BIODEGRADATION STUDY REPORT

The 35-Day Aerobic Biodegradation Study of PFOS

Thursday, March 29, 2001

PROJECT NUMBER

3M Project ID: LIMS E01-0444
Pace Contract Analytical Project ID: CA104

STUDY DIRECTOR

Cleston C. Lange, Ph.D.

CLIENT

James K. Lundberg, Ph.D.,
3M Environmental Laboratory
Bldg 2-3E-09, P.O. Box 33331,
St.Paul, MN 55133-3331

CONTRACT LABORATORY

Pace Analytical Services, Inc.
Contract Analytical Group
1700 Elm Street, Suite 200
Minneapolis, Minnesota 55414

PROJECT DATES

Project Initiation: September 20, 2000
Project Completion: April 1, 2001

AUTHOR

Cleston C. Lange, Ph.D.

Table of Contents

List of Tables & Figures

List of Appendices

Executive Summary

This screening study was undertaken to determine the aerobic biodegradation potential of perfluorooctanesulfonate (PFOS) when exposed to municipal wastewater treatment sludge. This study was conducted as a comparative six-sample point study that lasted for five weeks. Aerobic sludge from the Twin Cities Metro Wastewater Treatment Facility was used as the microbial inoculum in test cultures. The PFOS was tested at a target concentration of approximately 2.582 µg/mL (5.576 µM). Samples were prepared for quantitative analysis by solid phase extraction (SPE). Quantitative analysis of SPE extracts was conducted by LC/MS. Cultures containing municipal sludge were incubated for 35 days with no loss of PFOS over that period of time. Similarly, abiotic controls that did not contain sludge did not show a decrease in PFOS concentrations. The final comparative results showed that PFOS did not degrade during the 5 weeks of incubation. Recovery of PFOS throughout the study for all sample points was nearly 100%. Furthermore, expected biodegradation products, PFOA and PFOSulfinate, were not detected.

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	Analysts/Technicians	Ms. Angela L. Schuler
		Dr. Cleston C. Lange
1.3.4	HPLC/MS Analyst	Mr. Anthony E. Scales
1.3.5	Sample Custodian:	Dr. Cleston C. Lange
1.3.6	Report Author	Dr. Cleston C. Lange

2.0 Data Requirements

The sponsor representative desired an aerobic biodegradability screening study to be conducted using HPLC purified perfluorooctanesulfonate (PFOS) as the test substrate. The study was initiated on September 20, 2000 as a non-GLP study, and culture incubations proceeded through to October 25, 2000.

3.0 Project Objective

This study was conducted in order to elucidate whether or not PFOS is biodegradable under aerobic conditions using a microbial rich inoculum of municipal wastewater treatment sludge. The study design was copied from earlier studies conducted as part of Pace project CA058 [1] and CA097 [2] for 3M. The loss of parent material, determination of a biodegradation rate, and identification of products were critical parameters for the determination of biodegradation. The study was prepared as a six-sample point screening study, and the last cultures were harvested on the 35th day of incubation. The microbial inhibition by PFOS in aerobic sludge was not tested as part of this study, however, a previous activated sludge inhibition study for PFOS showed that PFOS is not inhibitory to microorganisms at the concentration tested here [3].

4.0 Test Article

The test article used for this study was HPLC purified PFOS (3M Std I.D. # TCR-00017-046), and was provided by the sponsor. The sponsor did not provide the purity of the test article. The test article was a white crystalline solid, supplied in a clear I-Chem 40 mL vial, and documentation provided with the material had an expiration date of January 1, 2010. Upon receipt at Pace, the material was given the test, control and reference (TCR) number CA-TCR02-014.

5.0 Reference Materials

Reference materials were received from the 3M Environmental Laboratory on June 8, 2000 for conduct of earlier studies. Approximately 1 gram of each of the following materials was received with accompanying purity information, MSDS, and chain of custody (COC # 14681). Upon receipt at Pace, each was given a Pace test, control, & reference number: Note: M556 was received from the 3M Environmental Laboratory as a methanol solution. An MSDS, and a chain of custody were not provided for this material.

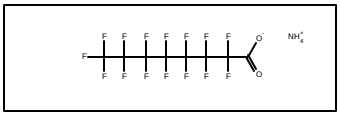
All test, control, and reference materials were stored at -85°C ± 15°C per Pace standard operating procedures. All final data in data tables for this report were calculated using the available purity information provided for PFOS (86.4% purity, below) since it was the only HPLC/MS target analyte detected.

NC: Purity analysis was not completed at the time of the study. All purity information regarding the control & reference articles was maintained under lock and key in the laboratory in a client specific binder.

5.1. Perfluorooctanoate Ammonium Salt (PFOA)

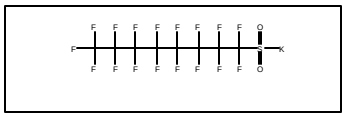
The purity was determined at **97-99%** by HPLC/MS as part of project CA058.

3M#: TCR-99131-37 Pace #: CA-TCR02-001



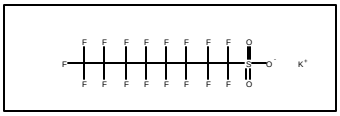
5.2. Perfluorooctanesulfinate Potassium Salt (PFOSulfinate)

Purity: **NC** 3M#: SD-007 Pace #: CA-TCR02-002



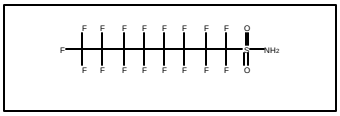
5.3. Perfluorooctanesulfonate Potassium Salt (PFOS)

Purity: **86.4%** 3M#: SD-009 Pace #: CA-TCR02-003



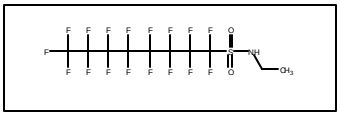
5.4. Perfluorooctanesulfonamide (FOSA)

Purity: **NC** 3M#: SD-029 Pace #: CA-TCR02-004



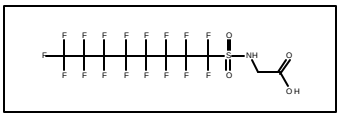
5.5. *N*-Ethyl perfluorooctanesulfonamide (*N*-EtFOSA)

Purity: **NC** 3M#: SD-012 Pace #: CA-TCR02-005



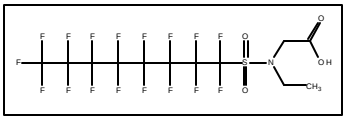
5.6. [2-(Perfluorooctanesulfonamido) acetic acid] (M556)

