

AR226-0187

OES-4

PROTOCOL

PFOS: A 96-HOUR TOXICITY TEST  
WITH THE FRESHWATER DIATOM (*Navicula pelliculosa*)

U.S. Environmental Protection Agency  
Series 850 - Ecological Effects Test Guidelines  
OPPTS Number 850.5400

3M Lab Request No. U2723

Submitted to

3M Corporation  
Environmental Laboratory  
935 Bush Avenue  
St. Paul, Minnesota 55106

***Wildlife International, Ltd.***

8598 Commerce Drive  
Easton, Maryland 21601  
(410) 822-8600

September 17, 1999

004498

PFOS: A 96-HOUR TOXICITY TEST  
WITH THE FRESHWATER DIATOM (*Navicula pelliculosa*)

SPONSOR:

3M Corporation  
Environmental Laboratory  
935 Bush Avenue  
St. Paul, Minnesota 55106

SPONSOR'S REPRESENTATIVE:

Rochelle R. Robideau

TESTING FACILITY:

Wildlife International, Ltd.  
8598 Commerce Drive  
Easton, Maryland 21601

STUDY DIRECTOR:

~~Kurt Dretter~~ Cary Sutherland HK 1/10/00  
Senior Aquatic Biologist

LABORATORY MANAGEMENT:

Henry O. Krueger, Ph.D.  
Director of Aquatic Toxicology & Non-Target Plants

FOR LABORATORY USE ONLY

|                                                                          |                                         |
|--------------------------------------------------------------------------|-----------------------------------------|
| Proposed Dates:                                                          |                                         |
| Experimental<br>Start Date: _____                                        | Experimental<br>Termination Date: _____ |
| Project No.: <u>454A-112</u>                                             |                                         |
| Test Concentrations: _____                                               |                                         |
| Test Substance No.: _____ Reference Substance No. (if applicable): _____ |                                         |

PROTOCOL APPROVAL

  
STUDY DIRECTOR

1/28/00  
DATE

  
LABORATORY MANAGEMENT

1/28/00  
DATE

  
SPONSOR'S REPRESENTATIVE

1/6/00  
DATE

004499

PROTOCOL NO.: 454/091799/NAV96/SUB454

3M LAB REQUEST NO. U2723

## INTRODUCTION

Wildlife International, Ltd. will conduct a five-day toxicity test with the freshwater diatom, *Navicula pelliculosa*, for the Sponsor at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. The study will be performed based on procedures in the U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.5400 (1). Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site, or at an alternative location to be specified in the final report.

## PURPOSE

The purpose of this study is to determine the toxicity of Perfluorooctane Sulfonic Acid, Potassium Salt (hereafter referred to as PFOS) to the freshwater diatom, *Navicula pelliculosa*.

## EXPERIMENTAL DESIGN

The freshwater diatom, *Navicula pelliculosa*, will be exposed to a geometric series of six test concentrations and a negative (culture medium) control for 96 hours. Target concentrations will not exceed 100 mg/L or the solubility limit of the test substance in water. Generally, the nominal concentration of each test substance used in the definitive test will be at least 50% of the next higher treatment, unless information concerning the concentration-effect curve indicates that a different dilution factor would be more appropriate.

Test solutions will be inoculated with 10,000 cells/mL. Three "biological" replicates per experimental group will be prepared for evaluating cell densities. One or more additional "analytical" replicates will be included in the test, as needed, to provide test solution for concentration verification on Day 3 of the exposure. An abiotic replicate at the highest concentration will be included in the test and will be sampled on Day 3 and Day 4. In order to control bias, the position of the flasks will be determined by indiscriminate draw daily during the exposure period. No other potential sources of bias are expected to affect the results of the study.

The response of the algae will be measured in terms of cell density, biomass expressed as area under the growth curve, and growth rate. An EC50 (i.e., the theoretical test concentration that produces a 50% reduction in the measured parameter) value for cell density will be calculated for each 24 hour interval. EC10, EC50 and EC90 values for cell density, biomass ( $E_bC$ ) and growth rate ( $E_rC$ ) will be calculated, if

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possible, at the 72 and 96 hour intervals. The no observed adverse effect concentration (NOAEC), which is the highest test concentration that induces no adverse inhibitory effect on growth, will be determined relative to each parameter at 72 and 96 hours based upon evaluation of the statistical results and the dose-response pattern. At the end of the 96-hour exposure, algistatic effects will be differentiated from algicidal effects in those treatments which are maximally inhibited.

