BIODEGRADATION STUDY REPORT

The Aerobic Biodegradation of *N*-EtFOSE Alcohol by the Microbial Activity Present in Municipal Wastewater Treatment Sludge

Thursday, November 02, 2000

PROJECT NUMBER

3M Project ID: LIMS E00-2252
Pace Contract Analytical Project ID: CA058
Pace Proposal ID: 000511.1

PRINCIPAL ANALYTICAL INVESTIGATOR

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. Science Solutions Division 1700 Elm Street, Suite 200 Minneapolis, Minnesota 55414

PROJECT DATES

Project Initiation: July 20, 2000 Project Completion: October 1, 2000

AUTHOR

Cleston C. Lange, Ph.D.

Report 2 of 2 for project CA058, Revised

Page 1 of 55

Table of Contents

Title	Page	1		
Tab	le of Contents	2		
List	of Tables & Figures	3		
List	of Appendices	3		
Exe	cutive Summary	4		
1.0	Project Personnel	5		
2.0	Data Requirements	5		
3.0	Project Objective	5-6		
4.0	Test Article	6		
5.0	Control & Reference Articles	7-8		
6.0	Receipt/Generation of Samples	g		
7.0	Methods	g		
	7.1 Sample Preparation	g		
	7.1.1 Collection of Sludge	9-10		
	7.1.2 Culture Setup			
	7.1.3 Solid Phase Extraction (SPE)	12-13		
	7.2 Instrumental Analysis	14		
	7.3 Data Transformations and Calculations	15		
	7.3.1 Molar Calculations	15		
	7.3.2 Conversion of ng/mL to micromolar and nanomolar			
	7.3.3 Mass Balance Calculations	16		
	7.4 Statistics	17		
	7.4.1 Formula for Standard Deviation	17		
	7.4.2 Formula for Relative Standard Deviation	17		
	7.5 Software Versions	17		
8.0	Results	18		
	8.1 Incubator, Refrigerator, and Freezer Temperature Log Data	18		
	8.2 Mishaps recorded during the study	19-20		
	8.3 Sludge Characterization Report	21		
	8.3.1 Metals	21		
	8.3.2 Wet Chemistry	21		
	8.4 Quality Control/Sample Matrix Spike Results			
	8.5 HPLC/MS Calibration and Calibration Verification	23-24		
	8.6 Blanks	24		
	8.7 Matrix Spikes	24		
	8.8 Surrogate Spikes	24		
	8.9 Sample Results	25		
	8.9.1 Results of incubations with 47 ng/mL N-EtFOSE alcohol	25-27		
	8.9.2 Results of incubations with 2,380 ng/mL N-EtFOSE alcohol	28-34		
9.0	Conclusions	34-36		
10.0	Sample and Data Retention	37		

List of Tables & Figures

Table 1: Solid phase extraction % recovery table for quality control matrix spike samples	25 26
Figure 4: Production of FOSA in cultures containing 47 ng/mL N-EtFOSE alcohol	27
Figure 5: Production of PFOS in cultures containing 47 ng/mL N-EtFOSE alcohol	27
Figure 6: Degradation curve of N-EtFOSE alcohol at a test concentration of 2,380 ng/mL (μM vs. tim	e) 28
Figure 7: Degradation curve of N-EtFOSE alcohol at a test concentration of 2,380 ng/mL (% vs. time)29
Figure 8: Second-Order relationship for N-EtFOSE alcohol biodegradation (1/conc vs. time)	30
Figure 9: Production of N-EtFOSAA in cultures containing 2,380 ng/mL of N-EtFOSE alcohol	31
Figure 10: Production of metabolite M556 in cultures containing 2,380 ng/mL of N-EtFOSE alcohol	32
Figure 11: Production of PFOSulfinate in cultures containing 2,380 ng/mL of N-EtFOSE alcohol	33
Figure 12: Production of FOSA in cultures containing 2,380 ng/mL of N-EtFOSE alcohol	33
Figure 13: Production of PFOS in cultures containing 2,380 ng/mL of N-EtFOSE alcohol	34
Figure 14: Production of PFOA in cultures containing 2,380 ng/mL of N-EtFOSE alcohol	34
Figure 15: Deduced metabolic pathway for degradation of N-EtFOSE alcohol	36
List of Appendices	
Appendix A: Signatures of Project Personnel	38
Appendix B: Table 1. Sample results presented as ng/mL (ppb)	39-47
Appendix B: Table 2. Sample results presented as molar values (µM)	18-50
Appendix C: Table 1. Biodegradation samples finalized data for 2,380 ng/mL samples	51
Table 2. Biodegradation samples finalized data for 47 ng/mL samples	51
Appendix D: Table 1. Sterilized/inhibited sludge controls finalized data for 2,380 ng/mL samples	52
Table 2. No-sludge controls finalized data for the 2,380 ng/mL samples	52
Table 3. Sterilized/inhibited sludge controls finalized data for the 47 ng/mL samples	53
Table 4. No-sludge controls finalized data for the 47ng/mL samples	53
Appendix E: Table 1. Percent Degradation data for the 2,380 ng/mL biodegradation samples	54
Appendix F: Table 1. Mass balance data table for all samples and control samples	55