Purity: **NC** 3M#: 00001-5-24 Pace#:CA-TCR02-006



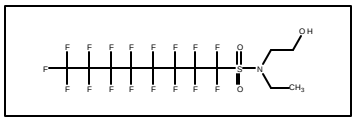
5.7. [2-(*N*-Ethyl-perfluorooctanesulfonamido) acetic acid]
(*N*-EtFOSAA) and was provided labeled as FC-129

Purity: **NC** 3M#: SE-038 Pace #: CA-TCR02-007



5.8. [2-(*N*-Ethyl-perfluorooctanesulfonamido) ethyl alcohol] (*N*-EtFOSE alcohol)

Purity: **99.9%** 3M#: SE-035 Pace #: CA-TCR02-008



6.0 Receipt/Generation of Samples

Samples were not received, but were generated as an inherent part of this study. Each of the thirty experimental cultures prepared for this study were extracted by solid phase extraction (SPE) methodology to generate ninety analytical samples. The SPE eluates collected for each sample were labeled as either SPE eluate 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. Quantitative HPLC/MS analysis was conducted for eluate 2 and eluate 3 only, and the resulting analytical data was used to determine whether biodegradation occurred.

7.0 Methods

7.1 Sample Preparation

7.1.1. Collection of Sludge.

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 Cites Metro Wastewater Treatment Facility (also referred to as Pig's Eye Sewer Treatment Plant) located in St. Paul, MN. Four liters of MLSS was collected by Pace laboratory personnel on September 18, 2000 and delivered as four 1-liter Nalgene polypropylene bottles containing MLSS, and were accompanied with a corresponding chain of custody (Pace C.O.C. # 528791). 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 4°C from September 18th to September 20th. On September 20th, the

settled solids "sludge" from bottle #1 was used to prepare cultures for this study. 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.

All cultures were initially prepared on September 20, 2000 and the last set of incubated cultures was harvested on October 25, 2000 (35 days total incubation). The culture preparation procedure described below was documented as Pace standard operating procedure (SOP) CAG-SP-03 [4].

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 Na_2HPO_4-$2H_2O$, 0.005 g NH_4Cl, 0.2175 g K_2HPO_4, and 0.085 KH_2PO_4, 0.0275 g $CaCl_2$-anhydrous, 0.0225 g $MgSO_4$-$7H_2O$, and 0.00025 g $FeCl_3$-$6H_2O$.

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.

Mineral Salts medium, un-sterilized and without sludge, was prepared. This mineral 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.

PFOS was added as the test substrate to cultures by transferring 6 µL of a 10,760 µg/mL methanol solution of PFOS (stock solution CA104-SS-001) to each culture flask, for a final target concentration of 2,582 ng/mL. Blank control cultures received 25 mL of equivalent mineral medium solution as test culture, but without PFOS addition.

The day zero samples were prepared and immediately placed in a –20°C freezer. All other cultures prepared were placed in temperature controlled shaking incubators that was maintained at 25°C $\pm$ 3°C. Samples were pulled from the incubator at designated time points of days 2, 5, 7, 14, and 35. Upon removal from the incubator, samples were either immediately frozen, or immediately prepared for analysis by solid phase extraction.

All sample preparation information, including times, analyte additions, etc. were recorded in sample preparation worksheets and signed and dated by the sample preparation analyst. All original data sheets were maintained in project specific binders labeled CA104.

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 [5].

All Sample 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 was packed with plug of quartz glass wool to deter plugging of the SPE filter. 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.

Frozen samples 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 Ichem 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 Ichem vial labeled eluate 2. Eluate 2 was expected to contain a majority of the analyte that was in the original culture sample. As a precaution that some analyte may be retained in the SPE cartridge, or in the culture flask, a second 25 mL methanol step was conducted in a similar fashion to that used for eluate 2, and collected in a third I-chem vial, 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°C.

7.1 Instrumental Analysis (HPLC/MS)

The quantitative LC/MS method described below was documented as Pace standard operating procedure (SOP) CAG-ORG-23 [6].

All quantitative analysis was conducted on an HP1100 high performance liquid chromatograph with mass spectrometer detector (HPLC/MSD) system. The HPLC/MSD system was set up with the same chromatography configuration as that described in method CAG-ORG-23, which utilized two columns fed into a pressure relief valve that served as a flow through splitter. The MSD was operated with electrospray ionization in negative ion mode and with selected ion monitoring (SIM). Ions monitored were: *m/z* 413 (PFOA); *m/z* 483 (PFOSulfinate); *m/z* 498 (FOSA); *m/z* 499 (PFOS); *m/z* 526 (*N*-EtFOSA); *m/z* 556 (M556); *m/z* 584 (*N*-EtFOSAA); and *m/z* 630 (*N*-EtFOSE alcohol). All analysis was quantitative, with 8 calibration standards prepared from approximately 5 to 1000 ng/mL (ppb) and containing each of the analytes being quantified. Typical injection volumes for samples and calibration standards were 50 µL, except where otherwise noted for instrumental dilutions in section 8.3 below. The 4.6 x 150 mm Betasil C8 column used for this study had serial number 0501383T, lot number P9G09 and the 4 x 35 mm NG1 column used had serial number 15123. 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.

MSD Settings:
Ionization mode: API-ES negative
Gas Temp: 300°C
Drying gas: 8.0 L/minute
Nebulizer Pressure: 30 psig
Vcap: 3500V
Fragmentor: 70V
EMV Gain: 2.0
Actual Dwell for each ion: 146
SIM resolution: high

HPLC Settings:
Flow: 1 mL/min, splitter used

Time (min).	%A	%B
0.00	97.0	3.0
0.50	97.0	3.0
6.00	5.0	95.0
8.50	5.0	95.0
8.51	97.0	3.0
10.50	97.0	3.0

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. The mole conversion values used for each of the analytes were as follows:

$PFOA^- NH4^+$ salt = 431 nanogram (ng) per nanomole (nmole)
$PFOSulfinate^- K^+$ salt = 522 ng/nmole
$PFOS^- K^+$ salt = 538 ng/nmole
FOSA = 499 ng/nmole
N-EtFOSA = 527 ng/nmole
M556 acid = 557 ng/nmole
N-EtFOSAA = 585 ng/nmole
N-EtFOSE alcohol = 571 ng/nmole

7.3.2 Conversion of ng/mL to micromolar (μM) and nanomolar (nM).

(Working Examples):

