TOXICITY TO AQUATIC PLANTS (e.g., Algae)

TEST SUBSTANCE

**Identity**: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

**Remarks:** The 3M production lot number was 37. The test sample is FC-143. It's purity was not sufficiently characterized, though current information indicates it is a mixture of 96.5 - 100% test substance and 0 – 3.5% $C_6$, $C_7$, and $C_9$ perfluoro analogue compounds.

METHOD:

**Method Followed:** Modified (modeled) after those described by USEPA – 600/9-78-018; ASTM-E-35.23 Draft No. 2; OECD; A.G. Payne.
**Type (test type):** Acute Static
**GLP:** No
**Year study performed:** 1981
**Species:** *Selenastrum capricornutum.*
**Source:** USEPA – ERL, Corv., Oregon (July 14, 1981).
**Analytical monitoring:** Algal cell counts (cells/ml), cell dry weights, and temperature.
**Exposure period:** 4, 7, 10, and 14 days
**Statistical Methods:** $EC_{50}$ values and 95% confidence limits were calculated utilizing 3M Sixcur, a linear regression model.
**Test orgamisms laboratory culture:** Algae from a 7-day-old stock culture
**Algal Nutrient Medium:** Sterile synthetic algal nutrient medium. This nutrient medium served as the diluent for all algal operations. The pH of this synthetic algal medium was adjusted to 7.5±0.1 prior to use in assays.
**Stock and Test Solution Preparation:** A primary stock solution was prepared in algal medium at a concentration of 5 g/L. After mixing, the primary stock solution was diluted with algal medium to prepare the six test concentraions.
**Exposure vessels:** Sterile 250 mL Erlenmeyer flasks containing 50 mL of test solution and stoppered with autoclaved foam plugs.
**Agitation:** Continuous platform shaking at 100 ± 10 rpm
**Number of replicates:** three
**Initial algal cell loading:** $1.0 \times 10^4$ cells/mL
**Number of concentrations:** six plus negative control
**Nominal concentrations:** Bk control, 100, 180, 320, 560, 1000, and 1800 mg/L
**Test conditions:**
**Temperature:** 23±2°C (70-77°F)
**Fluorescent illumination:** 400 ft. candles ± 10%

## RESULTS

**Algal Growth Response $EC_{50}$ values**

| Exposure (Contact) Days | Cell-Dry Weight [1] mg/L (95% C.I.) | Cell-Count [2] mg/L (95% C.I.) |
|---|---|---|
| 4 | 149 (57-340) | 49 (28-75) |
| 7 | 70 (34-118) | 30 (21-40) |
| 10 | 49 (15-96) | 27 (8-50) |
| 14 | 73 (25-147) | 43 (14-81) |

(1) Growth response parameter; cell-dry weight (mg/L), measured in triplicated sets of culture flasks.

(2) Growth response parameter; cell-count (number cells / mL), measured in triplicated sets of culture flasks.

**Algal Growth Response EC10 and EC90 values based on Cell-Count (number of cells / mL)**

| Exposure (Contact) Days | $EC_{10}$ mg/L (95% C.I.) | $EC_{90}$ mg/L (95% C.I.) |
|---|---|---|
| 4 | 5.3 (3-7) | 624 (C.I. not calculated) |
| 7 | 3.3 (2-4) | 283 (150-590) |
| 10 | 2.9 (1-5) | 386 (C.I. not calculated) |
| 14 | 5 (2-8) | 307 (C.I. not calculated) |

Element values based on nominal concentrations.

## CONCLUSIONS

Ammonium perfluorooctanoate exhibits a 14-day $EC_{50}$ (cell count) value of 43 mg/L with a 95% confidence interval of 14 to 81 mg/L.

## DATA QUALITY

**Reliability**: Klimisch ranking = 2. This study meets the criteria for quality testing at the time it was conducted. However, the study lacks information on purity of the test substance and actual measurements of the amount of test substance in solution.

## REFERENCES

3M Technical Report Summary, Multi-Phase Exposure / Recovery Algal Assay Test Method, Report Number 006, Project Number 9970030000, M. T. Elnabarawy, October 16, 1981.

