AR226-3755

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(CBI removed; copy restricted materials included as read only)

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15	Biodegradation studies of fluorotelomer-based polymers in soils	2	Subject to copyright – document is read only and may not be copied without author permission; Contact information to request author permission: Dr. Robert Buck (302-892-8935 robert.c.buck@usa.dupont.com)
16	Biodegradation studies of fluorotelomer-based polymers in activated sludge, soil, and sediments	27	Not for further distribution without author approval – document is read only and may not be copied without author permission; Contact information to request author permission: Dr. Robert Buck (302-892-8935 or Robert.C.Buck@usa.dupont.com)

Company Sanitized

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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 20th, 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,

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

Doc. 1

CONFIDENTIAL BUSINESS INFORMATION

DOCUMENT REMOVED

Document Number in Index: 2

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

APPENDIX I

Doc. 3

CONFIDENTIAL BUSINESS INFORMATION

DOCUMENT REMOVED

Document Number in Index: 4

Document Description: Cost Summary - Telomers

7

Doc. 4

CONFIDENTIAL BUSINESS INFORMATION

DOCUMENT REMOVED

Document Number in Index: 5

Document Description: Testing Invoices – Set 1

Doc. 5

CONFIDENTIAL BUSINESS INFORMATION

DOCUMENT REMOVED

Document Number in Index: 6

Document Description: Testing Invoices – Set 2

Doc. 6

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

Doc. 7

Wildlife International, Ltd.

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 Start Date: 15 MARCH 2005

Experimental Termination Date: 15 MARCH 2006

Project No.: 112E-108

Test Concentrations: 200 mg/kg ; 250 mg/kg ; 10 µg/kg

Test Substance No.: 6918 Reference Substance No. (if applicable): 6944, 6943, 6945

6826, 6E26, 6E29, 6E49

PROTOCOL APPROVAL

P.J.N. Schaefer — 10 MARCH 2005

STUDY DIRECTOR — DATE

[signature] — 3/09/05

LABORATORY MANAGEMENT — DATE

William A. Berti — 17-Feb-2005

SPONSOR'S REPRESENTATIVE — DATE

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

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:

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 ± 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 ± 2°C.

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

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}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}Co-sterilized soil with appropriate volumes of 8-2 TBA stock (250 µg/mL in ethanol) and fluorinated acid stocks (10 µg/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 $^{14}CO_2$ (aerobic and anaerobic conditions) and $^{14}CH_4$ (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.

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°C or lower. The C18 will subsequently extracted and analyzed. Sample extracts will be stored at –10°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°C or lower if analysis is delayed more than 24 hours. Extracts will be analyzed for:

1. $CF_3(CF_2)_7CH_2CH_2OH$ (perfluorooctyl ethanol, 8-2 TBA, CAS# 678-39-7)
2. $CF_3(CF_2)_7CH_2COOH$ (2-perfluorooctyl ethanoic acid, 8-2 Saturated Acid , CAS#27854-31-5)
3. $CF_3(CF_2)_6CF{=}CH_2COOH$ (2-H-hexadecafluoro-2-decenoic acid, 8-2 Unsaturated Acid, CAS# 70887-84-2)
4. $CF_3(CF_2)_6COOH$ (Octanoic acid, pentadecafluoro-; PFOA; CAS# 335-67-1)
5. Heptadecafluorononaoic Acid; CAS#375-95-1 (C9)
6. Nonadecafluorodecanoic Acid; CAS#335-76-2 (C10)

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

mg kg^{-1} (dry weight) and as mole kg^{-1} (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.

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

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.

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.

E. C. [signature]
STUDY DIRECTOR

6 May 2005
DATE

[signature]
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. Kaplan
STUDY DIRECTOR

25 Sept 2006
DATE

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.

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 Start Date: 15 MARCH 2005

Experimental Termination Date: 15 MARCH 2006

Project No.: 112E-109

Test Concentrations: 200 MG/KG ; 250 MG/KG ; 10MG/KG

Test Substance No.: 6919 Reference Substance No. (if applicable): 6944, 6943, 6945 6826, 6826, 6829, 6849

PROTOCOL APPROVAL

STUDY DIRECTOR — DATE: 10 MARCH 2005

LABORATORY MANAGEMENT — DATE: 3/09/05

SPONSOR'S REPRESENTATIVE — DATE: 12-Feb-2005

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.

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

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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.

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

METHODS AND MATERIALS

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

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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 ± 2°C.

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

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}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}Co-sterilized soil with appropriate volumes of 8-2 TBA stock (250 µg/mL in ethanol) and fluorinated acid stocks (10 µg/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 $^{14}CO_2$ (aerobic and anaerobic conditions) and $^{14}CH_4$ (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.

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°C or lower. The C18 will subsequently extracted and analyzed. Sample extracts will be stored at –10°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°C or lower if analysis is delayed more than 24 hours. Extracts will be analyzed for:

1. $CF_3(CF_2)_7CH_2CH_2OH$ (perfluorooctyl ethanol, 8-2 TBA, CAS# 678-39-7)
2. $CF_3(CF_2)_7CH_2COOH$ (2-perfluorooctyl ethanoic acid, 8-2 Saturated Acid , CAS#27854-31-5)
3. $CF_3(CF_2)_6CF{=}CH_2COOH$ (2-H-hexadecafluoro-2-decenoic acid, 8-2 Unsaturated Acid, CAS# 70887-84-2)
4. $CF_3(CF_2)_6COOH$ (Octanoic acid, pentadecafluoro-; PFOA; CAS# 335-67-1)
5. Heptadecafluorononaoic Acid; CAS#375-95-1 (C9)
6. Nonadecafluorodecanoic Acid; CAS#335-76-2 (C10)

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

mg kg^{-1} (dry weight) and as mole kg^{-1} (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.

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

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.

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 — DATE: 25 Sept 2006

LABORATORY MANAGEMENT — DATE: 5 Oct 06

Reviewed by QA
JHC 10-9-06

Wildlife International Ltd.

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. Lisa
STUDY DIRECTOR

25 Sept 2006
DATE

K. Kan
LABORATORY MANAGEMENT

5 Oct 06
DATE

APPENDIX II

Doc. 9

CONFIDENTIAL BUSINESS INFORMATION

DOCUMENT REMOVED

Document Number in Index: 10

Document Description: Testing Invoices

41

Doc. 10

APPENDIX III

Doc. 11

CONFIDENTIAL BUSINESS INFORMATION

DOCUMENT REMOVED

Document Number in Index: 12

Document Description: DuPont Investment in Fluorotelomer Environmental Fate Science

Doc. 12

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 thru 4Q1 .pdf **(DuPont Confidential Information)**

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

Soil Biodegradtion 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_04-2_Polymer Biodeg.pdf

Doc. 14

2006_SETAC NA_Polymer Biodeg Poster_RCB_DRAFT_One Page.pdf **(not confidential)**

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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_COAI_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 Anaylitical Summary.xls

Interpretation: 8_2006_11Oct_Interpretation Algorithm & AT Calculations.pdf

9_2006_10Oct_Interpretation Agorithm Workbook.xls

Modeling the Study Results: 11_Modeling of Fluoropolymer-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 Reference List_NOT CBI.pdf

C_8-2 FTOH Biodegradation Pathways_NOT CBI.pdf D_2006_JChromA_1110_pp117-124.pdf

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DuPont Presentation, 15 September, 2006 2006_15Sept_DD Review with USEPA.ppt

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DuPont Presentation, 16 August 2006 PMN Meeting

Premanufacture Notice slides 081606 final Company Sanitized.ppt EPA Meeting 081606 final 1.ppt

EPA Meeting 081606 final Company Sanitized.ppt EPA Meeting Introduction 081606 final 1.ppt


EPA Meeting Introduction 081506 final Company Sanitized.ppt


Premanufacture Notice Index 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 April05 EPA.ppt


EPA Meeting 08 April05.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 Alcohols.pdf


DuPont Supply Chain Review : Supply Chain Review 31Jan05 Final CBI.ppt

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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 :


US EPA DuPont Ongoing Research Summary_9Nov2004_NOCBI.ppt


US10 Nov2004 EPA _DuPont Ongoing Research Summary_CBI-RWR.ppt

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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

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DuPont Presentation, 30 April 2004

(FINAL CBI VERSION) DuPont PFOA Reduction R

DuPont PFOA Reduction Report I

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

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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 USEP

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 NonCB **(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 12Feb20 17Dec2001 DuPont EPA CBI 12Feb200

USEPA Updated Presentation Cover Lette

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

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

EPA Review DUPONT Presentation FEB21

EPA FILE TRP Presentation FEB

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

EPA Review DUPONT Presentation FEB21 2001 FINA

EPA FILE TRP Presentation FEB21 2001 mtg

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 [redacted] 226-0619.pdf

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

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Edit History:	Rev.	Editor	Edit Date
	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*

Biodegradation studies of fluorotelomer-based polymers in soils.

