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**BIODEGRADATION STUDY REPORT**

**Revision 1**

Wednesday, November 06, 2002

**Biodegradation Screen Study for Telomer-Type Alcohols**

**PROJECT NUMBER**

3M Projects ID: **E01-0684**  
Pace Contract Analytical Projects ID: **CA085**

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**PROJECT DATES**

Project Initiation: August 9, 2000  
Project Completion: September 18, 2001

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## Executive Summary

A screening study was undertaken to determine whether the fluorochemical telomer intermediate (telomer alcohol) biodegrades when exposed to municipal wastewater treatment sludge. The study included the preparation of cultures for a six sample-point comparative study, and included test cultures, blank cultures, and control cultures. The microbial inoculum for cultures was sludge obtained from the Twin Cities Metro Wastewater Treatment Facility. Telomer alcohol test substance was added to cultures at approximately 5  $\mu$ M concentrations. Cultures were incubated with shaking at 25°C. Solid phase extraction of cultures for recovery of biodegradation products and parent analytes was employed. An HPLC/MS analytical method was developed for analysis of the telomer alcohols and expected products. The HPLC/MS analysis of culture extracts provided strong evidence that the telomer alcohols were biodegraded. The observed loss of telomer alcohols occurred concomitant with the appearance of several expected perfluorinated carboxylic acids. Unexpectedly, transiently formed polyfluorinated  $\beta$ -oxidation intermediates were observed. The  $\beta$ -oxidation pathway was suspected to be the major route of biodegradation resulting in the observed even-numbered carbon chain length perfluorinated fatty acids. Minor end products, as odd-numbered carbon chain length perfluorinated fatty acids, were also observed and were likely the products of fatty acid  $\alpha$ -oxidation, a minor pathway of fatty acid metabolism usually observed in branched-chain fatty acids. After the 16day test period, perfluorinated carboxylic acids ranging from C<sub>5</sub> to C<sub>12</sub> were observed in the test cultures. These compounds were not in controls or blank cultures. Perfluorooctanoate (PFOA), was the only compound quantitatively analyzed and, based on mass balance data accounted for approximately 6-7% of the total telomer alcohols initially present in the test cultures.

## 1.0 Project Personnel

|       |                              |                       |
|-------|------------------------------|-----------------------|
| 1.1   | Sponsor Company              | 3M                    |
| 1.2   | Sponsor Representative       | Dr. James K. Lundberg |
| 1.3   | Contract Facility Personnel: |                       |
| 1.3.1 | Study Director               | Dr. Cleston C. Lange  |
| 1.3.2 | Laboratory Management:       | Mr. Bruce E. Warden   |
| 1.3.3 | Sample Preparation           | Ms. Angela L. Schuler |
|       |                              | Dr. Cleston C. Lange  |
| 1.3.4 | HPLC/MS Analyst              | Ms. Angela L. Schuler |
| 1.3.5 | Sample Custodian:            | Dr. Cleston C. Lange  |
| 1.3.6 | Report Author                | Dr. Cleston C. Lange  |

## 2.0 Data Requirements and Revision Justification

The sponsor representative desired an aerobic biodegradability screening study to be conducted using the telomer alcohol mixture as test substrate. The study was initiated on August 9, 2000 as a non-GLP study.

The report issued Friday, November 16, 2001 is revised to substitute "Dupont Zonyl BA type Telomer, or Zonyl type Telomer" with Telomer. The reason for the revision is to more accurately reflect the impact of results to the general class of telomer alcohols.

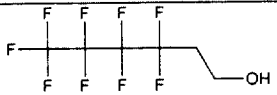
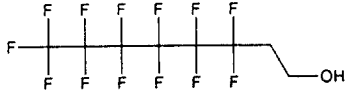
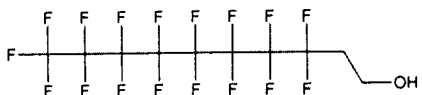
## 3.0 Project Objective

This study was conducted in order to elucidate whether the fluorotelomer intermediate (telomer alcohol) is biodegradable under aerobic conditions using a microbial rich inoculum of municipal wastewater treatment sludge. This study was similar to earlier studies conducted for 3M by Pace as Pace projects CA058 <sup>1</sup> and CA097 <sup>2</sup> and CA104 <sup>3</sup>, CA105 <sup>4</sup>, and CA132 <sup>5</sup>. The development of an analytical method and the analysis of parent analytes and possible products were critical parameters for the determination of biodegradation.

#### 4.0 Test Article

The test article used for this study was the Zonyl BA-type telomer alcohol fluorochemical surfactant intermediate. Two sources of test material were used for this study. The first source was provided by the sponsor company, 3M, as approximately 2.5 g of crystalline solid test material labeled Zonyl BA-N telomer alcohol, 3M tracability number TN-A-2186, on July 7, 2000. The sponsor did not provide an MSDS, a chain of custody, the purity information for the test article, nor an expiration date for the material. This material was given a test, control, and reference (TCR) number at Pace as CA-TCR02-009 and was stored at 4°C. Material CA-TCR02-009 was used primarily for method development purposes of the study, including the first set of cultures prepared and original LC/MS method development.

A commercial vendor, Aldrich Chemical Company, provided the second source of telomer alcohol used. Material was purchased as Zonyl BA-L fluorotelomer intermediate (1999 Aldrich Catalog number 42,151-0), also called perfluoroalkylethanol. The material characteristics were boiling point 145-245°C,  $F(CF_2)_nCH_2CH_2OH$  where  $n$  equals approximately 7 to 8, and molecular weight was reported as  $M_n$  ca. 443.70 wt. An expiration date and the purity, or percent composition, of the material were not provided. The purchased material was received at Pace on September 22, 2000. Upon receipt at Pace, the material was given tracability number CA-TNC00-254 and was stored at 4°C, or less. A representative chemical compilation of the Zonyl BA-type telomer alcohol is shown in Figure 1.

|                                                                                     |                       |                                                                                                                                                    |
|-------------------------------------------------------------------------------------|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
|    | <b>C<sub>6</sub></b>  | Molecular formula = C <sub>6</sub> H <sub>5</sub> F <sub>9</sub> O<br>Molecular Weight = 264.089<br>[M + CH <sub>3</sub> COO] <sup>+</sup> = 323   |
|    | <b>C<sub>8</sub></b>  | Molecular formula = C <sub>8</sub> H <sub>5</sub> F <sub>13</sub> O<br>Molecular Weight = 364.104<br>[M + CH <sub>3</sub> COO] <sup>+</sup> = 423  |
|    | <b>C<sub>10</sub></b> | Molecular formula = C <sub>10</sub> H <sub>5</sub> F <sub>17</sub> O<br>Molecular Weight = 464.119<br>[M + CH <sub>3</sub> COO] <sup>+</sup> = 523 |
|    | <b>C<sub>12</sub></b> | Molecular formula = C <sub>12</sub> H <sub>5</sub> F <sub>21</sub> O<br>Molecular Weight = 564.134<br>[M + CH <sub>3</sub> COO] <sup>+</sup> = 623 |
|  | <b>C<sub>14</sub></b> | Molecular formula = C <sub>14</sub> H <sub>5</sub> F <sub>25</sub> O<br>Molecular Weight = 664.149<br>[M + CH <sub>3</sub> COO] <sup>+</sup> = 723 |
|  | <b>C<sub>16</sub></b> | Molecular formula = C <sub>16</sub> H <sub>5</sub> F <sub>29</sub> O<br>Molecular Weight = 764.164<br>[M + CH <sub>3</sub> COO] <sup>+</sup> = 823 |

**Figure 1.** The telomer alcohols. Based on the HPLC/MS average peak area response observed in the six abiotic control cultures, the composition of the test material was 6.4% ± 0.3% as C<sub>6</sub> telomer alcohol, 39.3% ± 1.5% as C<sub>8</sub>, 27.7% ± 2.1% as C<sub>10</sub>, 17.5% ± 1.2% as C<sub>12</sub>, 7.2% ± 1.0% as C<sub>14</sub>, and 2.0% ± 0.2% as C<sub>16</sub>.



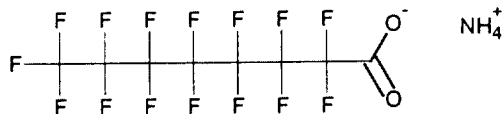
## 5.0 Reference Materials

The sponsor provided reference material. The neat material was stored at -80°C.

NS: Expiration date not specified

### 5.1. Perfluorooctanoate Ammonium Salt (PFOA)

Purity: **95.2 %**; 3M#: TCR-99131-37; Pace #: CA-TCR02-001; Expires: NS



**Figure 2.** Perfluorooctanoate ammonium salt

## 6.0 Receipt/Generation of Samples

Samples were not received, but were generated as an inherent part of this study. All of the experimental cultures prepared for each culture set were extracted by solid phase extraction (SPE) methodology to generate three analytical samples per culture. The analytical samples, as SPE eluates collected for each culture, were labeled as SPE eluates 1, 2 or 3. Of the three eluates generated for each sample, eluate 1 was the 25 mL aqueous sample eluate and eluates 2 and 3 were 25 mL methanol eluates from the SPE cartridge. Qualitative and semi-quantitative HPLC/MS analysis was conducted for eluate 2 only, and the resulting data was evaluated to determine whether biodegradation occurred.

## 7.0 Methods

### 7.1 Sample Preparation

#### 7.1.1. Collection of Sludge.

The sludge for this study was obtained from the Twin Cities Municipal waste treatment facility in a manner consistent with the sludge collected for other studies listed in section 3.0. The sludge used for the preparation of the first set of cultures was collected on July 31, 2000 and

delivered with Pace chain of custody (COC) form 465254. The sludge for the second set of cultures was collected on September 18, 2000 and was accompanied with COC # 528791. Sludge used for the second set of cultures in this study was also used in preparation of cultures for other fluorochemical biodegradation studies (CA105 <sup>4</sup> and CA132 <sup>5</sup>), and was shown to be active for biodegradation of other fluorochemical compounds in those studies.

