

**Sanitized Version for the  
AR-226 Public Electronic Docket  
(CBI and copy restricted documents removed)**

**Index**

|    | <b>DOCUMENT DESCRIPTION</b>                                                                            | <b>NO.<br/>OF<br/>PAGES</b> | <b>CBI OR OTHER RESTRICTION?</b>                                                                                                                                                                    |
|----|--------------------------------------------------------------------------------------------------------|-----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1  | March 7, 2007 letter from DuPont to EPA                                                                | 1                           | No restriction                                                                                                                                                                                      |
| 2  | DuPont Investment in Fluorotelomer<br>Environmental Fate Science 2004-2007                             | 1                           | CBI – removed                                                                                                                                                                                       |
| 3  | Appendix I Divider Sheet                                                                               | 1                           | No restriction                                                                                                                                                                                      |
| 4  | Cost Summary – Telomers                                                                                | 1                           | CBI – removed                                                                                                                                                                                       |
| 5  | Testing Invoices – Set 1                                                                               | 1                           | CBI – removed                                                                                                                                                                                       |
| 6  | Testing Invoices – Set 2                                                                               | 1                           | CBI – removed                                                                                                                                                                                       |
| 7  | Protocol H-26634                                                                                       | 15                          | No restriction                                                                                                                                                                                      |
| 8  | Protocol H-26665                                                                                       | 15                          | No restriction                                                                                                                                                                                      |
| 9  | Appendix II Divider Sheet                                                                              | 1                           | No restriction                                                                                                                                                                                      |
| 10 | Testing Invoices                                                                                       | 1                           | CBI – removed                                                                                                                                                                                       |
| 11 | Appendix III Divider Sheet                                                                             | 1                           | No restriction                                                                                                                                                                                      |
| 12 | DuPont Investment in Fluorotelomer<br>Environmental Fate Science                                       | 1                           | CBI – removed                                                                                                                                                                                       |
| 13 | Appendix IV Divider Sheet                                                                              | 1                           | No restriction                                                                                                                                                                                      |
| 14 | Summary of EPA Submissions (dated<br>07/11/2002)                                                       | 7                           | No restriction                                                                                                                                                                                      |
| 15 | Biodegradation studies of fluorotelomer-<br>based polymers in soils                                    | 1                           | Subject to copyright – removed;<br>Contact information to request<br>authorization to copy:<br>Dr. Robert Buck (302-892-8935<br>robert.c.buck@usa.dupont.com)                                       |
| 16 | Biodegradation studies of fluorotelomer-<br>based polymers in activated sludge, soil,<br>and sediments | 1                           | Not for further distribution without<br>author approval – removed;<br>Contact information to request<br>authorization to copy:<br>Dr. Robert Buck (302-892-8935 or<br>Robert.C.Buck@usa.dupont.com) |

**Company Sanitized**



DuPont Chemical Solutions Enterprise  
P. O. Box 80023  
Wilmington, DE 19880-0023

March 7, 2007

Dear Jim,

Thank you for your time in meeting with us February 20<sup>th</sup>, as well as for the good discussion that ensued. Enclosed please find the additional information you requested on:

- 1) The comprehensive, peer reviewed study commissioned by DuPont and conducted by Environ on the global fate and exposure potential from DuPont Fluorotelomer products and
- 2) Information on the studies that DuPont has conducted and commissioned on the biodegradation of fluorotelomer intermediates and products.

Specifically, you will find the Wildlife International invoices in Appendix I (outlined in the attached document pages 2 and 3), invoices from ENVIRON in Appendix II (outlined in the attached document page 4), and Academic Studies we have supported in Appendix III pages 9, 10, and 19-41 (outlined in the attached document page 5). As well, I have included a document in Appendix IV that reviews the information we have shared with the EPA and at various scientific meetings. Included in this Appendix is the most recent information shared at the SETAC meeting in November. If you would like any of these documents please don't hesitate to ask.

As a follow up I would like to arrange a time to discuss these items and their significance for future work with you at your earliest convenience.

With best regards,

A handwritten signature in black ink, appearing to read "Henry E. Bryndza".

Henry E. Bryndza  
Technology Directory  
DuPont Chemical Solutions Enterprise  
DuPont Central Research & Development

**CONFIDENTIAL BUSINESS INFORMATION**

**DOCUMENT REMOVED**

Document Number in Index: 2

Document Description: DuPont Investment in Fluorotelomer Environmental Fate Science 2004-2007

APPENDIX I

**CONFIDENTIAL BUSINESS INFORMATION**

**DOCUMENT REMOVED**

Document Number in Index: 4

Document Description: Cost Summary - Telomers

**CONFIDENTIAL BUSINESS INFORMATION**

**DOCUMENT REMOVED**

Document Number in Index: 5

Document Description: Testing Invoices – Set 1

**CONFIDENTIAL BUSINESS INFORMATION**

**DOCUMENT REMOVED**

Document Number in Index: 6

Document Description: Testing Invoices – Set 2

PROTOCOL

H-26634: TRANSFORMATION POTENTIAL  
IN AEROBIC AND ANAEROBIC SOILS

This protocol complies with the  
Organisation for Economic Cooperation and Development  
OECD Guideline 307 Adopted April 2002

Submitted to

E.I. du Pont de Nemours and Company  
Wilmington, Delaware 19898  
USA

***Wildlife International, Ltd.***

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

February 17, 2005



# Wildlife International, Ltd.

- 2 -

## H-26634: TRANSFORMATION POTENTIAL IN AEROBIC AND ANAEROBIC SOILS

SPONSOR: E.I. du Pont de Nemours and Company

SPONSOR'S REPRESENTATIVE: William Berti

TESTING FACILITY: Wildlife International, Ltd.  
8598 Commerce Drive  
Easton, Maryland 21601

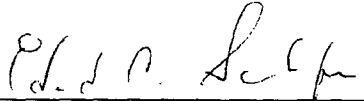
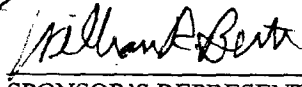
STUDY DIRECTOR: Edward C. Schaefer  
Wildlife International, Ltd.

LABORATORY MANAGEMENT: Henry O. Krueger, Ph.D.  
Director of Aquatic Toxicology and Non-Target Plants

### FOR LABORATORY USE ONLY

|                                                                                                                          |                                                        |
|--------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|
| Proposed Dates:                                                                                                          |                                                        |
| Experimental<br>Start Date: <u>15 MARCH 2005</u>                                                                         | Experimental<br>Termination Date: <u>15 MARCH 2006</u> |
| Project No.: <u>112E-108</u>                                                                                             |                                                        |
| Test Concentrations: <u>200 mg/kg ; 250 mg/kg ; 10 mg/kg</u>                                                             |                                                        |
| Test Substance No.: <u>6918</u> Reference Substance No. (if applicable): <u>6828, 6826, 6824, 6849, 6944, 6943, 6945</u> |                                                        |

### PROTOCOL APPROVAL

|                                                                                            |                      |
|--------------------------------------------------------------------------------------------|----------------------|
| <u></u> | <u>10 MARCH 2005</u> |
| STUDY DIRECTOR                                                                             | DATE                 |
| <u></u> | <u>3/09/05</u>       |
| LABORATORY MANAGEMENT                                                                      | DATE                 |
| <u></u> | <u>17-Feb-2005</u>   |
| SPONSOR'S REPRESENTATIVE                                                                   | DATE                 |

PROTOCOL NO.: 112/021705/SED-TRANSa/SUB112

# *Wildlife International, Ltd.*

---

- 3 -

## INTRODUCTION

Soil may be exposed to chemicals by direct application, spray drift, run-off, drainage, waste disposal, industrial or agricultural effluent and atmospheric deposition. This protocol describes a laboratory test method to assess the transformation of the test substance in aerobic and anaerobic soil systems. No bias is expected in this type of study.