Executive Summary

A study was undertaken to determine the biodegradability of N-EtFOSE alcohol [2-(N-ethylperfluorooctane sulfonamido) ethyl alcohol] when exposed to municipal wastewater treatment sludge. N-EtFOSE alcohol is a monomer used in the synthesis of fluorinated surfactant and protective chemical products. The results of this study elucidate the environmental fate of this compound and related compounds upon entry into the environment, either directly or through municipal waste treatment processes. The aerobic biodegradability of N-EtFOSE alcohol was tested during a 5-week, 8 time-point study. The study utilized municipal wastewater treatment sludge as microbial inoculum and employed test concentrations of 0.047 µg/mL (90 nM) and 2.38 µg/mL (4.168 µM) N-EtFOSE alcohol in a mineral salts medium. Analytical results demonstrate that N-EtFOSE alcohol is readily biodegraded, with 100% degradation of the 0.047μg/mL samples in 14 days and 90% of 2.38 μg/mL in 35 days. The major metabolites identified from test cultures were N-EtFOSAA and M556, which together accounted for 85% of the total products observed in the day 35 cultures. Observed minor metabolites included N-EtFOSA, FOSA, PFOSulfinate, PFOS and PFOA. Mass balance between parent material and measured products was achieved, implying that all significant degradation products of N-EtFOSE alcohol were identified and that PFOS and/or PFOA are the endpoints in the biodegradation. This work promises to serve as a model for studying the biotransformation of other perfluorooctanesulfonate and fluorinated telomer-alcohol based chemistries.

1.0 Project Personnel

1.1	Spons	sor	3M Company				
1.2	Sponsor Representative		Dr. James K. Lundberg				
1.3	Contra	act Facility Personnel:					
	1.3.1	Study Director	Dr. Cleston C. Lange				
	1.3.2	Laboratory Management:	Mr. Mark T. McCann				
	1.3.3	Sample Preparation Analysts	Ms. Angela L. Schuler				
			Dr. Cleston C. Lange				
	404	LIDLO/MC Analyst	Mr. Anthony E. Coolea				
	1.3.4	HPLC/MS Analyst	Mr. Anthony E. Scales				
	1.3.5	Sludge Characterization Report	Mr. Daryl K. Peterson				
	1.3.6	Sample Custodian:	Dr. Cleston C. Lange				
	1.3.7	Report Author	Dr. Cleston C. Lange				
	1.3.8	Report Reviewer	Mr. Dirk W. Hoogenboom				

2.0 Data Requirements

The sponsor representative desired a proposal for a biodegradation study to be conducted using *N*-EtFOSE Alcohol as the degradant. After brief discussions with Dr. James K. Lundberg and Dr. William K. Reagan of the 3M Environmental Laboratory concerning details of draft proposals, copies of a final proposal (Pace Proposal # 000511.1) were submitted to them on Tuesday, May 18, 2000. The study was to be conducted as a non-GLP study but with the understanding that good data quality objectives be met. Approval was given to proceed with the project via a letter received from Dr. James K. Lundberg and dated May 23, 2000.

