintained under the same conditions as the exposure jars, but containing no B0793.01, were established. One control jar contained the greatest amount of isopropyl alcohol present in any test jar (0.50 mL/L). All test solution temperatures were controlled by a system designed to maintain temperatures at 22 $\pm$ 1°C. Test solutions were not aerated. The photoperiod during testing was the same as that provided in the fish culture area.

Ten bluegill were impartially distributed to each test jar within 15 minutes after the test solutions had been

prepared. The resulting test organism loading concentration was 0.35 grams of biomass per liter of test solution. Fish were not fed during exposure.

Mortalities were recorded and removed from each test jar every 24 hours during exposure. Biological observations of the fish and observations of the physical characteristics of the test solutions were also made at each 24-hour interval. The pH's and dissolved oxygen concentrations were measured at 0, 24, 48, 72 and 96 hours in the control and all test concentrations. If mortality of 100% of the fish occurred in a test concentration, measurements were made at that time interval, but further measurements were discontinued. The temperature was measured in the control jar at the above-mentioned time intervals.

Total hardness concentrations presented in this report were measured by the EDTA titrimetric method and total alkalinity concentrations were determined by potentiometric titration to an endpoint of pH 4.5 (APHA et al., 1985). Specific conductivities were measured with a Yellow Springs Instrument Company (YSI) Model #33 salinity-conductivity-temperature meter and probe; the pH's were measured with an Instrumentation Laboratory Model #175 pH meter and combination electrode; the dissolved oxygen concentrations were measured with a YSI Model #57 dissolved oxygen meter and probe and the temperatures were measured with a Brooklyn

alcohol thermometer.

The concentrations tested and the corresponding mortality data derived from the toxicity test were used to estimate 24-, 48-, 72- and 96-hour median lethal concentrations (LC50) and 95% confidence intervals. The LC50 is defined as the concentration of the test material in dilution water which caused mortality of 50% of the test animal population at the stated exposure interval. If sufficient toxicant-related mortality occurred during the test (e.g., presence of at least one test concentration causing mortality of >50% of the animals in the test population), then a computer program (Stephan, 1982, personal communication) was used to calculate LC50 values. The computer program scanned the data base, identified the most appropriate statistical method and performed the analyses. Three statistical methods, in the following order of preference, were available in the computer program: moving average angle analysis, probit analysis, and binomial probability (Stephan, 1977). Binomial probability method calculates only the 95% confidence interval. In such a case, a point estimate of the LC50 is obtained by nonlinear interpolation (i.e., logarithm transformation of the concentration and the angle transformation of the percent dead; Stephan, 1982). The method selected was determined in the above order of preference and by the characteristics of the data base (e.g., presence or absence of several test

concentrations causing mortality of a partial number of animals in the respective test population). The LC50 values were empirically estimated when insufficient toxicant-related mortalities occurred.

RESULTS

The nominal test concentrations, the corresponding cumulative mortalities and the observations made during the toxicity test are presented in Table 1. The 96-hour LC50 for bluegill exposed to BO793.01 was estimated by nonlinear interpolation to be 2.1 mg/L with a 95% confidence interval calculated by binomial probability to be between 1.7 and 2.8 mg/L. Table 2 summarizes the 24-, 48-, 72- and 96-hour LC50's. The pH's, dissolved oxygen concentrations and temperatures measured during the toxicity test are presented in Table 3.

LITERATURE CITED

APHA, AWWA, WPCF. 1985. Standard methods for the examination of water and wastewater. 16th Edition, Washington, D.C., 1268 pp.

Stephan, C. E. 1977. Methods for calculating an LC50, Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, F. L. Mayer and J. L. Hamelink, Eds., American Society for Testing and Materials, pp. 65-84.

Stephan, C. E. 1982. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal communication to Dr. Lowell Bahner, Chairman ASTM Task Group on Calculating LC50's.

U.S. EPA. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecological Research Series (EPA-660/3-75-009), 61 pp.

Table 1. Concentrations tested and corresponding cumulative mortalities of bluegill (Lepomis macrochirus) exposed to BO793.01 for 24, 48, 72 and 96 hours. Test dilution water was collected from the Town River.

Nominal[a] concentration (mg/L)	Cumulative mortality (%)			
	24-hour	48-hour	72-hour	96-hour
5.0	70[b]	100	100	100
3.0	0[b]	50[c]	80[d]	80[d]
1.8	0[b]	10[d]	10[d]	20[d]
1.1	0[b]	0[b]	0[d]	10[d]
0.65	0	0[b]	0[d]	0[d]
0.39	0	0[b]	0[d]	0[d]
0.23	0	0[b]	0[d]	0[d]
0.14	0	0	0[e]	0[e]
solvent control	0	0	0	0
control	0	0	0	0

[a] All test solutions exhibited a film on the surface which was present throughout the exposure period.