## OBJECTIVE

The objective of the study is to assess the potential for transformation of the test substance in aerobic and anaerobic soils.

## EXPERIMENTAL DESIGN

The test will be conducted with four test soils under both aerobic and anaerobic condition. Four groups will be established for each soil type and condition (aerobic or anaerobic): (1) untreated (control) live soil; (2) 200 mg test substance/kg dw treated live soil; (3) 200 mg test substance/kg dw treated sterile soil; (4) 250 µg 8-2 telomer B alcohol/kg dw treated sterile soil; 10 µg 8-2 telomer B acid /kg dw; 10 µg 8-2 unsaturated telomer B acid/kg; and 10 µg perfluorooctanoic acid/kg dw, C9, C10, and C11 at 10 µg/kg dw all added to a sterile soil. The test systems will be incubated in sealed containers at approximately 20°C for up to 1 year. Two incubation chambers from each of the groups will be removed at appropriate time intervals and the soil samples will be extracted and analyzed for potential metabolites. In addition to the day zero samples, a minimum of eight additional samplings will be performed. Sufficient incubation chambers to allow for 3 additional sampling intervals will be prepared for the untreated control (1) and 200 mg test substance/kg dw treated live soil (2) groups. These chambers will be sampled as needed at the request of the study monitor.

Additional untreated and test substance treated soil chambers will be prepared for use as matrix fortification samples, biomass determinations, viability controls and to monitor aerobic conditions as necessary.

The test groups will be prepared using 25 grams (dry weight equivalent) of soil. While this amount of soil is less than the 50 to 200 grams specified in the OECD 307 guideline, it was chosen to allow for 1) the extraction of the entire test chamber contents and internal surfaces; 2) sufficient

## *Wildlife International, Ltd.*

---

- 4 -

headspace to minimize the need for active aeration which could result in a loss of potential transformation products.

### **METHODS AND MATERIALS**

The test system and study conditions are based on the OECD Guideline for Testing of Chemicals, Guideline 307, *Aerobic and Anaerobic Transformation in Soil* (1). As this guideline is designed for low molecular weight substances, but not for polymers, adaptations of the method may be necessary for feasibility reasons. Nevertheless, the study will be carried out in such a way that the recommendations given in the guideline will be followed as closely as possible.

#### **Test Substances**

Information on the characterization of test, control or reference substances is required by Good Laboratory Practice (GLP) Standards and Principles. The Sponsor is responsible for providing Wildlife International, Ltd. verification that the test substance has been characterized prior to its use in the study. If 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 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.

The test substance is not radiolabeled and has no single molecular formula. It is a mixture containing perfluoroalkyl groups of varying carbon numbers as polymer side chains.

#### **Test Soils**

Four freshly collected US soils will be used to evaluate the test substances under aerobic and anaerobic conditions. The soils will be collected from each of the following soil orders:

## *Wildlife International, Ltd.*

---

- 5 -

| Soil Order | Percent of US Land* |
|------------|---------------------|
| Alfisol    | 13                  |
| Mollisol   | 25                  |
| Inceptisol | 16                  |
| Ultisol    | 13                  |

\*Percent of total land in the US, from Foth, H.D. 1990. Fundamentals of Soil Science 8 ed. John Wiley & Sons, New York)

The soils will be processed as soon as possible after sampling. Vegetation, larger soil fauna and stones will be removed prior to passing the soil through a 2 mm sieve. The soil used in the sterile groups will be sterilized by cobalt irradiation. The soils may be stored in the dark at  $4 \pm 2$  °C, if necessary. However, storage and pre-incubation time together will not exceed 3 months.

Characterization of the soils will be conducted by Agvise Laboratories (Northwood, North Dakota, USA). The following is a list of the minimum physicochemical properties of the soil to be determined.

- Texture (i.e., percentage of sand, silt and clay)
- pH
- Organic carbon
- Bulk density
- Field moisture capacity
- Cation exchange capacity

The percent moisture and water holding capacity of each of the soil types will be determined. In addition, the microbial biomass of a live sample of each soil type will be determined. The soil microbial biomass will be determined at the beginning of the test prior to adding the test substance and in test and control soil samples at four months, at six months, and at 12 months using the fumigation-extraction method.

### **Test Apparatus and Conditions**

The test chambers will be glass serum bottles with foil lined closures and will be identified by project number, test substance ID, test concentration, and unique identifier. The chambers will be incubated statically at approximately  $20 \pm 2$  °C.

## *Wildlife International, Ltd.*

---

- 6 -

### **Soil Pre-Incubation**

The soil moisture of each test soil content will be adjusted to 40 to 60% of water holding capacity (which is equivalent to a pF of between 2.0 and 2.5, or from 0.1 to 0.33 bar). The test soil then will be allowed to warm to the test temperature for a least 2 days. A pre-incubation period of at least 7 days for the aerobic soils and 14 days for the anaerobic soils will be performed under the appropriate conditions (i.e. temperature, soil moisture content, aerobic or anaerobic). Approximately 25 grams dry weight equivalent of test soil will be added to the test chambers. Sterile soil treatments will be dosed at approximately 200 mg/kg (dw) with both chloramphenicol and cycloheximide to inhibit microbial growth. The chambers to be incubated under aerobic conditions will be sealed with septa and incubated. The chambers to be incubated under anaerobic conditions will be flushed with anaerobic mixed gas, sealed with septa and incubated. The soil moisture content should be maintained in the optimal microbial growth range of 40 to 60% water holding capacity (WHC). The soil moisture content should be checked and adjusted, if necessary, at least once during the pre-incubation period by weighing the test vessels. Sterile-filtered demineralized water (degassed sterile-filtered demineralized water for the anaerobic soils) will be added as necessary to compensate for water losses.

### **Preparation of the Test Chambers**

The soil moisture content will be checked and adjusted, if necessary, prior to the addition of the test substance. Sterile-filtered demineralized water (degassed sterile-filtered demineralized water for the anaerobic soils) will be added as necessary to compensate for water. Anaerobic treatments and controls will be prepared within an anaerobic environment. The test substance will be applied to the soil within the test chambers as described below.

Background Blank Control: Soils with no test substance added will be tested in duplicate to check for background concentrations of analytes (see Sampling and Measurements). The entire bottle will be extracted after a sample of the headspace has been taken. The methods and procedures used will be documented in the study records and in the final report.

Test Substance: The test substance dosed treatments will be prepared by dosing approximately 25 grams (dry weight equivalent) of soil with sufficient test substance to deliver 200 mg/kg dry weight. The test substance will be administered by direct weight addition. The test chambers will be sealed with septum lined with aluminum foil, mixed and incubated. The entire bottle

## *Wildlife International, Ltd.*

---

- 7 -

will be extracted after a sample of the headspace has been taken. The methods and procedures used will be documented in the study records and in the final report.

Abiotic Controls: The abiotic controls will be prepared by dosing approximately 25 grams (dry weight equivalent) of  $^{60}\text{Co}$ -sterilized soil with sufficient test substance to deliver 200 mg/kg dry weight. The test substance will be administered by direct weight addition. The entire bottle will be extracted after a sample of the headspace has been taken. The methods and procedures used will be documented in the study records and in the final report.

Spike Recovery Controls: The spike recovery controls will be prepared by dosing approximately 25 grams (dry weight equivalent) of  $^{60}\text{Co}$ -sterilized soil with appropriate volumes of 8-2 TBA stock (250  $\mu\text{g/mL}$  in ethanol) and fluorinated acid stocks (10  $\mu\text{g/mL}$  in water). The stocks will be injected directly into the soil using a glass microsyringe. The test chambers will be immediately sealed with septum lined with aluminum foil and the content of the chambers will be mixed. The entire bottle will be extracted after a sample of the headspace has been taken. The methods and procedures used will be documented in the study records and in the final report.