3.0 **Project Objective**

The objective of the proposed study was to determine whether *N*-EtFOSE alcohol will degrade under aerobic conditions using the inherent microbial populations of municipal waste treatment plant mixed liquor suspended solids "sludge" as the inoculum. The proposed study incorporated EPA-published guidelines OPPTS 835.3200, OPPTS

Page 5 of 55

835.3210, and OPPTS 835.5045. Degradation rates, identification of product analytes, and quantitation of all suspected products and the parent material for mass balance determinations was proposed.

Initially, a 3-day preliminary study was conducted that indicated that *N*-EtFOSE alcohol was readily biodegraded. This preliminary study showed the production of expected perfluorinated intermediates with the concomitant decrease in *N*-EtFOSE alcohol concentration. Subsequently, a 2-week multiple sample point screening study was conducted to work out difficulties in the analytical procedures that might prove costly if encountered in a larger study. The 2-week screening study provided direct evidence that *N*-EtFOSE alcohol is biodegraded at a test concentration well above its suspected water solubility, that the test concentration of 2.5 mg/L is not inhibitory to microbial growth, and that the compound degrades to predictable products in a stoichiometric fashion. Furthermore, that study confirmed that the extraction methods and analytical procedures efficiently recovered products and parent analyte from the culture matrix. A report for the 2-week screening study was provided to 3M on August 11, 2000, and provided the first direct evidence that this compound was biotransformed to smaller molecular weight fluorinated compounds.

This 35-day study was conducted in order to elucidate the biodegradation pathway of *N*-EtFOSE alcohol; to determine the sequence of events leading to the observed products; and to determine whether the perfluorooctanoate (PFOA), which was detected below the MQL near the end of the 2-week screening study, is truly a product of the biodegradation of *N*-EtFOSE alcohol.

4.0 Test Article

The test article used was HPLC purified *N*-EtFOSE Alcohol (3M Std I.D. # SE-035). The 3M Environmental Laboratory provided approximately 3 grams of *N*-EtFOSE alcohol for testing on June 8, 2000. The *N*-EtFOSE alcohol was delivered accompanied by a Material Safety and Data Sheet (MSDS) and a chain of custody (COC) as 3M Environmental Lab. COC # 14681. Upon receipt at Pace, the material was given a test, control and reference (TCR) number CA-TCR02-008. The purity of the material was not provided at the time the study was conducted, but an interim certificate of analysis was provided on October 31, 2000 and the purity was reported at 99.9%.

Page 6 of 55

5.0 Control & Reference Articles

Reference materials were received from the 3M Environmental Laboratory on June 8, 2000. 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.

NC: purity analysis not completed. All purity information provided for control & reference articles was archived with the raw data for the study.

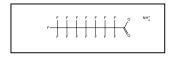
All test, control, and reference materials were stored at -77° C \pm 0.2°C.

All final data in data tables for this report are calculated assuming 100% purity of test and reference materials because purity information was not complete for all compounds.

5.1. Perfluorooctanoate Ammonium Salt (PFOA)

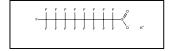
The purity was determined at 97-99% by HPLC/MS as part of this study.

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



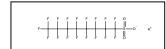
5.2. Perfluorooctane sulfinate Potassium Salt (PFOSulfinate)

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



5.3. Perfluorooctane sulfonate Potassium Salt (PFOS)

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



Page 7 of 55

5.4. Perfluorooctane sulfonamide (FOSA)

Purity: NC

3M#: SD-029

Pace #: CA-TCR02-004



5.5. *N*-Ethyl perfluorooctane sulfonamide (*N*-EtFOSA)

Purity: NC

3M#: SD-012

Pace #: CA-TCR02-005



5.6. [2-(Perfluorooctane sulfonamido) acetic acid] (M556).