[b] All of the surviving bluegill were lethargic and respiring rapidly.

[c] All of the surviving bluegill were respiring rapidly at the surface of the test solution, were lethargic and exhibited a darkened pigmentation.

[d] All of the surviving bluegill were lethargic and respiring rapidly at the surface of the test solution.

[e] All of the surviving bluegill were respiring rapidly.

Table 2. The 24-, 48-, 72- and 96-hour LC50 values and 95% confidence intervals for bluegill (Lepomis macrochirus) exposed to BO793.01. The test dilution water was collected from the Town River.

LC50 (mg/L)			
24-hour	48-hour	72-hour	96-hour
4.4[a] (3.0-5.0)[b]	2.7[c] (2.3-3.5)	2.4[c] (2.0-3.0)	2.1[c] (1.7-2.8)

[a] LC50 value estimated by nonlinear interpolation.

[b] 95% confidence limits calculated by binomial probability.

[c] LC50 value and 95% confidence interval calculated by moving average angle analysis.

Table 3. The pH's, dissolved oxygen concentrations and temperatures measured during the 96-hour exposure of bluegill (Lepomis macrochirus) to BO793.01. The test dilution water was collected from the Town River.

	Nominal concentration (mg/L)	0-hour	24-hour	48-hour	72-hour	96-hour
pH	5.0	6.5	6.1	5.7	---[b]	---[b]
	3.0	6.4	6.0	5.8	5.8	5.9
	1.8	6.5	5.9	5.7	5.9	5.9
	1.1	6.4	5.9	5.6	5.8	5.8
	0.65	6.4	5.9	5.7	5.9	5.9
	0.39	6.4	5.9	5.7	5.8	5.8
	0.23	6.5	6.0	5.7	5.8	5.8
	0.14	6.5	6.1	5.8	5.9	5.8
	solvent control	6.3	6.1	5.8	6.1	5.9
	control	6.3	6.2	5.8	5.9	5.9
Dissolved oxygen (mg/L)	5.0	8.9(101)[a]	8.4(95)	2.0(23)		
	3.0	8.9(101)	7.3(83)	2.9(33)	2.1(24)	1.1(12)
	1.8	8.9(101)	5.6(64)	2.9(33)	2.1(24)	1.1(12)
	1.1	8.9(101)	6.0(68)	3.0(34)	2.7(31)	1.2(14)
	0.65	8.9(101)	5.9(67)	3.3(38)	2.7(31)	1.6(18)
	0.39	8.9(101)	5.3(60)	3.2(36)	2.6(30)	1.4(16)
	0.23	8.7(99)	5.2(59)	3.3(38)	2.2(25)	1.8(20)
	0.14	8.8(100)	5.2(59)	3.3(38)	3.1(35)	1.7(19)
	solvent control	8.9(101)	4.6(52)	4.5(51)	4.3(49)	3.9(44)
	control	9.0(102)	6.2(70)	5.4(61)	4.6(52)	4.1(46)
Temperature (^{o}C)	control	22	22	22	22	22

[a] % of saturation.

[b] Measurement not required due to 100% mortality of test fish population.

The data contained in this report were audited by the Quality Assurance Unit to assure compliance with the protocols, standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations on the following dates: 15 May, 11 and 20 September, and 6 November, 1985. If discrepancies were found, reports were made immediately to the Study Director and management. It is the opinion of this Unit that these data accurately reflect the raw data generated during this study.

R. E. Bentley 18 Nov. '85

Robert E. Bentley
Director, Quality Assurance and
Special Projects

SUBMITTED BY:

Springborn Bionomics, Inc.
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
October, 1985

PREPARED BY:

Richard B. Nicholson

Richard B. Nicholson 11/12/85

Aquatic Toxicologist

STUDY DIRECTOR:

Joseph V. Sousa

Joseph V Sousa 11/12/85

Aquatic Toxicologist

APPROVED BY:

Donald C. Surprenant

D C Surprenant 11/12/85

Director, Aquatic Toxicology

DATA AUDITED BY:

Robert E. Bentley

R. E. Bentley 18 Nov. '85

Director, Quality Assurance Unit

THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

P.O. BOX 39175
CINCINNATI, OHIO 45247

March 13, 1985

Mr. Robert Bentley
Springborn Bionomics, Inc.
790 Main St.
Wareham, MA 02571

Dear Mr. Bentley:

This is to authorize you to carry out the following study according to the attached protocol, and in conformance with the stipulations of our current Laboratory Services Agreement.