Viability Controls (untreated): The metabolic activity of untreated test soil will be assessed on a monthly basis. Duplicate incubation chambers for each test soil and condition (aerobic and anaerobic) will be dosed at approximately 100 mg/kg dw with a combination of radiolabeled and non-labelled glucose. The evolved  $^{14}\text{CO}_2$  (aerobic and anaerobic conditions) and  $^{14}\text{CH}_4$  (anaerobic conditions) will be measured and the percent biodegradation calculated. The methods and procedures used will be documented in the study records and in the final report.

Aerobic Controls (treated): The aerobic controls will be prepared by dosing approximately 25 grams (dry weight equivalent) of soil with sufficient test substance to deliver 200 mg/kg dry weight. The test substance will be administered by direct weight addition. The test chambers will be sealed with septum lined with aluminum foil, mixed and incubated. The oxygen content of a sacrificial chamber will be measured at monthly intervals. The methods and procedures used will be documented in the study records and in the final report.

### **Maintenance of Test Chambers**

The soil moisture content should be maintained in the optimal microbial growth range of 40 to 60% water holding capacity (WHC; a pF of between 2.0 and 2.5, or from 0.1 to 0.33 bar). The soil moisture content should be checked and adjusted, if necessary, at regular 1 to 2 week intervals by weighing the test vessels. Adding sterile-filtered demineralized water (degassed sterile-filtered demineralized water for the anaerobic soils) will compensate water losses.

## *Wildlife International, Ltd.*

---

- 8 -

The oxygen content in the headspace of two aerobic control chambers will be assessed on a monthly basis to ensure aerobic conditions. If the mean measured oxygen content within the control chambers is less than 19%, aerobic test chambers will be aerated with filter-sterilized, oil-free air.

If test chambers have to be opened to add water or for monitoring purposes, a sample of the head space will be taken first using a C18 cartridge as described below. The chamber will then be recapped as soon as possible. The septum and cap previously removed will be stored at  $-10^{\circ}\text{C}$  or lower. The C18 will subsequently be extracted and analyzed. Sample extracts will be stored at  $-10^{\circ}\text{C}$  or lower if analysis is delayed more than 24 hours.

### **Sampling and Measurements**

Duplicate chambers from each of the control, treated and spike recovery soils will be extracted (e.g., acetonitrile or other suitable solvent) and analyzed. Proposed sampling intervals will be at 0, 1 and 2 weeks and 1, 2, 4, 6, 9, and 12 months (9 sampling times), however the actual sampling intervals will be documented in the study records and in the final report. More or less frequent intervals may be conducted at the discretion of the Study Director. Potential volatile transformation products in the headspace of the soils chambers will be collected using an appropriate trap (i.e. C18 cartridge). At each sampling time prior to opening the test vessel, the septum will be pierced using a needle connected to a C18 cartridge and syringe. A volume of headspace gas from within the chamber will be pulled through the C18 cartridge using the attached syringe. The C18 will be subsequently extracted and analyzed. Sample extracts will be stored at  $-10^{\circ}\text{C}$  or lower if analysis is delayed more than 24 hours. Extracts will be analyzed for:

1.  $\text{CF}_3(\text{CF}_2)_7\text{CH}_2\text{CH}_2\text{OH}$  (perfluorooctyl ethanol, 8-2 TBA, CAS# 678-39-7)
2.  $\text{CF}_3(\text{CF}_2)_7\text{CH}_2\text{COOH}$  (2-perfluorooctyl ethanoic acid, 8-2 Saturated Acid, CAS#27854-31-5)
3.  $\text{CF}_3(\text{CF}_2)_6\text{CF}=\text{CH}_2\text{COOH}$  (2-H-hexadecafluoro-2-decenoic acid, 8-2 Unsaturated Acid, CAS# 70887-84-2)
4.  $\text{CF}_3(\text{CF}_2)_6\text{COOH}$  (Octanoic acid, pentadecafluoro-; PFOA; CAS# 335-67-1)
5. Heptadecafluorononanoic Acid; CAS#375-95-1 (C9)
6. Nonadecafluorodecanoic Acid; CAS#335-76-2 (C10)

## *Wildlife International, Ltd.*

---

- 9 -

7. Perfluoroundecanoic Acid; CAS#4234-23-5 (C11)

### **Analytical Methods**

Methods of analysis will be verified prior to the start of the study. Analytical reference standards that are used to aid the identification of the test substance and its potential degradation products will be documented in the study records. All chemicals and solvents will be reagent grade or purer. Certificates of analysis (COA) will be provided for all test substances, reference standards, chemicals and solvents, when available. Sources will be documented in the study records. Demineralized water will be used in the study.

### **Quality Criteria**

Recovery for a given sampling time point should range between 70 to 120% for the analysis of the 8-2 TBA, 8-2 Saturated Acid, 8-2 Unsaturated Acid, C9, C10, C11, and PFOA from the spike recovery control samples. These ranges should be interpreted as targets and should not be used as criteria for acceptance of the test. If recoveries and analysis do not meet the criteria of between 70 to 120%, then the study director will consult with the Sponsor's Representatives.

### **8-2 TBA**

Liquid Chromatography/ Mass Spectrometry (LC/MS), Gas Chromatography/Mass Spectrometry (GC/MS), or other suitable method can be used to analyze 8-2 TBA in the extracts of soil and headspace samples. Sample collection, preparation, and analytical methods used will be documented in the study records and the final report.

### **8-2 Saturated Acid, 8-2 Unsaturated Acid, C9, C10, C11, and PFOA**

Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) or other suitable method, will be used to analyze 8-2 Saturated Acid, 8-2 Unsaturated Acid, C9, C10, C11 and PFOA in the extracts of soil samples. Sample collection, preparation, and analytical methods used will be documented in the study records and the final report.

### **EVALUATION OF THE RESULTS**

Statistical methods including means, standard deviations, and regression lines, will be used as appropriate. The concentration of the transformation products in the soil and headspace will be given as



## *Wildlife International, Ltd.*

---

- 10 -

mg kg<sup>-1</sup> (dry weight) and as mole kg<sup>-1</sup> (dry weight) for each sampling interval. Transformation products listed in soil and headspace samples will be plotted against time.

### **HEALTH AND SAFETY**

It is advisable to read the MSDS for the reference chemical and test substance when available. If an MSDS is not available (e.g., the substance is a product of research and development) it is important to review what is known about the substance with a person knowledgeable about the safe handling of the substance, such as the person supplying the substance for testing.

It is advisable to follow guidelines on Storage and Handling of Chemicals; Personal Protective Equipment, Waste Disposal Guide.

### **TEST SUBSTANCE DISPOSAL**

After the issuance of the final report, the remaining test substance will be stored at the testing lab until its expiration date and then destroyed, unless other arrangements are made between the Sponsor and the testing lab.

### **RECORDS TO BE MAINTAINED**

Records to be maintained will include, but not limited to, the following:

1. A copy of the signed protocol.
2. Identification and characterization of the test substance as provided by Sponsor.
3. Study initiation and termination dates.
4. Experimental initiation and termination dates.
5. Test and reference substance preparation and dosing calculations.
6. Soil source and pretreatment data.
7. Results of analytical methods performed.
8. Temperature range recorded during test period.
9. Copy of final report.

## *Wildlife International, Ltd.*

---

- 11 -

### **FINAL REPORT**

A final report of the results of the study will be prepared by Wildlife International, Ltd. and submitted to the Study Monitor no later than one month after the conclusion of the definitive study. The report will include, but not be limited to the following, when applicable:

1. Name and address of facility performing the study.
2. Dates on which the study was initiated and completed.
3. Objectives and procedures stated in the approved protocol, including any changes in the original protocol.
4. Identification and characterization of the test substance as provided by Sponsor.
5. A summary and analysis of the data .
6. A description of the transformations and calculations performed on the data.
7. A description of the methods used and reference to any standard method employed.
8. A description of the test system.
9. A description of the preparation of the test solutions, the testing concentrations, and the duration of the test.
10. A description of all circumstances that may affect the quality or integrity of the data.
11. The name of the study director, the names of other scientists or professionals, and the names of all supervisory personnel, involved in the study.
12. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
13. The location where the raw data and final report are to be stored.