500 ng/mL PFOSulfinate = (500 ng/mL)*(1nmole/522 ng) = 0.958 nmole/mL = 0.958 μmole/liter = 0.958 μM

50 ng/mL PFOSulfinate = (50 ng/mL) * (1 nmole/522 ng)
= 0.0958 nmole/mL = 0.0958 μmole/L = 0.0958 μM = 95.8 nM

7.3.3 Molar Mass Balance Calculations:

Convert all ng/mL values to their corresponding molar concentrations as µM or nM (see section 7.3.2, above). Then, sum the quantified molar values (µM) for all analytes in eluate 2 and eluate 3. 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 *N*-EtFOSE alcohol was at 1800 µM
And, after incubation, the following were determined:

PFOA was detected at 50 µM
PFOS was detected at 100 µM
N-EtFOSAA was detected at 500 µM
N-EtFOSE alcohol was detected at 1000 µM

Then, the mass balance is as follows:
Mass balance = [(50µM + 100µM + 500µM + 1000µM)/1800µM] X 100%
Mass balance = (1650µM/1800µM) X 100% = **91.7%**

7.4 Statistics

7.4.1 Formula used for determination of Standard Deviation (SD):

$$SD = (\Sigma\chi^2/(n-1))^{1/2} = [\Sigma (X - \bar{X})^2/(n-1)]^{1/2}$$

$\Sigma\chi^2$ = the sum of the squared differences of deviations between the values of individual data points and the mean: $= \Sigma [(X_i - \bar{X})^2]$

Σ = The sum of all values in a population

X_i = the value of an individual data point

n = the total number of samples, where one sample is represented as (i).

$\bar{X}$ = mean of all X in the sample population = $(\Sigma X) / n$

7.4.2 Formula used for Relative Standard Deviation (RSD): The RSD is a ratio of the standard deviation (SD) to the arithmetic mean of the replicate analysis and expressed as a percent.

RSD = (SD / mean of Xn) * 100%

7.5 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.
Chemstation for the LC/MSD was used for data collection and integration.

8.0 Results

8.1 Incubator, Refrigerator, and Freezer Temperature Log Data.

During this study, one reciprocal shaker incubator was used. The Pace ID number for the incubator-shaker was 0269. Samples were shaken at 200 rpm, but the speed was not regularly verified.

A –20 °C freezer, Norlake freezer, was used during the study to store the cultures until they could be prepared by solid phase extraction. The freezer ID was 0050 and was situated at Pace/3M Tier-2 facility in location SL-R6.

One IsoTemp ultra-low freezer (-80°C) was used for storage of test, control and reference materials. The freezer ID was 0241 and was situated at Pace in location SL-R4.

Two refrigeration units, a Carroll walk-in cooler and a True refrigerator, were used to store SPE-prepared samples and stock and calibration standards during the study. The coolers were identified as ID 0140 (location SL-R1) and ID 0213 (location SL-R8), respectively.

The average temperatures $\pm$ the standard deviation for each incubator, refrigerator, and freezer are shown below for the dates in which they were used. The average temperatures and standard deviations are based on readings that were recorded on a daily basis, excluding weekends and holidays.

Incubator-shaker, ID 0269, from dates 9/20/00 to 10/25/00:	**27°C $\pm$ 1°C**
Norlake Freezer, ID 0050, from dates 9/20/00 to 10/25/00:	**-20°C $\pm$ 7° C**
IsoTemp Freezer, ID 0241, from dates 9/20/00 to 10/25/00:	**-76.0°C $\pm$ 19°C**
Carroll walk-in cooler, ID 0140, from dates 9/20/00 to 10/25/00:	**4°C $\pm$ 2°C**
True refrigerator, ID 0213, from dates 9/20/00 to 10/25/00:	**4°C $\pm$ 1°C**

8.2 Mishaps recorded during the Study.

One sample, CA104-0920-028, was found to contain *N*-EtFOSE alcohol and some of its expected biodegradation intermediates. This sample was supposed to be a day-14 no sludge control (abiotic control) for PFOS. It is probable that the technician, upon transferring the sample to an auto-vial, mistakenly retrieved the wrong sample from storage and used a sample from another study. Since *N*-EtFOSE alcohol was never used for this study, there is no other logical explanation. Furthermore, the concentration of PFOS in the sample was not at the level expected of an abiotic control for this study (expected at approximately 2.5 µg/mL, but measured at approximately 10 ng/mL). While the data for this sample is included in the final data tables in Appendix B, it is not discussed in the final conclusions and is excluded from discussions of PFOS biodegradation.

8.3 Sludge Characterization

Chemical analysis of the mixed liquor suspended solids used for this study was not conducted. However, the sludge was obtained from the Twin Cities Municipal waste treatment facility in manner consistent with sludge collected for project CA058 [1] and was used immediately within 2 days. The sludge used for the preparation of samples in this study was also used for the preparation of samples in other biodegradation studies, and in some of those other studies, preliminary results have shown positive biological activity for degradation of fluorochemicals [7,8]. Hence, it was assumed that active microorganisms were present in the sludge used for this study.

8.4 Quality Control/Sample Matrix Spike Results.

The determination of analyte recoveries from sample matrix spikes was not included as part of this screening study. However, PFOS was recovered at expected concentrations, and previous duplicated results obtained during project CA058 [1] showed excellent recoveries from sludge matrices for all analytes pertinent to this study by the SPE method.

8.5 HPLC/MS Calibration and Calibration Verification.

All HPLC/MS analyses were conducted in analytical sequence run D102600.s on Pace instrument "Dutch".

Although this study targeted the quantitative analysis of PFOA, PFOS and PFOSulfinate, the HPLC/MS multi-component calibration standards used in this study contained PFOA, PFOSulfinate, PFOS, FOSA, *N*-EtFOSA, M556, *N*-EtFOSAA and *N*-EtFOSE alcohol. Eight calibration standards were prepared and contained each of the analytes at nominal concentrations of 5, 10, 20, 40, 100, 200, 500 and 1000 ng/mL. The calibration standards used were labeled from low concentration to high concentration as CA105-MW-001 to CA105-MW-008, respectively. The calibration standards were prepared for use in projects CA105 and CA132 at Pace and preparation sheets can be cross-referenced to those projects. For final quantitation of unknowns, the exact concentration of the standards were used in calibration curves. Calibration standards were prepared in methanol in 10 mL quantities and were stored in clear Ichem vials at 4°C in refrigerator ID 0213. Aliquots were transferred to autovials and capped for use in HPLC/MS runs. Continuing calibration verification standards (CCVs) were used in sequence runs, and included both CA105-MW-004 (approximately 20 ng/mL) and CA105-MW-006 (approximately 200 ng/mL). CCVs were used for verifying the integrity of the calibration curves for quantitation throughout the sequence run.