### MATERIALS AND METHODS

#### Test Substance

Information on the characterization of test, control or reference substances is required by Good Laboratory Practice Standards (GLP). The Sponsor is responsible for providing Wildlife International, Ltd. written verification that the test substance has been characterized according to GLPs prior to its use in the study. If written verification of GLP test substance characterization is not provided to Wildlife International, Ltd., it will be noted in the compliance statement of the final report. The attached form IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR (Appendix I) is to be used to provide information necessary for GLP compliance.

The Sponsor is responsible for all information related to the test substance and agrees to accept any unused test substance and/or test substance containers remaining at the end of the study.

#### Test Solution Preparation

The desired concentrations of PFOS will be obtained by preparing a single stock solution in medium and diluting the appropriate volume of stock solution with medium. The test solutions may also be prepared using secondary dilutions of the primary stock, based upon the concentration desired.

The test substance will be administered to the test organism in algal medium. This route of administration was selected because it represents the most likely route of exposure to algae, which exist suspended in a water column.

#### Test Organism

The test species will be the freshwater diatom, *Navicula pelliculosa*. This species is representative of an important group of algae and was selected for use in the test based upon past use and ease of handling in the laboratory. Stock cultures, obtained from the Culture Collection of Algae at the University of Texas

at Austin or another supplier, will be maintained in culture medium at Wildlife International, Ltd. for a minimum of two weeks prior to use in a toxicity test. Diatoms used in toxicity tests will be in exponential growth phase, which is defined as the period of growth when algal cells are dividing at a constant rate.

Just prior to beginning the test, an inoculum of the stock culture will be prepared so that each milliliter of inoculum contains enough cells to provide an initial cell density of approximately 10,000 cells/mL in each replicate.

## Culture Medium

Culture medium prepared according to Wildlife International, Ltd. Standard Operating Procedures will be used as dilution water. The concentrations of the components in the medium are presented in Table 1 (2). Stock nutrient solutions will be prepared by adding reagent-grade or better chemicals to Wildlife International, Ltd. well water purified by reverse-osmosis. Appropriate volumes of the stock nutrient solutions will then be diluted with purified well water to prepare the medium. The medium will be filter sterilized (0.22  $\mu$ m) or autoclaved prior to use. Analyses will be performed at least once annually to determine the concentrations of selected organic and inorganic constituents of the well water and results of the most recent GLP compliant analyses will be summarized in the final report.

Specifications for acceptable levels of contaminants have not been established for culture medium. However, there are no levels of contaminants reasonably expected to be present in the medium that are considered to interfere with the purpose or conduct of the study.

## Test Apparatus

Test chambers will be sterile, 250-mL polycarbonate Erlenmeyer flasks plugged with foam stoppers and containing 100 mL of test solution. The test chambers will be indiscriminately positioned on a mechanical shaker table in an environmental chamber and will be shaken continuously at approximately 100 rpm. Test chambers will be labelled with the project number, test concentration and replicate.

## Environmental Conditions

Test flasks will be held at  $24 \pm 2^\circ\text{C}$  under continuous cool-white fluorescent lighting at an intensity of  $4300 \pm 10\%$  lux. Light intensity will be measured at five locations surrounding the test flasks on Day 0

of the test. Temperature will be measured twice daily in a container of water adjacent to the test flasks in the environmental chamber using a calibrated, hand-held mercury thermometer.

The pH of each treatment and control group will be measured at test initiation and terminating using a Fisher Accumet Model 915 pH meter or equivalent. Samples for pH measurement at test initiation will be collected from the individual batches of test solution prepared for each treatment and control group. At test termination, pH will be measured in pooled samples of test solution collected from each of the three replicates of each respective treatment and control group.