Purity: NC

3M#: 00001-5-24

Pace#:CA-TCR02-006

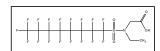


5.7. [2-(*N*-Ethyl perfluorooctane sulfonamido) acetic acid] (*N*-EtFOSAA)

Purity: NC

3M#: SE-038

Pace #: CA-TCR02-007



5.8. [2-(*N*-Ethyl perfluorooctane sulfonamido) 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 for this project, but were generated as an inherent part of this study. Each of the one hundred and ninety-two experimental cultures were extracted by solid phase extraction (SPE) methodology to generate five hundred and seventy-six analytical samples, and referred to as SPE eluates 1, 2 and 3. Of the three eluates generated for each of the samples, eluate 1 was the aqueous sample wash and eluates 2 and 3 were separately collected 25 mL methanol elutions of the sample washed SPE cartridges. Data obtained for eluates 2 and 3 from the 2-week screening study demonstrated quantitative recovery of target compounds, and thus it was expected that no analytes are lost to the aqueous eluate 1. Because degradation of *N*-EtFOSE alcohol was observed, the analysis of the positive-control cultures containing sodium dodecyl sulfate (SDS) as a degradant, was deemed unnecessary.

7.0 Methods

7.1 Sample Preparation

7.1.1. Collection of Sludge.

To prepare the test 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. Six liters of MLSS was collected by Pace laboratory personnel on July 31, 2000 and delivered as six 1-liter Nalgene polypropylene bottles containing MLSS, and were accompanied with a corresponding chain of custody (Pace C.O.C. # 465254). Upon receipt, individual bottles were labeled #1 through #6. Bottle #1 was subsequently delivered to Mr. Daryl Peterson, of the Minnesota Environmental Laboratory at Pace Analytical Services, for sludge characterization. The sludge characterization report was received on August 16, 2000 and is summarized in section 8.3.

Page 9 of 55

The MLSS in bottles #2 and #3 were allowed to settle overnight and the settled sludge was used to prepare cultures for the 5-week study. At the time that the 5-week study was begun, the settled MLSS had been stored 12 hours at 4°C. The settled sludge in each bottle constituted approximately 20% of the volume, or approximately 200 mL in a 1-liter sample, based on visual inspections.

7.1.2. Culture Setup.

All cultures were prepared on August 1, 2000 and the final sample collection occurred on September 5, 2000. Slight modifications to the original proposed methods (Pace proposal 05011.1) were deemed necessary based on findings from the 3-day and 2-week screening studies. Changes were made with regard to culture volume (changed from 20 mL to 25 mL) and the number of test concentrations (only two concentrations were tested, not three as originally proposed).

Cultures were prepared using a mineral salts medium defined in EPA Guideline OPPTS 835.3200. The mineral salts medium pH was 7.4 and contained per liter, 0.334 g Na₂HPO₄-2H₂O, 0.005 g NH₄Cl, 0.2175 g K_2 HPO₄, and 0.085 KH_2 PO₄, 0.0275 g CaCl₂-anhydrous, 0.0225 g MgSO₄-7H₂O, and 0.00025 g FeCl₃-6H₂O.

Per one liter of mineral salts medium in a 2 L Erlenmeyer flask, 50 mL of settled sludge was added. This mineral salts medium plus sludge mixture was then used to prepare the 25 mL sample cultures.

A separate 500 mL of mineral salts medium plus sludge was prepared as described above and was sterilized in an autoclave at 121°C for 30 minutes and then cooled to ambient room temperature. Upon cooling, this sterile sludge medium was treated with 100 μg/mL of chloramphenicol as a microbial growth inhibitor, and used to prepare the 25 mL sterile/inhibited control cultures.

Page 10 of 55

Mineral Salts medium, un-sterilized and without sludge, was used to

prepare the 25 mL no sludge controls.

Individual cultures and controls were prepared by dispensing 25 mL of

appropriate mineral salts medium solution into sterile 125 mL Nalgene polycarbonate culture flasks containing labels with appropriate

information. Mineral salts medium containing sludge had to be swirled

to homogenize the mixture before dispensing.