Instrument calibration was performed for each analyte by use of either the low 5 calibration points (5 to 100 ng/mL) or the high 5 calibration points (40 to 1000 ng/mL). Analyte concentrations below 100 ng/mL were reported based on the value given for bw calibration curve data and residuals defining the quantitation limits were 100% $\pm$ 25% of the actual value. Values reported between 100 and 1000 ng/mL were determined from high calibration curve data, and residuals defining the quantitation limits were 100% $\pm$ 25% of the actual value.

For samples with concentrations determined to be above 1000 ng/mL, the samples were re-injected as 10 µL injections and quantified off of calibration curves in which 50 µL of calibrant was injected, creating an instrumental dilution

factor of 5. Final concentrations of dilution injections were corrected for the dilution factor automatically by the instrument, and final reported values were used. Final quantitative data for dilutions was obtained using the high five standards (40-1000 ng/mL), residual values in the range of 100% ± 15%. The minimum limit of quantitation (LOQ) for the targeted analytes for analysis conducted were: PFOA (11.0 ng/mL), PFOS (4.4 ng/mL-adjusted for purity 86.4%), PFOSulfinate (5.5 ng/mL), FOSA (5.3 ng/mL), *N*-EtFOSA (5.9 ng/mL), M556 (5.0 ng/mL), *N*-EtFOSE acid (5.3 ng/mL), and *N*-EtFOSE alcohol (6.0 ng/mL-adjusted for purity 99.9%).

Calibration check and verification standards (CCVs) were used after 15 sample injections and included both calibration standards for verification of low and high range curves. CCVs were flanked by methanol blanks. All quantitation was conducted using averaged calibration curves flanking no more than 30 samples, one set of CCVs, and the accompanying solvent blanks. All calibration curves were slightly quadratic and had R-values of at least 0.990. All data was collected as external standard calibration, without the use of an internal standard. Instrumental sequence runs were setup as described in method CAG-ORG-23.

8.6 Analytical Blanks

Methanol blanks were injected onto the HPLC/MS column and quantitatively analyzed to determine the background analyte concentration in the methanol used for sample preparation.

Sample blanks, consisting of either mineral salts medium or mineral salts medium containing sludge, and no analyte addition. Sample blanks were prepared and incubated in an identical manner to true samples. The sample blank results were used to determine whether the sample matrix contained any of the analytes of interest. No fluorochemical analytes were detected in the sample blanks.

8.7 Matrix Spikes

Sample matrix spikes were not employed for this study.

8.8 Surrogate Spikes

Surrogate spikes were not employed during this study.

8.9 Sample and Control Sample Results

8.9.1 Result of Incubations with PFOS.

The final reported data were calculated using the sponsor-provided purity information from the Centre Analytical Laboratories, Inc. certificate of analysis # 023-018B for PFOS lot 171 (3M I.D. SD-009). The PFOS lot 171 material was used as reference material for preparation of calibration standards (PFOS reference material at 86.4% purity). In the test material PFOS (HPLC purified, 3M Std I.D. # TCR-00017-046), no significant impurities were detected in samples for this study. From the graphs in **Figure 1** and **Figure 2,** it is obvious that degradation of PFOS did not occur during the 35-day incubation experiment. Furthermore, PFOA and

PFOSulfinate, expected products from biodegradation of PFOS, were not detected (**Table 1, Appendix B**).

The average concentration of PFOS for cultures containing sludge throughout the study was 2,553 ng/mL ± 102 ng/mL, for an RSD of 4.0% during 35 days of incubation. The average concentration of PFOS for abiotic control cultures was 2,654 ng/mL ± 83 ng/mL, for an RSD of 3.1%. The average PFOS concentration determined for all test and control cultures was 2,601 ng/mL ± 105 ng/mL, with an RSD of 4.0%. The data presented in **Appendix B** and **Figure 1** and **Figure 2**, below, demonstrate that PFOS was not degraded in any fashion during the course of this screening study.

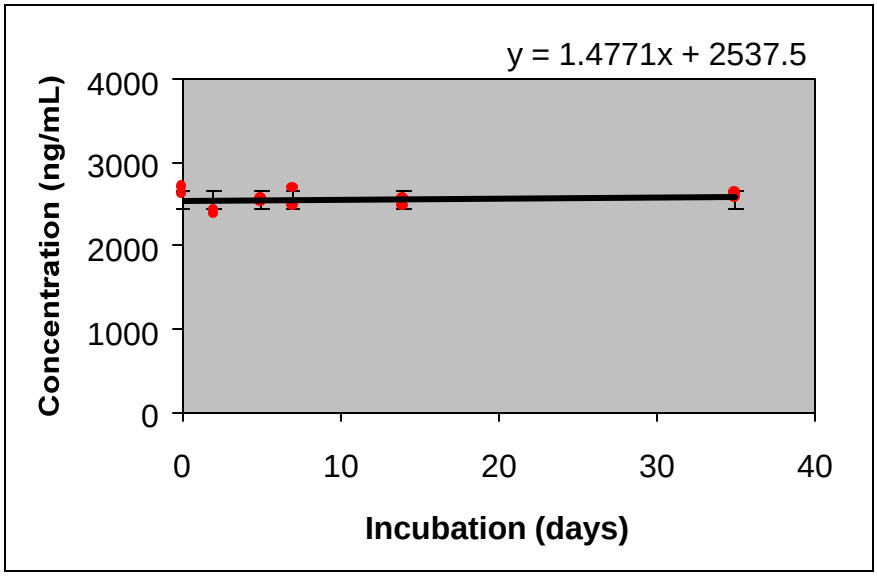


Figure 1. The data plot for the 35-day incubation of PFOS with aerobic sludge showing that no significant biodegradation occurred. The data is fit with a linear equation and error bars representing one standard deviation from the mean. The slope of the fit line is slightly positive in nature, while degradation would produce a negative slope (due to loss of PFOS).