### **CHANGES TO THE FINAL REPORT**

If it is necessary to make corrections or additions to the 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 study that is being amended and the reasons for the alteration. Amendments shall be signed and dated by the Study Director and Laboratory QA.

### **CHANGES TO PROTOCOL**

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and approved by 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

## *Wildlife International, Ltd.*

---

- 12 -

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; will be consistent with OECD Principles of Good Laboratory Practice. 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). When the final report is completed, original copies of the study data and magnetically encoded records generated by Wildlife International, Ltd. for this study will be sent to the Sponsor. A certified copy will be retained in the archives of Wildlife International, Ltd.

*Wildlife International, Ltd.*

---

- 13 -

#### REFERENCES

- 1 **Organisation for Economic Cooperation and Development.** April 2002. *Aerobic and Anaerobic Transformation in Soil.* OECD Guideline 307.

# Wildlife International, Ltd.

## AMENDMENT TO STUDY PROTOCOL

**STUDY TITLE:** H-26634: TRANSFORMATION POTENTIAL IN AEROBIC AND ANAEROBIC SOILS

**PROTOCOL NO:** 112/021705/SED-TRANSa/SUB112 **AMENDMENT NO.:** 1

**SPONSOR:** E.I du Pont de Nemours and Company **PROJECT NO.:** 112E-108

**EFFECTIVE DATE:** 06 May 2005

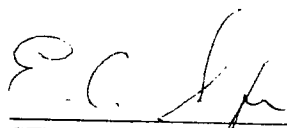
---

**AMENDMENT:** Maintenance of Test Chambers, Page -7-

**CHANGE:** The soil moisture content should be checked and adjusted, if necessary, at regular 1 to 2 week intervals by weighing the test vessels.

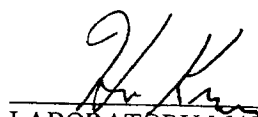
**TO:** The soil moisture content should be checked and adjusted, if necessary, at monthly intervals by weighing the test vessels.

**REASON:** The frequency at which the soil moisture content was checked was reduced based on observed losses.



STUDY DIRECTOR

6 May 2005  
DATE



LABORATORY MANAGEMENT

5 Oct 06  
DATE

Reviewed by QA.  
JHC 10-9-06

Wildlife International Ltd.**DEVIATION TO STUDY PROTOCOL**

**STUDY TITLE:** H-26634: TRANSFORMATION POTENTIAL IN AEROBIC AND ANAEROBIC SOILS

**PROTOCOL NO:** 112/021705/SED-TRANSa/SUB112

**DEVIATION NO.:** 1

**SPONSOR:** E.I du Pont de Nemours and Company

**PROJECT NO.:** 112E-108

**DEVIATION:** Preparation of Test Chambers; Viability Controls (untreated), Page -7-

The protocol indicated that duplicate incubation chambers for each test soil and condition (aerobic and anaerobic) would be dosed at approximately 100 mg/kg dw with a combination of radiolabelled and non-labelled glucose. The test chambers were actually dosed at 3000 mg/kg dw with a combination of radiolabelled and non-labelled glucose.

**REASON:** Oversight by study personnel.

**IMPACT:** In the best judgment of the Study Director, this deviation did not impact the integrity of study. A concentration of 2000 to 4000 mg glucose per kg dry weight soil is a common for the determination of glucose-induced respiration rates.

E.C. Scip  
STUDY DIRECTOR

25 SEPT 2006  
DATE

H. K.  
LABORATORY MANAGEMENT

5 Oct 06  
DATE

PROTOCOL

H-26665: TRANSFORMATION POTENTIAL  
IN AEROBIC AND ANAEROBIC SOILS

This protocol complies with the  
Organisation for Economic Cooperation and Development  
OECD Guideline 307 Adopted April 2002

Submitted to

E.I. du Pont de Nemours and Company  
Wilmington, Delaware 19898  
USA

***Wildlife International, Ltd.***

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

February 17, 2005

# Wildlife International, Ltd.

- 2 -

## H-26665: TRANSFORMATION POTENTIAL IN AEROBIC AND ANAEROBIC SOILS

SPONSOR: E.I. du Pont de Nemours and Company

SPONSOR'S REPRESENTATIVE: William Berti

TESTING FACILITY: Wildlife International, Ltd.  
8598 Commerce Drive  
Easton, Maryland 21601

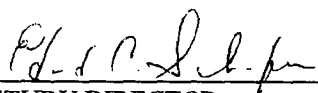
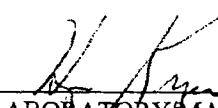
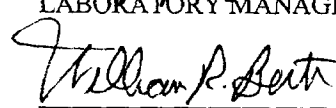
STUDY DIRECTOR: Edward C. Schaefer  
Wildlife International, Ltd.

LABORATORY MANAGEMENT: Henry O. Krueger, Ph.D.  
Director of Aquatic Toxicology and Non-Target Plants

### FOR LABORATORY USE ONLY

|                                                                                              |                                                                  |
|----------------------------------------------------------------------------------------------|------------------------------------------------------------------|
| Proposed Dates:                                                                              |                                                                  |
| Experimental<br>Start Date: <u>15 MARCH 2005</u>                                             | Experimental<br>Termination Date: <u>15 MARCH 2006</u>           |
| Project No.: <u>112E-109</u>                                                                 |                                                                  |
| Test Concentrations: <u>200 mg/Kg ; 250 mg/Kg , 1000 mg/Kg</u> <u>682E, 6826, 6829, 6849</u> |                                                                  |
| Test Substance No.: <u>6919</u>                                                              | Reference Substance No. (if applicable): <u>6944, 6943, 6945</u> |

### PROTOCOL APPROVAL

|                                                                                            |                      |
|--------------------------------------------------------------------------------------------|----------------------|
| <u></u> | <u>10 MARCH 2005</u> |
| STUDY DIRECTOR                                                                             | DATE                 |
| <u></u> | <u>3/09/05</u>       |
| LABORATORY MANAGEMENT                                                                      | DATE                 |
| <u></u> | <u>17-Feb-2005</u>   |
| SPONSOR'S REPRESENTATIVE                                                                   | DATE                 |

PROTOCOL NO.: 112/021705/SED-TRANSb/SUB112

104



## *Wildlife International, Ltd.*

---

- 3 -

### INTRODUCTION

Soil may be exposed to chemicals by direct application, spray drift, run-off, drainage, waste disposal, industrial or agricultural effluent and atmospheric deposition. This protocol describes a laboratory test method to assess the transformation of the test substance in aerobic and anaerobic soil systems. No bias is expected in this type of study.

### OBJECTIVE

The objective of the study is to assess the potential for transformation of the test substance in aerobic and anaerobic soils.

### EXPERIMENTAL DESIGN

The test will be conducted with four test soils under both aerobic and anaerobic condition. Four groups will be established for each soil type and condition (aerobic or anaerobic): (1) untreated (control) live soil; (2) 200 mg test substance/kg dw treated live soil; (3) 200 mg test substance/kg dw treated sterile soil; (4) 250 µg 8-2 telomer B alcohol/kg dw treated sterile soil; 10 µg 8-2 telomer B acid /kg dw; 10 µg 8-2 unsaturated telomer B acid/kg; and 10 µg perfluorooctanoic acid/kg dw, C9, C10, and C11 at 10 µg/kg dw all added to a sterile soil. The test systems will be incubated in sealed containers at approximately 20°C for up to 1 year. Two incubation chambers from each of the groups will be removed at appropriate time intervals and the soil samples will be extracted and analyzed for potential metabolites. In addition to the day zero samples, a minimum of eight additional samplings will be performed. Sufficient incubation chambers to allow for 3 additional sampling intervals will be prepared for the untreated control (1) and 200 mg test substance/kg dw treated live soil (2) groups. These chambers will be sampled as needed at the request of the study monitor.