OTHER

**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

**Last changed:** 5/24/00

Form 6747 11-8

# TECHNICAL REPORT SUMMARY

Date: 11/16/81

TO: TECHNICAL COMMUNICATIONS CENTER – 201-2CN

*(Important – If report is printed on both sides of paper, send two copies to TCC.)*

Division: Environmental Laboratory - EE & PC - 2-3E

Dept. Number: 0535

Project: New Methods Development - FC-143, Lot 37

Project Number: 9970030000

Report Title: Multi-Phase Exposure/Recovery Algal Assay Test Method

Report Number: 006

To: D. Bacon/R. Bohon/[redacted]/A. Welter

Author(s): M. T. Elnabarawy

Employee Number(s): 46981

Notebook Reference:

No. of Pages Including Coversheet:

SECURITY ▶ ☒ Open ☐ Closed (Special Authorization)

3M CHEMICAL REGISTRY ▶ New Chemicals Reported ☐ Yes ☒ No

KEYWORDS: (Select terms from 3M Thesaurus. Suggest other applicable terms.)

EE & PC-Div. (Env. Lab)

CURRENT OBJECTIVE:

1. To evaluate the algal growth response of freshwater green algae "Selenastrum capricornutum" over several generations as they may be affected by exposure to the fluorochemical FC-143, lot 37.

2. To develop an algal test protocol based on variable exposure or recovery periods to the test compound.

REPORT ABSTRACT: (200-250 words) This abstract information is distributed by the Technical Communications Center to alert 3M'ers to Company R&D.


In these triplicated four-phase exposure/recovery assays, the initial effects on organisms following varied exposures to FC-143 were assessed over several generations. Test results gave an indication of the possible effects on algal populations. Algal biomass was assessed quantitatively in terms of cell-dry weight (mg/l) and cell-count (No./ml). The following biomass indicators were measured and calculated for:

A. Verification of inhibitory effects

1. Median growth response $EC_{50}$ (mg/l) from varied exposures.
2. $EC_{10}$ and $EC_{90}$ (mg/l) for same exposure periods.
3. No-effect levels

B. Verification of algicidal effects

1. Resumption or absence of logarithmic growth in subcultures during varied periods of recovery.


Information Liaison Initials:

003794

## CONCLUSIONS

1) Materials possessing an $EC_{50}$ in the range of 10-100 mg/l are considered to be slightly phytotoxic to algae and aquatic plant species.

2) The algal growth response in all subcultures following varied periods of recovery indicated minimal algicidal effects (death of algal cells) at concentrations tested. The algal cells recovered and resumed logarithmic growth when resuspended in fresh nutrient medium in the absence of the test material. The median growth response ($EC_{50}$) was therefore interpreted as basically indicative of an inhibitory effect as opposed to an algicidal effect. In the former case, photosynthesis, cell-growth, and cell-division, are reduced whereas an algicidal effect causes direct cell-destruction and cell-death.

3) Exposure/recovery results indicated that extended exposures (e.g., >7 days) induced greater inhibition and consequently exhibited slower recovery rates in the subcultures.

## SUMMARY

A four-phase exposure/recovery algal assay was developed to evaluate algal growth response of the freshwater unicellular green algae "Selenastrum capricornutum" over several generations as they may be affected by exposure to the fluorochemical, FC-143. This multiphase algal assay evaluated algal growth responses during four consecutive exposure/recovery combinations: 4/10, 7/7, 10/4, and 14 days. As is clearly indicated, the duration of exposure periods increased, while respective recovery periods decreased. The combined duration of each exposure/recovery phase was fixed at 14 days. Algal growth response was measured in terms of an increase in biomass as cell-dry weight (mg/l) and cell-count (No./ml). Dry-weight and cell-count results used for the calculation of $EC_{50}$'s (mg/l) were averages of triplicated sets of test culture flasks.

In these triplicated multiphase multigeneration algal assays, the calculated $EC_{50}$ values (mg/l) for freshwater green algae "Selenastrum capricornutum" are presented in Table 1. These values represent the median growth response ($EC_{50}$ mg/l) following exposure to the test material for 4, 7, 10, and 14 days.

The values of $EC_{10}$ and $EC_{90}$ (mg/l) for each of the exposure periods listed earlier were calculated in terms of cell-count and are presented in Table 2.

No test concentrations were defined as a no-effect level.