To prepare cultures, sludge was obtained from the primary municipal waste treatment facility in the Twin Cities area. Arrangements were made for Pace personnel to retrieve fresh mixed liquor suspended solids (MLSS) from the aeration units at the Twin Cities Metro Wastewater Treatment Facility located in St. Paul, MN. Typically, Four liters of MLSS was collected by Pace laboratory personnel and delivered as four 1-liter Nalgene polypropylene bottles containing MLSS, and were accompanied with a corresponding chain of custody with date collected. Upon receipt at Pace Science Solutions, the individual bottles were labeled #1 through #4. The suspended solids in the bottles were allowed to settle at least 24 hours at 4°C  $\pm$  3°C. The settled solids "sludge" were then used to prepare MLSS plus sludge for use in preparing test cultures. A sludge characterization analysis was not conducted as part of this screening study.

The settled sludge in each bottle constituted approximately 20% of the volume, or approximately 200 mL volume in a 1-liter bottle, based on visual observations-and was consistent with observation of earlier sludge collections.

### 7.1.2. Culture Preparation.

The first set of cultures were prepared August 9, 2000 and incubation of those cultures continued with intermittent culture harvests until the final harvest on September 13, 2000, for a total incubation time of 35 days. The second set of cultures were prepared on September 27, 2000 and incubation of those cultures continued with intermittent culture harvests until the final harvest on October 12, 2000, for a total of 16 days incubation. The culture preparation procedure described below was used, and was documented as Pace standard operating procedure (SOP) CAG-SP-03<sup>6</sup>.

Cultures were prepared using a mineral salts medium defined by EPA Guideline OPPTS 835.3200. The mineral salts medium pH was 7.4 and contained per liter, 0.334 g  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ , 0.005 g  $\text{NH}_4\text{Cl}$ , 0.2175 g  $\text{K}_2\text{HPO}_4$ , and 0.085 g  $\text{KH}_2\text{PO}_4$ , 0.0275 g  $\text{CaCl}_2$ -anhydrous, 0.0225 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.00025 g  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ .

Two liters of a mineral salts medium containing 100 mL of settled sludge was prepared and contained 1 mL of methanol. To each test culture was added 25 mL of this mineral medium containing sludge.

A mineral salts medium, un-sterilized and without sludge, was prepared. This mineral salts medium without sludge was used to prepare the 25 mL no-sludge (abiotic) control cultures.

All cultures were prepared by dispensing 25 mL of appropriate mineral salts medium solution into clear sterile 125 mL Nalgene polycarbonate culture flasks containing labels with appropriate identification information. Note: The mineral salts medium that contained sludge had to be swirled regularly during dispensing in order to keep the mixture homogenous and prevent the sludge from settling out of the solution.

The test substance, either Zonyl BA-N telomer alcohol for culture set one (Stock ID CA058-SS-004 at 10,280  $\mu\text{g/mL}$  in methanol) or Zonyl BA-L

telomer alcohol for culture set two (Stock ID CA085-SW-001 at 7,288 µg/mL in methanol), was added to the test cultures by transferring 5 µL and 8µL, respectively, to the appropriate test cultures. The final test concentration in cultures was 2.056 µg/mL of Zonyl BA-N telomer alcohol for set one, and 2.332 µg/mL of Zonyl BA-L telomer alcohol for set two. Blank control cultures received 25 mL of mineral medium solution, as did the test culture, but without addition of the test substrate.

All of the day zero cultures were prepared and immediately frozen at -20°C for storage until SPE preparation could be conducted. All other cultures were placed in temperature controlled shaking incubators that were maintained at 25°C ± 3°C. Cultures were removed from the incubators at designated time points. Upon removal from the incubator, cultures were either immediately frozen, or immediately prepared for analysis by solid phase extraction.

All culture preparation information, including times, analyte additions, etc. were recorded in sample preparation worksheets and signed and dated by the preparation analyst. All original data sheets were maintained in project specific binder labeled as Project CA085.

#### **7.1.3. Solid Phase Extraction of Cultures**

The solid phase extraction procedure described below was documented as Pace standard operating procedure (SOP) CAG-SP-04 <sup>7</sup>.

All cultures and control cultures were prepared by solid-phase extraction methodology using SEP-VAC C18 6cc SPE cartridges from Waters Corporation (Part No. WAT036905). A sample label was applied to each SPE cartridge prior to use, and each cartridge was packed with plug of quartz glass wool to deter plugging. Each SPE cartridge was washed prior to use by drawing 5 mL of methanol and then 5 mL of aqueous 1% acetic acid solution through the cartridge. These wash solution eluates were discarded to waste. All of the SPE eluates for this study were collected in clear I-Chem vials with labels that identified them as eluate 1, 2 or 3, as defined below.

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Frozen cultures were thawed at ambient room temperature before extraction. Following thawing, and prior to solid phase extraction, 0.25 mL of glacial acetic acid was added to each of the cultures yielding a final concentration of 1% acetic acid. The content of each acidified culture was swirled to mix, and then drawn by vacuum through the appropriately labeled SPE cartridge by carefully pouring the contents of the culture flask into the SPE cartridge. The aqueous eluate was collected in an I-Chem vial labeled eluate 1, removed from the vacuum manifold, and capped. Then, 25 mL of methanol was added to the culture flask, the flask sealed, and vigorously shaken. The cap was then removed from the flask, and the methanol content (25 mL) drawn through the SPE cartridge, collected in an I-Chem vial labeled eluate 2. Eluate 2 was expected to contain a majority of the analyte that was in the original culture.

As a precaution that some analyte may be retained in the SPE cartridge or in the culture flask, a second 25 mL methanol eluate was collected in a similar fashion to that collected for eluate 2, and was labeled eluate 3. Aliquots of eluates 2 and 3 were transferred to autovials, capped, and then quantitatively analyzed by HPLC/MS. The remaining volume of each eluate was stored at  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ .

The final SPE extractions of the day 7, 14 and 16 day cultures from set two occurred on September 24, 2001.

## **7.2 Instrumental Analysis (LC/MS)**

### **7.2.1 Instrument Parameters**

The HPLC/MS method used was a modified version of Pace method CAG-ORG-23<sup>8</sup>, as described below.

Analysis of culture extracts from culture set one, prepared with Zonyl BA-N telomer alcohol, was conducted using instrument "STING" which included an HP1100 HPLC pump with Gilson 215 liquid handling system in line with a Micromass Quattro II triple-quadrupole mass spectrometer detector.

Analysis of culture extracts from culture set two, prepared with Zonyl BA-L telomer alcohol, were analyzed both on instrument "STING" and instrument "10LCMS03" which consisted of a Waters 2690 HPLC system in line with a Micromass Quattro II triple-quadrupole mass spectrometer detector. Typical conditions for analysis of telomer alcohols and fluorochemical acid products were as follows:

**Instrument conditions for HPLC/MS analysis.**

**Mass Spectrometer**

Ionization mode: API-ES negative  
 Desolvation Temperature: 200°C  
 Source Block Temperature: 150°C  
 RF lens: 0.2  
 Extractor: 3  
 Cone: 8  
 Capillary: 3.50  
 LM Resolution: 14  
 HM Resolution: 14  
 Ion Energy: 2.5  
 Lens 6: 2  
 MS1 Multiplier: 650  
 MS/MS: Collision Energy-varied from 10 to 40 V for individual Experiments,  
 MS/MS Collision Gas: Argon  
 MS2 Multiplier: 750

**HPLC**

| Time (min.) | %A   | %B   |
|-------------|------|------|
| 0.00        | 97.0 | 3.0  |
| 0.50        | 97.0 | 3.0  |
| 5.00        | 5.0  | 95.0 |
| 11.00       | 5.0  | 95.0 |
| 11.50       | 97.0 | 3.0  |
| 15.00       | 97.0 | 3.0  |

Flow: 1 mL/min, splitter approx. 3:1  
 Solvent A= 2 mM ammonium acetate  
 Solvent B= Methanol  
 Column Temperature: ambient

Typical injection volumes for samples and calibration standards were 50 µL. The 4.6 x 150 mm Betasil C8 column used for the quantitation of extracts from culture set two had serial number 1101567H and the 4 x 35 mm NG1 column used had serial number 15104. A pressure-regulated splitter was used with an approximate split ratio of 2:1 (Waste:MS)

**7.2.2 Qualitative Parent Analyte and Products Analysis.**

| Parent Analyte                                 | Expected Ions (m/z) |
|------------------------------------------------|---------------------|
| C <sub>8</sub> telomer alcohol acetate adduct  | 423                 |
| C <sub>10</sub> telomer alcohol acetate adduct | 523                 |
| C <sub>12</sub> telomer alcohol acetate adduct | 623                 |
| C <sub>14</sub> telomer alcohol acetate adduct | 723                 |
| C <sub>16</sub> telomer alcohol acetate adduct | 823                 |

**Table 1.** Parent telomer alcohols analyzed.

To conduct the analysis of parent compounds and expected biodegradation products, the HPLC/MS system was set up with the same chromatographic configuration as that described in method CAG-ORG-23<sup>8</sup>. The method utilized two columns in tandem with flow to a pressure relief valve that serves as a flow through splitter to the mass spectrometer Z-spray source.