Additional untreated and test substance treated soil chambers will be prepared for use as matrix fortification samples, biomass determinations, viability controls and to monitor aerobic conditions as necessary.

The test groups will be prepared using 25 grams (dry weight equivalent) of soil. While this amount of soil is less than the 50 to 200 grams specified in the OECD 307 guideline, it was chosen to allow for 1) the extraction of the entire test chamber contents and internal surfaces; 2) sufficient

## *Wildlife International, Ltd.*

---

- 4 -

headspace to minimize the need for active aeration which could result in a loss of potential transformation products.

### **METHODS AND MATERIALS**

The test system and study conditions are based on the OECD Guideline for Testing of Chemicals, Guideline 307, *Aerobic and Anaerobic Transformation in Soil* (1). As this guideline is designed for low molecular weight substances, but not for polymers, adaptations of the method may be necessary for feasibility reasons. Nevertheless, the study will be carried out in such a way that the recommendations given in the guideline will be followed as closely as possible.

#### **Test Substances**

Information on the characterization of test, control or reference substances is required by Good Laboratory Practice (GLP) Standards and Principles. The Sponsor is responsible for providing Wildlife International, Ltd. verification that the test substance has been characterized prior to its use in the study. If 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 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.

The test substance is not radiolabeled and has no single molecular formula. It is a mixture containing perfluoroalkyl groups of varying carbon numbers as polymer side chains.

#### **Test Soils**

Four freshly collected US soils will be used to evaluate the test substances under aerobic and anaerobic conditions. The soils will be collected from each of the following soil orders:

## *Wildlife International, Ltd.*

---

- 5 -

| Soil Order | Percent of US Land* |
|------------|---------------------|
| Alfisol    | 13                  |
| Mollisol   | 25                  |
| Inceptisol | 16                  |
| Ultisol    | 13                  |

\*Percent of total land in the US, from Foth, H.D. 1990. Fundamentals of Soil Science 8 ed. John Wiley & Sons, New York)

The soils will be processed as soon as possible after sampling. Vegetation, larger soil fauna and stones will be removed prior to passing the soil through a 2 mm sieve. The soil used in the sterile groups will be sterilized by cobalt irradiation. The soils may be stored in the dark at  $4 \pm 2$  °C, if necessary. However, storage and pre-incubation time together will not exceed 3 months.

Characterization of the soils will be conducted by Agvise Laboratories (Northwood, North Dakota, USA). The following is a list of the minimum physicochemical properties of the soil to be determined.

- Texture (i.e., percentage of sand, silt and clay)
- pH
- Organic carbon
- Bulk density
- Field moisture capacity
- Cation exchange capacity

The percent moisture and water holding capacity of each of the soil types will be determined. In addition, the microbial biomass of a live sample of each soil type will be determined. The soil microbial biomass will be determined at the beginning of the test prior to adding the test substance and in test and control soil samples at four months, at six months, and at 12 months using the fumigation-extraction method.

### **Test Apparatus and Conditions**

The test chambers will be glass serum bottles with foil lined closures and will be identified by project number, test substance ID, test concentration, and unique identifier. The chambers will be incubated statically at approximately  $20 \pm 2$  °C.

## *Wildlife International, Ltd.*

---

- 6 -

### **Soil Pre-Incubation**

The soil moisture of each test soil content will be adjusted to 40 to 60% of water holding capacity (which is equivalent to a pF of between 2.0 and 2.5, or from 0.1 to 0.33 bar). The test soil then will be allowed to warm to the test temperature for a least 2 days. A pre-incubation period of at least 7 days for the aerobic soils and 14 days for the anaerobic soils will be performed under the appropriate conditions (i.e. temperature, soil moisture content, aerobic or anaerobic). Approximately 25 grams dry weight equivalent of test soil will be added to the test chambers. Sterile soil treatments will be dosed at approximately 200 mg/kg (dw) with both chloramphenicol and cycloheximide to inhibit microbial growth. The chambers to be incubated under aerobic conditions will be sealed with septa and incubated. The chambers to be incubated under anaerobic conditions will be flushed with anaerobic mixed gas, sealed with septa and incubated. The soil moisture content should be maintained in the optimal microbial growth range of 40 to 60% water holding capacity (WHC). The soil moisture content should be checked and adjusted, if necessary, at least once during the pre-incubation period by weighing the test vessels. Sterile-filtered demineralized water (degassed sterile-filtered demineralized water for the anaerobic soils) will be added as necessary to compensate for water losses.

### **Preparation of the Test Chambers**

The soil moisture content will be checked and adjusted, if necessary, prior to the addition of the test substance. Sterile-filtered demineralized water (degassed sterile-filtered demineralized water for the anaerobic soils) will be added as necessary to compensate for water. Anaerobic treatments and controls will be prepared within an anaerobic environment. The test substance will be applied to the soil within the test chambers as described below.

**Background Blank Control:** Soils with no test substance added will be tested in duplicate to check for background concentrations of analytes (see Sampling and Measurements). The entire bottle will be extracted after a sample of the headspace has been taken. The methods and procedures used will be documented in the study records and in the final report.

**Test Substance:** The test substance dosed treatments will be prepared by dosing approximately 25 grams (dry weight equivalent) of soil with sufficient test substance to deliver 200 mg/kg dry weight. The test substance will be administered by direct weight addition. The test chambers will be sealed with septum lined with aluminum foil, mixed and incubated. The entire bottle

# *Wildlife International, Ltd.*

---

- 7 -

will be extracted after a sample of the headspace has been taken. The methods and procedures used will be documented in the study records and in the final report.

Abiotic Controls: The abiotic controls will be prepared by dosing approximately 25 grams (dry weight equivalent) of  $^{60}\text{Co}$ -sterilized soil with sufficient test substance to deliver 200 mg/kg dry weight. The test substance will be administered by direct weight addition. The entire bottle will be extracted after a sample of the headspace has been taken. The methods and procedures used will be documented in the study records and in the final report.

Spike Recovery Controls: The spike recovery controls will be prepared by dosing approximately 25 grams (dry weight equivalent) of  $^{60}\text{Co}$ -sterilized soil with appropriate volumes of 8-2 TBA stock (250  $\mu\text{g/mL}$  in ethanol) and fluorinated acid stocks (10  $\mu\text{g/mL}$  in water). The stocks will be injected directly into the soil using a glass microsyringe. The test chambers will be immediately sealed with septum lined with aluminum foil and the content of the chambers will be mixed. The entire bottle will be extracted after a sample of the headspace has been taken. The methods and procedures used will be documented in the study records and in the final report.

Viability Controls (untreated): The metabolic activity of untreated test soil will be assessed on a monthly basis. Duplicate incubation chambers for each test soil and condition (aerobic and anaerobic) will be dosed at approximately 100 mg/kg dw with a combination of radiolabeled and non-labelled glucose. The evolved  $^{14}\text{CO}_2$  (aerobic and anaerobic conditions) and  $^{14}\text{CH}_4$  (anaerobic conditions) will be measured and the percent biodegradation calculated. The methods and procedures used will be documented in the study records and in the final report.

Aerobic Controls (treated): The aerobic controls will be prepared by dosing approximately 25 grams (dry weight equivalent) of soil with sufficient test substance to deliver 200 mg/kg dry weight. The test substance will be administered by direct weight addition. The test chambers will be sealed with septum lined with aluminum foil, mixed and incubated. The oxygen content of a sacrificial chamber will be measured at monthly intervals. The methods and procedures used will be documented in the study records and in the final report.