Protocol: Static Acute Freshwater Fish Toxicity Study of B0793.01 in River Water
Date: 3/5/85
Sponsor's Principal Investigator:

Notice: The stipulations of this protocol are to be implemented in conformance with EPA Good Laboratory Practice Regulations (40 CFR, Part 792).

Please have the Study Director approve and complete both copies of the attached protocol by adding your study or project number, estimated starting and reporting dates, and date the test material was received. Retain one copy for your files and return the other to me.

Please return all unused portions of the test material to the Sponsor's Principal Investigator at the following address:

The Procter & Gamble Company
Sharon Woods Technical Center
11520 Reed Hartman Hwy.
Cincinnati, OH 45241

Please use the same test materials from previous acute studies with Daphnia submitted 1/24/85. Verbal results are needed by April 5, 1985.

We understand the estimated cost for this study is $900. Invoices are also to be sent to the Principal Investigator.

Matters involving the scientific aspects of the work can be handled directly with the Sponsor's Principal Investigator. Feel free to contact me at if you have any other questions or concerns.

Sincerely,

THE PROCTER & GAMBLE COMPANY

Approved:

Attachments
cc:

Laboratory Study No. 1011.0385.6136.110

PROTOCOL

SPONSOR: The Procter & Gamble Company; Cincinnati, Ohio

LABORATORY: Springborn Bionomics, Inc.
790 Main St.
Wareham, MA 02571

TITLE: Static Acute Freshwater Fish Toxicity Study of B0793.01 in River Water

OBJECTIVE: To determine the 96 hr LC_{50} of the test material to a freshwater fish species.

JUSTIFICATION FOR TEST SYSTEM: The bluegill (Lepomis macrochirus) is a readily available freshwater fish species, on which a large amount of toxicity data exists, and is recommended by the USEPA[1] as a standard test organism.

TEST MATERIAL:

Sample Code B0793.01 Color Opaque Form Gel/liquid

%Active 100 Density -- Solubility 5% in IPA

Expiration Date in progress Other --

Storage Conditions - room temperature

Safe Handling Precautions - Caution: May be irritating to eyes and skin. If exposed rinse with plenty of water. Strong fishy odor - use hood.

The Sponsor accepts full responsibility for appropriate characterization and stability verification of this test material.

TEST MATERIAL ADDITION/PREPARATION: All calculations and measurements are to be based on the active ingredient. The maximum concentration to be tested is 1000 mg/L active ingredient. Add test material from IPA stock solution. Use appropriate IPA controls. Maximum IPA concentration as per laboratory SOP.

TEST ORGANISM:

Species - Bluegill (Lepomis macrochirus)
Age - All fish from the same year class.
Weight - Average is not to exceed 1.2 g.
Length - The largest fish is to be no more than twice the standard length of the shortest fish.

All fish must be in holding for a minimum of 14 days prior to starting the test. Fish are to be acclimated to the test temperature for a minimum of 48 hours in the "dilution water" or water of similar chemical composition to the "dilution water". Fish are not to be fed during this acclimation period or during the test period. A group of fish must not be used if individuals appear to be diseased or otherwise stressed or if more than 3% die during the 48 hours.

[1]USEPA, Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. EPA-660/3-75-009.

TEST CONTAINERS: Wide mouth glass jars (approximately 20 L capacity) containing 15 L of test solution.

DILUTION WATER: Town River water collected above the sewage treatment plant.

TEST CONDITIONS:

(1) Maintain temperature at 22 ± 1°C.
(2) The dissolved oxygen concentration in each test container must be at least 90% of saturation on Day 0, and for the control, must remain greater than 40% of saturation throughout the test.
(3) The biomass to water ratio must not exceed 0.8 g/L.
(4) A control and at least five concentrations of the test material are to be used. Except for the control, the concentration of test material in each treatment must be at least 56% of the next higher one.

OPERATION:

Ten fish are randomly assigned to each test container, following the laboratory's standard operating procedures, within 30 minutes after the addition of the test material. One treatment must have killed or affected more than 63% of the fish, and one treatment (not the control) must have killed or affected less than 35% of the exposed fish. The test is conducted for 96 hours, commencing when the test fish are first exposed to the test material.

If any additive (e.g. solvent) is used to solubilize the test material, another control containing the greatest amount of solvent present in any other container is also required. A test is not valid if there is greater than 10% mortality in either control.