Specific MS conditions for mass analysis of the telomer alcohols and acid products were developed during this study. The mass spectrometer, Micromass triple-quadrupole mass spectrometer with Z-spray ion source, was operated with electrospray ionization in either selected ion-recording (SIR) mode or with mass-range (150-1000 m/z) scanning on MS1. Discrete chromatographic peaks with singly charged negative ions, (M + Acetate)<sup>-</sup> for parent telomer alcohols and (M - H<sup>+</sup>)<sup>-</sup> for expected acid biodegradation products, were observed and monitored:

| Expected Product                               | Expected anion structure                                                         | Expected anions (m/z) |
|------------------------------------------------|----------------------------------------------------------------------------------|-----------------------|
| Perfluorobutyrate (C <sub>4</sub> )            | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>2</sub> COO <sup>-</sup>                 | 213, 169              |
| Perfluoropentanoate (C <sub>5</sub> )          | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>3</sub> COO <sup>-</sup>                 | 263, 219              |
| Perfluorohexanoate (C <sub>6</sub> )           | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>4</sub> COO <sup>-</sup>                 | 313, 269              |
| Perfluoroheptanoate (C <sub>7</sub> )          | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>5</sub> COO <sup>-</sup>                 | 363, 319              |
| Perfluorooctanoate (C <sub>8</sub> , PFOA)     | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>6</sub> COO <sup>-</sup>                 | 413, 369              |
| Perfluorononanoate (C <sub>9</sub> )           | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>7</sub> COO <sup>-</sup>                 | 463, 419              |
| Perfluorodecanoate (C <sub>10</sub> )          | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>8</sub> COO <sup>-</sup>                 | 513, 469              |
| Perfluoroundecanoate (C <sub>11</sub> )        | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>9</sub> COO <sup>-</sup>                 | 563, 519              |
| Perfluorododecanoate (C <sub>12</sub> )        | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>10</sub> COO <sup>-</sup>                | 613, 569              |
| 2H, 2H-perfluorooctanoate (C <sub>8</sub> )    | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>5</sub> CH <sub>2</sub> COO <sup>-</sup> | 377                   |
| 2H, 2H-perfluorodecanoate (C <sub>10</sub> )   | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>7</sub> CH <sub>2</sub> COO <sup>-</sup> | 477                   |
| 2H, 2H-perfluorododecanoate (C <sub>12</sub> ) | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>9</sub> CH <sub>2</sub> COO <sup>-</sup> | 577                   |

**Table 2.** Expected products, and expected anions for each.

### 7.2.3 Quantitative Analysis.

Quantitative analysis was conducted only for perfluorooctanoate (PFOA) and only on extracts of culture set two. Quantitative analysis was performed by the external standard method using SPE extracted calibration standards (eluate 2 only) and quadratic calibration curves. The HPLC/MS analysis of culture set one extracts was used primarily for method development purposes.

Typical injection volumes for samples and calibration standards were 50  $\mu\text{L}$ . The 4.6 x 150 mm Betasil C8 column used for the quantitation of extracts from culture set two had serial number 1101567H and the 4 x 35 mm NG1 column used had serial number 15104. The pressure-regulated splitter had no identifying number to distinguish it. The part number for ordering the pressure relief valve (splitter) was Alltech catalog part number 39025.

## 7.3 Data Transformations and Calculations

### 7.3.1 Molar Calculations:

Because all data was collected on an ng/mL basis (part per billion, ppb), a transformation from ng/mL to molar concentrations had to be conducted to obtain mass balance information when applicable. The mole conversion values for each analyte are as follows:

PFOA molecular weight, as ammonium salt, is 431.10

Zonyl BA-L telomer alcohol average molecular weight is 434.70.

### 7.3.2 Conversion of ng/mL to micromolar ( $\mu\text{M}$ ) and nanomolar (nM).

(Working Examples):

$2,232 \text{ ng/mL telomer alcohol} = (2,232 \text{ ng/mL}) * (1 \text{ nmole} / 434.7 \text{ ng}) =$   
 $5.135 \text{ nmole/mL} = 5.135 \text{ }\mu\text{mole/liter} = 5.135 \text{ }\mu\text{M}$ —assuming 100% purity.

$150 \text{ ng/mL PFOA (NH}_4^+ \text{ salt)} = (150 \text{ ng/mL}) * (1 \text{ nmole} / 431.1 \text{ ng})$   
 $= 0.3479 \text{ nmole/mL} = 0.3479 \text{ }\mu\text{mole/L} = 0.3479 \text{ }\mu\text{M} = 347.9 \text{ nM}$

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### 7.3.3 Molar Mass Balance Calculations (Theoretical Yield):

Convert all ng/mL values to their corresponding molar concentrations as  $\mu\text{M}$  or nM (see section 7.3.2, above). Divide the sum of the analyte concentrations by the known concentration of starting compound and represent the final result as a percentage of the known starting concentration.

#### (Working Example):

If, the starting concentration of BA-L telomer alcohol was at  $5.135 \mu\text{M}$

And, after incubation, the following was determined:

If, PFOA was detected at  $0.3479 \mu\text{M}$

Then, the mass balance is as follows:

$$\text{Mass balance} = [(0.3479 \mu\text{M}) / 5.135 \mu\text{M}] \times 100\%$$

$$\text{Mass balance} = [0.0678] \times 100\% = \mathbf{6.78\%}$$

Or, 6.78% of the telomer BA-L alcohol was oxidized to form PFOA.

## 7.4 Software Versions

Microsoft™ Excel 2000 was used for data processing and producing tables.

Microsoft™ Word 2000 was used for processing the analytical report text.

Adobe Acrobat 4.0 was used for generation of the final electronic report.

Masslynx version 3.2 was used for data collection and peak integration.

ACD ChemsSketch version 4.0 was used for chemical drawings.

## **8.0 Results**

### **8.1 Sludge Characterization**

A chemical analysis of the mixed liquor suspended solids used for the samples prepared on August 9, 2000 was conducted, and characterization information can be found in the final report for project CA058<sup>1</sup>. The sludge for samples prepared on September 27, 2000 was not characterized, but was obtained from the Twin Cities Municipal waste treatment facility in a manner consistent with the sludge collected and used for project CA058.

### **8.2 Quality Control/Sample Matrix Spike Results.**

The determination of the analyte recoveries from sample matrix spikes was not included as part of this screening study. Recoveries are only reflected in the ability to achieve molar mass balance based on expected parent and product yields from samples and by the use of a sludge-extracted curve for semi-quantitative analysis of PFOA.

### **8.3 Analytical Blanks**

Methanol solvent blanks were injected onto the HPLC/MS column and quantitatively analyzed to determine the instrument background analyte concentration and carry over during the analysis.

The no-sludge (abiotic) controls containing mineral salts medium with test analyte, and culture blanks, containing mineral salts medium with test analyte and no sludge, were prepared and analyzed. Abiotic controls and blank cultures were prepared and incubated in an identical manner to biodegradation test cultures. The results were used to determine whether the sample matrix contained any of the analytes of interest and whether biodegradation could be attributed to the bioactivity of the sludge. Fluorochemical analytes were not detected in blanks and biodegradation did not occur in abiotic controls.

## 8.4 HPLC/MS Analysis Results

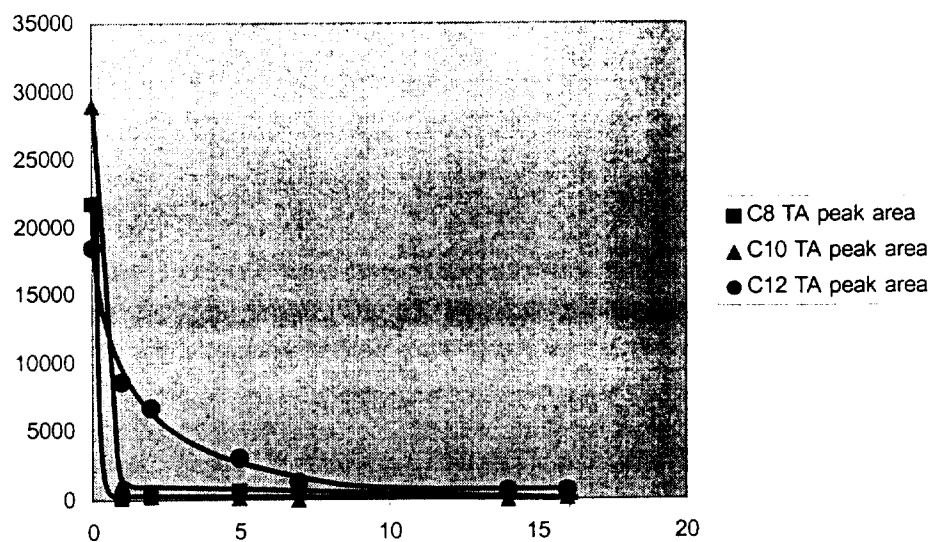
Semi-quantitative analysis was conducted only for the SPE eluate 2 extracts from the second set of cultures, prepared September 27, 2000, and were performed on September of 2001 as sequence CA085\_092401b.spl (with full scan MS data), and CA085\_092701a.spl (SIR data) on instrument 10LCMS03 in the Bio-Analytical services group at Pace. The qualitative HPLC/MS analyses and HPLC/MS method development for this study were conducted in September and October of 2000 as analytical sequence runs S091500.spl, S091800.spl, S092000.spl, S092100.spl, S092600.spl, S1000200.spl, and S100400.spl on Pace instrument "STING".

### 8.4.2 Qualitative HPLC/MS and HPLC/MS/MS Results

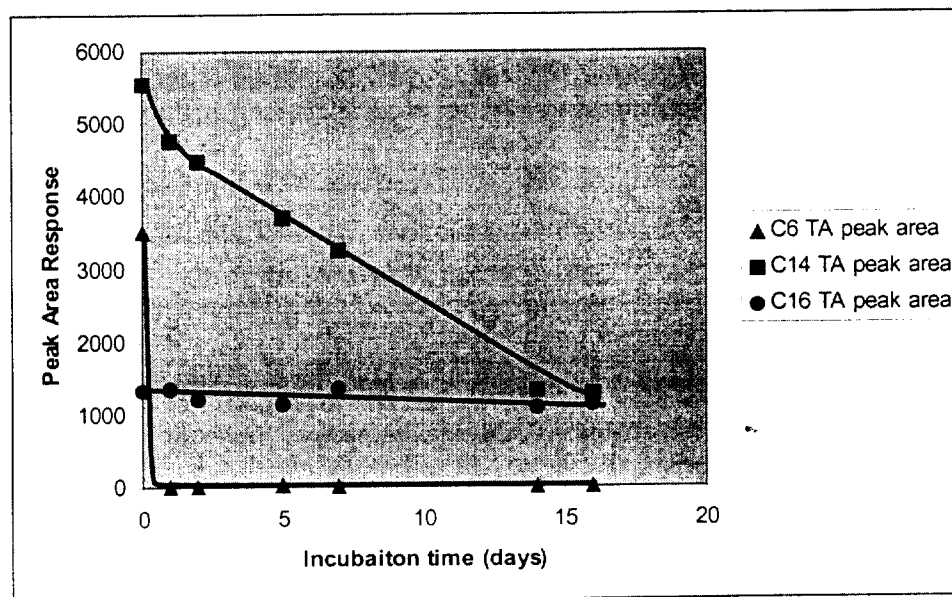
The first set of test cultures containing sludge and BA-N telomer alcohol substrate provided evidence that biodegradation occurred. The HPLC/MS data showed near total loss of all of the telomer alcohol peaks in the HPLC/MS chromatogram, with formation of chromatographic peaks that had mass spectra consistent with perfluorinated acids. However, at that time, the analytical test method was not complete. Concerns about the integrity of the test material resulted in a second set of test cultures being prepared using commercially purchased Zonyl BA-L telomer intermediate.