To verify algicidal effects, and to assess possible resumption of growth in the absence of the test material subcultures were established from each triplicated set of test culture flasks combined at the end of each exposure period. Algal recovery response was evaluated following respective recovery periods of 10, 7, and 4 days.

## INTRODUCTION

In testing the possible effects of chemical substances on the aquatic environment, unicellular algae are recommended as a model system for evaluating the influence of chemicals on algal growth (aquatic primary producers), aquatic plants, and phytoplankton.

Toxic effects are tested on growing algal cultures that undergo cell-division during the test. These growth responses may be: a) stimulatory, b) inhibitory and/or algicidal.

## MATERIAL AND EXPERIMENTAL DESIGN

The test substance (FC-143, lot 37) is a fine white powder, soluble in water at ambient room temperature. The bioassays performed on this material evaluated its potential algal toxicity and the data generated form the basis of this report.

The test protocol utilized for this study was modified after those described by USEPA - 600/9-78-018; ASTM-E-35.23 Draft No. 2; OECD; A. G. Payne. (1), (2), (3), (4).

### Test Species:

A bacteria-free culture of the freshwater planktonic green algae (Chlorophyceae), Selenastrum capricornutum of the order chlorococcales was obtained from USEPA - ERL, Corv., Oregon (February 18, 1981). The test algae are non-motile unicellular cells, having the appearance of a new moon. The algal culture was stored in the dark at 4°C.

### Inoculum:

Algae from a 7-day-old stock culture were used as inoculum to give a starting optimum inoculum level of green algae Selenastrum capricornutum $1 \times 10^4$ cells/ml. The use of a 7 to 10-day-old stock culture insured the presence of a sufficient number of viable algal cells in the exponential growth phase. The initial algal cell count in the stock culture was determined using a hemocytometer (277,000 cells/ml).

Algal Nutrient Medium:

Mineral (inorganic) standard nutrient medium for culturing and testing algae was prepared as outlined in Attachment #1. This nutrient medium provided all mineral nutrients essential for algal growth and also served as the diluent for all algal operations including the preparation of stock solutions. The pH of this synthetic algal medium was adjusted to 7.5±.1 prior to use in assays.

Culture Flasks:

Each 250 ml Erlenmeyer containing 50 ml of test solution comprised a test flask. Culture flasks and all other glassware were specially prepared as described in Attachment #2. Autoclaved foam plugs used as flask closures, permitted free gaseous exchange to occur.

Range-Finding Test (Exploratory):

The exploratory test consisted of determining the 4-day algal biomass in triplicated flasks containing standard nutrient medium plus the test material at concentrations covering several orders of magnitude: 100, 250, 500, 750, 1,000, and 1500 mg/l.

Full-Scale Test (Definitive):

All definitive algal assays were carried out in triplicate using 250 ml Erlenmeyer flasks containing 50 ml of test solution. The definitive assay consisted of four simultaneous exposure/recovery tests (4/10, 7/7, 10/4, and 14/0 days. The purpose of the exposure (contact) stage was to verify inhibitory effects. The recovery (subculture) stage evaluated the viability of growth-inhibited algae and verified algicidal effects.

The following FC-143 logarithmic concentrations: 100, 180, 320, 560, 1000, and 1800 mg/l were used to initiate the exposure stage. This dose range of dilutions was selected to bracket the 4-day $EC_{50}$ values predicted from preliminary exploratory testing. Three flasks containing 100% fresh algal nutrient medium plus algal cells comprised the nontreated controls. A fresh stock solution of the test substance was prepared in the algal nutrient medium immediately prior to testing. The initial pH of this stock solution was 7.4. Procedure and steps for preparation of algal test flasks are outlined in Attachment #3.

Each recovery stage was initiated by subculturing (0.5/50 ml) in the absence of the test material. Algal cells from each triplicated set of culture flasks were combined and then resuspended into fresh nutrient medium. Subculture methods are briefly described in Attachment #4.

Test Conditions:

All algal operations were carried out under aseptic conditions, in order to avoid contamination with bacteria and other algae. Algal cultures were maintained in an environmental chamber under the following standard growth conditions:

Temperature - 23 ± 2°C (70-77°F)

Fluorescent illumination - 400 ft.C. ± 10%

Free gas exchange - Continuous Platform Shaking 100 ± 10 rpm.