WATER CHEMISTRY: Dissolved oxygen (DO) and pH are determined at 0, 24, 48, 72, and 96 hours in all test concentrations, including the controls. When 100% mortality is observed in a test concentration, determinations are to be made, but further determinations for that concentration are discontinued.

SPECIAL INSTRUCTIONS (If any, for operation, sampling, analyses, or monitoring): Analysis of suspended solids in dilution water should be included in final report.

RANGE-FINDING TEST: To be conducted at the discretion of the laboratory unless otherwise specified.

OBSERVATIONS: Observations concerning mortality, morbidity, and behavior are to be recorded for all test concentrations. Of specific interest, but not exclusive to, are those discussed in USEPA[1], p. 38-39. Observations are made at 24, 48, 72, and 96 hours, at which time dead fish are removed.

CALCULATIONS: Test results are used to calculate the 96 hour LC_{50}. The 24, 48, and 72 hour LC_{50}'s are calculated when possible. The LC_{50} is defined as the calculated concentration of the test material which causes 50% mortality in populations of test fish at the specified time of exposure. Results are to be calculated and reported on the basis of added (nominal) test concentrations or from concentrations confirmed by actual analysis, if requested.

RECORDS TO BE MAINTAINED: All records necessary to reconstruct the study and demonstrate adherence to the Protocol.

PROTOCOL CHANGES: If a change in the approved protocol becomes necessary, verbal agreement should be made between the Study Director and Sponsor's Principal Investigator. As soon as practical thereafter, this change and the reasons for it should be put in writing, approved by both persons, and attached to the protocol as an addendum.

REPORTING: The report is to be a typed document in triplicate, describing the results of the study and is to be signed and dated by the Study Director, Quality Assurance Officer and Laboratory Manager. It is to include, but is not limited to, the following:

1) Identification of test material by sample code, percent active, color, form, and date received.
2) Procedures followed for test material preparation and addition.
3) Reference to laboratory notebook or other file containing raw data.
4) Date definitive test was conducted.
5) Species tested, source, mean and range of the length and weight.
6) Percentage mortality in all test containers, including the control.
7) Calculated LC_{50} values, 95% confidence intervals, and reference to the method used to calculate these values.
8) Description of dilution water used, including a range of the measured pH, hardness, alkalinity, and conductivity.
9) All temperature, pH, and DO determinations and all visual observations.
10) Laboratory Study Number.
11) Reference to Protocol (title, author, and date) and addenda if made, and any analytical procedures used.
12) Any Protocol deviations and their implications.
13) Results of reference toxicant and date conducted.
14) Description of the quality assurance methods used to insure the quality of the data.

ALTERNATE PRINCIPAL INVESTIGATOR _____ PHONE: _____

NOTED: _____ 3/4/85 PHONE: _____
Operations Logistics Date

APPROVED: _____ 3/4/85 PHONE: _____
Principal Investigator Date

TO BE COMPLETED BY STUDY DIRECTOR:

Study No. 1011-0385-6136-110

Estimated Starting Date 21 March 1985

Estimated Reporting Date 13 May 1985 (verbal 5 April)

Date Test Material Received 25 January 1985

Approved _____ 3/21/85 PHONE: 617-295-2550
Study Director Date

/kd

Test Substance Identification Number (TSIN): [illegible]
Safety Test Request Number:
Principle Investigator:

Product or Ingredient:
Physical Description: White Solid/Yeast Solid Solubility: NK pH: NK
Recommended Storage Conditions: Room Temperature Expiration Date: > 1 year
Hazards (i.e. flammability, toxic gases): Non-Hazardous
Dept. of Transportation Hazard Classification: Non-Hazardous CAS No.(a): 124-28-7

Formulated Composition (b)

Component (c)	Mol. Wt.	Nominal Level (% by Wt.)	Acceptable Range	Stock Code No.	Supplier	Lot Number (MB-Ref.)
Di-Methyl Stearyl Amine	297	100.00		NK	Sherex	4069-S-666334

(a) Include CAS number(s) for the three most major components of a formulation or for single chemical products. Footnote to the material with which the respective number is associated.

(b) If information requested is not known, then the symbol NK will be entered.

(c) Chemical names which are inconveniently long may be abbreviated in tables but should be listed in full in referenced footnotes. Non-chemical names, such as Tergitol 15-S-9 or Yellow Dye #10, may not be acceptable but should be previewed with the responsible toxicologist. Nondefinitive identification (e.g. Arquad, DC-base) is not acceptable.