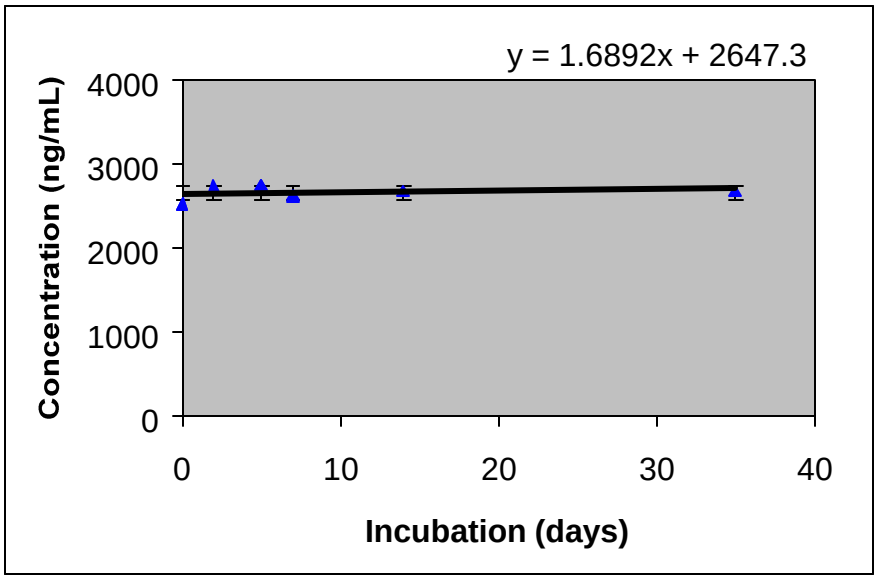


Figure 2. The data plot for the 35-day incubation of PFOS in abiotic controls, showing that no significant degradation occurred. The data is fit with a linear equation and error bars representing one standard deviation from the mean. The slope of the fit line is slightly positive, while degradation would produce a negative slope (due to loss of PFOS).

9.0 Conclusions

This study determined that PFOS is not biodegraded by the inherent microbial populations of municipal waste treatment facilities under these defined test conditions and at the tested concentration of 2.582µg/mL (5.576 µM) of PFOS. The PFOS concentration in all test cultures and control cultures remained unchanged throughout the 35 days of incubation. The measured concentration of PFOS in all cultures was always within 100 ± 7% of the expected concentration of 2.582 µg/mL after adjusting for reference material purity.

The results of this study support the postulation that PFOS may be an end-point metabolite in the biodegradation of fluorochemical polymers and fluorochemical monomers (see final reports for project CA058 [1] and CA097 [2]). The results of this study corroborate those of project CA097, which showed no biodegradation of PFOS, but did show biodegradation of other perfluorooctanesulfonyl-based chemistries [2].

Although toxicity was not addressed as part of this study, the microbial respiration inhibition by PFOS has been tested previously, and PFOS was found to not be a significant inhibitor of microbial respiration processes at (13% inhibition at 2.7 μg/mL) [3].

10.0 References

1. Final Reports for Pace Project CA058, "2-Week N-EtFOSE Alcohol Biodegradation Screen Study Report" and "Aerobic Biodegradation of N-EtFOSE alcohol Study Report". 3M LIMS Project Number: E00-2252. Author: Cleston C. Lange, Ph.D.

2. Final report for Pace Project CA097, "The 18-Day Aerobic Biodegradation Study of Perfluorooctanesulfonyl-based Chemistries." 3M LIMS Project Number: not available. Author: Cleston C. Lange, Ph.D.

3. Activated Sludge Respiration Inhibition Test for PFOS. Robust Summary for 3M Environmental Laboratory Lab Request number U2723. Test Conducted under GLP requirements for 3M by Wildlife International, Ltd. Easton, MD.

4. Pace method CAG-SP-03. "Culture Preparation for Assessment of Aerobic Biodegradability of Fluorochemicals Using Municipal or Industrial Sludge as Microbial Inoculum."

5. Pace method CAG-SP-04. "C18 Solid Phase Extraction Procedure for Fluorochemicals Recovery from Aqueous/Sludge Matrices."

6. Pace method CAG-ORG-23 "Quantitative Analysis of Fluorochemicals by High Performance Liquid Chromatography with Mass Spectrometric Detection."

7. Project CA105, "35-Day Fluorochemical Adipate Biodegradation Screen Study". Initiated September 2001—in progress, Study Director: Cleston C. Lange, Ph.D.

8. Project CA132, "35-Day FC807-Diester Biodegradation Screen Study ". Initiated September 2001—in progress, Study Director: Cleston C. Lange, Ph.D.

9.0 Sample and Data Retention

Original facility records and original data and a copy of the signed final report will be retained in the Pace Analytical-Tier 2 data archives for a period of 2 years after completion of the project. At the end of 2 years, or upon request before that time, the data will be sent to the 3M Environmental Laboratory for ultimate long-term archiving.

The following will be provided to 3M personnel:

- The original signed analytical report and one copy of the signed original
- All original Data, correspondence, chromatograms, sample & standards prep sheets, etc., upon request.

Upon request of original data and records, copies of all original raw data in the form of chromatograms, reduced data, and written records concerning this project will be prepared and retained in the Pace Analytical-Tier 2 data archives.. All electronic copies of the instrumental raw data will be archived onto CD disks (read-only) and a copy provided to 3M upon request, or after 2 years, which ever is first.

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

The remaining sample extracts will be retained at the Pace Analytical facility for a period of 1 year 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.

APPENDIX A

SIGNATURES OF PROJECT PERSONNEL

Project Title: 5-Week *N*-EtFOSE Alcohol Aerobic Biodegradation Study

Client Project ID: LIMS E01-044

Contract Analytical Project Number: CA104

The following individuals participated in the conduct of this project:

Study Director:

Cleston C. Lange _____ _____
Signature Date

Laboratory Management:

Bruce E Warden _____ _____
Signature Date

Report Author:

Cleston C. Lange _____ _____
Signature Date

Sample Preparation Analyst:

Angela L. Schuler _____ _____
Signature Date

HPLC/MS Analysis Conducted by:

Anthony E. Scales _____ _____
Signature Date

Angela L. Schuler _____ _____
Signature Date

Appendix B
Final Concentration Results: PFOS Adjusted for Purity

Table 1. Final data results for sample cultures during this 35-day screening study. PFOS was the only target analyte detected in sample cultures, and the mean concentration during the entire study for sample cultures, plus/minus the standard deviation, was 2,553 ng/mL ± 102 ng/mL, and with a relative standard deviation of 4.0%. The final concentration values shown are adjusted for the PFOS reference material purity reported at 86.4%. The PFOS measured versus the expected concentration (2,582 ng/mL) is presented as a percentage in the last column