Algal Biomass Monitoring:

The algal growth response was appraised quantitatively by using either algal cell-dry weight (mg/l) and/or by cell counts (cells/ml). Both growth measurements were made with algal cultures after each of the exposure and recovery stages. Both procedures for algal biomass measurements are briefly described in Attachment #5.

$EC_{50}$ values and 95% confidence limits were calculated utilizing 3M SIXCUR, a TRAC System for regression models of experimental data.

## TEST RESULTS

The results of biomass measurements of the green algae Selenastrum capricornutum used in these studies are self-explanatory and are detailed in the attached data sheets. Calculated $EC_{50}$ (mg/l) values indicating algal growth response to FC-143, lot 37, following exposure periods of 4, 7, 10, and 14 days are presented in Table 1. The values of $EC_{10}$ and $EC_{90}$ for each of the exposure periods listed above were calculated in terms of cell-count and are presented in Table 2. Recovery data and resumption of logarithmic growth in subcultures are summarized in Table 3.

To illustrate the action of test material and to give an overview of what has happened in the exposure experiment, toxicity curves are shown in Figures 1 and 2. Utilizing the $EC_{50}$ values, toxicity curves were constructed against time on semilog plots.

The data reported herein are based on studies developed and performed by M. T. Elnabarawy and R. R. Robideau. Accompanying data sheets comprise original data.

It should be noted that all the reported EC values were calculated on the basis of the initial concentrations of FC-143, lot 37, in test solutions at the beginning of the bioassay.

## REFERENCES

(1) Miller, W. E., J. C. Greene, and T. Shiroyama. 1978. The Selenastrum Capricornutum Printz Algal Assay Bottle Test: Experimental design, application, and data interpretation. U.S. Environmental Protection Agency, Corvallis, Oregon. EPA-600/9-78-018. 125 p.

(2) ASTM-E-35.23. 1981. Proposed Standard Practice For Conducting Toxicity Tests with Freshwater and Saltwater Algae. Draft No. 2.

(3) OECD Guidelines for Testing of Chemicals (1981) Section 2, Effects on Biotic Systems, Test 201 "Algae, Growth Inhibition Test," Adopted May 12, 1981.

(4) Payne, A. G. and R. H. Hall, 1979. A Method for Measuring Algal Toxicity and Its Application to the Safety Assessment of New Chemicals. ASTM STP #667, p. 171-180.

TABLE 1

Algal Growth Response to
FC-143, Lot 37
$EC_{50}$'s (mg/l)(1)

| Exposure (Contact) Days | Cell-Dry Weight(2) | Cell-Count(3) |
|---|---|---|
| 4 | 149(57-341)(4) | 49(28-75) |
| 7 | 70(34-118) | 30(21-40) |
| 10 | 49(15-96) | 27(8-50) |
| 14 | 73(25-147) | 43(14-81) |

(1) Method of $EC_{50}$ calculation: 3M SIXCUR, a TRAC system for regression models of experimental data.

(2) Growth response parameter; cell-dry weight (mg/l), measured in triplicated sets of culture flasks.

(3) Growth response parameter; cell-count (no. cells/ml), measured in triplicated sets of culture flasks.

(4) 95% Confidence Limits.

TABLE 2

Algal Growth Response to
FC-143, Lot 37
$EC_{10}$'s and $EC_{90}$'s (mg/l)
Based on Cell-Count (no. cells/ml)

| Exposure (Contact) Days | $EC_{10}$ | $EC_{90}$ |
|---|---|---|
| 4 | 5.3(3-7) | 624(No Limits) |
| 7 | 3.3(2-4) | 283(150-590) |
| 10 | 2.9(1-5) | 386(No Limits) |
| 14 | 5(2-8) | 307(60-No Limits) |