R. C. Buck[1], W. R. Berti[1], M. H. Russell[1], B. Szostek[1], N. Wang[1], E. Schaefer[2], R. Van Hoven[2]
1 E. I. duPont de Nemours & Co., Inc. Wilmington, Delaware U.S.A.
2 Wildlife International, Ltd., Easton, Maryland, U.S.A

Poster presented at SETAC North American Meeting
November 2006 Montréal, Canada

Abstract

Biodegradation of fluorotelomer-based polymers in the environment is of interest to determine if they may be a potential source of perfluorocarboxylic acids (PFCAs) We have conducted studies on a fluorotelomer-based acrylate polymeric product in four soils to determine whether the fluorotelomer moieties covalently bonded in these polymers can be liberated and transformed to PFCAs

Treatments included an untreated control, live (non-sterile) treatments containing the test substance, sterile treatments with the test substance, and sterile treatments spiked with several analytes to assess recoveries The test vessels were incubated statically for one year. Separate headspace and soil samples were collected and analyzed

The polymeric test substance contained residual fluorotelomer raw materials that are known to degrade to the target analytes The experimental data after 15 months were analyzed using a kinetic degradation model PFCAs quantified in the test systems originated exclusively from transformation of the residual fluorotelomer raw materials There was no indication that polymer degradation contributed to PFCAs observed The estimated fluoroacrylate polymer degradation half-life was > 10,000 years

Introduction

Perfluoro carboxylates (PFCAs) have been identified as widely present in the environment [1] Recently a complete picture of PFCA sources has been reviewed [2] Direct emissions of PFCAs are the largest source of global historic PFCA emissions Indirect PFCA sources, or "precursors", included perfluoroalkyl sulfonyl- and fluorotelomer-based substances For these indirect sources, residual unreacted raw materials and impurities present in sales products were identified as a potential source of PFCAs A key uncertainty in the assessment of indirect PFCA sources was whether fluorotelomer-based products, largely comprised of acrylic polymers, or perfluoroalkylsulfonyl-based products degrade in the environment to form PFCAs In order for this to occur, the polymer backbone and/or the covalent bond or "linkage" between the fluorotelomer or perfluoroalkyl sulfonyl functionality and the polymer backbone would have to be severed

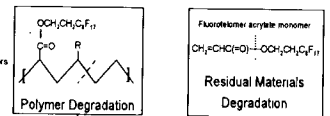
Biodegradation is a transformation route that will determine polymer environmental fate A limited number of experimental biodegradation studies have been conducted on fluorotelomer alcohol (FTOH), a residual raw material, which have identified transformation products that can be used to probe polymer degradation [3,4]

The present study was designed to determine whether and to what extent a fluoroacrylate polymer derived from fluorotelomer alcohol degraded in aerobic soils to form perfluorooctanoate (PFO)

Test Substance & Degradation Pathways

Fluorotelomer-based Acrylate Polymeric Product Test Substance

- An aqueous dispersion of fluoroacrylate polymer in water
  - Fluorotelomer acrylate monomer emulsion polymerized with other monomers
  - Product by process
- Composition
  - Fluoroacrylate polymer
  - Hydrocarbon surfactants
  - Water
  - Residual Raw Materials and Impurities
    - Fluorotelomer Alcohol $F(CF_2)_nCH_2CH_2OH$
      - n = 8, 10, 12+ (55%, 30%, 15%)
    - Fluorotelomer acrylate monomer $F(CF_2)_nCH_2CH_2OC(O)CH=CH_2$
    - Etc

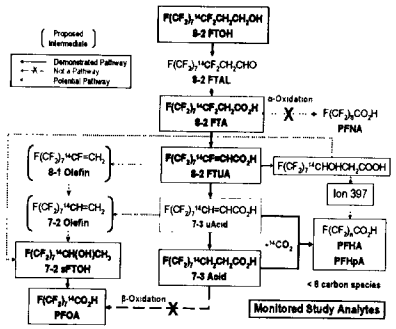


Cleave Ester Linkage (hydrolysis)

Polymer Backbone Degradation to Oligomers

Cleave Ester Linkage (hydrolysis)

$C_8F_{17}CH_2CH_2OH$
8-2 Fluorotelomer Alcohol



Study Analytes

8-2 Fluorotelomer Alcohol (FTOH)	$F(CF_2)_4CH_2CH_2OH$
8-2 Fluorotelomer Acid (FTA)	$F(CF_2)_4CH_2COOH$
8-2 Fluorotelomer Unsaturated Acid (FTUA)	$F(CF_2)_4CF=CHCOOH$
7-3 Acid	$F(CF_2)_4CH2CH2COOH$
7-2 sFTOH	$F(CF_2)_4CH(OH)CH_3$
PFO, PFN, PFD PFU (PFCAs)	$F(CF_2)_nCOOH$, n = 7, 8, 9, 10

Study Methodology

OECD 307 as a guideline

- 4 soils, aerobic and anaerobic conditions initially.
- Measurements at 0, 1, 2 weeks; 1, 2, 4, 6, 9, 12, 15, 18, 24 months
- Analyzed 12 and 15 month samples for 7-3 Acid and 7-2 sFTOH.
- Completing 18 months sampling and analysis. Final samples to be taken at 24 months.

Test Soils

1. Alfisol: Howard County, Missouri Sandy loam (11% clay), pH 5.8, Organic carbon 1.4%
2. Inceptisol: Deer Park (Spokane Co.), Washington Loam (23% clay), pH 6.6, Organic carbon 2.0%
3. Mollisol: Grand Forks County, North Dakota Sandy clay loam (23% clay), pH 6.9, Organic carbon 2.1%
4. Ultisol: New Castle Co, Delaware Sandy loam (14% clay), pH 4.8, Organic carbon 2.9%

General Experimental Design

- Background Blank Control: no test item added
- Test Item: test item concentration of 200 mg active ingredient kg-1 dry wt. soil
- Abiotic Control: sterilized test medium with added test item concentration of 200 mg a.i. kg-1 DW soil.
- Spike Recovery Controls: sterilized medium spiked with following analytes: 8-2 FTOH, 8-2 FTA, 8-2 FTUA, PFO, PFN, PFD, PFU, 7-2 sFTOH, 7-3 FTA
- Test vessels are glass serum bottles with aluminum foil-lined closures incubated statically at 20°C.
- Soil moisture content assessed regularly by weighing each bottle and adding water as needed to maintain a level of 40 to 60% water-holding capacity.
- A headspace sample from each vessel is passed through a C18 cartridge. The entire contents of the test vessel are then extracted first using acetonitrile and second with an aliquot of a 200 mM NaOH solution.