		PFOS	PFOA	PFOS Sulfinate	m/z 556	*N*-EtFOSE acid	FOSA	*N*-EtFOSE Alcohol	*N*-EtFOSA	*PFOS Recovery*
Sample ID	**Sample Information**	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	%
CA104-0920-SA-001	Day 0 PFOS biodegradation sample	2620	ND	ND	ND	ND	ND	ND	ND	101.5%
CA104-0920-SA-002	Day 0 PFOS biodegradation duplicate	2710	ND	ND	ND	ND	ND	ND	ND	105.0%
CA104-0920-SA-003	Day 2 PFOS biodegradation sample	2374	ND	ND	ND	ND	ND	ND	ND	91.9%
CA104-0920-SA-004	Day 2 PFOS biodegradation duplicate	2425	ND	ND	ND	ND	ND	ND	ND	93.9%
CA104-0920-SA-005	Day 5 PFOS biodegradation sample	2522	ND	ND	ND	ND	ND	ND	ND	97.7%
CA104-0920-SA-006	Day 5 PFOS biodegradation duplicate	2558	ND	ND	ND	ND	ND	ND	ND	99.1%
CA104-0920-SA-007	Day 7 PFOS biodegradation sample	2485	ND	ND	ND	ND	ND	ND	ND	96.3%
CA104-0920-SA-008	Day 7 PFOS biodegradation duplicate	2695	ND	ND	ND	ND	ND	ND	ND	104.4%
CA104-0920-SA-009	Day 14 PFOS biodegradation sample	2477	ND	ND	ND	ND	ND	ND	ND	95.9%
CA104-0920-SA-010	Day 14 PFOS biodegradation duplicate	2559	ND	ND	ND	ND	ND	ND	ND	99.1%
CA104-0920-SA-011	Day 35 PFOS biodegradation sample	2585	ND	ND	ND	ND	ND	ND	ND	100.1%
CA104-0920-SA-012	Day 35 PFOS biodegradation duplicate	2626	ND	ND	ND	ND	ND	ND	ND	101.7%

LOQ for PFOS 4.4 ng/mL LOQ for PFOA 11.0 ng/mL
LOQ for PFOSulfinate 5.5 ng/mL ND: peak not detected

Table 2. Final data results for the abiotic control cultures during this 35-day screening study. PFOS was the only analyte detected, and the mean concentration plus/minus the standard deviation determined for all abiotic controls through the study was 2,653 ng/mL ± 83 ng/mL, with a relative standard deviation of 3.1%. Note: Sample CA104-0920-028 results were invalid because of a sample preparation error—this sample is likely a positive control culture with *N*-EtFOSE alcohol as a test substrate, which was prepared for another study. The final concentration values are adjusted for PFOS reference material purity at 86.4% pure.

		PFOS	PFOA	PFOS Sulfinate	m/z 556	*N*-EtFOSE acid	FOSA	*N*-EtFOSE Alcohol	*N*-EtFOSA	*PFOS Recovery*
Sample ID	**Sample Information**	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	%
CA104-0920-SA-019	Day 0 PFOS abiotic control	2522	ND	ND	ND	ND	ND	ND	ND	97.7%
CA104-0920-SA-020	Day 0 PFOS abiotic duplicate	2530	ND	ND	ND	ND	ND	ND	ND	98.0%
CA104-0920-SA-021	Day 2 PFOS abiotic control	2704	ND	ND	ND	ND	ND	ND	ND	104.7%
CA104-0920-SA-022	Day 2 PFOS abiotic duplicate	2744	ND	ND	ND	ND	ND	ND	ND	106.3%
CA104-0920-SA-023	Day 5 PFOS abiotic control	2744	ND	ND	ND	ND	ND	ND	ND	106.3%
CA104-0920-SA-024	Day 5 PFOS abiotic duplicate	2751	ND	ND	ND	ND	ND	ND	ND	106.5%
CA104-0920-SA-025	Day 7 PFOS abiotic control	2610	ND	ND	ND	ND	ND	ND	ND	101.1%
CA104-0920-SA-026	Day 7 PFOS abiotic duplicate	2635	ND	ND	ND	ND	ND	ND	ND	102.1%
CA104-0920-SA-027	Day 14 PFOS abiotic control	2681	ND	ND	ND	ND	ND	ND	ND	103.8%
CA104-0920-SA-028	Invalid Sample	4.3	ND	ND	9.9	86.6	7.6	150	ND	Not applicable
CA104-0920-SA-029	Day 35 PFOS abiotic control	2588	ND	ND	ND	ND	ND	ND	ND	100.2%
CA104-0920-SA-030	Day 35 PFOS abiotic duplicate	2683	ND	ND	ND	ND	ND	ND	ND	103.9%

LOQ for PFOS 4.4 ng/mL LOQ for PFOA 11.0 ng/mL
LOQ for PFOSulfinate 5.5 ng/mL ND: peak not detected

Table 3. The final data results for sludge blanks during the 35-day study. Target analytes were not detected in any of the sludge blanks.

		PFOS	PFOA	PFOS Sulfinate	m/z 556	*N*-EtFOSE acid	FOSA	*N*-EtFOSE Alcohol	*N*-EtFOSA
Sample ID	**Sample Information**	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL
10260017.D	Day 0 PFOS sludge blank	ND	ND	ND	ND	ND	ND	ND	ND
10260022.D	Day 2 PFOS sludge blank	ND	ND	ND	ND	ND	ND	ND	ND
10260051.D	Day 5 PFOS sludge blank	ND	ND	ND	ND	ND	ND	ND	ND
10260056.D	Day 7 PFOS sludge blank	ND	ND	ND	ND	ND	ND	ND	ND
10260085.D	Day 14 PFOS sludge blank	ND	ND	ND	ND	ND	ND	ND	ND
10260090.D	Day 35 PFOS sludge blank	ND	ND	ND	ND	ND	ND	ND	ND

ND: peak not detected

Appendix C
Integrated Chromatogram Peak Area Data

Table 1. Actual integrated LC/MS peak area data for calibration standards, samples, controls, blanks, and CCVs. Data obtained from LC/MS batch reports for sequence D102600.S run on LC/MS instrument "Dutch". Peaks that were not detected resulted in a reported peak area of 0.00. The results for SPE eluate 2 are designated by E2 and results for SPE eluate 3 are designated by E3.