The above information provided by:

(Name) (Signature) 12/13/84 (Date)

The above information reviewed and accepted by:

Principle Investigator:

(Name) (Signature) 12/18/84 (Date)

LMB909/lcd

TEST SUBSTANCE CHARACTERIZATION REPORT (TSCR)

Side 2 of 2

Test Substance Identification Number (TSIN):

Analyzed Composition

Date Submitted	Submitter Code	Analysis Code/Analysis	Estimated Value	Measured Value	Testing Laboratory

Analytical Information Verified By:

(Signature) Date:

This test substance is suitable for ani

Principle Investigator:

Date: 2/5/86

This test substance is suitable for human (clinical) safety testing.

Principle Investigator:

(Signature) Date:

CONFIDENTIAL
• ONLY SPECIMEN MAY COPY
• MUST BE STRICTLY CONTROLLED

Date: 5/22/85

LOGISTICS MONITORING REPORT

Monitor(s):

Date of Visit: 5/16 & 5/17/85

TSIN 4B0793.01

Test: Bluegill acute in lab & river waters; Daphnia acute in river water

Laboratory Project No.: 1011.0385.6134 thru 6139

Date Started: 3/26/85

Date Ended: 4/13/85

Principal Investigator:

Location: Springborn Bionomics

Study Director: J. Sousa for fish tests; J. Hoberg for Daphnia tests.

Discussed With: D. Surprenant (aquatic lab manager); R Bentley (QAU)

Phases Audited/Inspected:

1) Protocol
2) Test material tracking and usage
3) Study records
4) Final reports

Findings are discussed in Attachment I.

(signature) 5/22/85 (date)

CIRCULATION (Do not copy. Please initial and date):

0146

(1)

ENVIRONMENTAL SAFETY TEST SUMMARY REVIEW

Test Material Name: CAS RN 124-28-7

Test Substance Identification Number: B0793.2 **project # suffix):** B0793.01-07

Type of Study: Microbial Toxicity **Report or Project #:** 85-0498-11

Name of Originator:

Laboratory Involved: Hill Top

Date Report Written: 7-3-85 **Date Received by Operations Section:** 7-17-85

This report has been received and found in agreement with the Protocol and there appear to be no inaccuracies in the numerical data or written portions with the following exceptions:

None

Logistics Reviewer Date 8/6/85

This report has been reviewed for scientific quality and is summarized with the following comments:

Principal Investigator Date 8/12/85

This report is rec

Corp. Date 8/20/85

File approved for

Date 8/21/85

(Return to)

Entered into Safety Data System: Date _____ By _____

Microfilming Completed: Date _____ By _____

Copy returned to: _____ Date _____

TEST SUMMARY SHEET

A.

B. Test: (Note - tabulations must be limited to 71 characters in width)

Test Type: Microbial Toxicity

Test Protocol: Bacterial Toxicity Test on B0729.0, 3/5/85

Sludge Source: O'Bannon Creek STP

TSS: 7937 mg/l
VSS: 4562 mg/l
Temperature: 21 degrees C

Results:

Prior to Normalization for VSS

HA50, mg/l	95% Conf. Interval	R^2
>120	---	---

After Normalization for VSS

HA50, mg/gram VSS	95% Conf. Interval	
>2.0	---	---

Comments: No inhibition seen at the highest conc. tested of 120 mg/l. Material solubilized with EPH.
Material tested as 100% active.

Test Method: ESD Standard Method VIII-D-1, Issue 3, 2/9/82

C. Project Status Sheet Code No. B0729.0H7

D. Test Type: MCTX (KEYSTONE) Microbial Toxicity

E. Test Material: Di-Methyl-Stearyl Amine

F. Title/Date/ESD File No. Bacterial Toxicity Test on B0729.0/7-12-85 ~~as determined by Inhibition of Heterotrophic Activity/~~

G. Test Location: Mill Top H. Lab Project No.: 85-0498-11

I. Date: 85/07/26 (YY MM DD)

K. Prepared by:

M. TSIN: B0729.01

N. Date Work Done: 7/2/85

Hill Top Research, Inc.

P.O. Box 42501, Cincinnati, Ohio 45242 (513) 831-3114

Ref. 85-0498-11

July 3, 1985

BACTERIAL TOXICITY TEST ON B0793.01
For: The Procter and Gamble Company

PURPOSE

To determine the bacterial toxicity of the test material to a mixed microbial population.