AE Mass Balance Interpretation

Alcohol Equivalents (AE)

Definitions

- AE = Moles of 8-2 FTOH equivalents or "Alcohol Equivalents" derived from 8-2 substances (e.g. 8-2 fluorotelomer acrylate) present in the Test Substance (TS)
- Residuals = The sum of raw materials and impurities identified and present in the Test Substance

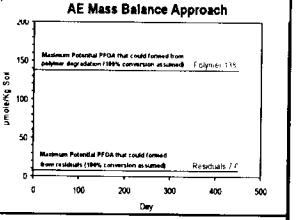
Input Values

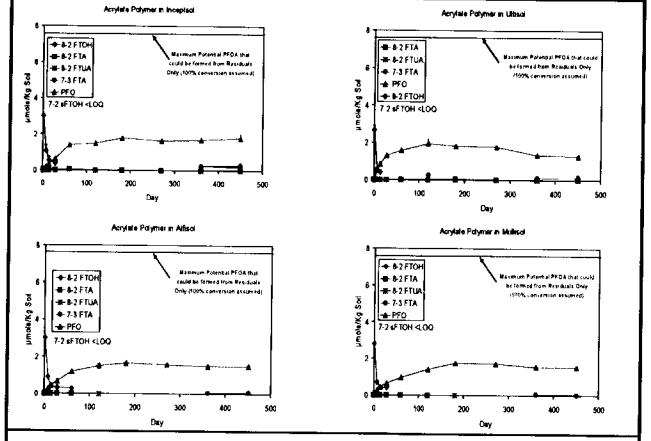
- X1 = weight percent (wt%) Fluorine in the Test Substance (TS)
- X2 = weight percent (wt%) Fluorine in the Fluorotelomer Alcohol (FTOH) Raw Material used to make the Test Substance
- X3 = weight percent(wt%) 8-2 Fluorotelomer Alcohol (8-2 FTOH) in the Fluorotelomer Alcohol Raw Material

$$\text{Polymer AE} = \frac{\text{nmol AE}}{\text{mg Test Substance}} \left(\frac{X1}{X2}\right) \cdot X3 = \frac{172 \text{ nmol AE}}{\text{mg TS}}$$

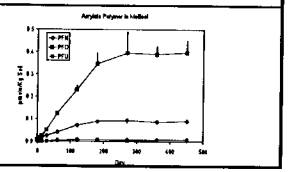
- 172 nmol AE in Polymer per mg of Test Substance
- 9.45 nmol AE in Residuals per mg of Test Substance

AE Mass Balance Approach





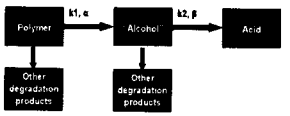
- PFN, PFD and PFU levels observed are consistent with expectations based on test substance composition and known degradation pathways (e.g. PFN ~1% of PFO)



Kinetic Modeling

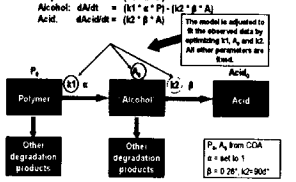
Polymer Degradation Kinetic Model

k1 = degradation rate of polymer
k2 = degradation rate of alcohol / intermediate
α = fraction of polymer reacting to form alcohol / intermediate
β = fraction of alcohol / intermediate reacting to form acid

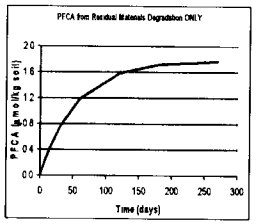


Conceptual Polymer Degradation Pathway Equations

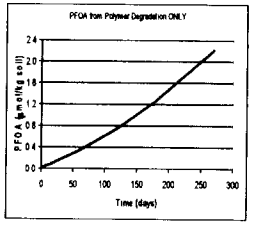
Polymer: $dP/dt = -(k1 * \alpha * P)$
Alcohol: $dA/dt = (k1 * \alpha * P) - (k2 * \beta * A)$
Acid: $dAcid/dt = (k2 * \beta * A)$



Optimize ONLY degradation of Residual Materials to form PFCA



Optimize ONLY degradation of Polymer to form PFCA



The time course of residual fluorotelomer alcohol degradation provides a much more reasonable fit to the experimental data than the corresponding time course of polymer degradation

Soil	Polymer half-life (years)	Residual Materials half-life (days)
alfisol	> 10,000	33
inceptisol	> 10,000	38
mollisol	> 10,000	45
ultisol	> 10,000	16

Kinetic Modeling Summary

- Primary source of PFCA fits with a model for degradation of Residual Materials alone.
- Calculated degradation half-life of Residual Materials ranges between 16 and 45 days in the four soils.
- Calculated Acrylate Polymer degradation half-life is greater than 10,000 years.

Summary & Conclusions

- Degradation analytes determined in the test system can be exclusively attributed to degradation of residual fluorotelomer raw materials and impurities in the test substance.
- The results obtained from kinetic modeling the degradation kinetics of the fluoroacrylate polymer further support the conclusion that PFO formation is dominantly due to the transformation of the residual materials.
- The degradation of the polymeric structure appears to be negligible with calculated degradation half-lives that are > 10,000 years.
- PFN, PFD and PFU levels observed are consistent with expectations based on test substance composition and known degradation pathways. (e.g PFN ~1% of PFO)
- Demonstrated analytical methods & study operation for over 15 months.

References

1 Houde et al. *Environ Sci Technol* 40(11), 3463 2 Prevedouros et al *Environ Sci Technol* 40(1) 32 3 Wang et al *Environ Sci Technol* 39(2) 531 4 Wang et al *Environ Sci Technol* 39(19), 7516

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Biodegradation studies of fluorotelomer-based polymers in activated sludge, soil, and sediments.

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E. I. duPont de Nemours & Co., Inc.

E. Schaefer and R.L. Van Hoven
Wildlife International

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Outline

- Introduction
- Biodegradation studies in activated sludge
- Biodegradation studies in soils
- Biodegradation studies in sediments
- Biodegradation studies in anaerobic digester sludge
- Summary

Summary

Study results to date

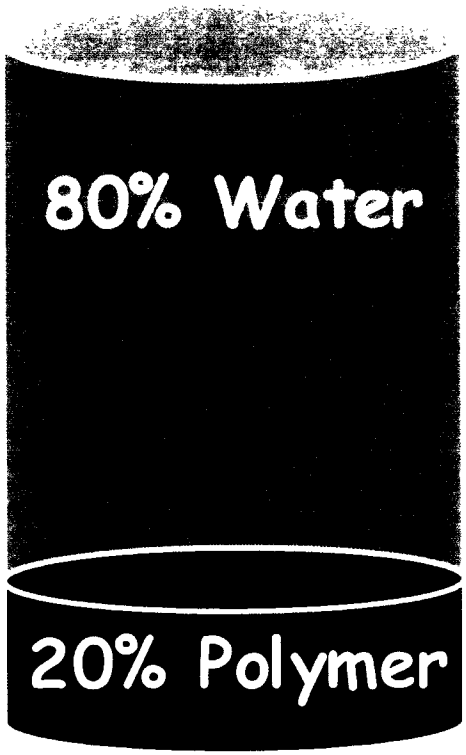
- Soil and Sludge methodologies and analytical methods have been demonstrated
- No evidence of polymer transformation observed
  - Inherent Biodegradation in Sludge – 21 Days
  - Biodegradation in four Soils – 180 Days

Ongoing studies

- Soil Studies continuing to one-year
- Sediment studies commenced continuing to one year
- Anaerobic sludge studies to commence shortly

Polymeric Product Dispersion

- Aqueous Dispersion of….
- Polymeric Particles 100-200 nm
- Hydrocarbon Surfactant(s)



"Typical" Polymeric Product

- wt.% Active Ingredient (~ 20-30 %)
- wt.% Fluorine (~ 6-10%)
- ~ 500 ppm Residual Raw Materials
  - Telomer B Alcohol
  - Telomer B Olefin
  - Telomer Acrylate Monomer
- Polymer Mw ≥ 10,000

Product Routes to the Environment

- Soil (e.g. landfill)
  - Municipal and Industrial Waste Landfill
- Waste-Water
  - Sludge & Sediment
- Incineration
  - Municipal and Industrial Waste
  - No PFOA formed under typical municipal waste incinerator conditions
    - Yamada et al. Chemosphere 2005, 61, 974-984.