Although each sample, control, and blank for the second set of cultures was prepared and incubated in duplicate, analysis was conducted for SPE eluate 2 extracts from one sample, control and blank per time point.

The data showed that the sample test cultures underwent rapid loss of the C<sub>6</sub>, C<sub>8</sub>, C<sub>10</sub>, and C<sub>12</sub>-telomer alcohols, and moderate loss of the C<sub>14</sub>-telomer alcohol over the 16 days of incubation, as determined by decreasing peak area response for each telomer alcohol ion at the sequential sampling time points (**Figures 3 and 4**). The C<sub>16</sub>-telomer alcohol was degraded very slowly and showed little loss.



**Figure 3.** HPLC/MS integrated peak areas for the most abundant telomer alcohols (TA). Near complete degradation of all three was observed. Data plotted with the best manual fit of the data.



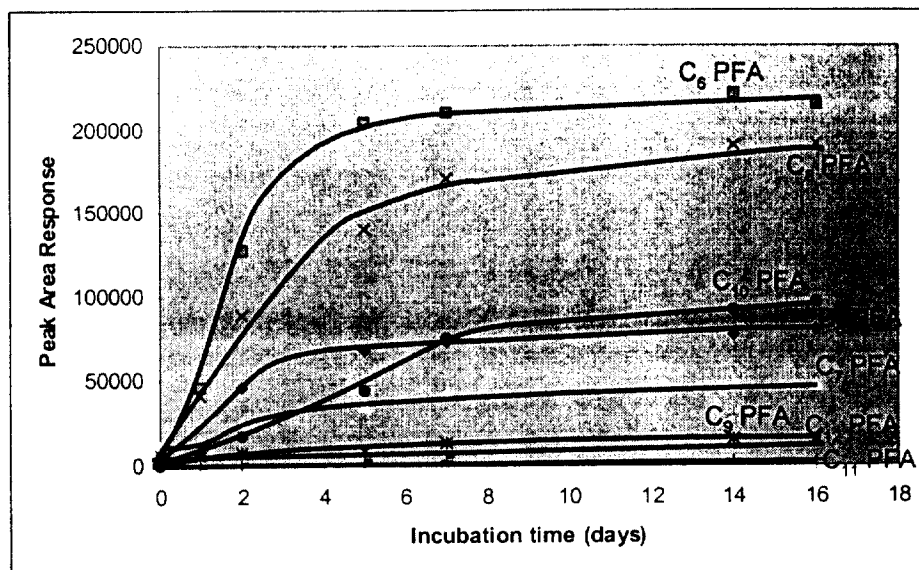
**Figure 4.** HPLC/MS integrated peak areas for the less abundant telomer alcohols (TA). Near complete degradation was observed for the C<sub>6</sub>-TA. Moderate degradation of the C<sub>14</sub>-TA was observed and little degradation of the C<sub>16</sub>-TA was observed. Data plotted with the best manual fit of the data.

Concomitant with the loss in telomer alcohols was the formation of compounds with HPLC/MS ions and retention times consistent with perfluorinated acids (PFAs), including perfluorooctanoate (PFOA, C<sub>8</sub>-PFA), which was confirmed as an end product. The transformation of telomer alcohols to PFAs was rapid, with a large increase in all of the PFA peak area responses at day 1, and continuing increase through day-16 (**Figure 5**). No degradation to form PFAs was observed in the abiotic no-sludge controls.

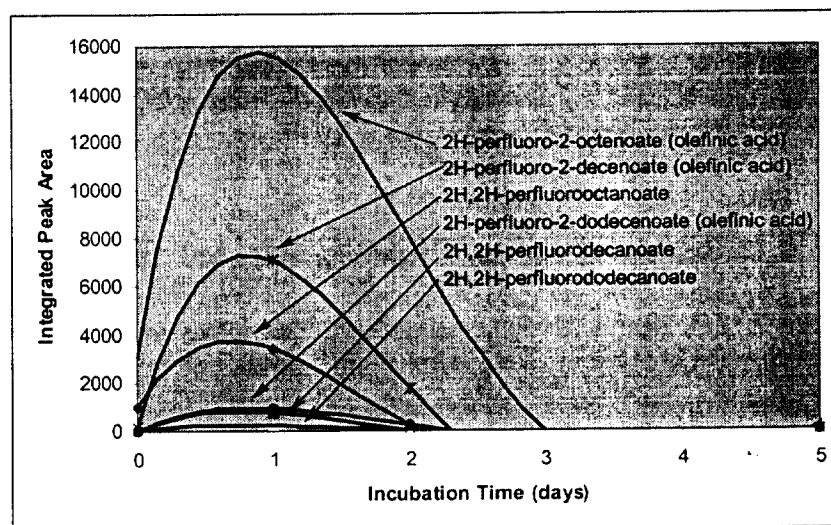
Although PFOA was accurately quantified, many other perfluorinated acids were qualitatively observed and their relative concentrations determined based on the observed increasing peak area response. The observed perfluorinated fatty acid end products were: perfluoropentanoic acid (C<sub>5</sub> PFA), perfluorohexanoic acid (C<sub>6</sub>-PFA), perfluoroheptanoic acid (C<sub>7</sub>-PFA), perfluorooctanoic acid (C<sub>8</sub>-PFA, PFOA), perfluorononanoic acid (C<sub>9</sub>-PFA), perfluorodecanoic acid (C<sub>10</sub>-PFA), perfluoroundecanoic acid (C<sub>11</sub>-PFA) and perfluorododecanoic acid (C<sub>12</sub>-PFA).

Transiently formed intermediate compounds were also observed at early time points following the initial exposure of the sludge to the telomer alcohol substrate (**Figure 6**). The transient compounds were suspected to be: 2H, 2H-perfluorooctanoate; 2H, 2H-perfluorodecanoate; 2H, 2H-perfluorododecanoate and the possible  $\beta$ -oxidation pathway intermediates: 2H-perfluoro-2-octenoate, 2H-perfluoro-2-decenoate, and 2H-perfluoro-2-dodecenoate. **Table 3** shows the MS ions and retention times observed for the polyfluorinated and perfluorinated acid products and the telomer alcohol parent substrates.

HPLC/MS/MS Data (**Table 4**) support the identification of the suspected metabolites..



**Figure 5.** Integrated peak area responses for the perfluorinated fatty acids (PFAs) observed in test culture extracts. Even numbered carbon chain length carboxylic acids were the most abundant carboxylic acid peaks and were: perfluorohexanoate (C<sub>6</sub>-PFA), perfluorooctanoate (C<sub>8</sub>-PFA) and perfluorodecanoate (C<sub>10</sub>-PFA).



**Figure 6.** Transient polyfluorinated fatty acid intermediates observed. The 2H-perfluorinated olefinic fatty acids are suspected  $\beta$ -oxidation intermediates that formed rapidly upon initial exposure to telomer alcohols, and then were transformed to their corresponding perfluorinated carboxylic acids end products

| Analytes Observed                 | Chemical Formula                                                                                                       | Retention Time | LC/MS anions observed |
|-----------------------------------|------------------------------------------------------------------------------------------------------------------------|----------------|-----------------------|
| C <sub>6</sub> -telomer alcohol*  | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> OH...CH <sub>3</sub> COO <sup>-</sup>  | 7.63           | 323                   |
| C <sub>8</sub> -telomer alcohol*  | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>5</sub> CH <sub>2</sub> CH <sub>2</sub> OH...CH <sub>3</sub> COO <sup>-</sup>  | 8.13           | 423                   |
| C <sub>10</sub> -telomer alcohol* | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>7</sub> CH <sub>2</sub> CH <sub>2</sub> OH...CH <sub>3</sub> COO <sup>-</sup>  | 8.50           | 523                   |
| C <sub>12</sub> -telomer alcohol* | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>9</sub> CH <sub>2</sub> CH <sub>2</sub> OH...CH <sub>3</sub> COO <sup>-</sup>  | 8.96           | 623                   |
| C <sub>14</sub> -telomer alcohol* | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>11</sub> CH <sub>2</sub> CH <sub>2</sub> OH...CH <sub>3</sub> COO <sup>-</sup> | 9.53           | 723                   |
| C <sub>16</sub> -telomer alcohol* | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>13</sub> CH <sub>2</sub> CH <sub>2</sub> OH...CH <sub>3</sub> COO <sup>-</sup> | 10.25          | 823                   |
| 2H, 2H-perfluorooctanoate*        | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>5</sub> CH <sub>2</sub> COO <sup>-</sup>                                       | 7.17           | 377                   |
| 2H, 2H-perfluorodecanoate*        | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>7</sub> CH <sub>2</sub> COO <sup>-</sup>                                       | 7.38           | 477                   |
| 2H, 2H Perfluorododecanoate*      | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>9</sub> CH <sub>2</sub> COO <sup>-</sup>                                       | 7.49           | 577                   |
| 2H-perfluoro-2-octenoate*         | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>4</sub> CF=CHCOO <sup>-</sup>                                                  | 7.14           | 357,293               |
| 2H-perfluoro-2-decenoate*         | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>6</sub> CF=CHCOO <sup>-</sup>                                                  | 7.35           | 457,393               |
| 2H-Perfluoro-2-dodecenoate*       | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>8</sub> CF=CHCOO <sup>-</sup>                                                  | 7.52           | 557,493               |
| Perfluoropentanoate               | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>3</sub> COO <sup>-</sup>                                                       | 6.62           | 263, 219              |
| Perfluorohexanoate                | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>4</sub> COO <sup>-</sup>                                                       | 6.88           | 313, 269              |
| Perfluoroheptanoate               | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>5</sub> COO <sup>-</sup>                                                       | 7.07           | 363, 319              |
| Perfluorooctanoate                | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>6</sub> COO <sup>-</sup>                                                       | 7.22           | 413, 369              |
| Perfluorononanoate                | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>7</sub> COO <sup>-</sup>                                                       | 7.30           | 463, 419              |
| Perfluorodecanoate                | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>8</sub> COO <sup>-</sup>                                                       | 7.41           | 513, 469              |
| Perfluoroundecanoate              | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>9</sub> COO <sup>-</sup>                                                       | 7.48           | 563, 519              |
| Perfluorododecanoate              | CF <sub>3</sub> (CF <sub>2</sub> ) <sub>10</sub> COO <sup>-</sup>                                                      | 7.52           | 613, 569              |

\*; Only anions corresponding to even chain length molecules were observed.