TEST MATERIAL

The test material, identified as B0793.01 was received from the Procter and Gamble Company on March 18, 1985 for use in this study. Sample receipt and handling was performed according to SOP No. 11-BIOL-14-0001A. The Sponsor provided the following information regarding the test material:

• Color	White
• Form	Liquid/gel
• % Active	100
• Density	-
• Solubility	5% in IPA
• Expiration Date	In progress
• Storage Conditions	room temperature
• Safe Handling Precautions	May cause skin or eye irritation - strong fishy odor, use hood when handling

Preparation of test material stock solutions for the range finding and definitive phases of the study are presented in Table 1. The various concentrations of test material were added to each test beaker using an Eppendorf pipette set to deliver 0.1 ml.

TABLE 1

Preparation of Stock Solutions of B0793.01

Desired Concentration in 25 ml Volume	Working Stock Solution Concentration	Prepared by adding
0.1 ppm	25 ppm	5 ml of 250 ppm working stock, q.s. to 50 ml w/IPA
0.5 ppm	125 ppm	5 ml of 1250 ppm working stock, q.s. to 50 ml w/IPA
1.0 ppm	250 ppm	5 ml of 2500 ppm working stock, q.s. to 50 ml w/IPA
5.0 ppm	1250 ppm	25 ml of 2500 ppm working stock, q.s. to 50 ml w/IPA
7.0 ppm	1750 ppm	35 ml of 2500 ppm working stock, q.s. to 50 ml w/IPA
10.0 ppm	2500 ppm	0.2520 g of B0793.01 q.s. to 100 ml w/IPA

TEST SCHEDULE

The study was started and completed on July 2, 1985.

PROCEDURE

A. Test Method
The test was performed according to the protocol entitled Bacterial Toxicity Test on B0793.01 provided by R.H. Hall, Principal Investigator for the Sponsor. The protocol effective date was March 5, 1985.

B. Sludge Source
The secondary activated sludge used in this study was collected from the O'Connon Creek Sewage Treatment Plant on July 2, 1985. A total and volatile suspended solids was performed on the sludge according to SOP No. 11-BIOL-20-0022A. The average total suspended solids and average volatile suspended solids was 7537 mg/l and 4963 mg/l, respectively. The temperature of the sludge at the time of the definitive study was 21°C.

C. ^{14}C-Glucose Stock Solution
The solution was prepared by adding 0.0510g of cold anhydrous glucose to 100 ml of deionized water, followed by the addition of 15 ul of 240 mCi/mmol Uniform ^{14}C-Glucose. The solution was sterilized through a 0.22 um membrane filter and collected in a sterile serum bottle. One, (1) ml aliquot was placed in a liquid scintillation vial containing 14 ml of Beckman MP Scintillation cocktail. The activity was 0.0360 uCi/ml as determined by liquid counting.

D. Range Finding Phase
The selected concentrations that were tested in the range finding phase of the study were 0.1, 0.5, 1.0, 5.0, 7.0, and 10.0 ppm active B0793.01. The range finding phase was performed according to SOP No. 11-BIOL-20-0026A.

E. Definitive Phase
From the data obtained in range finding phase, the selected concentrations to test in the definitive phase were 0.1, 0.5, 1.0, 5.0, 7.0 and 10.0 ppm.

PROCEDURE (Cont.)

F. Scintillation Counting
Samples were prepared for scintillation counting according to SOP No. 11-BIOL-20-0023A. All liquid scintillation counting was performed according to SOP No. 11-BIOL-20-0025A. The determination of counting efficiency and calculation of DPM of samples generated in the definitive study was performed according to SOP No. 11-BIOL-20-0024A.

RESULTS

The results of the range finding and definitive phases are presented in Summary Tables I and II, respectively.

RETENTION OF DATA

All data is retained in the archives according to SOP No. 11-BIOL-24-0001A.

STUDY CONTRIBUTORS

The following members of Hill Top Research, Inc. contributed to the conduct and reporting of this study:

Name	Title	Function
Warren H. Pence, B.S.	Section Head, Biodegradation	Study Director, Conduct and Reporting of Study

STATEMENT OF COMPLIANCE

This study was performed in compliance to EPA Good Laboratory Practices (40 CFR 792).