Study Objectives : Sludge, Soils, and Sediments

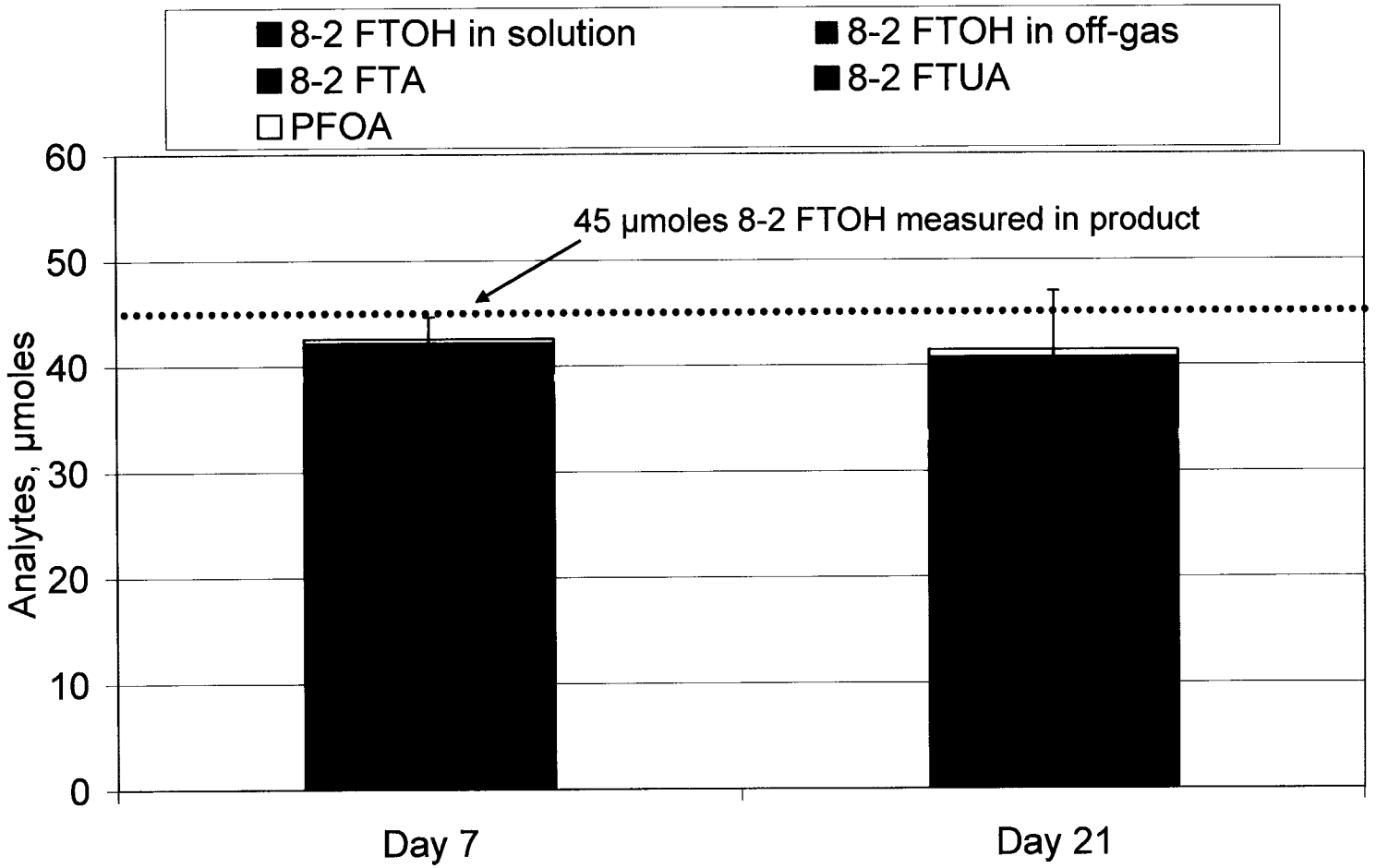
- Preliminary studies :
  - Develop, demonstrate and optimize experimental methods and analytical procedures.
- Main studies
  - Assess degradation potential of fluorotelomer-based polymers by monitoring formation of potential products of degradation
    - 8-2 Fluorotelomer Alcohol (FTOH): $F(CF_2)_8CH_2CH_2OH$
    - 8-2 Fluorotelomer Acid (FTA): $F(CF_2)_8CH_2COOH$
    - 8-2 Fluorotelomer Unsaturated Acid (FTUA): $F(CF_2)_7CF{=}CHCOOH$
    - Perflurorooctanoic Acid (PFOA): $F(CF_2)_7COOH$

Aerobic Activated Sludge Studies

- Used experimental set-up for a Modified Zahn-Wellens Study (OECD 302B)
- 300 mg DOC as test substance/L
- 1 g sludge (D.W.)/L
- Up to 28 days
- Measured Dissolved Organic Carbon, F-, and specific metabolites
  - Trapped gasses in C18 resin column
  - Initial experiments to examine polymer degradation in sludge

Inherent Biodegradability : Acrylate Polymer

- No evidence of polymer transformation observed after 21 days



General Experimental Design : Soil Studies

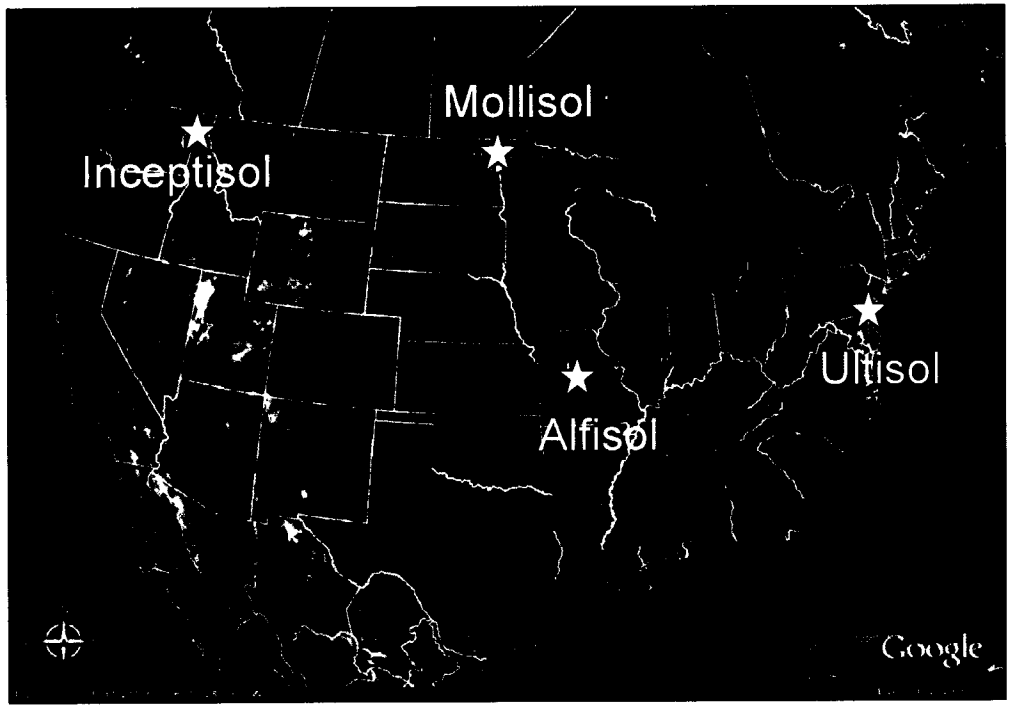
- Background Blank Control: no test item added
- Test Item: test item concentration of 200 mg active ingredient kg^{-1} dry wt.
- Abiotic Control: sterilized test medium with added test item concentration of 200 mg a.i. kg^{-1} DW soil.
- Spike Recovery Controls: sterilized medium spiked with following analytes:
  - 8-2 FTOH, 8-2 FTA, 8-2 FTUA, PFOA

Soil Studies : OECD 307 as a guideline

- Preliminary Study
  - Two test items and one soil under aerobic conditions for 28 days
    - Sandy clay loam from North Dakota; pH 7.9; 1.2 % organic carbon
  - Demonstrated experimental and analytical capabilities, recoveries of analytical spikes.
  - No evidence of polymer transformation observed