**Table 3.** Parent and products anions observed in by HPLC/MS analysis of SPE eluate 2 extracts from test cultures containing sludge and telomer alcohol substrate. The parent anion signals decreased in test cultures over time concomitant with increases in product signals. Many of the telomer alcohol signals decreased below detection limits in test cultures.

| Product Analytes Observed   | Chemical Formula                                     | Retention Time | LC/MS/MS Parent anion | LC/MS/MS daughter anions observed |
|-----------------------------|------------------------------------------------------|----------------|-----------------------|-----------------------------------|
| 2H,2H-perfluorooctanoate*   | $\text{CF}_3(\text{CF}_2)_5\text{CH}_2\text{COO}^-$  | 7.17           | 377                   | 333,313,293                       |
| 2H,2H-perfluorodecanoate*   | $\text{CF}_3(\text{CF}_2)_7\text{CH}_2\text{COO}^-$  | 7.38           | 477                   | 413,393                           |
| 2H,2H Perfluorododecanoate* | $\text{CF}_3(\text{CF}_2)_9\text{CH}_2\text{COO}^-$  | 7.49           | 577                   | --                                |
| 2H-perfluoro-2-octenoate*   | $\text{CF}_3(\text{CF}_2)_4\text{CF}=\text{CHCOO}^-$ | 7.14           | 357                   | 313,293,243,143,119               |
| 2H-perfluoro-2-decenoate*   | $\text{CF}_3(\text{CF}_2)_6\text{CF}=\text{CHCOO}^-$ | 7.35           | 457                   | 413,393,343                       |
| 2H-Perfluoro-2-dodecenoate* | $\text{CF}_3(\text{CF}_2)_8\text{CF}=\text{CHCOO}^-$ | 7.52           | 557                   | 493                               |

**Table 4. HPLC/MS/MS data for suspected -oxidation products.** Parent and daughter anions from the HPLC/MS/MS analysis of the SPE eluate 2 extract from the 1-day biodegradation sample (CA085-0927-SA-033 E2). The parent anions were observed as transiently formed anions in early sample point biodegradation samples only, with the most intense signals for the ions observed at day one. The MS/MS data collected for each product was consistent with the predicted chemical formula. Common mass loss from the parent anion, attributed to loss of  $\text{CO}_2$  (mass 44) and HF (mass 20), were observed.

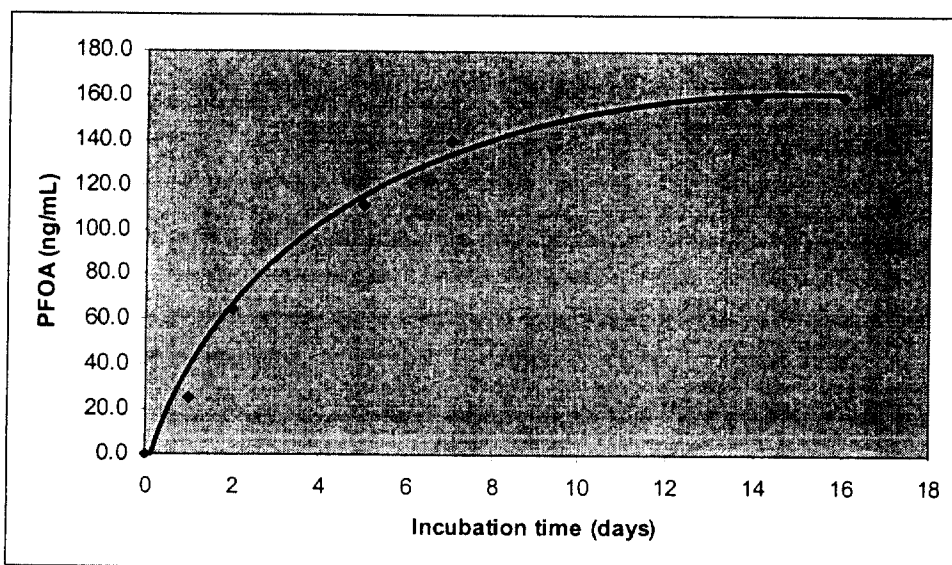
#### 8.4.2 Quantitative Analysis of PFOA

The HPLC/MS multi-component calibration standards used in this study for product quantitation contained the target analytes: PFOA, PFOSulfinate, PFOS, FOSA, *N*-MeFOSA, M556, M570, and *N*-MeFOSE alcohol. Five calibration standards were prepared by addition of a known amount of perfluorinated analytes to a culture medium with sludge, and then extraction by solid phase extraction in manner identical to the treatment of samples. The PFOA was the only target analyte of that mixture that was used for this study. The exact concentrations of the standards were used in calibration curves for quantitative analysis.



All calibration standards and sample eluates were stored at 4°C in refrigerator ID 0213 until analysis. Aliquots were transferred to autovials and capped for use in HPLC/MS runs. The Instrument calibrations were performed for PFOA by use of a five point calibration with quadratic fit of the data. The LLOQ was 11.0 ng/mL PFOA, and the coefficient of determination ( $r$ ) for PFOA was 0.965. Quantitative sequence runs contained calibration standards at the beginning and end of the run.

The biodegradation of the telomer alcohols resulted in an increase in the measured levels of PFOA during the 16-day study (Figure 7).



**Figure 7.** The measured perfluorooctanoate (PFOA) in cultures containing sludge and fluorotelomer alcohol. The PFOA was not measurable in cultures that did not contain sludge. Data plotted with the best manual fit of the data and concentrations shown were not adjusted for reference material purity of 95.2%.



## 9.0 Conclusions

This study has demonstrated that fluorochemical telomer alcohols are biodegradable and are transformed to perfluorinated acid end products. Although the telomer alcohols are supplied only as even numbered carbon chain compounds, the end product perfluorinated acids consist of both even number and odd number carbon chains, with even numbered carbon chain length acids predominating as the major end products. This observation suggests that, following the initial oxidation of the hydroxyl-carbon to form the primary carboxylic acid (telomer carboxylate), two oxidation mechanisms exist as shown in **Figure 8, III**. The first, less utilized route (**Figure 8, IIIa**), involves oxidation of the  $\alpha$ -carbon concomitant with decarboxylation (oxidative decarboxylation) to form an odd chain length perfluorinated carboxylic acid, which does not undergo further biotransformation. The second observed route of oxidation (**Figure 8, IIIb**) is the more common route of fatty acid  $\beta$ -oxidation, as evidenced by the formation of detectable transient  $\beta$ -oxidation polyfluorinated fatty acid intermediates and the more abundant even number carbon chain length carboxylic acids

Although toxicity of the telomer alcohols was not addressed as part of this study, the microbial degradation observed here over the entirety of the 16-day study suggests that microorganisms present in the sludge were not significantly inhibited by the tested concentration of the test material.

## 8.0 Literature Cited

1. Final Reports for Pace Project CA058 (3M # E00-2252), "2-Week *N*-EtFOSE Alcohol Biodegradation Screen Study Report" and "Aerobic Biodegradation of *N*-EtFOSE alcohol Study Report". Author: Cleston C. Lange, Ph.D.
2. Final report for Pace Project CA097 (3M # E01-0415), "The 18-Day Aerobic Biodegradation Study of Perfluorooctanesulfonyl-based Chemistries." Author: Cleston C. Lange, Ph.D.
3. Final report for Pace Project CA104 (3M # E01-0444), "The 35-Day Aerobic Biodegradation Study of Perfluorooctanesulfonate (PFOS)." Author: Cleston C. Lange, Ph.D.
4. Final Report for Pace Project CA105 (3M # E01-0683), "Fluorochemical Adipate (L15468) Biodegradation Screen Study." Final report issued July 10, 2001. Study Director: Cleston C. Lange, Ph.D.
5. Final Report for Pace Project CA132 (3M # E01-0682), "The Aerobic Biodegradation Study of the Fluorochemical FC807-Diester". Final report issued May 29, 2001. Study Director: Cleston C. Lange, Ph.D.
6. Pace method CAG-SP-03. "Culture Preparation for Assessment of Aerobic Biodegradability of Fluorochemicals Using Municipal or Industrial Sludge as Microbial Inoculum."
7. Pace method CAG-SP-04. "C18 Solid Phase Extraction Procedure for Fluorochemicals Recovery from Aqueous/Sludge Matrices."
8. Pace method CAG-ORG-23 "Quantitative Analysis of Fluorochemicals by High Performance Liquid Chromatography with Mass Spectrometric Detection."

## 9.0 Sample and Data Retention

At a minimum, one copy of all pertinent raw data and one copy of the signed final report will be retained in the Pace Analytical-Tier 2 data archives for a minimum period of 2 years after completion of the project. The remaining sample extracts will be retained at the Pace Analytical facility for a period of 2 years after completion of the project at 4°C in the Carroll walk-in cooler (Pace ID 0140) located in the Pace Analytical-Tier 2 facility.

The following will be provided to the sponsor (3M):

- The original signed analytical report and one copy of the signed original.
- The final scanned report (read only) and pertinent electronic data on a CD.
- All original Data, correspondence, chromatograms, sample & standards prep sheets, etc.
- Upon request before 2 years, the stored samples may be sent to the sponsor.