## **Maintenance of Test Chambers**

The soil moisture content should be maintained in the optimal microbial growth range of 40 to 60% water holding capacity (WHC; a pF of between 2.0 and 2.5, or from 0.1 to 0.33 bar). The soil moisture content should be checked and adjusted, if necessary, at regular 1 to 2 week intervals by weighing the test vessels. Adding sterile-filtered demineralized water (degassed sterile-filtered demineralized water for the anaerobic soils) will compensate water losses.

## *Wildlife International, Ltd.*

---

- 8 -

The oxygen content in the headspace of two aerobic control chambers will be assessed on a monthly basis to ensure aerobic conditions. If the mean measured oxygen content within the control chambers is less than 19%, aerobic test chambers will be aerated with filter-sterilized, oil-free air.

If test chambers have to be opened to add water or for monitoring purposes, a sample of the head space will be taken first using a C18 cartridge as described below. The chamber will then be recapped as soon as possible. The septum and cap previously removed will be stored at  $-10^{\circ}\text{C}$  or lower. The C18 will subsequently be extracted and analyzed. Sample extracts will be stored at  $-10^{\circ}\text{C}$  or lower if analysis is delayed more than 24 hours.

### **Sampling and Measurements**

Duplicate chambers from each of the control, treated and spike recovery soils will be extracted (e.g., acetonitrile or other suitable solvent) and analyzed. Proposed sampling intervals will be at 0, 1 and 2 weeks and 1, 2, 4, 6, 9, and 12 months (9 sampling times), however the actual sampling intervals will be documented in the study records and in the final report. More or less frequent intervals may be conducted at the discretion of the Study Director. Potential volatile transformation products in the headspace of the soils chambers will be collected using an appropriate trap (i.e. C18 cartridge). At each sampling time prior to opening the test vessel, the septum will be pierced using a needle connected to a C18 cartridge and syringe. A volume of headspace gas from within the chamber will be pulled through the C18 cartridge using the attached syringe. The C18 will be subsequently extracted and analyzed. Sample extracts will be stored at  $-10^{\circ}\text{C}$  or lower if analysis is delayed more than 24 hours. Extracts will be analyzed for:

1.  $\text{CF}_3(\text{CF}_2)_7\text{CH}_2\text{CH}_2\text{OH}$  (perfluorooctyl ethanol, 8-2 TBA, CAS# 678-39-7)
2.  $\text{CF}_3(\text{CF}_2)_7\text{CH}_2\text{COOH}$  (2-perfluorooctyl ethanoic acid, 8-2 Saturated Acid, CAS#27854-31-5)
3.  $\text{CF}_3(\text{CF}_2)_6\text{CF}=\text{CH}_2\text{COOH}$  (2-H-hexadecafluoro-2-decenoic acid, 8-2 Unsaturated Acid, CAS# 70887-84-2)
4.  $\text{CF}_3(\text{CF}_2)_6\text{COOH}$  (Octanoic acid, pentadecafluoro-; PFOA; CAS# 335-67-1)
5. Heptadecafluorononanoic Acid; CAS#375-95-1 (C9)
6. Nonadecafluorodecanoic Acid; CAS#335-76-2 (C10)

## *Wildlife International, Ltd.*

---

- 9 -

### 7. Perfluoroundecanoic Acid; CAS#4234-23-5 (C11)

#### **Analytical Methods**

Methods of analysis will be verified prior to the start of the study. Analytical reference standards that are used to aid the identification of the test substance and its potential degradation products will be documented in the study records. All chemicals and solvents will be reagent grade or purer. Certificates of analysis (COA) will be provided for all test substances, reference standards, chemicals and solvents, when available. Sources will be documented in the study records. Demineralized water will be used in the study.

#### **Quality Criteria**

Recovery for a given sampling time point should range between 70 to 120% for the analysis of the 8-2 TBA, 8-2 Saturated Acid, 8-2 Unsaturated Acid, C9, C10, C11, and PFOA from the spike recovery control samples. These ranges should be interpreted as targets and should not be used as criteria for acceptance of the test. If recoveries and analysis do not meet the criteria of between 70 to 120%, then the study director will consult with the Sponsor's Representatives.

#### **8-2 TBA**

Liquid Chromatography/ Mass Spectrometry (LC/MS), Gas Chromatography/Mass Spectrometry (GC/MS), or other suitable method can be used to analyze 8-2 TBA in the extracts of soil and headspace samples. Sample collection, preparation, and analytical methods used will be documented in the study records and the final report.

#### **8-2 Saturated Acid, 8-2 Unsaturated Acid, C9, C10, C11, and PFOA**

Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) or other suitable method, will be used to analyze 8-2 Saturated Acid, 8-2 Unsaturated Acid, C9, C10, C11 and PFOA in the extracts of soil samples. Sample collection, preparation, and analytical methods used will be documented in the study records and the final report.

### **EVALUATION OF THE RESULTS**

Statistical methods including means, standard deviations, and regression lines, will be used as appropriate. The concentration of the transformation products in the soil and headspace will be given as

## *Wildlife International, Ltd.*

---

- 10 -

mg kg<sup>-1</sup> (dry weight) and as mole kg<sup>-1</sup> (dry weight) for each sampling interval. Transformation products listed in soil and headspace samples will be plotted against time.

### **HEALTH AND SAFETY**

It is advisable to read the MSDS for the reference chemical and test substance when available. If an MSDS is not available (e.g., the substance is a product of research and development) it is important to review what is known about the substance with a person knowledgeable about the safe handling of the substance, such as the person supplying the substance for testing.

It is advisable to follow guidelines on Storage and Handling of Chemicals; Personal Protective Equipment, Waste Disposal Guide.

### **TEST SUBSTANCE DISPOSAL**

After the issuance of the final report, the remaining test substance will be stored at the testing lab until its expiration date and then destroyed, unless other arrangements are made between the Sponsor and the testing lab.

### **RECORDS TO BE MAINTAINED**

Records to be maintained will include, but not limited to, the following:

1. A copy of the signed protocol.
2. Identification and characterization of the test substance as provided by Sponsor.
3. Study initiation and termination dates.
4. Experimental initiation and termination dates.
5. Test and reference substance preparation and dosing calculations.
6. Soil source and pretreatment data.
7. Results of analytical methods performed.
8. Temperature range recorded during test period.
9. Copy of final report.



## *Wildlife International, Ltd.*

---

- 11 -

### **FINAL REPORT**

A final report of the results of the study will be prepared by Wildlife International, Ltd. and submitted to the Study Monitor no later than one month after the conclusion of the definitive study. The report will include, but not be limited to the following, when applicable:

1. Name and address of facility performing the study.
2. Dates on which the study was initiated and completed.
3. Objectives and procedures stated in the approved protocol, including any changes in the original protocol.
4. Identification and characterization of the test substance as provided by Sponsor.
5. A summary and analysis of the data .
6. A description of the transformations and calculations performed on the data.
7. A description of the methods used and reference to any standard method employed.
8. A description of the test system.
9. A description of the preparation of the test solutions, the testing concentrations, and the duration of the test.
10. A description of all circumstances that may affect the quality or integrity of the data.
11. The name of the study director, the names of other scientists or professionals, and the names of all supervisory personnel, involved in the study.
12. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
13. The location where the raw data and final report are to be stored.

### **CHANGES TO THE FINAL REPORT**

If it is necessary to make corrections or additions to the 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 study that is being amended and the reasons for the alteration. Amendments shall be signed and dated by the Study Director and Laboratory QA.

### **CHANGES TO PROTOCOL**

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and approved by 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

## *Wildlife International, Ltd.*

---

- 12 -

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; will be consistent with OECD Principles of Good Laboratory Practice. 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). When the final report is completed, original copies of the study data and magnetically encoded records generated by Wildlife International, Ltd. for this study will be sent to the Sponsor. A certified copy will be retained in the archives of Wildlife International, Ltd.

## *Wildlife International, Ltd.*

---

- 13 -

### REFERENCES

- 1 **Organisation for Economic Cooperation and Development.** April 2002. *Aerobic and Anaerobic Transformation in Soil.* OECD Guideline 307.