TABLE 3

Recovery Stage Data Summary
Algal Cell County, Cells/ml (MEAN VALUES)(1)

| Treatment | Initial Algal Cell Loading(2) 4 days | 7 Days | 10 Days | Final Algal Cell-Count(3) 10 Days | 7 Days | 4 Days |
|---|---|---|---|---|---|---|
| Control | 2,017 | 2,430 | 2,453 | 380,000 | 256,000 | 136,000 |
| 1 100 mg/l | 643 | 580 | 587 | 372,000 | 226,000 | 48,000 |
| 2 180 mg/l | 530 | 290 | 280 | 348,000 | 204,000 | 20,000 |
| 3 320 mg/l | 383 | 273 | 380 | 300,000 | 214,000 | 20,000 |
| 4 560 mg/l | 237 | 137 | 290 | 236,000 | 200,000 | 18,000 |
| 5 1,000 mg/l | 57 | 60 | 103 | 310,000 | 174,000 | 18,000 |
| 6 1,800 mg/l | 73 | 73 | 73 | 182,000 | 54,000 | 18,000 |

(1) Based on 0.5 ml/50 ml subcultures established from each triplicated sets of culture flasks combined.

(2) Measured at the end of each exposure period.

(3) Measured at the end of each recovery period.

FIGURE 1. TOXICITY CURVE - ALGAL GROWTH RESPONSE TO FC 143 LOT 3

● $EC_{50}$'s (mg/l) based on cell-dry weight (mg/l)