All electronic copies of the instrumental raw data will be archived onto CD disks and one copy provided to 3M and one copy retained at Pace.

Facility data will be retained for a period of 10 years. Facility data is available for inspection and includes the following records:

- Training records
- Controlled storage temperature logs
- Standard preparation logs
- Calibration and maintenance logs
- Chemical and solvent traceability logs
- Standard Operating Procedures
- Methods pertaining to the conduct of this project

**APPENDIX A**

**APPROVAL SIGNATURES**

**Project Title:** Biodegradation Screen Study for Telomer Alcohols

**Client Project ID:** E01-0684

**Contract Analytical Project Number:** CA085

Report Revised by:

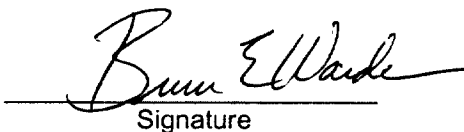
Mourad Rahi

  
Signature

11-7-02  
Date

Laboratory Management:

Bruce E Warden

  
Signature

11/7/02  
Date

## Appendix B

### Final Quantitative Results for PFOA in samples, controls and blanks

**Table 1.** The measured PFOA concentrations in test cultures during the 16-day screening study for telomer alcohol aerobic biodegradability.

| Sample                                              | Concentration (ng/mL) [Eluate 2] |
|-----------------------------------------------------|----------------------------------|
|                                                     | PFOA                             |
| Day 0 biodegradation sample, CA085-0927-SA-031, E2  | 0.0                              |
| Day 1 biodegradation sample, CA085-0927-SA-033, E2  | 25.2                             |
| Day 2 biodegradation sample, CA085-0927-SA-035, E2  | 85.0                             |
| Day 5 biodegradation sample, CA085-0927-SA-037, E2  | 111.4                            |
| Day 7 biodegradation sample, CA085-0927-SA-039, E2  | 139.8                            |
| Day 14 biodegradation sample, CA085-0927-SA-041, E2 | 158.8                            |
| Day 16 biodegradation sample, CA085-0927-SA-043, E2 | 180.0                            |

**Table 2.** The measured PFOA concentrations in the abiotic control cultures during the 16-day screening study for telomer alcohol aerobic biodegradability.

| Sample                                          | Concentration (ng/mL) [Eluate 2] |
|-------------------------------------------------|----------------------------------|
|                                                 | PFOA                             |
| Day 0 no sludge control, CA085-0927-SA-053, E2  | 0.0                              |
| Day 1 no sludge control, CA085-0927-SA-055, E2  | 0.0                              |
| Day 2 no sludge control, CA085-0927-SA-056, E2  | 0.0                              |
| Day 5 no sludge control, CA085-0927-SA-059, E2  | 0.0                              |
| Day 7 no sludge control, CA085-0927-SA-060, E2  | 0.0                              |
| Day 14 no sludge control, CA085-0927-SA-062, E2 | 0.0                              |
| Day 16 no sludge control, CA085-0927-SA-064, E2 | 0.0                              |

**Table 3.** The final data results for sludge blanks during the 16-day screening study for telomer alcohol aerobic biodegradability. PFOA was not detected in any of the sludge blanks.

| Sample                                     | Concentration (ng/mL) [Eluate 2] |
|--------------------------------------------|----------------------------------|
|                                            | PFOA                             |
| Day 0 sludge blank, CA085-0927-SA-045, E2  | 0.0                              |
| Day 1 sludge blank, CA085-0927-SA-046, E2  | 0.0                              |
| Day 2 sludge blank, CA085-0927-SA-047, E2  | 0.0                              |
| Day 5 sludge blank, CA085-0927-SA-048, E2  | 0.0                              |
| Day 7 sludge blank, CA085-0927-SA-049, E2  | 0.0                              |
| Day 14 sludge blank, CA085-0927-SA-050, E2 | 0.0                              |
| Day 16 sludge blank, CA085-0927-SA-051, E2 | 0.0                              |

## Appendix C

### Integrated Chromatogram Peak Area Data for telomer alcohols

**Table 1.** Integrated LC/MS peak area raw data for the parent telomer alcohols substrates. Data acquired from sequence run CA085\_092701a.spl as SIR data.

| Sample                                              | File ID           | Telomer Alcohol Integrated Peak Areas |       |       |       |      |      | SUM  |
|-----------------------------------------------------|-------------------|---------------------------------------|-------|-------|-------|------|------|------|
|                                                     |                   | C6                                    | C8    | C10   | C12   | C14  | C16  |      |
| Day 0 biodegradation sample, CA085-0927-SA-031, E2  | CA085_092701a_008 | 3503                                  | 21704 | 28940 | 18446 | 5558 | 1328 | 1328 |
| Day 1 biodegradation sample, CA085-0927-SA-033, E2  | CA085_092701a_009 | ND                                    | 91    | 1309  | 8633  | 4786 | 1340 | 1340 |
| Day 2 biodegradation sample, CA085-0927-SA-035, E2  | CA085_092701a_010 | ND                                    | 268   | 262   | 8677  | 4463 | 1213 | 1213 |
| Day 5 biodegradation sample, CA085-0927-SA-037, E2  | CA085_092701a_011 | 12                                    | 690   | 103   | 3070  | 3701 | 1140 | 1140 |
| Day 7 biodegradation sample, CA085-0927-SA-039, E2  | CA085_092701a_012 | ND                                    | 631   | ND    | 1263  | 3248 | 1343 | 1343 |
| Day 14 biodegradation sample, CA085-0927-SA-041, E2 | CA085_092701a_013 | ND                                    | 594   | ND    | 670   | 1324 | 1093 | 1093 |
| Day 16 biodegradation sample, CA085-0927-SA-043, E2 | CA085_092701a_014 | ND                                    | 643   | 117   | 885   | 1278 | 1137 | 1137 |
|                                                     |                   |                                       |       |       |       |      |      |      |
| Day 0 no sludge control, CA085-0927-SA-053, E2      | CA085_092701a_016 | 5072                                  | 30841 | 22594 | 13734 | 5014 | 1492 | 1492 |
| Day 1 no sludge control, CA085-0927-SA-055, E2      | CA085_092701a_017 | 4891                                  | 31305 | 20373 | 11273 | 4712 | 1477 | 1477 |
| Day 2 no sludge control, CA085-0927-SA-056, E2      | CA085_092701a_018 | 5198                                  | 31882 | 24414 | 13866 | 5379 | 1462 | 1462 |
| Day 5 no sludge control, CA085-0927-SA-059, E2      | CA085_092701a_019 | 4383                                  | 27486 | 22470 | 12856 | 4941 | 1393 | 1393 |
| Day 7 no sludge control, CA085-0927-SA-060, E2      | CA085_092701a_020 | 3515                                  | 21480 | 13621 | 10429 | 4252 | 1095 | 1095 |
| Day 14 no sludge control, CA085-0927-SA-062, E2     | CA085_092701a_021 | 3319                                  | 21284 | 14366 | 10226 | 4992 | 1265 | 1265 |
| Day 16 no sludge control, CA085-0927-SA-064, E2     | CA085_092701a_022 | 4802                                  | 27329 | 17948 | 12215 | 5230 | 1478 | 1478 |
|                                                     |                   |                                       |       |       |       |      |      |      |
| Day 0 sludge blank, CA085-0927-SA-045, E2           | CA085_092701a_044 | ND                                    | 47    | ND    | ND    | ND   | ND   | ND   |
| Day 1 sludge blank, CA085-0927-SA-046, E2           | CA085_092701a_045 | ND                                    | ND    | ND    | ND    | ND   | ND   | ND   |
| Day 2 sludge blank, CA085-0927-SA-047, E2           | CA085_092701a_046 | ND                                    | ND    | ND    | ND    | ND   | ND   | ND   |
| Day 5 sludge blank, CA085-0927-SA-048, E2           | CA085_092701a_047 | ND                                    | ND    | ND    | ND    | ND   | ND   | ND   |
| Day 7 sludge blank, CA085-0927-SA-049, E2           | CA085_092701a_048 | 37                                    | 162   | ND    | ND    | ND   | ND   | ND   |
| Day 14 sludge blank, CA085-0927-SA-050, E2          | CA085_092701a_049 | ND                                    | 211   | ND    | ND    | ND   | ND   | ND   |
| Day 16 sludge blank, CA085-0927-SA-051, E2          | CA085_092701a_050 | 17                                    | 68    | ND    | 269   | ND   | ND   | ND   |

**Table 2.** Percentage of integrated peak area remaining at different time points with respect to the peak area at time zero for their respective culture types. Near complete degradation of several telomer alcohols (TA) was observed in test cultures only. Near 90% remained in control cultures for all except C<sub>10</sub> TA, which was 79.4%.