#### Biological Measurements

Cell densities will be monitored during the test by conducting cell counts using a hemacytometer and a microscope. One sample will be collected from each replicate of the treatment and control groups at approximate 24-hour intervals during the exposure period. The samples will be evaluated immediately or will be held in the dark at approximately 4°C until cell counts can be performed. Each sample will be diluted using an electrolyte solution (Isoton®), as needed, to maintain counting accuracy. A small amount of each sample will be loaded onto a hemacytometer and the total number of cells in 10 grids will be counted. The cell density of the sample will be calculated based on the mean number of cells per grid.

Microscopic examination of algae will be made to assess changes in gross morphology. Algae will be observed to assess changes in cell shape, cell color, and aggregation (clumping) of the cells. Observations will be used to determine whether treatment-related effects include those changes in gross morphology.

#### Sampling for Analytical Measurements

Samples of the exposure solutions will be collected on Days 0, 3 and 4 and analyzed for test substance concentrations. Day 0 samples will be taken from each batch of test solution prior to their distribution among the replicate test chambers. Day 3 samples will be collected from the extra replicate test chamber(s) included in the test to provide sample for that analysis and from the abiotic replicates. If more than one replicate is included for the Day 3 sample analysis, then the solutions from each replicate will be composited to provide the sample for that treatment. Day 4 samples will be composited solutions from the three replicates remaining at the end of the test.

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Samples collected on Days 3 and 4 may be centrifuged or filtered to remove algal cells prior to analysis. Samples will be analyzed immediately or placed in an appropriate storage container (e.g., polypropylene or polyethylene bottle) and stored until analyzed. The sample scheme is summarized below:

PROPOSED NUMBERS OF VERIFICATION SAMPLES

| Experimental Group                     | Day 0 | Day 3          | Day 4          |
|----------------------------------------|-------|----------------|----------------|
| Control                                | 1     | 1              | 1              |
| Solvent Control (if needed)            | 1     | 1              | 1              |
| Level 1-Low Concentration              | 1     | 1              | 1              |
| Level 2                                | 1     | 1              | 1              |
| Level 3                                | 1     | 1              | 1              |
| Level 4                                | 1     | 1              | 1              |
| Level 5                                | 1     | 1              | 1              |
| Level 6-High Concentration             | 1     | 2 <sup>1</sup> | 2 <sup>1</sup> |
| Totals                                 | 8     | 9              | 9              |
| <sup>1</sup> Includes abiotic samples. |       |                |                |

The above numbers of samples represent those collected from the test and do not include quality control (QC) samples such as matrix blanks and fortifications prepared and analyzed during the analytical chemistry phase of the study.

#### Analytical Chemistry

Chemical analysis of the samples will be performed by Wildlife International Ltd. The analytical method used will be based upon methodology provided by the Sponsor and identified in Appendix II. Modifications made to the analytical method will be documented in the raw data and described in the final report.

#### Statistical Analyses

Biomass expressed as area under the growth curve will be calculated for the treatment and control groups using the following formula:

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$$A = ((N_1 - N_0)/2)(t_1) + ((N_1 + N_2 - 2N_0)/2)(t_2 - t_1) + \dots + ((N_{n-1} + N_n - 2N_0)/2)(t_n - t_{n-1})$$

Where:

- A = Area under the growth curve
- N<sub>0</sub> = Measured number of cells/mL at t<sub>0</sub>
- N<sub>1</sub> = Measured number of cells/mL at t<sub>1</sub>
- N<sub>2</sub> = Measured number of cells/mL at t<sub>2</sub>
- N<sub>n</sub> = Measured number of cells/mL at t<sub>n</sub>
- t<sub>1</sub> = Time of first measurement after beginning of test (hours)
- t<sub>2</sub> = Time of second measurement after beginning of test (hours)
- t<sub>n</sub> = Time of nth measurement after beginning of test (hours)

Growth rates (μ) will be calculated for the treatment and control groups using the following formula:

$$\mu = \frac{\ln N_n - \ln N_0}{t_n - t_0}$$

- μ = Average specific growth rate
- N<sub>0</sub> = Measured number of cells/mL at t<sub>0</sub>
- N<sub>n</sub> = Measured number of cells/mL at t<sub>n</sub>
- t<sub>0</sub> = Time of beginning of test (hours)
- t<sub>n</sub> = Time of nth measurement after beginning of test (hours)

Percent inhibition will be calculated for each treatment group based upon mean cell density, biomass and growth rate using the following formula:

$$\text{Percent Inhibition} = \frac{\text{Mean Control - Mean Treatment Response}}{\text{Mean Control Response}} \times 100$$

EC values will be determined, when possible, using linear interpolation or other suitable techniques with treatment response (i.e. cell densities, biomass and growth rate) and exposure concentration data for each 24-hour interval of the test.