Main soil studies : In-progress

- Four soils
  - Alfisol: Howard County, Missouri
    - Sandy loam (11% clay), pH 5.8, Organic carbon 1.4%
  - Inceptisol: Deer Park (Spokane Co.), Washington
    - Loam (23% clay), pH 6.6, Organic carbon 2.0%
  - Mollisol: Grand Forks County, North Dakota
    - Sandy clay loam (23% clay), pH 6.9, Organic carbon 2.1%
  - Ultisol: New Castle Co, Delaware
    - Sandy loam (14% clay), pH 4.8, Organic carbon 2.9%



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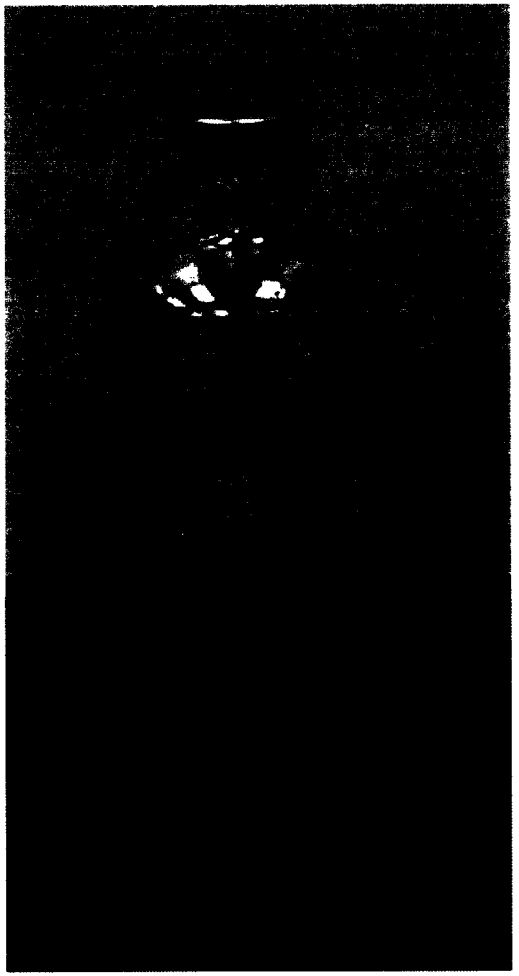
Soils Studies

- Test vessels are glass serum bottles with aluminum foil-lined closures incubated statically at 20°C.
- Soil moisture content assessed regularly by weighing each bottle and adding water as needed to maintain a level of 40 to 60% water-holding capacity.
- A headspace sample from each vessel is passed through a C18 cartridge. The entire contents of the test vessel are then extracted first using acetonitrile and second with an aliquot of a 200 mM NaOH solution.

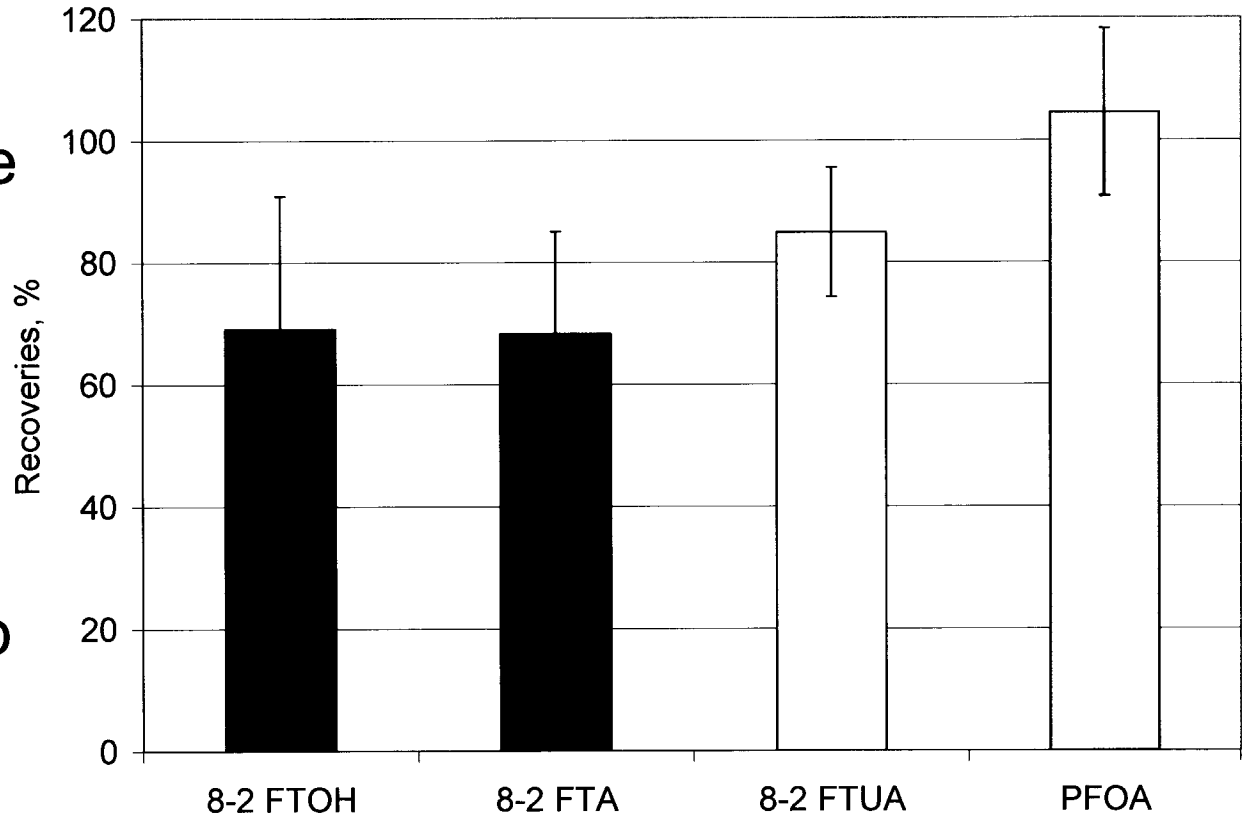


Soils Studies : Main Studies in-progress

- Measurements at 0, 1, and 2 weeks, and 1, 2, 4, and 6 months
  - All analytes extracted from control (untreated), treated live, treated sterile, and spiked (with all analytes, no test substance) sterile soils, each replicated twice
  - 8-2 FTOH measurements of headspace samples
  - Moisture content, viability assessments, O_2 content of treatments, biomass (months 0, 4 and 6)
- Status
  - Test systems working well; fast mineralization of 14C-glucose; moisture content constant (~50% WHC)
  - Changing to LC/MS to determine 8-2 FTOH in soil extracts
  - No analytes in controls; 8-2 FTOH not measured in headspace samples