| Sample Description                                  | Day | % TA Remaining versus Day 0 |                   |                    |                    |                    |                    |
|-----------------------------------------------------|-----|-----------------------------|-------------------|--------------------|--------------------|--------------------|--------------------|
|                                                     |     | C <sub>6</sub> TA           | C <sub>8</sub> TA | C <sub>10</sub> TA | C <sub>12</sub> TA | C <sub>14</sub> TA | C <sub>16</sub> TA |
| Day 0 biodegradation sample, CA085-0927-SA-031, E2  | 0   | 100.0%                      | 100.0%            | 100.0%             | 100.0%             | 100.0%             | 100.0%             |
| Day 1 biodegradation sample, CA085-0927-SA-033, E2  | 1   | 0.0%                        | 0.4%              | 4.5%               | 46.8%              | 85.8%              | 101.1%             |
| Day 2 biodegradation sample, CA085-0927-SA-035, E2  | 2   | 0.0%                        | 1.2%              | 0.9%               | 36.2%              | 80.7%              | 91.5%              |
| Day 5 biodegradation sample, CA085-0927-SA-037, E2  | 5   | 0.3%                        | 3.2%              | 0.4%               | 16.6%              | 66.6%              | 86.0%              |
| Day 7 biodegradation sample, CA085-0927-SA-039, E2  | 7   | 0.0%                        | 2.9%              | 0.0%               | 6.8%               | 58.4%              | 101.3%             |
| Day 14 biodegradation sample, CA085-0927-SA-041, E2 | 14  | 0.0%                        | 2.7%              | 0.0%               | 3.6%               | 23.8%              | 82.4%              |
| Day 16 biodegradation sample, CA085-0927-SA-043, E2 | 16  | 0.0%                        | 3.0%              | 0.4%               | 3.7%               | 23.0%              | 85.7%              |
|                                                     |     |                             |                   |                    |                    |                    |                    |
| Day 0 no sludge control, CA085-0927-SA-053, E2      | 0   | 100.0%                      | 100.0%            | 100.0%             | 100.0%             | 100.0%             | 100.0%             |
| Day 1 no sludge control, CA085-0927-SA-055, E2      | 1   | 96.4%                       | 101.5%            | 90.2%              | 82.1%              | 94.0%              | 99.0%              |
| Day 2 no sludge control, CA085-0927-SA-056, E2      | 2   | 102.5%                      | 103.3%            | 108.1%             | 101.0%             | 107.3%             | 98.0%              |
| Day 5 no sludge control, CA085-0927-SA-059, E2      | 5   | 86.4%                       | 89.1%             | 99.5%              | 93.6%              | 98.5%              | 93.4%              |
| Day 7 no sludge control, CA085-0927-SA-060, E2      | 7   | 69.3%                       | 69.6%             | 60.3%              | 75.9%              | 84.8%              | 73.4%              |
| Day 14 no sludge control, CA085-0927-SA-062, E2     | 14  | 65.4%                       | 69.0%             | 63.6%              | 74.5%              | 99.6%              | 84.8%              |
| Day 16 no sludge control, CA085-0927-SA-064, E2     | 16  | 90.7%                       | 88.6%             | 79.4%              | 88.9%              | 104.3%             | 99.1%              |



## Appendix D

### Integrated Peak Area Data for Perfluorinated Acid End Products

**Table 1.** Integrated LC/MS peak area raw data for the end products formed. Data acquired from sequence run CA085\_092701a.spl as SIR data.

| Sample                                              | File ID           | Integrated Peak Areas |                     |                      |                     |                   |                     |                       |                       |
|-----------------------------------------------------|-------------------|-----------------------|---------------------|----------------------|---------------------|-------------------|---------------------|-----------------------|-----------------------|
|                                                     |                   | Perfluoro-pentanoate  | Perfluoro-hexanoate | Perfluoro-heptanoate | Perfluoro-octanoate | Perfluoro-nonoate | Perfluoro-decanoate | Perfluoro-undecanoate | Perfluoro-dodecanoate |
| Day 0 biodegradation sample, CA085-0927-SA-031, E2  | CA085_092701a_008 | 276                   | 3578                | 12127                | 2715                | 8818              | 253                 | 1569                  | 375                   |
| Day 1 biodegradation sample, CA085-0927-SA-033, E2  | CA085_092701a_009 | 13796                 | 46183               | 15484                | 41821               | 8386              | 6428                | 1281                  | 395                   |
| Day 2 biodegradation sample, CA085-0927-SA-035, E2  | CA085_092701a_010 | 46384                 | 127207              | 25841                | 88508               | 8267              | 17566               | 1378                  | 1192                  |
| Day 5 biodegradation sample, CA085-0927-SA-037, E2  | CA085_092701a_011 | 68648                 | 204221              | 35747                | 140409              | 9375              | 44419               | 1641                  | 2865                  |
| Day 7 biodegradation sample, CA085-0927-SA-039, E2  | CA085_092701a_012 | 72870                 | 210569              | 43227                | 170923              | 13751             | 75377               | 2644                  | 6047                  |
| Day 14 biodegradation sample, CA085-0927-SA-041, E2 | CA085_092701a_013 | 76976                 | 221543              | 47751                | 190903              | 15382             | 92188               | 3071                  | 11078                 |
| Day 16 biodegradation sample, CA085-0927-SA-043, E2 | CA085_092701a_014 | 79826                 | 215870              | 47315                | 192075              | 15699             | 96834               | 3278                  | 11603                 |
|                                                     |                   |                       |                     |                      |                     |                   |                     |                       |                       |
| Day 0 no sludge control, CA085-0927-SA-053, E2      | CA085_092701a_016 | 193                   | 861                 | 9244                 | 1057                | 4382              | 184                 | 1283                  | 49                    |
| Day 1 no sludge control, CA085-0927-SA-055, E2      | CA085_092701a_017 | 288                   | 1722                | 16885                | 1695                | 5516              | 233                 | 1692                  | 51                    |
| Day 2 no sludge control, CA085-0927-SA-058, E2      | CA085_092701a_018 | 351                   | 1437                | 17394                | 1649                | 5958              | 164                 | 1178                  | 21                    |
| Day 5 no sludge control, CA085-0927-SA-059, E2      | CA085_092701a_019 | 475                   | 1744                | 17948                | 1683                | 7420              | 278                 | 1928                  | 61                    |
| Day 7 no sludge control, CA085-0927-SA-060, E2      | CA085_092701a_020 | 457                   | 1643                | 15212                | 1583                | 6328              | 173                 | 1015                  | 25                    |
| Day 14 no sludge control, CA085-0927-SA-062, E2     | CA085_092701a_021 | 561                   | 1731                | 16978                | 1680                | 7129              | 214                 | 1675                  | 86                    |
| Day 16 no sludge control, CA085-0927-SA-064, E2     | CA085_092701a_022 | 611                   | 1851                | 17910                | 1837                | 7203              | 233                 | 1342                  | 34                    |
|                                                     |                   |                       |                     |                      |                     |                   |                     |                       |                       |
| Day 0 sludge blank, CA085-0927-SA-045, E2           | CA085_092701a_044 | 618                   | 525                 | 31                   | 117                 | 18                | 0                   | 0                     | 0                     |
| Day 1 sludge blank, CA085-0927-SA-046, E2           | CA085_092701a_045 | 663                   | 152                 | 76                   | 166                 | 262               | 0                   | 0                     | 0                     |
| Day 2 sludge blank, CA085-0927-SA-047, E2           | CA085_092701a_046 | 685                   | 510                 | 51                   | 228                 | 722               | 0                   | 0                     | 48                    |
| Day 5 sludge blank, CA085-0927-SA-048, E2           | CA085_092701a_047 | 672                   | 320                 | 86                   | 223                 | 392               | 0                   | 0                     | 0                     |
| Day 7 sludge blank, CA085-0927-SA-049, E2           | CA085_092701a_048 | 737                   | 248                 | 62                   | 581                 | 247               | 0                   | 24                    | 42                    |
| Day 14 sludge blank, CA085-0927-SA-050, E2          | CA085_092701a_049 | 676                   | 237                 | 86                   | 731                 | 314               | 0                   | 24                    | 29                    |
| Day 16 sludge blank, CA085-0927-SA-051, E2          | CA085_092701a_050 | 634                   | 285                 | 81                   | 645                 | 397               | 0                   | 21                    | 0                     |

## Appendix E

### Integrated Peak Area Data for Transiently formed Polyfluorinated Acid Products

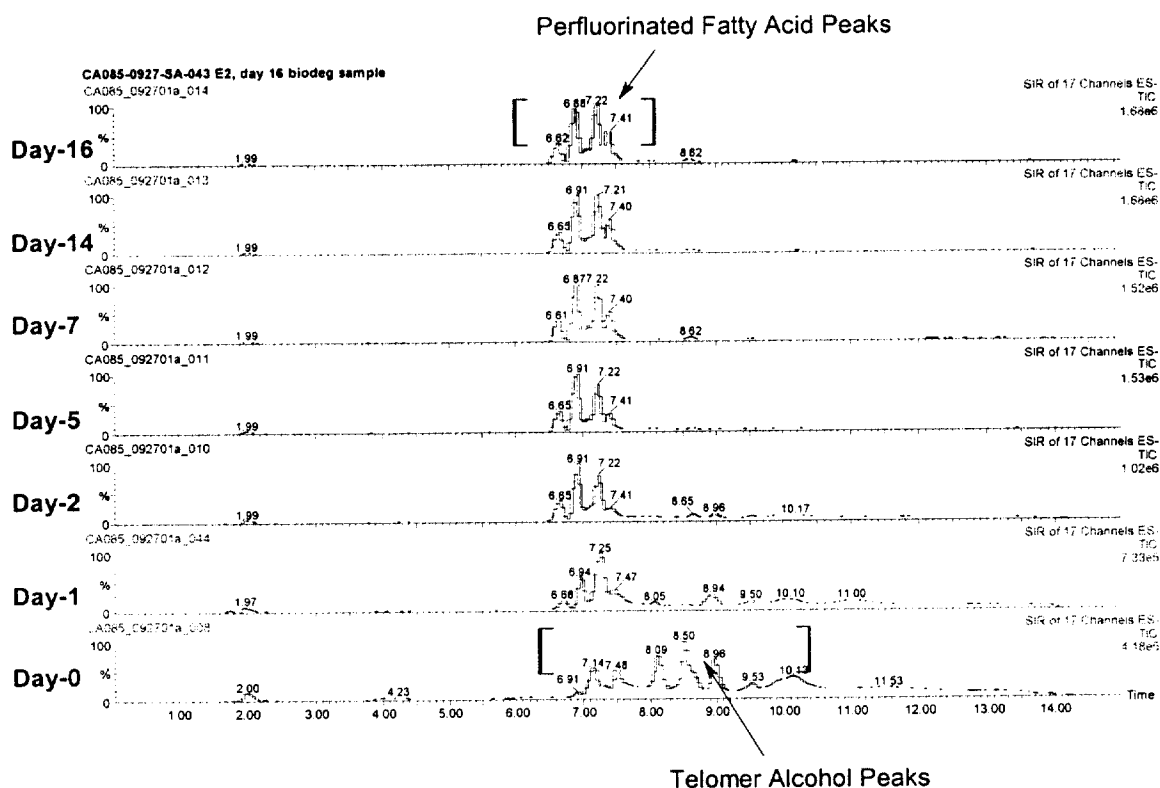
**Table 1.** Integrated LC/MS peak area data for transient products. Data from sequence CA085\_092401b.spl as full scan MS data from  $m/z$  100 to  $m/z$  1000.