A no observed adverse effect concentration (NOAEC) will be selected based on an evaluation of the dose-response pattern and the results of the statistical analysis using individual replicate values of the cell density, biomass and growth rate. Statistically significant differences between the control and the treatment groups will be identified using analysis of variance (ANOVA) and a test to compare treatment mean

responses to the control response (e.g., Dunnett's test or Bonferroni's t-test). Data will be assessed for normality and homogeneity of variances prior to performing the ANOVA. Transformations will be used to correct any condition of non-normality or unequal error variances, if possible. If a solvent control is used in addition to the negative control, their responses will be compared using Student's t-test. Data from the two control groups will be pooled for treatment level analyses if no statistical differences exist. Otherwise, all comparisons will be made to the solvent control group. Statistical comparisons for the ANOVA and the comparison of mean responses will be carried out using TOXSTAT® V3.5 software (4) or equivalent.

#### **RECORDS TO BE MAINTAINED**

Records to be maintained for data generated at Wildlife International, Ltd. will include, but not be limited to:

1. Copy of signed protocol.
2. Identification and characterization of the test substance, if provided by Sponsor.
3. Dates of initiation and termination of the test.
4. Source of algae.
5. Culture conditions.
6. Growth measurements.
7. Calculation and preparation of test concentrations.
8. Observations.
9. If applicable, the methods used to analyze test substance concentrations and the results of analytical measurements.
10. Statistical calculations.
11. Test conditions and physical/chemical measurements.
12. Copy of final report.

#### **FINAL REPORT**

A final report of the results of the study will be prepared by Wildlife International, Ltd. The report will include, but not be limited to the following, when applicable:

1. Name and address of the facility performing the study.

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2. Dates on which the study was initiated and completed. It is the responsibility of the Sponsor to provide the final date that data are recorded for chemistry pathology and/or supporting evaluations that may be generated at other laboratories.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Objectives and procedures stated in the approved protocol, including any changes in the original protocol.
5. The test substance identified by name, chemical abstract number or code number, strength, purity, composition, and other characteristics provided by the Sponsor.
6. Stability and solubility of the test substances under the conditions of administration, if provided by the Sponsor.
7. A description of the methods used to conduct the test.
8. A description of the test system, including the source of the test organisms and their scientific name.
9. A description of the preparation of the test solutions, methods used to allocate organisms to the test chambers and begin the test, numbers of organisms and chambers per treatment, and duration of the test.
10. A description of circumstances that may have affected the quality or integrity of the data.
11. The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study.
12. A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the biological data and analytical chemistry data, and a statement of the conclusions drawn from these analyses.
13. Statistical methods employed for analyzing the data.
14. The signed and dated reports of each of the individual scientists or other professionals involved in the study.
15. The location where raw data and final report are to be stored.
16. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and the dates of any findings reported to the Study Director and Management.
17. If it is necessary to make corrections or additions to a final report after it has been accepted, such changes shall be made in the form of an amendment issued by the Study Director. The amendment

shall clearly identify the part of the final report that is being amended and the reasons for the alteration. Amendments shall be signed and dated by the Study Director.

#### CHANGING OF PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations signed by the Study Director and filed with the raw data. All changes to the protocol will be indicated in the final report.

#### GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 160 and/or Part 792); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau). Each study conducted by Wildlife International, Ltd. is routinely examined by the Wildlife International, Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International, Ltd. The Sponsor will be responsible for compliance with Good Laboratory Practices for procedures performed by other laboratories (e.g., residue analyses or pathology). Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site, or at an alternative location to be specified in the final report.