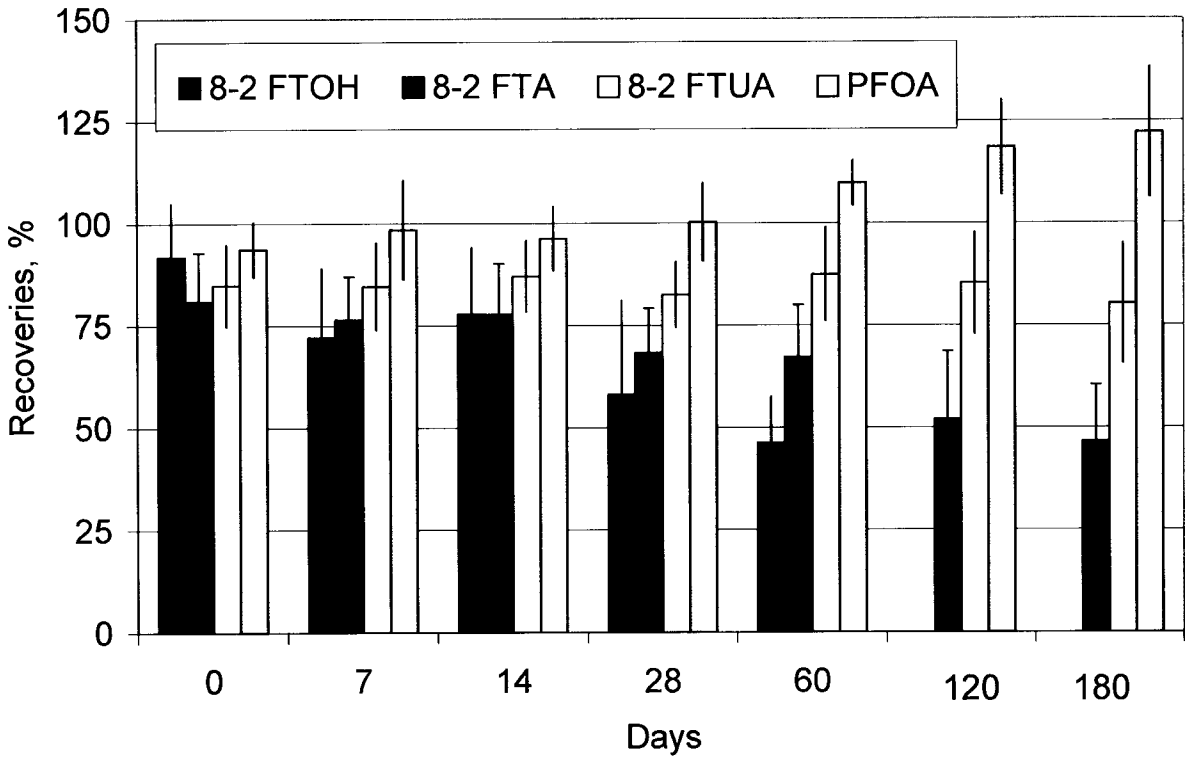
Recoveries from four soils

- Includes all four soils incubated under sterile conditions
- Spikes
  - 8-2 FTOH: 250 ug/Kg
  - Acids: 10 ug/Kg
- Average recoveries up to 6 months
  - 8-2 FTOH up to 60 days
  - ~70% and greater.



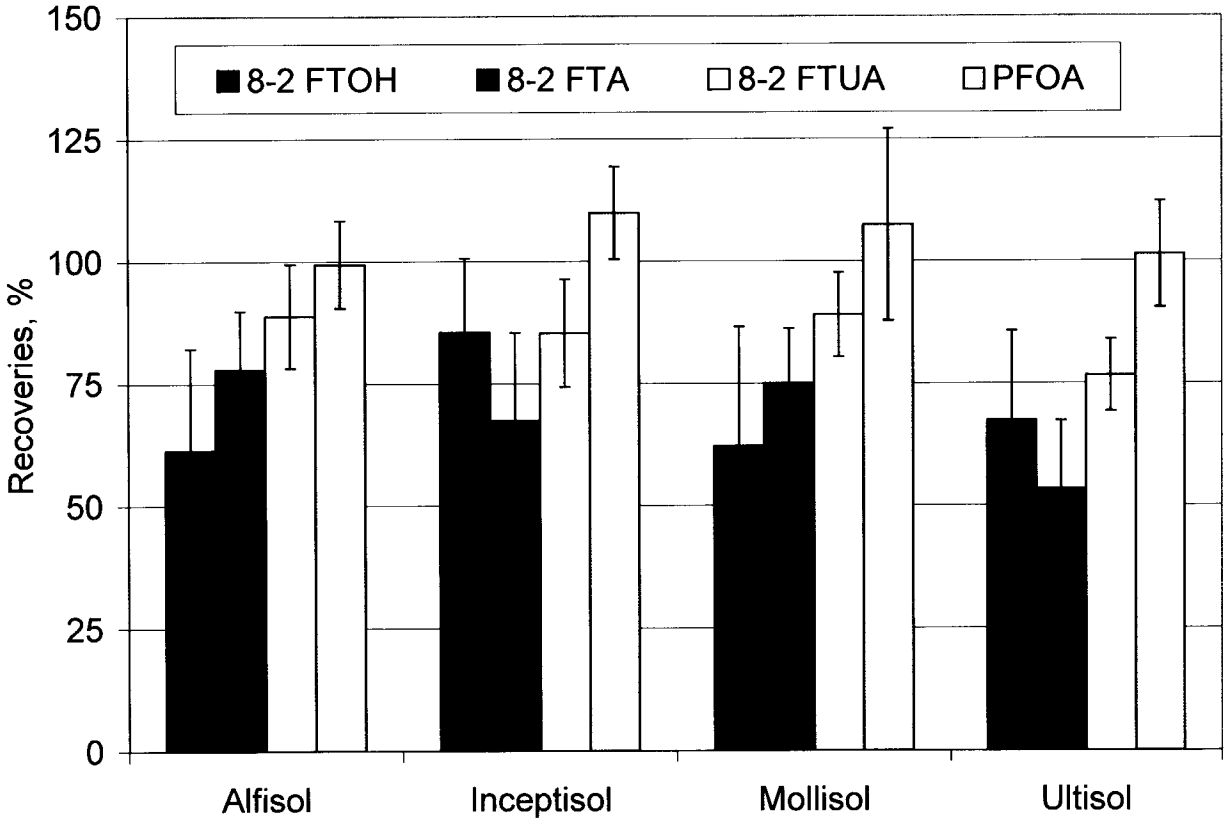
Recoveries from Soils up to 180 days

- Four soils, sterile conditions
- Recoveries of PFOA and 8-2 FTUA > 80%
- 8-2 FTOH and 8-2 FTA recoveries decreasing with time.
  - Not recovered – bound residue



Recoveries from Soils up to 180 days

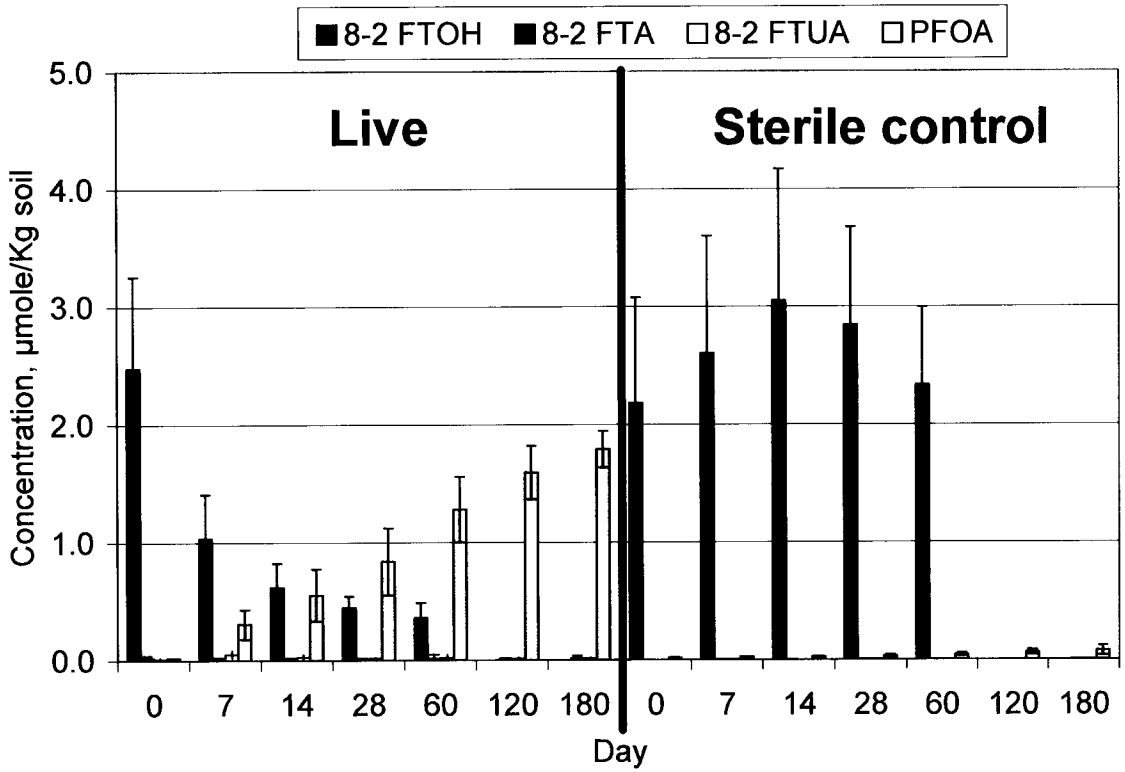
- Average recoveries up to 6 months under sterile conditions
  - 60 days for 8-2 FTOH.
- Recoveries of 8-2 FTOH variable
- Differences by soil type