Warren H. Pence 7-12-85
Warren H. Pence, B.S. Date
Study Director

Study Approved for: HILL TOP RESEARCH, INC,

By: Warren H. Pence 7-12-85
Warren H. Pence, B.S. Date
Study Director

By: [signature] FOR 7-15-85
Gayle K. Mulberry, M.S. Date
Director of Technical Services
Microbiological Services Division

By: L. Lea Johnston 7-14-85
L. Lea Johnston, M.A. Date
Director, Quality Assurance

SUMMARY TABLE I

RESULTS OF RANGE FINDING PHASE

Beaker No.	Concentration	CPM/ml
1.	HCl Control	3125
2.	0 ppm	2170
3.	0.1 ppm B0793.01	2261
4.	0.5 ppm B0793.01	2223
5.	1.0 ppm B0793.01	2289
6.	5.0 ppm B0793.01	2268
7.	7.0 ppm B0793.01	2248
8.	10.0 ppm B0793.01	2270
9.	IPA Control	2239

SUMMARY TABLE II

RESULTS OF DEFINITIVE PHASE FOR B0793.01

Test Concentration	Replicate *DPM Values		
	A	B	C
HCl Control	3359	3311	3325
0 ppm	2508	2292	2354
0.1 ppm B0793.01	2270	2334	2341
0.5 ppm B0793.01	2336	2243	2259
1.0 ppm B0793.01	2406	2533	2490
5.0 ppm B0793.01	2456	2596	2534
7.0 ppm B0793.01	2509	2502	2567
10.0 ppm B0793.01	2505	2620	2581
IPA Control	2427	2535	2491

*Efficiency/Quench corrected

Ref.: 85-0498-11

July 3, 1985

STUDY REVIEW RECORD

REFERENCE: 85-0498-11

DATE OF STUDY INITIATION: July 2, 1985

DATE OF STUDY COMPLETION: July 2, 1985

STUDY DIRECTOR: Warren H. Pence, B.S.

DISPOSITION OF REMAINING TEST MATERIAL:

Unused sample(s) returned to sponsor on July 3, 1985.

LOCATION OF RAW DATA: Archives of Hill Top Research, Inc.

LOCATION OF FINAL REPORT: Archives of Hill Top Research, Inc.

QUALITY ASSURANCE UNIT STATEMENT

Date(s) Study Inspected: 7-2-85

Date Report Reviewed: 7-10-85

Date(s) Findings Reported to Management: 7-3-85

Date(s) Findings Reported to Study Director: 7-3-85

Mary A. Weimer
Quality Assurance Auditor

7-12-85

L. Lee Johnston
Quality Assurance Director

7-15-85

0153

THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

P. O. BOX 39175
CINCINNATI, OHIO 45247

March 13, 1985

Mr. James R. Agin
Hill Top Research, Inc.
Miamiville, OH 45147

Dear Mr. Agin:

This is to authorize you to carry out the following study according to the attached protocol, and in conformance with the stipulations of our current Laboratory Services Agreement.

Protocol: Bacterial Toxicity Test on B0793.01
Date: 3/5/85
Sponsor's Principal Investigator:

Notice: The stipulations of this protocol are to be implemented in conformance with EPA Good Laboratory Practice Regulations (40 CFR, Part 792).

Please have the Study Director approve and complete both copies of the attached protocol by adding your study or project number, estimated starting and reporting dates, and date the test material was received. Retain one copy for your files and return the other to me.

Please return all unused portions of the test material to the Sponsor's Principal Investigator at the following address:

The Procter & Gamble Company
Sharon Woods Technical Center
11520 Reed Hartman Hwy.
Cincinnati, OH 45241

We understand the estimated cost for this study is $570. Invoices are also to be sent to the Principal Investigator.

Matters involving the scientific aspects of the work can be handled directly with the Sponsor's Principal Investigator. Feel free to contact me at . if you have any other questions or concerns.

Sincerely,

THE PROCTER & GAMBLE COMPANY

Approved:

.sion

Attachments
cc:

0154

Laboratory Project No. 85-0498-11

PROTOCOL

Proj. No. 85-0498-11
Page No. 1

SPONSOR: The Procter & Gamble Company; Cincinnati, Ohio

LABORATORY: Hill Top Research, Inc.
Cincinnati, OH 45242

TITLE: Bacterial Toxicity Test on B0793.01

OBJECTIVE: To determine the bacterial toxicity of the test material to a mixed microbial population.

JUSTIFICATION FOR TEST SYSTEM: Most of our products are disposed of through wastewater treatment systems where microorganisms can biodegrade the organic product components. Activated sludge is a common wastewater treatment process and contains a variety of microbial species.

TEST MATERIAL:

Sample Code B0793.01 Color White Form liquid/gel

% Active 100 Density — Solubility 5% in IPA

Expiration Date in progress Other _____

Storage Conditions - room temperature

Safe Handling Precautions - Caution: May cause skin or eye irritation. If exposed rinse with plenty of water. Strong fishy odor - use hood.
The Sponsor accepts full responsibility for appropriate characterization and stability verification of this test material.