+ Lower limit

* Upper limit

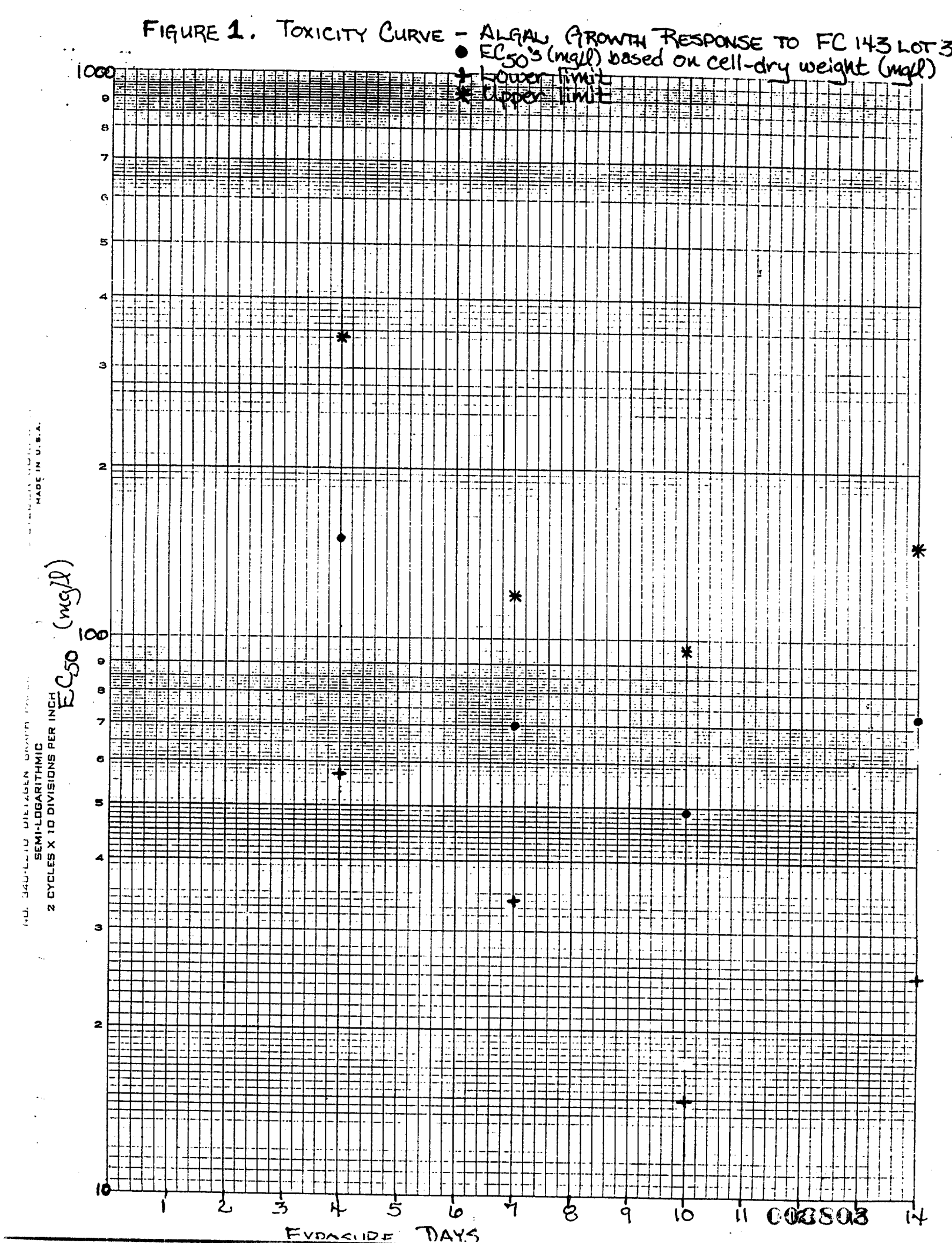
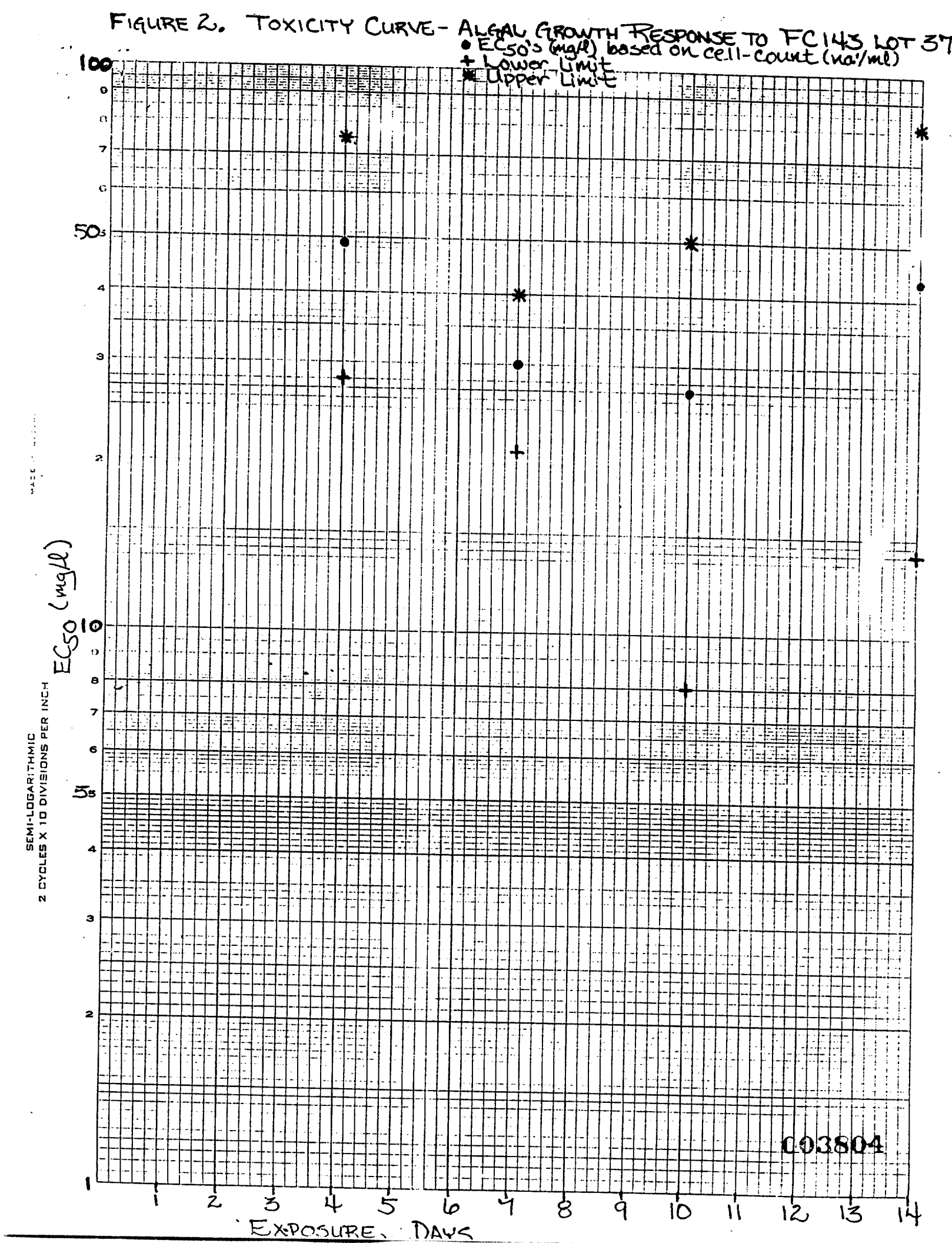