Soil : Acrylate Polymer Product

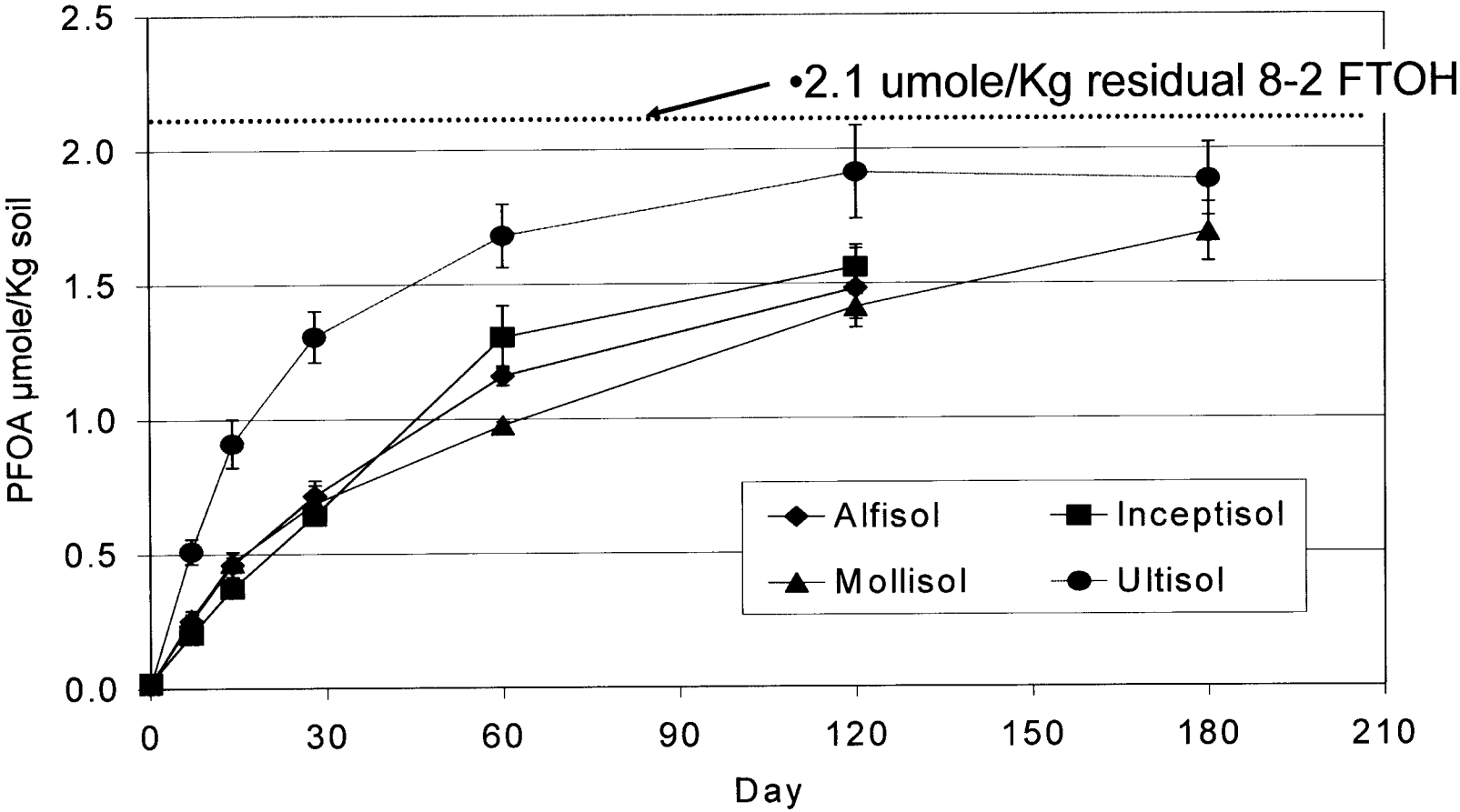
- 8-2 FTOH concentration decreases indicating biodegradation
- After 180 days, PFOA is less than the 8-2 FTOH added to soil as an residual
- Month 6 samples taken. Continue to one year
- **No evidence of polymer transformation observed**

Four soils combined



- 2.1 µmoles 8-2 FTOH•Kg^{-1} soil added as residual
- 130 µmole equiv.8-2 FTOH•Kg^{-1} soil within polymer
- LOQ of 8-2 FTA and 8-2 FTUA < ~0.004 µmoles•Kg^{-1}
- 8-2 FTOH not measured at 120 and 180 days

[redacted] **Acrylate Polymer Product** [redacted]oval.



- **PFOA formed appears to be leveling off at the concentration of residual 8-2 FTOH.**
- **PFOA formation is *only* from the degradation of residual 8-2 FTOH and *not* from the polymer.**
- **No evidence of polymer transformation observed**

Summary – Soil Studies

- No evidence of polymer degradation observed thus far
- Demonstrated analytical methods & study operation
- Sterile spike recoveries
  - Good overall recoveries, especially for PFOA and 8-2 FTUA
  - Validating alternative method and will reanalyze samples for 8-2 FTOH
- 8-2 FTOH was not found in the head space of any samples,
  - including sterile soils to which it had been fortified at 0.54 µmole kg^{-1} (250 µg kg^{-1}, LOQ < 0.0125 µg mL^{-1} headspace).
- Acrylic Polymer
  - 8-2 FTOH, 8-2 FTA, 8-2 FTUA, and PFOA extracted from soils can be accounted for by transformation of fluorinated residuals alone.
  - Similar results for other polymer products under study

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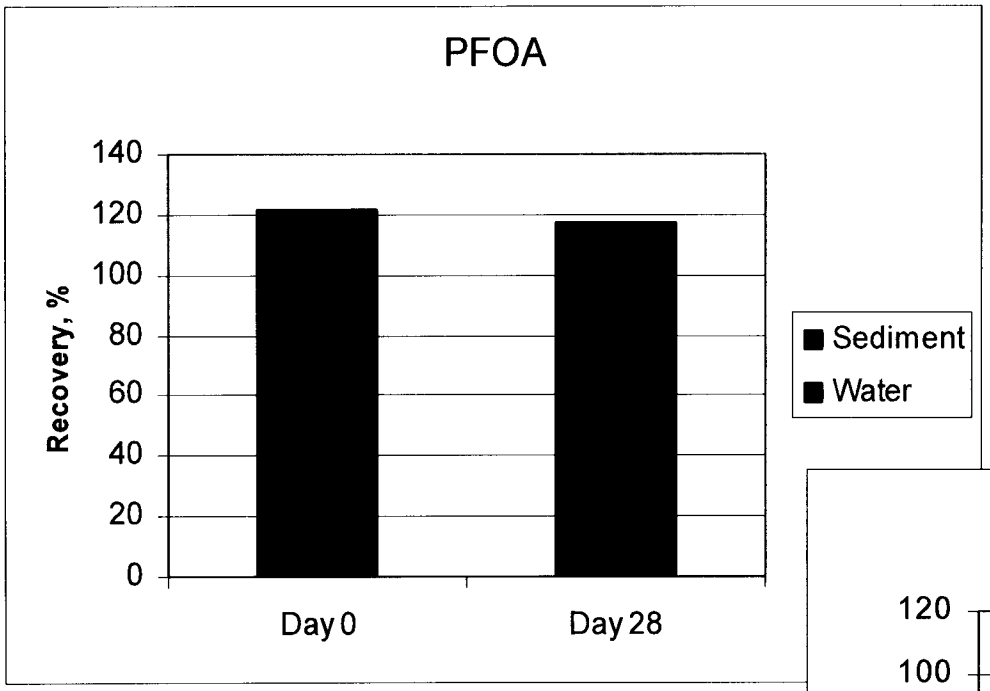
Sediment Studies

- Treatments and methods for the sediment similar to soil studies.
- Sediment and water added to each test vessel
  - water:sediment volume ratio between 3:1 and 4:1 ; the minimum sediment layer is ~ 2 cm.
- Headspace gases continuously purged a low air flow rate; passed through a C18 cartridge.
- Anaerobic test vessel conditions are imposed as in the soil test.
- In addition to headspace and sediment extract samples, separate aqueous samples are collected and analyzed.