| Sample                                              | File ID           | Integrated Peak Areas    |                          |                            |                           |                           |                             |
|-----------------------------------------------------|-------------------|--------------------------|--------------------------|----------------------------|---------------------------|---------------------------|-----------------------------|
|                                                     |                   | 2H-perfluoro-2-octenoate | 2H-perfluoro-2-decenoate | 2H-perfluoro-2-dodecenoate | 1H,1H-perfluoro-octanoate | 1H,1H-perfluoro-decanoate | 1H,1H-perfluoro-dodecanoate |
| Day 0 biodegradation sample, CA085-0927-SA-031, E2  | CA085_092401b_008 | 1040                     | ND                       | ND                         | 3085                      | 211                       | ND                          |
| Day 1 biodegradation sample, CA085-0927-SA-033, E2  | CA085_092401b_009 | 3429                     | 780                      | 249                        | 15825                     | 7055                      | 958                         |
| Day 2 biodegradation sample, CA085-0927-SA-035, E2  | CA085_092401b_010 | 223                      | ND                       | ND                         | 8020                      | 1802                      | 283                         |
| Day 5 biodegradation sample, CA085-0927-SA-037, E2  | CA085_092401b_011 | ND                       | ND                       | ND                         | 238                       | ND                        | ND                          |
| Day 7 biodegradation sample, CA085-0927-SA-039, E2  | CA085_092401b_012 | ND                       | ND                       | ND                         | 157                       | ND                        | ND                          |
| Day 14 biodegradation sample, CA085-0927-SA-041, E2 | CA085_092401b_013 | ND                       | ND                       | ND                         | 86                        | ND                        | ND                          |
| Day 16 biodegradation sample, CA085-0927-SA-043, E2 | CA085_092401b_014 | ND                       | ND                       | ND                         | 128                       | ND                        | ND                          |
| Day 0 no sludge control, CA085-0927-SA-053, E2      | CA085_092401b_016 | ND                       | ND                       | ND                         | ND                        | ND                        | ND                          |
| Day 1 no sludge control, CA085-0927-SA-055, E2      | CA085_092401b_017 | ND                       | ND                       | ND                         | ND                        | ND                        | ND                          |
| Day 2 no sludge control, CA085-0927-SA-058, E2      | CA085_092401b_018 | ND                       | ND                       | ND                         | ND                        | ND                        | ND                          |
| Day 5 no sludge control, CA085-0927-SA-059, E2      | CA085_092401b_019 | ND                       | ND                       | ND                         | ND                        | ND                        | ND                          |
| Day 7 no sludge control, CA085-0927-SA-060, E2      | CA085_092401b_020 | ND                       | ND                       | ND                         | ND                        | ND                        | ND                          |
| Day 14 no sludge control, CA085-0927-SA-062, E2     | CA085_092401b_021 | ND                       | ND                       | ND                         | ND                        | ND                        | ND                          |
| Day 16 no sludge control, CA085-0927-SA-064, E2     | CA085_092401b_022 | ND                       | ND                       | ND                         | ND                        | ND                        | ND                          |
| Day 0 sludge blank, CA085-0927-SA-045, E2           | CA085_092401b_030 | ND                       | ND                       | ND                         | ND                        | ND                        | ND                          |
| Day 1 sludge blank, CA085-0927-SA-046, E2           | CA085_092401b_031 | ND                       | ND                       | ND                         | ND                        | ND                        | ND                          |
| Day 2 sludge blank, CA085-0927-SA-047, E2           | CA085_092401b_032 | ND                       | ND                       | ND                         | ND                        | ND                        | ND                          |
| Day 5 sludge blank, CA085-0927-SA-048, E2           | CA085_092401b_033 | ND                       | ND                       | ND                         | ND                        | ND                        | ND                          |
| Day 7 sludge blank, CA085-0927-SA-049, E2           | CA085_092401b_034 | ND                       | ND                       | ND                         | ND                        | ND                        | ND                          |
| Day 14 sludge blank, CA085-0927-SA-050, E2          | CA085_092401b_035 | ND                       | ND                       | ND                         | ND                        | ND                        | ND                          |
| Day 16 sludge blank, CA085-0927-SA-051, E2          | CA085_092401b_036 | ND                       | ND                       | ND                         | ND                        | ND                        | ND                          |

## Appendix F

### Typical Total Ion Chromatograms for SIR Data Showing Telomer Alcohols and Polyfluorinated Fatty Acid Products from Day-0 to Day-16 for HPLC/MS Analysis of Biodegradation Culture SPE eluate 2.

SIR of 17 anions:  $m/z$  263, 313, 363, 413, 463, 513, 563, and 613 for perfluorinated acids;  $m/z$  377, 477 and 577 for polyfluorinated acids; and  $m/z$  323, 423, 523, 623, 723, and 823 for telomer alcohols.



## Appendix G

### SIR data Showing Ions for Telomer Alcohols at Day-0 and Day-16 from Analysis of the Biodegradation Culture SPE eluate 2.

CA085-0927-SA-031 E2, day 0 biodeg sample

CA085\_092701a\_008

100

%

CA085\_092701a\_008

100

%

CA085\_092701a\_008

100

%

CA085\_092701a\_008

100

%

CA085\_092701a\_008

100

%

CA085\_092701a\_008

100

%

CA085\_092701a\_008

100

%

Time

SIR of 17 Channels ES-  
423.00 1.00Da  
5.00e5

SIR of 17 Channels ES-  
723.00 1.00Da  
5.00e5

SIR of 17 Channels ES-  
823.00 1.00Da  
5.00e5

SIR of 17 Channels ES-  
523.00 1.00Da  
5.00e5

SIR of 17 Channels ES-  
423.00 1.00Da  
5.00e5

SIR of 17 Channels ES-  
323.00 1.00Da  
5.00e5

SIR of 17 Channels ES-  
TIC  
5.00e5

CA085-0927-SA-043 E2, day 16 biodeg sample

CA085\_092701a\_014

100

%

CA085\_092701a\_014

100

%

CA085\_092701a\_014

100

%

CA085\_092701a\_014

100

%

CA085\_092701a\_014

100

%

CA085\_092701a\_014

100

%

CA085\_092701a\_014

100

%

Time

SIR of 17 Channels ES-  
823.00 1.00Da  
5.00e5

SIR of 17 Channels ES-  
723.00 1.00Da  
5.00e5

SIR of 17 Channels ES-  
823.00 1.00Da  
5.00e5

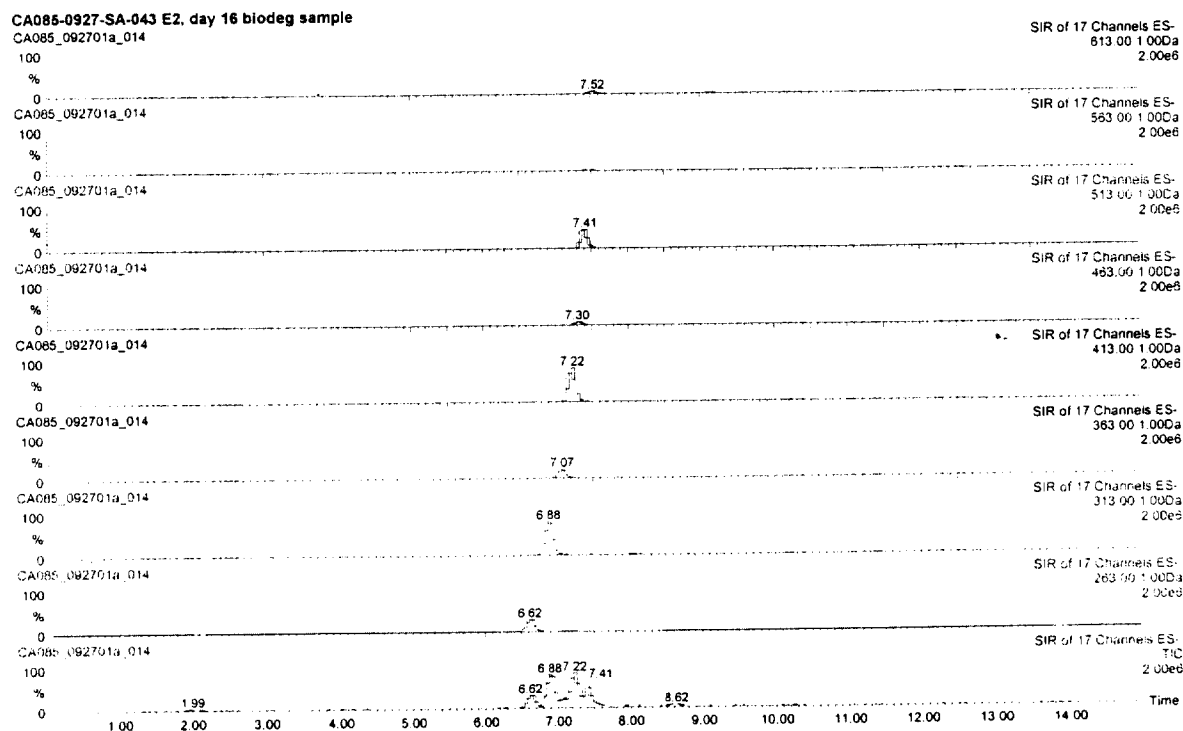
SIR of 17 Channels ES-  
523.00 1.00Da  
5.00e5

SIR of 17 Channels ES-  
423.00 1.00Da  
5.00e5

SIR of 17 Channels ES-  
323.00 1.00Da  
5.00e5

SIR of 17 Channels ES-  
TIC  
5.00e5

**SIR data Showing Ions for Perfluorinated Fatty Acids at Day-0 and Day-16 from Analysis of the Biodegradation Culture SPE eluate 2.**



000039

## Appendix I

Chromatograms of Extracted ions  $m/z$  377, 477, and 577 and Mass Spectra for Each Peak for Polyfluorinated Acid Intermediates Formed Transiently in Biodegradation Cultures at Day-0, Day-1, Day-2 and Day-5. Suspected  $\beta$ -Oxidation olefinic acid intermediates.