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REFERENCES

- 1 U.S. Environmental Protection Agency. 1996. Series 850- Ecological Effects Test Guidelines (draft), OPPTS Number 850.5400: Algal Toxicity, Tiers I and II.
- 2 ASTM Standard Guide 1218-90E. 1990. *Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae*. American Society for Testing and Materials. Philadelphia, Pennsylvania.
- 3 Norberg-King, T.J. 1993. *A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (IC<sub>50</sub>) Approach (Version 2.0)*. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, Minnesota.
- 4 West, Inc. and D. D. Gulley. 1996. *TOXSTAT Release 3.5*. Western EcoSystems Technology, Inc. Cheyenne, Wyoming.

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TABLE 1  
FRESHWATER ALGAL MEDIUM WITH SILICA CONSTITUENTS

| Component                                           | Nominal Concentration |
|-----------------------------------------------------|-----------------------|
| MgCl <sub>2</sub> •6H <sub>2</sub> O                | 12.16 mg/L            |
| CaCl <sub>2</sub> •2H <sub>2</sub> O                | 4.40 mg/L             |
| H <sub>3</sub> BO <sub>3</sub>                      | 0.1856 mg/L           |
| MnCl <sub>2</sub> •4H <sub>2</sub> O                | 0.416 mg/L            |
| ZnCl <sub>2</sub>                                   | 3.28 g/L              |
| FeCl <sub>3</sub> •6H <sub>2</sub> O                | 0.1598 mg/L           |
| CoCl <sub>2</sub> •6H <sub>2</sub> O                | 1.428 g/L             |
| Na <sub>2</sub> MoO <sub>4</sub> •2H <sub>2</sub> O | 7.26 g/L              |
| CuCl <sub>2</sub> •2H <sub>2</sub> O                | 0.012 g/L             |
| Na <sub>2</sub> EDTA•2H <sub>2</sub> O              | 0.300 mg/L            |
| NaNO <sub>3</sub>                                   | 25.50 mg/L            |
| MgSO <sub>4</sub> •7H <sub>2</sub> O                | 14.70 mg/L            |
| K <sub>2</sub> HPO <sub>4</sub>                     | 1.044 mg/L            |
| NaHCO <sub>3</sub>                                  | 15.00 mg/L            |
| Na <sub>2</sub> SiO <sub>3</sub> •9H <sub>2</sub> O | 20.0 mg/L             |
| Na <sub>2</sub> SeO <sub>3</sub> •5H <sub>2</sub> O | 0.010 mg/L            |

The pH of the medium will be adjusted, as necessary, to 7.5 ± 0.1 using 0.1 N NaOH or 10% HCl.

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APPENDIX I

IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

- I. Test Substance Identity (name to be used in the report): PFOS (Perfluorooctane Sulfonic Acid Potassium Salt)  
Reference Standard (if applicable): Analytical Standard: N/A

Internal Standard: 1,1,2,2H,H,H,H Perfluorooctane Sulfonic Acid

Test Substance Sample Code or Batch Number: Lot 217

Test Substance Purity (% Active Ingredient): 98.9 Expiration Date: 2008

II. Test Substance Characterization

Have the identity, strength, purity and composition or other characteristics which appropriately define the test substance and reference standard been determined prior to its use in this study in accordance with GLP Standards? Yes x No

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International, Ltd.

Ambient

Has the stability of the test substance under these storage conditions been determined in accordance with GLP Standards? Yes x No

Other pertinent stability information:

IV. Test Concentrations:

x Adjust test concentration to 100% a.i. based upon the purity (%) given above.

Do not adjust test concentration to 100% a.i. Test the material AS IS.

V. Toxicity Information:

Mammalian: Rat LD50 251 mg/kg Mouse LD50 N/A

Aquatic:

Invertebrate Toxicity (EC/LC50)

Fish Toxicity (LC50)

Daphnia magna: 27 mg/L

Rainbow Trout: 11 mg/L

Daphnia magna: 50 mg/L

Fathead Minnow: 38 mg/L

Other Toxicity Information (including findings of chronic and subchronic tests):

Please see MSDS

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APPENDIX II

Analytical Method Provided by Sponsor

Samples will be analyzed based upon procedures provided by the Sponsor in the following analytical methods:

1. Liquid Chromatography Mass Spectrometry (LCMS) Method for the Determination of Perfluorooctane Sulfonic Acid Potassium Salt (PFOS) In Freshwater, Saltwater and Algal Medium

A copy of the above method will be maintained in the raw data. The actual methodology used to analyze the test samples will be documented in the raw data and summarized in the final report.