		PFOS	PFOA	PFOSulfinate	FOSA	M556	N-EtFOSE acid	*N*-EtFOSE Alcohol	*N*-EtFOSA
Sample ID	**File ID**	**AREA**	**AREA**	**AREA**	**AREA**	**AREA**	**AREA**	**AREA**	**AREA**
Methanol Blank	10260005.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA105-MW-001	10260006.D	14935.93	17005.95	11417.06	32261.59	12498.49	6516.98	21793.25	34484.85
CA105-MW-002	10260007.D	30181.21	18006.10	21815.46	51792.79	27038.68	13797.29	34541.48	71779.78
CA105-MW-003	10260008.D	60707.00	39103.28	43160.50	107790.25	51526.13	25951.37	68317.75	145654.98
CA105-MW-004	10260009.D	136328.78	78302.48	91770.55	240400.95	105243.81	58180.79	148115.28	309278.94
CA105-MW-005	10260010.D	305656.84	192285.58	212681.84	589225.94	244543.95	150302.11	394311.94	829537.06
CA105-MW-006	10260011.D	648404.88	383602.31	442822.63	1215682.00	494728.31	296591.75	745335.31	1610657.00
CA105-MW-007	10260012.D	1436899.38	810410.25	926250.81	2680574.00	949330.00	650015.88	1667151.00	3691885.25
CA105-MW-008	10260013.D	2749046.50	1568670.38	1687577.50	5255131.00	1658535.88	1103419.25	3026545.75	6929020.50
Methanol Blank	10260014.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-001 E2	10260015.D	6957170.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-002 E2	10260016.D	6305939.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-013 E2	10260017.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-019 E2	10260018.D	6291996.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-020 E2	10260019.D	6393484.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-003 E2	10260020.D	5646777.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-004 E2	10260021.D	6028145.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-014 E2	10260022.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-021 E2	10260023.D	6741408.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-022 E2	10260024.D	6487929.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methanol Blank	10260025.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA105-MW-004	10260026.D	119812.42	74300.85	82843.96	210264.64	98867.90	63633.68	144413.31	308571.63
CA105-MW-006	10260027.D	613307.56	374440.19	395532.88	1170395.00	438835.00	290054.69	787338.31	1637672.38
Methanol Blank	10260028.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-001 E3	10260029.D	396809.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-002 E3	10260030.D	824812.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-013 E3	10260031.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-019 E3	10260032.D	1115791.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-020 E3	10260033.D	900586.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-003 E3	10260034.D	1364811.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-004 E3	10260035.D	1301216.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-014 E3	10260036.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-021 E3	10260037.D	811296.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-022 E3	10260038.D	1358693.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methanol Blank	10260039.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA105-MW-001	10260040.D	13926.68	16257.32	9813.04	26289.82	12067.14	8051.56	22119.35	36496.52
CA105-MW-002	10260041.D	30499.03	19643.56	20608.71	56804.00	25511.25	15130.46	37607.71	72426.15
CA105-MW-003	10260042.D	57042.59	35963.19	43763.26	109354.09	49171.96	31349.53	66553.86	138753.38
CA105-MW-004	10260043.D	115962.44	72333.11	84334.57	219859.73	100589.99	62632.75	146629.98	303927.13
CA105-MW-005	10260044.D	306848.22	184666.11	211118.88	584366.00	245054.34	155416.72	360967.19	762607.75
CA105-MW-006	10260045.D	624188.44	362352.81	423281.03	1196495.38	460255.28	334414.94	780334.31	1613388.25
CA105-MW-007	10260046.D	1394744.88	812106.06	907407.63	2672052.00	959887.38	682568.50	1736950.75	3800280.25
CA105-MW-008	10260047.D	2612717.25	1473658.50	1696326.63	5336807.00	1724157.38	1200336.75	3244554.00	7224467.50
Methanol Blank	10260048.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-005 E2	10260049.D	6786659.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-006 E2	10260050.D	6006282.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-015 E2	10260051.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-023 E2	10260052.D	6788747.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-024 E2	10260053.D	6410242.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-007 E2	10260054.D	6488784.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-008 E2	10260055.D	6665772.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-016 E2	10260056.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-025 E2	10260057.D	6924641.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-026 E2	10260058.D	6109320.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methanol Blank	10260059.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA105-MW-004	10260060.D	119866.02	70442.73	85432.81	222467.50	97611.95	59434.46	150306.63	306529.22
CA105-MW-006	10260061.D	598445.38	366501.22	407539.69	1179220.63	501073.41	325603.88	786183.63	1615856.13
Methanol Blank	10260062.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-005 E3	10260063.D	845802.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-006 E3	10260064.D	1369521.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Appendix C
Integrated Chromatogram Peak Area Data

Table 1, continued.