FIGURE 2. TOXICITY CURVE - ALGAL GROWTH RESPONSE TO FC143 LOT 37

● EC50's (mg/l) based on cell-count (no./ml)
+ Lower Limit
* Upper Limit

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ATTACHMENT 1

# NUTRIENT MEDIUM FOR FRESHWATER ALGAE

A. MACRONUTRIENTS STOCK SOLUTIONS (CONCENTRATED)
(Prepared separately with deionized water.)

1) 25.500 gm $NaNO_3$ in 1 liter DI water.

2) 1.044 gm $K_2HPO_4$ in 1 liter DI water.

3) 12.159 gm $MgCl_2 \cdot 6H_2O$ in 1 liter DI water.

4) 14.700 gm $MgSO_4 \cdot 7H_2O$ in 1 liter DI water.

5) 4.410 gm $CaCl_2 \cdot 2H_2O$ in 1 liter DI water.

6) 15.000 gm $NaHCO_3$ in liter DI water.

B. MICRONUTRIENTS STOCK SOLUTION (CONCENTRATED)
(Combined in a single one-liter stock mix.)

1) 185.5 mg $H_3BO_3$

2) 415.6 mg $MnCl_2 \cdot 4H_2O$

3) 3.27 mg $ZnCl_2$

4) 1.43 mg $CoCl_2 \cdot 6H_2O$

5) 0.01 mg $CuCl_2$

6) 7.26 mg $Na_2MoO_4 \cdot 2H_2O$

7) 96 mg $FeCl_3$

8) 300 mg $Na_2EDTA \cdot 2H_2O$

## PREPARATION OF SYNTHETIC ALGAL NUTRIENT MEDIUM

- Add one ml of each macronutrient stock solution plus one ml micronutrients stock mix per 1 liter of deionized water;
- sonicate and then filter through 0.22 µm membrane;
- adjust pH to 7.5 ± 0.1;
- store in the dark at 4°C.

ATTACHMENT 2

## PREPARATION OF ALGAL CULTURE FLASKS

All flasks used in maintaining and testing algae were made of borosilicate glass (KIMAX).

- flasks were brushed inside with a stiff bristle brush;
- washed with non-phosphate detergent (MICRO) and rinsed 3 times with tap water;
- rinsed with a 10% HCl solution;
- rinsed 3 times with tap water and 3 times with D.I. water;
- dried in an oven at 70°C for 2 hours (placed inverted);
- and autoclaved with foam plugs inserted at 121°C for 20 minutes.

ATTACHMENT 3

## PREPARATION OF ALGAL TEST FLASKS (IN CHRONOLOGICAL ORDER)

| ALGAL FLASKS | CONTENTS | | | | | | |
|---|---|---|---|---|---|---|---|
| | ALGAL MEDIUM (ml) | + | TEST MATERIAL (ml) or (mg)* | + | ALGAL INOCULUM (ml) | = | TOTAL (ml) |
| CONTROL | 48.2 | + | NONE | + | 1.8 | = | 50 |
| 1 100 mg/l (5mg/50ml) | 47.2 | + | 1.0 ml | + | 1.8 | = | 50 |
| 2 180 mg/l (9) | 46.4 | + | 1.8 ml | + | 1.8 | = | 50 |
| 3 320 mg/l (16) | 45.0 | + | 3.2 ml | + | 1.8 | = | 50 |
| 4 560 mg/l (28) | 42.6 | + | 5.6 ml | + | 1.8 | = | 50 |
| 5 1000 mg/l (50) | 38.2 | + | 10 ml | + | 1.8 | = | 50 |
| 6 1800 mg/l (90) | 30.2 | + | 18 ml | + | 1.8 | = | 50 |
| 7 | | + | | + | | = | 50 |

1) Determine initial algal cell count in the stock culture.

2) Adjust pH of algal nutrient medium to 7.5 + 0.1 ml.

3) Make up stock solutions with algal nutrient medium as desired.

A) Control flasks preparations:

1) Add inoculum (volume predetermined); initial cell loading of $1 \times 10^4$ cells/ml;

2) Add algal nutrient medium to bring volume to 50ml; swirl flasks, and place autoclaved foam plugs.