REFERENCES: Procter & Gamble Environmental Standard Test Method VIII-D-1, Issue 3 (2-9-82).

TESTING PERIOD: Conduct the test for 15 minutes following the test material addition period of 5 minutes.

TEST MATERIAL ADDITION/PREPARATION: All calculations and measurements are to be based on the active ingredient. The test concentration range is at the discretion of the Study Director. The range of interest for this material is 0.1-10.0 mg/l. Add test material from IPA stock. All test and control vessels must contain the same level of IPA (highest level tested). Also include control w/o IPA.

CALCULATION-STATISTICAL ANALYSIS: Calculations will be performed by the Sponsor, including HA_{50}, 95% confidence limits, R^2 and HA_{50} normalization for VSS.

RECORDS TO BE MAINTAINED: All records necessary to reconstruct the study and demonstrate adherence to Protocol.

PROTOCOL CHANGES: If a change in the approved protocol becomes necessary, verbal agreement should be made between the Study Director and Sponsor's Principal Investigator. As soon as practical thereafter, this change and the reasons for it should be put in writing, approved by both persons, and attached to the protocol as an addendum.

REPORTING: The report is to be a typed document in triplicate, describing the results of the study and is to be signed by the Study Director, Quality Assurance Officer and Laboratory Manager. It is to include, but is not limited to, the following:

1. Identification of test material by sample code, percent active, color, form, and date received.
2. Procedures followed for test material preparation and addition.
3. Reference to the Protocol (title, author and date) and addenda if made, Test Methods, and any analytical or standard operating procedures used.
4. Any Protocol deviations and their implications.
5. Reference to laboratory notebook or other file containing raw data.
6. Laboratory Project Number.
7. Starting and ending dates of study.
8. Activated sludge source, temperature, VSS & TSS.
9. Efficiency - and quench - corrected values of radioactivity of triplicate samples of glucose stock solution, test flasks and control flasks.
10. Description of the quality assurance methods used to insure the quality of the data.

ALTERNATE PRINCIPAL INVESTIGATOR

HONE:

NOTE: 3/11/85 Date PHONE:

APPROVED:
Principal Investigator 3/8/85 Date PHONE:

TO BE COMPLETED BY STUDY DIRECTOR:

Project No. 85-0498-11

Estimated Starting Date 7-2-85

Estimated Reporting Date 7-21-85

Date Test Material Received 3-18-85

Approved Warren H. Penn 7-2-85 PHONE: 513-831-3114
Study Director Date

LABORATORY: Hill Top Research PHONE: Same as above

0156

Test Substance Identification Number (TSIN): 80793.01
Safety Test Request Number:
Principle Investigator:

Product or Ingredient:
Physical Description: White Solid/Semi Solid Solubility: NK pH: NK
Recommended Storage Conditions: Room Temperature Expiration Date: > 1 year
Hazards (i.e. flammability, toxic gases): Non-Hazardous
Dept. of Transportation Hazard Classification: Non-Hazardous CAS No.(a): 124-28-7

Formulated Composition (b)

Component (c)	Mol. Wt.	Nominal Level (% by Wt.)	Acceptable Range	Stock Code No.	Supplier	Lot Number (NB-Ref.)
Di-Methyl Stearyl Amine	297	100.00		NK	Sherex	4069-S-666334

(a) Include CAS number(s) for the three most major components of a formulation or for single chemical products. Footnote to the material with which the respective number is associated.

(b) If information requested is not known, then the symbol NK will be entered.

(c) Chemical names which are inconveniently long may be abbreviated in tables but should be listed in full in referenced footnotes. Non-chemical names, such as Tergitol 15-S-0 or Yellow Dye #10, may not be acceptable but should be previewed with the responsible toxicologist. Nondefinitive identification (e.g. Arquad, DC-base) is not acceptable.

12/18/84
(Date)

The above information r

Principle Investigator:

12/18/84
(Date)

LMS009/lad

0157

Test Substance Identification Number (TSIN):

Analysed Composition

Date Submitted	Submitter Code	Analysis Code/Analysis	Estimated Value	Measured Value	Testing Laboratory

[illegible]

Analytical Information Verified By:

(Signature) Date:

This test substance is suitable for ~~animal (non-clin.~~ [illegible]

Principle Investigator:

Date: 3/5/85

This test substance is suitable for human (clinical) safety testing.

Principle Investigator:

(Signature) Date:

0158

AMTEK CORP.

CERTIFICATE OF AUTHENTICITY

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