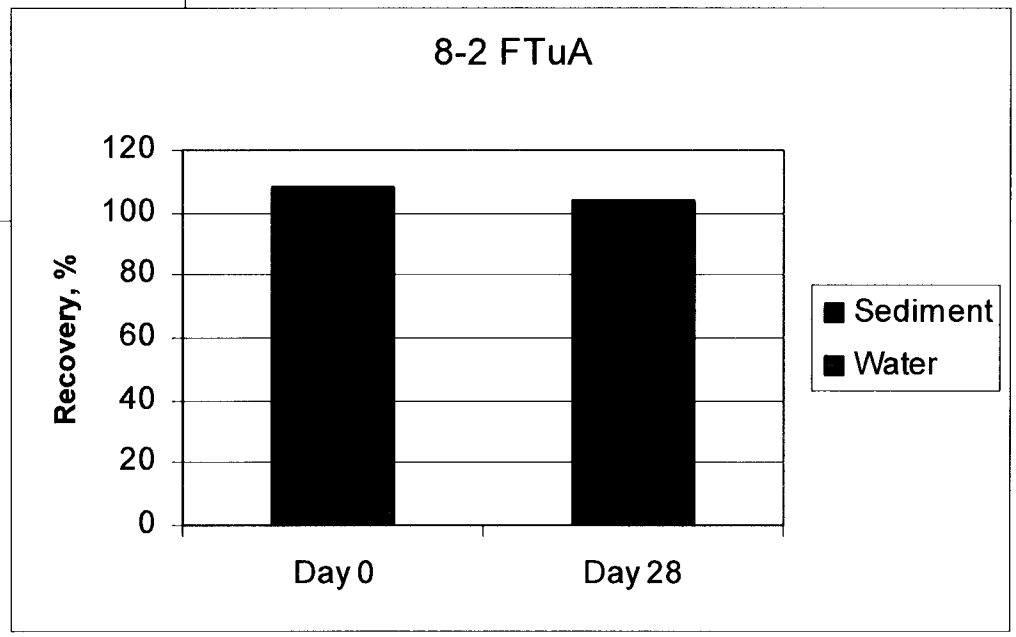
Sediment System Studies : OECD 308 Guideline

- Preliminary Studies : *complete*
  - In-life completed (28 days)
  - Anaerobic sediment from Turkey Creek (Talbot County, MD USA)
    - High organic carbon content (6.4% OC), pH 5.6, and fine texture (loam)
  - Changing to LC-MS (from GC-MS) for 8-2 FTOH to improve analytical after validating method
- Main Study
  - Review preliminary study results for recoveries once 8-2 FTOH analysis complete and revise protocol
  - 2 sediments
    - Aerobic sediment from Choptank River (Denton, MD USA)
      - Low organic carbon content (0.5% OC) and a course texture (sand).
    - Same source of anaerobic sediment as that used in preliminary study
  - 12 month duration; may extend to 18 months depending on data from soil studies

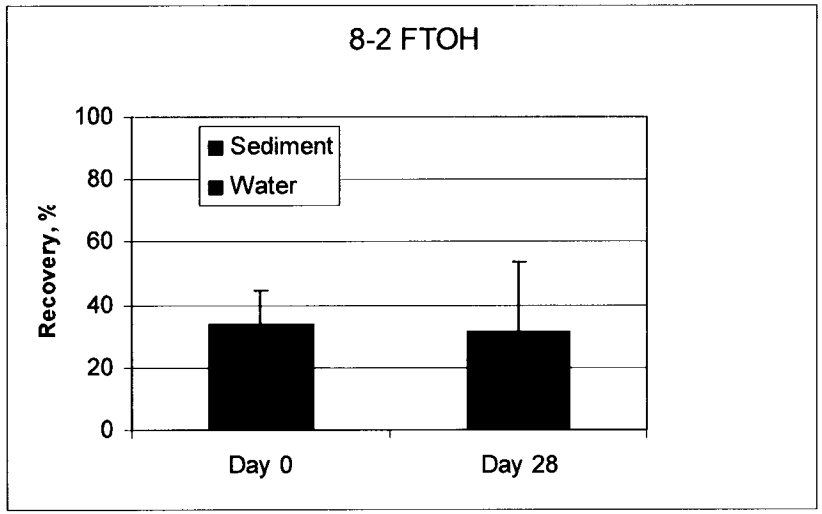
Preliminary studies in anaerobic aquatic sediments: Spike Recoveries



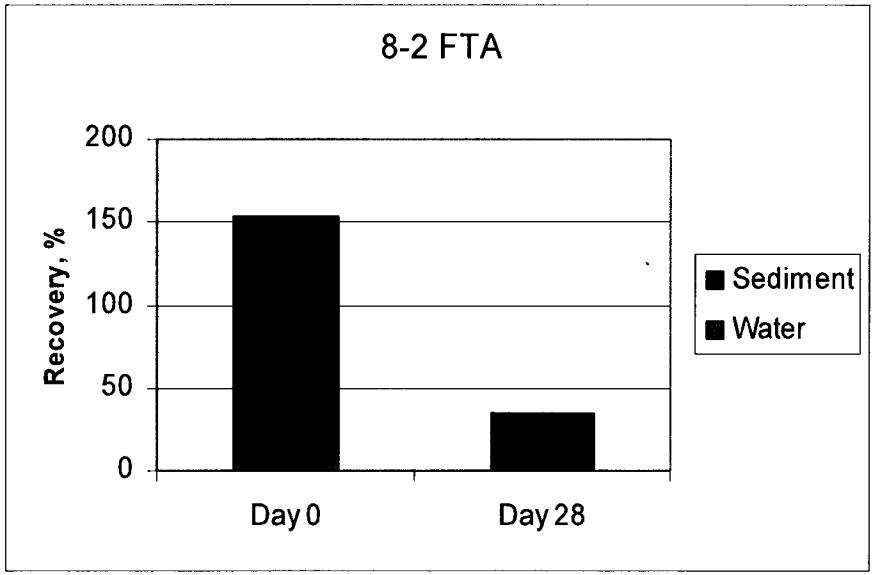
- One sediment, sterile conditions
- Good recoveries of PFOA and 8-2 FTUA



Preliminary studies in anaerobic aquatic sediments: Spike Recoveries



- One sediment, sterile conditions
- Low recoveries of 8-2 FTOH
- Recoveries of 8-2 FTA inconsistent



Summary - Preliminary sediment studies

- No evidence of polymer degradation observed
- Low recoveries of 8-2 FTOH from spike recovery control systems
  - Validating LC-MS method (from GC-MS) for 8-2 FTOH and reanalyze samples
- Revising and finalizing protocol for main study
  - Assess ability of test vessels and systems to contain analytes of interest, especially 8-2 FTOH

Anaerobic Biodegradability in Digester Sludge Study

- OECD 311 Guideline
- Preliminary Study
  - –Develop and validate analytical methods in digester sludge
  - –Protocol under review
    - 1 anaerobic digester sludge
    - Incubate at 35 degrees C
    - Sample on days 0, 14, and 28

Summary

Study results to date

- Soil and Sludge methodologies and analytical methods have been demonstrated
- No evidence of polymer transformation observed
  - Inherent Biodegradation in Sludge – 28 Days
  - Biodegradation in four Soils – 180 Days

Ongoing studies

- Soil Studies continuing to one-year
- Sediment studies commenced continuing to one year
- Anaerobic sludge studies to commence shortly