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AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PFOS: A 96-HOUR TOXICITY TEST WITH THE FRESHWATER DIATOM  
(*Navicula pelliculosa*)

PROTOCOL NO: 454/091799/NAV96/SUB454

AMENDMENT NO.: 1

SPONSOR: 3M Corporation

PROJECT NO.: 454A-112

EFFECTIVE DATE: February 23, 2000

3M LAB REQUEST NO.: U2723

AMENDMENT: Page 2

Add: Experimental Start Date: February 25, 2000  
Experimental Termination Date: February 29, 2000  
Test Concentrations: Negative Control, 70.0, 92.5, 125, 167, 225, 300 and 395 mg a.i./L  
Test Substance No.: 4675

REASON: The above information was not known when the protocol was signed by the Study Director.

AMENDMENT: Experimental Design, Page 3

Change: Target concentrations will not exceed 100 mg/L or the solubility limit of the test substance in water.

To: Target concentrations will not exceed 1000 mg/L or the solubility limit of the test substance in water.

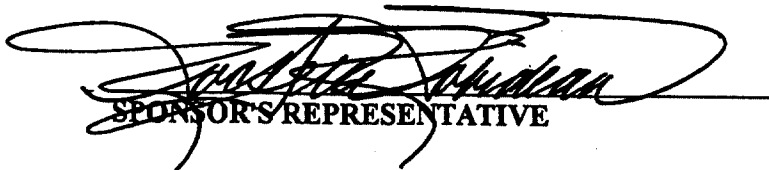
REASON: Nominal test concentrations exceed 100 mg/L.

  
STUDY DIRECTOR

2/29/00  
DATE

  
LABORATORY MANAGEMENT

2/29/00  
DATE

  
SPONSOR'S REPRESENTATIVE

3/2/00  
DATE

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QA  
KH 2-29-00

## AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PFOS: A 96-HOUR TOXICITY TEST WITH THE FRESHWATER DIATOM  
(*Navicula pelliculosa*)

PROTOCOL NO: 454/091799/NAV96/SUB454

AMENDMENT NO.: 2

SPONSOR: 3M Corporation

PROJECT NO.: 454A-112

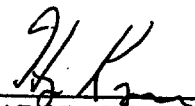
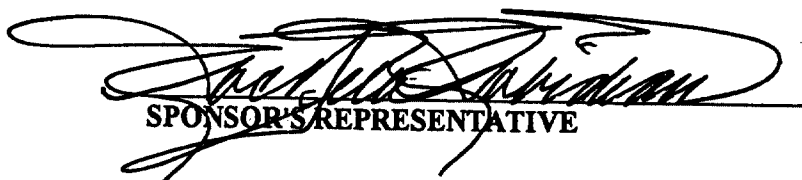
EFFECTIVE DATE: February 28, 2000

3M LAB REQUEST NO.: U2723

AMENDMENT: Biological Measurements Page 6

Add: At the end of the test, algistatic effects, which result in the inhibition of cell growth, will be differentiated from algicidal effects, which result in the death of cells. Aliquots (0.5 mL) of test solution will be taken from each replicate of those treatment groups where growth is maximally inhibited. Maximally inhibited treatments are those in which it cannot be visually determined if live algal cells are present (e.g., absence of color to the test solution). These aliquots will be pooled by concentration and diluted with untreated culture medium to a concentration of the test substance that theoretically should not affect growth. A negative control will be prepared by diluting a 0.5-mL aliquot from one negative control replicate to 100 mL with culture medium. Growth of these subcultures will be monitored for a maximum of 9 days to determine whether inhibition of growth observed during the test is reversible. Samples will be collected for cell counts at recovery phase initiation, termination and at approximately 3-day intervals between initiation and termination. The recovery phase may be terminated once algal growth is sufficient to indicate that the algal cells collected from the treatment groups have fully recovered from effects due to the test substance. This will be determined through visual examination of the recovery test solutions.

REASON: To add a recovery phase to the protocol.

  
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