Samples were spiked with either 5 µL of a 11,900 µg/mL solution of N-

EtFOSE alcohol in methanol (CA058-SS-001) or 5 μL of a 238 μg/mL

solution of N-EtFOSE alcohol prepared in methanol (CA058-SS-012) to

prepare the 2.380 μ g/ mL (4.168 μ M) samples and 0.047 μ g/mL (90 nM)

samples, respectively. Blank controls received only the appropriate

mineral medium and no analyte.

The day zero samples were prepared and immediately placed in a

freezer that was maintained at -19° C \pm 7° C. All other cultures were

placed in temperature controlled shaking incubators that were

maintained at 28°C ± 2°C. Samples were pulled from incubators at

designated time points of days 1, 3, 7, 14, 21, 28 and 35. Following the

first week of incubation, the samples to be collected on days 14, 21, 28

and 35, received weekly additions of 1 mL of mineral salts medium plus

sludge (stored at 4°C) in order to add fresh sludge as a food source to

maintain microbial activity. The differences in volume were not a factor

in the analysis because the entire sample volumes were extracted using

SPE at the end of the incubation period.

Upon removal from the incubator, samples were either immediately

frozen, or immediately prepared 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.

Page 11 of 55

Aerobic Biodegradation of *N*-EtFOSE alcohol Cleston C. Lange, Ph.D.,

The positive control cultures containing sodium dodecyl sulfate (SDS) were prepared by adding 25 μ L of a stock solution of SDS (50,043 mg/L) to make a final concentration of 50.043 mg/L (173.5 μ M), as suggested by EPA guideline OPPTS 835.3200.

Two sets of QC test control samples were prepared in mineral salts medium containing sludge. Each set consisted of five independently prepared 25 mL cultures containing mineral salts medium plus sludge in a 125 mL flask, to which a multi-component standard containing each of the analytes of interest was added. The final concentrations of the five QC control samples were approximately 10, 50, 200, 500 and 1000 ng/mL (exact amounts are accounted for in data calculations). These QC control samples were allowed to sit for 15 minutes at room temperature and were then extracted by the SPE methodology described for this study. The data obtained from HPLC/MS analysis of eluate 2 and eluate 3 from the QC control samples was used to determine the efficiency of analyte recovery from the culture matrix.

7.1.3. Solid Phase Extraction of Cultures

A change was made from the original proposed procedure with regard to the solid phase extraction procedure. Different types of SPE cartridges were used.

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.

Page 12 of 55

Samples that were frozen had to be thawed before extraction. Following thawing, and prior 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 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.

7.2 Instrumental Analysis

All quantitative analysis was conducted on an HP1100 high performance liquid chromatograph with mass spectrometer detector (HPLC/MSD) system. HPLC/MSD system was set up with the same chromatography configuration as that described in method ETS 8-136.0 which utilizes a dual-column setup followed by a pressure relief valve serving as a flow through splitter. The MSD was operated in electrospray ionization in negative ion mode using selected ion Ions monitored were: m/z 413 (PFOA); m/z 483 monitoring (SIM). (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). Other expected products, such as analytes II, V and VI in Figure 15, were not monitored, as reference standards for those compounds were not available. All analysis was quantitative, with 8 calibration standards prepared from 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. Part numbers for ordering can be found in method ETS 8-136.0

MSD Settings:	HPLC Settings:				
Ionization mode: API-ES negative	Flow: 1 mL/min	mL/min, splitter used			
Gas Temp: 300°C	Time (min).	<u>%A</u>	<u>%B</u>		
Drying gas: 8.0 L/minute	0.00	97.0	3.0		
Nebulizer Pressure: 30 psig	0.50	97.0	3.0		
Vcap: 3500V	6.00	5.0	95.0		
Fragmentor: 70V	8.50	5.0	95.0		
EMV Gain: 2.0	8.51	97.0	3.0		
Actual Dwell for each ion: 146 SIM resolution: high	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

N-EtFOSAA = 585 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 PFOS = (500 ng/mL)*(1nmole/522 ng) = 0.958 nmole/mL = 0.958 μ mole/liter = 0.958 μ M

50 ng/mL PFOS = (50 ng/mL) * (1 nmole/522 ng) = 0.0958 nmole/mL = 0.0958 μ mole/L = 0.0958 μ M = 95.8 nM

7.3.3 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 as a percentage of the known starting concentration.