# Wildlife International, Ltd.

---

## AMENDMENT TO STUDY PROTOCOL

**STUDY TITLE:** H-26665: TRANSFORMATION POTENTIAL IN AEROBIC AND ANAEROBIC SOILS

**PROTOCOL NO:** 112/021705/SED-TRANSb/SUB112

**AMENDMENT NO.:** 1

**SPONSOR:** E.I du Pont de Nemours and Company

**PROJECT NO.:** 112E-109

**EFFECTIVE DATE:** 06 May 2005

---

**AMENDMENT:** Maintenance of Test Chambers, Page -7-

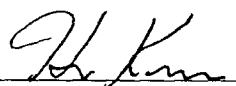
**CHANGE:** The soil moisture content should be checked and adjusted, if necessary, at regular 1 to 2 week intervals by weighing the test vessels.

**TO:** The soil moisture content should be checked and adjusted, if necessary, at monthly intervals by weighing the test vessels.

**REASON:** The frequency at which the soil moisture content was checked was reduced based on observed losses.

  
\_\_\_\_\_  
STUDY DIRECTOR

25 Sept 2006  
DATE

  
\_\_\_\_\_  
LABORATORY MANAGEMENT

5 Oct 06  
DATE

Reviewed by QA  
JHC 10-9-06

~~Confidential~~

### DEVIATION TO STUDY PROTOCOL

**STUDY TITLE:** H-26665: TRANSFORMATION POTENTIAL IN AEROBIC AND ANAEROBIC SOILS

**PROTOCOL NO:** 112/021705/SED-TRANSb/SUB112

**DEVIATION NO.:** 1

**SPONSOR:** E.I du Pont de Nemours and Company

**PROJECT NO.:** 112E-109

**DEVIATION:** Preparation of Test Chambers; Viability Controls (untreated), Page -7-

The protocol indicated that duplicate incubation chambers for each test soil and condition (aerobic and anaerobic) would be dosed at approximately 100 mg/kg dw with a combination of radiolabelled and non-labelled glucose. The test chambers were actually dosed at 3000 mg/kg dw with a combination of radiolabelled and non-labelled glucose.

**REASON:** Oversight by study personnel.

**IMPACT:** In the best judgment of the Study Director, this deviation did not impact the integrity of study. A concentration of 2000 to 4000 mg glucose per kg dry weight soil is a common for the determination of glucose-induced respiration rates.

E. C. L. L. L.  
STUDY DIRECTOR

25 Sept 2006  
DATE

L. K.  
LABORATORY MANAGEMENT

5 Oct 06  
DATE

APPENDIX II

**CONFIDENTIAL BUSINESS INFORMATION**

**DOCUMENT REMOVED**

Document Number in Index: 10

Document Description: Testing Invoices

APPENDIX III



**CONFIDENTIAL BUSINESS INFORMATION**

**DOCUMENT REMOVED**

Document Number in Index: 12

Document Description: DuPont Investment in Fluorotelomer Environmental Fate Science

APPENDIX IV



# U.S. EPA - DuPont

Created By: Robert C Buck on 07/11/2002 at 02:50 PM

Security

Workgroup: Product Stewardship  
Category: General  
Sub Category: General

## U.S. ENVIRONMENTAL PROTECTION AGENCY

4 March 2007 :

### 1) DuPont Investment in Fluorotelomer Environmental Fate Science:



DuPont Investment in Fluorotelomer Environmental Fate Science 2004-06.pdf



**Presentation:** 2007\_7March\_USEPA Slides\_RCB.pdf (DuPont Confidential Information)



### 2) Wildlife Intl a) Invoices: 2007\_Feb\_2005-06 Wildlife Intl Invoices.pdf b) Quote for 307/311



2006\_WLI\_Costs to date and Est Future Studies Costs.pdf

(DuPont Confidential Information)



### 3) ENVIRON Invoices: Environ invoices, DEM Study 2005-2006\_ALL.pdf (DuPont Confidential Information)



**Historical Archive Document of EPA Presentations :** Surface Protections Solutions -.pdf

### Soil Biodegradation Study Protocols:



Urethane Polymer Protocol: 112E-108protocol\_CBI.pdf



Acrylate Polymer Protocol: 112E-109protocol\_CBI.pdf

*Note: The studies started with a suite of 7 analytes. Two additional analytes were added at 15 months.*



**SETAC Presentations:** SETAC NA 2005\_642\_Polymer Biodeg.pdf



2006\_SETAC NA\_Polymer Biodeg Poster\_RCB\_DRAFT\_One Page.pdf (not confidential)

### 3 November 2006 : Preliminary Aerobic Soil Studies Information

sent as DuPont Confidential Information. Contains TSCA CBI. Subject to Copyright.

#### Contents of CD 1 of 3



**Cover Letter:** 2006\_3 Nov\_Cover Letter Soil Studies\_USEPA\_CBI.pdf



**Draft Study Reports:** 2006\_02 Nov\_H26665\_DRAFT REPORT\_RPT109\_CBI.pdf



2006\_02 Nov\_H26634\_DRAFT REPORT\_RPT108\_CBI.pdf



**Raw Data Index:** 2006\_02 Nov\_DRAFT\_Soil Studies Records Index\_CBI.pdf



**Analytical Data Summary:** 2006\_02 Nov\_DRAFT 12 Month Analytical Summary\_CBI.pdf



**Test Substance Certificates of Analysis:** 4\_2006\_11Oct\_DRAFT\_H-26665\_COA\_CBI.pdf



3\_2006\_11Oct\_DRAFT\_H-26634\_COA\_CBI.pdf

#### Contents of CD 2 of 3

#### Contents of CD 3 & 3

### DuPont Soil Biodegradation Study Update, October 2006, Washington, DC

#### CBI Documents Provided in Advance:



1\_1\_Cover Letter for CBI Documents\_12Oct2006\_CBI.pdf

#### **Test Substance COAs:**



1\_H26634\_COA\_17 Oct2005.pdf 2\_H26665\_COA\_17Oct2005.pdf



3\_2006\_11Oct\_DRAFT\_H-26634\_COA.pdf 4\_2006\_11Oct\_DRAFT\_H-26665\_COA.pdf



**Study Protocols:** 5\_H26634\_Soil Study\_112E-108protocol.pdf 6\_H26665\_Soil Study\_112E-109protocol.pdf



**Analytical Metehods:** 7\_Soil Study Analytical Methods.pdf 12\_Analytical Method\_Added analytes.pdf



**Data:** 10\_Mollisol Raw Data Anyltical Summary.xls



**Interpretation:** 8\_2006\_11Oct\_Interpretation Algortihm & AE Calculations.pdf



9\_2006\_10Oct\_Interpretation Algorithm Workbook.xls



**Modeling the Study Results:** 11\_Modeling of Fluorotelomer-based Polymeric Products\_Soil Biodeg Studies\_DRAFT.pdf

Non-CBI Documents Provided in Advance:



A\_12 October 2006 Email to D Lynch EPA\_NOT CBI.pdf



B\_2006\_11 Oct\_Publication & Report References\_NOT CBI.pdf



C\_8-2 FTOH Biodegradation Pathways\_NOT CBI.pdf



D\_2006\_JChromA\_1110\_pp117-124.pdf



**DuPont Presentation, 15 September, 2006**

2006\_15Sept\_DD Review with USEPA.ppt

**DuPont Presentation, 16 August 2006 PMN Meeting**



Premanufacture Notice slides 081606 final Company Sanitized.ppt EPA Meeting 081606 final CBI.ppt



EPA Meeting 081606 final Company Sanitized.ppt EPA Meeting Introduction 081506 final CBI.ppt





EPA Meeting Introduction 081506 final Company Sanitized.ppt Premanufacture Notice slides 081606 final CBI.ppt