		PFOS	PFOA	PFOSulfinate	FOSA	M556	N-EtFOSE acid	*N*-EtFOSE Alcohol	*N*-EtFOSA
Sample ID	File ID	AREA	AREA	AREA	AREA	AREA	AREA	AREA	AREA
CA104-SA-015 E3	10260065.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-023 E3	10260066.D	498743.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-024 E3	10260067.D	1086776.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-007 E3	10260068.D	768729.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-008 E3	10260069.D	447635.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-016 E3	10260070.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-025 E3	10260071.D	801625.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-026 E3	10260072.D	1471700.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methanol Blank	10260073.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA105-MW-001	10260074.D	13982.04	16699.33	9979.43	25697.01	12133.76	7161.94	22058.21	35894.19
CA105-MW-002	10260075.D	29987.14	19143.72	21326.87	55001.61	25088.65	16386.03	35591.88	75564.77
CA105-MW-003	10260076.D	57815.73	36066.05	41587.84	111607.10	50632.96	32367.63	72765.41	146495.67
CA105-MW-004	10260077.D	120780.75	73131.43	85434.55	225726.55	97380.48	61900.77	146651.70	302634.66
CA105-MW-005	10260078.D	281840.94	175630.80	201832.98	538672.31	234026.91	156350.09	345086.19	753119.63
CA105-MW-006	10260079.D	582970.25	344841.03	394586.31	1127954.38	451452.41	302950.56	753909.56	1621473.00
CA105-MW-007	10260080.D	1360512.75	778203.38	905614.13	2775788.75	966946.69	694380.88	1755616.00	3769292.50
CA105-MW-008	10260081.D	2716866.75	1538752.63	1670244.88	5418179.50	1755200.00	1224527.13	3052786.50	7069280.00
Methanol Blank	10260082.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-009 E2	10260083.D	6546132.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-010 E2	10260084.D	6927869.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-017 E2	10260085.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-027 E2	10260086.D	6125243.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-028 E2	10260087.D	12923.94	0.00	0.00	35557.76	24310.33	103052.61	394497.41	0.00
CA104-SA-011 E2	10260088.D	7176159.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-012 E2	10260089.D	6938688.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-018 E2	10260090.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-029 E2	10260091.D	6555500.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-030 E2	10260092.D	6113930.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methanol Blank	10260093.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA105-MW-004	10260094.D	114039.99	72349.25	83337.26	228457.66	102876.83	68451.39	149993.77	314206.38
CA105-MW-006	10260095.D	590903.75	347965.78	380184.41	1120460.75	443270.28	304462.94	769036.19	1652037.25
Methanol Blank	10260096.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-009 E3	10260097.D	523538.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-010 E3	10260098.D	278158.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-017 E3	10260099.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-027 E3	10260100.D	1810332.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-028 E3	10260101.D	0.00	0.00	0.00	0.00	0.00	27767.98	60579.23	0.00
CA104-SA-011 E3	10260102.D	244567.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-012 E3	10260103.D	251377.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-018 E3	10260104.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-029 E3	10260105.D	245937.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-030 E3	10260106.D	997470.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methanol Blank	10260107.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA105-MW-001	10260108.D	13783.06	16971.07	10400.72	23816.95	10909.54	6845.91	19189.26	31411.06
CA105-MW-002	10260109.D	29314.46	16183.18	19628.93	51018.12	24116.90	15959.31	33522.69	73106.06
CA105-MW-003	10260110.D	50766.63	35860.38	37017.05	106005.91	49735.05	31492.54	69082.69	149169.80
CA105-MW-004	10260111.D	116922.48	76600.59	77199.96	231666.81	95368.06	66819.37	136360.42	305432.00
CA105-MW-005	10260112.D	290955.97	173415.73	197817.11	511399.22	236238.25	158163.89	364269.91	790519.06
CA105-MW-006	10260113.D	610388.56	368883.84	381316.00	1124824.63	449826.97	322770.94	722861.75	1547735.88
CA105-MW-007	10260114.D	1420859.75	811055.88	880515.69	2824799.25	972162.31	658313.31	1587305.25	3652949.75
CA105-MW-008	10260115.D	2440706.50	1431169.13	1573230.13	5090895.00	1639724.38	1193133.25	3142575.75	7039522.00
Methanol Blank	10260116.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-001 E2	10260117.D	1567058.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-002 E2	10260118.D	1543427.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-013 E2	10260119.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-019 E2	10260120.D	1382376.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-020 E2	10260121.D	1426916.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-003 E2	10260122.D	1248381.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-004 E2	10260123.D	1290877.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Appendix C
Integrated Chromatogram Peak Area Data

Table 1, continued.

		PFOS	PFOA	PFOSulfinate	FOSA	M556	N-EtFOSE acid	*N*-EtFOSE Alcohol	*N*-EtFOSA
Sample ID	File ID	AREA	AREA	AREA	AREA	AREA	AREA	AREA	AREA
CA104-SA-014 E2	10260124.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-021 E2	10260125.D	1542179.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-022 E2	10260126.D	1465341.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methanol Blank	10260127.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA105-MW-004	10260128.D	115161.10	72027.19	77033.35	224267.55	99030.34	65506.07	134408.08	289903.72
CA105-MW-006	10260129.D	569541.31	345411.16	401612.88	1078812.50	474330.34	322461.78	730636.06	1518741.38
Methanol Blank	10260130.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-005 E2	10260131.D	1425852.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-006 E2	10260132.D	1347554.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-015 E2	10260133.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-023 E2	10260134.D	1615440.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-024 E2	10260135.D	1512463.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-007 E2	10260136.D	1419311.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-008 E2	10260137.D	1596666.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-016 E2	10260138.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-025 E2	10260139.D	1484539.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-026 E2	10260140.D	1372794.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methanol Blank	10260141.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA105-MW-004	10260142.D	116771.37	70822.80	78991.95	222036.23	98022.34	64854.18	143022.80	294664.34
CA105-MW-006	10260143.D	570884.63	340879.81	393441.50	1135857.38	452548.31	312251.16	758448.81	1639711.25
Methanol Blank	10260144.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-009 E2	10260145.D	1459985.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-010 E2	10260146.D	1548178.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-017 E2	10260147.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-027 E2	10260148.D	1330363.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-028 E2	10260149.D	0.00	0.00	0.00	0.00	0.00	19335.43	72679.98	0.00
CA104-SA-011 E2	10260150.D	1568615.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-012 E2	10260151.D	1591005.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-018 E2	10260152.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-029 E2	10260153.D	1570424.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA104-SA-030 E2	10260154.D	1492012.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methanol Blank	10260155.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CA105-MW-001	10260156.D	13339.08	17440.71	9950.26	25193.90	11523.59	7477.15	0.00	0.00
CA105-MW-002	10260157.D	25805.36	17887.09	18693.97	50273.33	23402.56	14347.88	32397.41	65195.19
CA105-MW-003	10260158.D	51243.50	34595.46	39270.14	105613.48	44237.55	30264.75	61003.66	135146.89
CA105-MW-004	10260159.D	114950.23	74509.45	78962.37	230707.58	100317.29	70063.81	149303.59	310741.59
CA105-MW-005	10260160.D	285749.47	173712.80	205597.61	569895.75	243329.03	154189.81	339701.94	760203.31
CA105-MW-006	10260161.D	612202.88	377687.50	419857.09	1123617.13	484254.34	340038.13	652429.19	1479341.63
CA105-MW-007	10260162.D	1336394.25	816368.25	883441.13	2664414.75	940157.13	686594.19	1667519.50	3684853.75
CA105-MW-008	10260163.D	2668366.75	1505736.25	1665229.50	5137724.00	1700229.00	1182218.63	2862431.75	6919559.00
Methanol Blank	10260164.D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

APPENDIX A

SIGNATURES OF PROJECT PERSONNEL

Project Title: 5-Week *N*-EtFOSE Alcohol Aerobic Biodegradation Study

Client Project ID: LIMS E01-044

Contract Analytical Project Number: CA104

The following individuals participated in the conduct of this project:

Study Director:

Cleston C. Lange _____ Signature _____ 3/29/01 Date

Laboratory Management:

Bruce E Warden _____ Signature _____ 3/29/01 Date

Report Author:

Cleston C. Lange _____ Signature _____ 3/29/01 Date

Sample Preparation Analyst:

Angela L. Schuler _____ Signature _____ 5/29/01 Date

HPLC/MS Analysis Conducted by:

Anthony E. Scales _____ Signature _____ 3/29/01 Date

Angela L. Schuler _____ Signature _____ 3/29/01 Date

Original