(Working Example):

If, the starting concentration of *N*-EtFOSE alcohol was at 1800 μ M And, after incubation, the following were determined:

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

Then, the mass balance is as follows:

Mass balance = $[(50\mu\text{M} + 100\mu\text{M} + 500\mu\text{M} + 1000\mu\text{M})/1800\mu\text{M}] \text{ X } 100\%$

Mass balance = $(1650\mu M/1800\mu M) \times 100\% = 91.7\%$

7.4 Statistics

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

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

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

 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).

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

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.

$$RSD = (SD / \overline{mean of Xn}) * 100\%$$

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.

8.0 Results

8.1 Incubator, Refrigerator, and Freezer Temperature Log Data.

During this study, three reciprocal shaker incubators were used. Pace ID numbers for the incubator-shakers were 0267, 0268 and 0269. Shaker Speeds on incubators were set to 200 rpm, but were not regularly calibrated.

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. 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 0267, from dates 7/31/00 to 8/8/00:	27°C <u>+</u> 1°C
Incubator-shaker, ID 0268, from dates 7/31/00 to 8/21/00:	28° <u>+</u> 2°C
Incubator-shaker, ID 0269, from dates 7/31/00 to 9/5/00:	28°C <u>+</u> 1°C
Norlake Freezer, ID 0050, from dates 7/31/00 to 9/5/00:	-19°C <u>+</u> 7° C
IsoTemp Freezer, ID 0241, from dates 7/31/00 to 9/5/00:	-77.0°C <u>+</u> 0.2°C
Carroll walk-in cooler, ID 0140, from dates 7/31/00 to 9/5/00:	4°C <u>+</u> 2°C
True refrigerator, ID 0213, from dates 7/31/00 to 9/5/00:	2.5°C <u>+</u> 1.3°C

8.2 Mishaps recorded during the Study.

During the study, two incubator failures occurred as a result of shaker motor failure and overheating. These failures were addressed immediately upon that finding by transferring all cultures contained within the incubator to another functional incubator. On occasion, incubator temperatures rose above 30°C as a result of building air conditioning failure. Low incubator temperatures are highly dependent on ambient room temperature since only 1 of 5 incubators used was refrigerated (ID 0269). Specific temperature data can be found in the incubator logs as part of the facility records. The occasional temperature spikes were not expected to have a profound effect on the study. Below is an account of the mishaps recorded.

On August 21, 2000, incubator 0267 failed with a high temp weekend reading of 40 °C but had returned to 27°C and shaking had been maintained. The study was not affected by this since all cultures in that incubator (days 1, 3 and 7) had been collected and prepared by August 8, 2000.

On August 21, 2000, incubator 0268 failed, also with a high temp weekend reading of 36°C and a shaker motor failure. Shaking had ceased for an unknown time, but less than 3 days. The study may have been affected by this mishap since cultures for days 21, 28 and a subset of day 35 samples were contained in that incubator when it failed. Immediately upon discovery of the failure on August 21, 2000, all affected cultures were transferred to functioning incubators 0274 and 0269 for the remainder of the study. Day 21 cultures (CA058-0801-SA-106 to -126) were transferred to incubator ID 0274, day 28 cultures (CA058-0801-SA-127 to -147), and the subset of day 35 cultures (CA058-0801-SA-148 to -156) were transferred to incubator ID 0269.

During the study, all of the long-term test cultures (longer than 7-day incubations) were to receive weekly additions of fresh sludge, with the exception of the sterile/inhibited controls and the no-sludge control cultures. On August 8, 2000, two of the sterile/inhibited control cultures mistakenly received fresh sludge. Because active sludge was added to these negative controls, biodegradation of *N*-EtFOSE alcohol occurred for two weeks. The Identification numbers for those

Page 19 of 55

two controls were CA058-0801-SA-113 and CA058-0801-SA-114. The data for those samples was not used when determining mass balance. Oddly, no biodegradation products were observed in those two samples even though a reduced concentration of the *N*-EtFOSE alcohol was observed.