---



**DuPont Presentation, 8 September 2005 :** EPA Mtg 9-8-05-CBI.ppt

---

**DuPont Presentation, 8 April 2005 :**



Supply Chain Review 08 April'05 EPA.ppt



EPA Meeting 08 April'05.ppt



GDEM April 2005 SHK.ppt



Paper PS Summary April 2005 SHK.ppt

---

**DuPont Presentation, 31 January 2005 :**



**DuPont Global Strategy Presentation :** 31Jan2005 DuPont Presentation to USEPA\_AR226-1914.pdf



**DuPont Supply Chain Review :** Supply Chain Review 31 Jan'05 Final\_CBI.ppt

---

**DuPont Presentation, 15 December 2004 : DuPont Biodegradation Studies :**



15Dec2004\_DuPont-EPA Meeting Presentation.ppt



15Dec2004\_DuPont-EPA Meeting Agenda.pdf

---

**DuPont Presentation, 10 November 2004 :**



USEPA\_DuPont Ongoing Research Summary\_9Nov2004\_NOCBI.ppt



US10 Nov2004\_EPA\_DuPont Ongoing Research Summary\_CBI-RWR.ppt

---

---

**DuPont Presentation, 17 June 2004 : DuPont Consumer Article Exposure & Risk Characterization (FINAL VERSION)**



DuPont Risk Assessment - Article Presentation\_USEPA\_17June2004.pdf



DuPont Risk Assessment - Article Presentation for EPA\_17June2004.ppt

---

**DuPont Presentation, 30 April 2004**



**(FINAL CBI VERSION)** DuPont PFOA Reduction Report 04300: DuPont PFOA Reduction Report (



**NON-CBI VERSION** DuPont PFOA Reduction Report 04300: DuPont Report 30April2004 n

---

**U.S. EPA-OPPTS**



**DuPont Presentation, 16 March 2004 (FINAL: CBI VERSION)** Brand Presentation US EPA 16 Ma

---

**U.S. EPA-OPPTS**

**DuPont Presentation, 25 November 2002**



**(FINAL: CBI VERSION)** 25Nov2002 USEPA DuPont PS Update



**(FINAL: NON-CBI VERSION)** 25Nov2002 USEPA DuPont PS Update F

**Copy of the Non-CBI Document in the Public Record (AR-226) :**



25Nov2002 DuPont Presentation to USFF

---

**U.S. EPA-OPPTS DuPont Presentation, 17 December 2001**



17 DEC 2001 DUPONT 17Dec2001 DuPont EPA CBI (CBI VERSION : 17 Dec 2001 RCB)



17 DEC 2001 DUPONT 17Dec2001 DuPont EPA NonCBI(NON-CBI VERSION : 17 Dec 2001 RCB)  
17Dec2001 EPA Presentation with Post Meeting Corrections/Updates



Dec2001 DuPont EPA Presentation Conv w P

**U.S. EPA-OPPTS DuPont Presentation, 17 December 2001, RESUBMITTED 12 FEB, 2002**



17Dec2001 DuPont EPA NonCBI 12Feb2001 17Dec2001 DuPont EPA CBI 12Feb2001



USEPA Updated Presentation Cover Letter



U.S. EPA-OPPTS TRP Presentation (20 JUN 2000) 20 June 2000 Mtg TRP mtg\_w EPA Charts



U.S. EPA-OPPTS TRP Presentation (23 OCT 2000) EPA Review Presentation OCT23\_2000\_fina

**U.S. EPA-OPPTS Presentations DuPont & TRP (21 Feb 2001) (DO NOT MAKE ANY CHANGES)**



EPA Review DUPONT Presentation FEB21



EPA FII F TRP Presentation FEB21

(CHARTS GIVEN TO THE EPA-OPPTS on 21 FEB 2001 non-CBI Versions of the Above)



EPA Review DUPONT Presentation FEB21 2001 FINA



EPA FII F TRP Presentation FEB21 2001 nor





U.S. EPA-ORD, Duluth, MN, 7 June 2001 (R. C. Buck) 7Jun2001 EPA\_ORD Discussion.

---

Miscellaneous Presentations to and by U.S. EPA



August 3. 2000 EPA Staff Presentation on PFOS 226-0619.pdf



SPI-FMG Presentation to U.S. EPA 23 April 2002 Final No CBI April 23 EPA Presentation

---

| Edit History: | <u>Rev.</u> | <u>Editor</u> | <u>Edit Date</u>       |
|---------------|-------------|---------------|------------------------|
|               | 70.         | Robert C Buck | 03/08/2007 03:48:37 PM |
|               | 69.         | Robert C Buck | 03/07/2007 04:32:26 PM |
|               | 68.         | Robert C Buck | 03/07/2007 07:53:07 AM |
|               | 67.         | Robert C Buck | 03/07/2007 07:48:16 AM |
|               | 66.         | Robert C Buck | 03/07/2007 07:47:59 AM |

*\*Only past five edits are shown*

**COPY RESTRICTED DOCUMENT****DOCUMENT REMOVED**

Document Number in Index: 15

Document Description: Biodegradation studies of fluorotelomer-based polymers in soils

Comment: DuPont has provided a Read Only copy of this document to EPA for inclusion in the AR-226 printed paper file. The document may be viewed (but not copied) from the EPA public printed paper copy file located at the EPA Public Reading Room. Information from the EPA web-site on the Public Reading Room is provided below. Note that this information reflects information on EPA's website as of the date of this letter. Readers should verify with EPA that the information has not changed.

The EPA/DC Public Reading Room is located in the EPA Headquarters West Building, Room 3334, located at 1301 Constitution Ave., NW, Washington, DC. The EPA/DC Public Reading Room hours of operation are 8:30 a.m. to 4:30 p.m. Eastern Standard Time, Monday through Friday, excluding federal holidays. Please visit the EPA/DC website (<http://www.epa.gov/epahome/dockets.htm>) for the latest status concerning the Public Reading Room and public access to docket materials.

**Pollution Prevention and Toxics Docket**

Email: [oppt.ncic@epa.gov](mailto:oppt.ncic@epa.gov)

Phone Number: 202-566-0280

Fax Number: 202-566-9744

Mail Code: 2822T

DuPont Contact Information: Contact information to request author permission to copy the document -

Dr. Robert Buck  
302-892-8935  
[robert.c.buck@usa.dupont.com](mailto:robert.c.buck@usa.dupont.com)

130

Doc. 15

**COPY RESTRICTED DOCUMENT****DOCUMENT REMOVED**

Document Number in Index: 16

Document Description: Biodegradation studies of fluorotelomer-based polymers in activated sludge, soil, and sediments

Comment: DuPont has provided a Read Only copy of this document to EPA for inclusion in the AR-226 printed paper file. A printed copy of the document may be viewed (but not copied) from the EPA public printed paper copy file located at the EPA Public Reading Room. Information from the EPA web-site on the Public Reading Room is provided below. Note that this information reflects information on EPA's website as of the date of this letter. Readers should verify with EPA that the information has not changed.

The EPA/DC Public Reading Room is located in the EPA Headquarters West Building, Room 3334, located at 1301 Constitution Ave., NW, Washington, DC. The EPA/DC Public Reading Room hours of operation are 8:30 a.m. to 4:30 p.m. Eastern Standard Time, Monday through Friday, excluding federal holidays. Please visit the EPA/DC website (<http://www.epa.gov/epahome/dockets.htm>) for the latest status concerning the Public Reading Room and public access to docket materials.

**Pollution Prevention and Toxics Docket**

Email: [oppt.ncic@epa.gov](mailto:oppt.ncic@epa.gov)

Phone Number: 202-566-0280

Fax Number: 202-566-9744

Mail Code: 2822T

DuPont Contact Information: Contact information to request author permission to copy the document:

Dr. Robert Buck  
302-892-8935  
[robert.c.buck@usa.dupont.com](mailto:robert.c.buck@usa.dupont.com)

COPY

1/16/00

131

Dec. 16