B) Test flasks:

1) Add algal nutrient medium;

2) Add test material (dissolved in algal medium if required);

3) Swirl flasks;

4) Add algal inoculum;

5) Swirl flasks, and place autoclaved foam plugs.

* STOCK SOLUTION: 5g/l (5,000mg/1000ml). EACH 1 ml = 5mg.

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ATTACHMENT 4

## SUBCULTURE METHOD IN ALGAL ASSAYS
## (0.5 ml/50 ml)

OBJECTIVE: To determine algal recovery response in absence of test substance.

PROCEDURE:

- Transfer 0.5ml from each of the triplicate culture flasks into Corning-15ml sterile centrifuge tubes.
- Centrifuge at 10,000 rpm for 5 minutes.
- Decant supernatant liquid and save the precipitated algal cells.
- Using the same centrifuge tube, resuspend algal cells in algal nutrient medium to a fixed volume: 15ml.
- Mix thoroughly and decant the top 10ml of algal cell suspension, and transfer the remaining volume (5ml) of algal cell suspension into a clean 250ml Erlenmeyer culture flask.
- Using the same centrifuge tubes, transfer 45ml of fresh algal nutrient medium into the above Erlenmeyer culture flask, to bring total volume to 50 ml.

ATTACHMENT 5

## ALGAL BIOMASS MONITORING

### A. GRAVIMETRIC CELL DRY WEIGHT (1)

The filter recommended is Millipore type BD with an 0.6 micrometer pore size.
The method is as follows:

- Dry filters for two hours at 70°C in an oven;
- Cool filters in a desiccator containing desiccant, for at least two hours before weighing;
- Filter a suitable measured aliquot of the culture under a vacuum or pressure not to exceed 8psi;
- Rinse the filter funnel with D.I. water;
- Dry the filter to constant weight at 70°C, cool in a desiccator for two hours and weigh.

Basic instrument used: Analytical Balance; Mettler ME 30.

### B. SPECTROPHOTOMETRIC DETERMINATION OF CHLOROPHYLL a (2)

Basic instrument used: Stectrophotometer; Bausch & Lomb, Spectronic 20.

### C. IMPROVED NEUBAUER 0.1 mm DEEP HEMOCYTOMETER COUNTING CHAMBER AND OPTICAL MICROMETER (Used to measure diameter of algal MCV). (3)


(1). Miller, W. E., J. C. Greene, and T. Shiroyama. 1978. Selenastrum capricornutum Printz Algal Assay Bottle Test: Experimental Design, Application, and Data Interpretation Protocol. Ecol. Res. Series EPA-600/9-78-018. Corvallis, Oregon. pp. 27.

(2). APHA-AWWA-WPCF (1975). "Standard Methods for the Examination of Water and Wastewater," (M. Franson, manag. ed.), 14th edition, pp. 1030-1031.

(3). Brite-Line (R), American Optical Corporation, Buffalo, New York.

ENVIRONMENTAL LABORATORY
ALGAL ASSAY : BOTTLE TEST (AA:BT)
ALGAL GROWTH RESPONSE TO

EC-143 LOT 34

$EC_{50}$'s

| ), YS | EXPOSURE (1) CELL-DRY WEIGHT (mg/l) | EXPOSURE (1) CELL COUNT (no. cells/ml) | NO. DAYS | RECOVERY (2) CELL-DRY WEIGHT (mg/l) | RECOVERY (2) CELL COUNT (no. cells/ml) |
|---|---|---|---|---|---|
| | 148.7 (56.8-344) | 49.4 (28.1-75.1) | 10 | | |
| | 69.7 (34.1-117.9) | 29.7 (20.16-39.16) | 7 | | |
| | 49 (14.7-96) | 27.1 (8.2-49.7) | 4 | | |
| | 72.7 (24.16-146.5) | 43.4 (14.3-81.3) | | | |

SEQUENTIAL COMBINATIONS OF EXPOSURE/RECOVERY PERIODS (DAYS):

4 + 10
7 + 7
10 + 4
14 + 0

(1) Productivity is based on percent growth reduction; values indicate inhibitory response.

(2) Productivity is based on percent growth reduction; values indicate recovery response.