8.3 Sludge Characterization Report

Analysis of the mixed liquor suspended solids used for this study yielded the following. Sludge was collected on August 1, 2000 and was digested on August 3, 2000. The Pace project number for the sludge characterization report was 1035161. 5-Day BOD determinations were not performed, however, for future studies it is recommended.

8.3.1 Metals:

Boron: 600 μg/L Cadmium: $12.9 \mu g/L$ Calcium: 124,000 μg/L Chromium: 273 μg/L Cobalt: 15.6 μg/L Copper: $1,450 \mu g/L$ Iron: $31,000 \mu g/L$ Lead: 140 μg/L $29,700 \mu g/L$ Magnesium: 12,200 μg/L Manganese: Molybdenum: 60 μg/L Nickel: 123 μg/L Selenium: 28 μg/L Potassium: $30,500 \mu g/L$

8.3.2. Wet Chemistry:

Zinc:

Sulfate: 57.1 mg/L
Orthophosphate: 6.8 mg/L
Phosphorous: 56.7 mg/L
Ammonia: 1.3 mg/L
Kjeldahl Nitrogen: 197 mg/L

 $1,320 \mu g/L$

Chemical Oxygen Demand (COD): 3,440 mg/L
Total Organic Carbon (TOC): 120 mg/L
Total suspended solids: 2,280 mg/L
Total solids: 3,070 mg/L

Page 21 of 55

Aerobic Biodegradation of *N*-EtFOSE alcohol Cleston C. Lange, Ph.D., Pace Analytical Services, Science Solutions Division Wednesday November 1, 2000

8.4 Quality Control/Sample Matrix Spike Results.

Analyte recovery from sample matrices was determined by conducting solid phase extraction (SPE) of spiked sludge matrix samples. The percent recovery was calculated for each analyte based on the known amount spiked into the sample matrix. The recoveries were determined for 5 analyte concentrations ranging from 10 to 1000 ng/mL. SPE quality control samples were prepared in duplicate and labeled CA058-MW-023A to CA058-MW-027A and CA058-MW-023B to CA058-MW-027B. The summed quantitative results of the HPLC/MS evaluation of eluate 2 and eluate 3 from each sample are listed below in Table 1. All analytes, except M556, were recovered at near 90 to 100%. M556 was not recovered from the 10 ppb sample. Recovery from the higher concentrations of M556 averaged 73.1%.

		Recovery (%)							
Sample ID	<u>Description</u>	PFOA	<u>PFOS</u>	PFOSulfinate	M556	N-EtFOSAA	FOSA	N-EtFOSE- alcohol	N-EtFOSA
CA058-MW-023A	SPE QC SAMPLE, 10 PPB IN SLUDGE FOR RECOVERY	91.2%	97.4%	91.6%	0.0%	86.8%	91.9%	96.7%	85.4%
CA058-MW-023B	SPE QC DUPLICATE SAMPLE, 10 PPB IN SLUDGE FOR RECOVERY	101.2%	97.3%	94.1%	0.0%	89.4%	93.2%	91.1%	84.1%
CA058-MW-024A	SPE QC SAMPLE, 50 PPB IN SLUDGE FOR RECOVERY	107.1%	110.9%	107.9%	97.4%	112.1%	110.1%	102.1%	100.8%
CA058-MW-024B	SPE QC DUPLICATE SAMPLE, 50 PPB IN SLUDGE FOR RECOVERY	105.4%	113.9%	107.6%	89.8%	112.7%	111.3%	108.3%	103.2%
CA058-MW-025A	SPE QC SAMPLE, 200 PPB IN SLUDGE FOR RECOVERY	100.2%	106.9%	104.5%	69.2%	104.3%	103.2%	103.3%	98.3%
CA058-MW-025B	SPE QC DUPLICATE SAMPLE, 200 PPB IN SLUDGE FOR RECOVERY	107.0%	108.0%	105.3%	72.1%	104.6%	102.1%	102.2%	96.4%
CA058-MW-026A	SPE QC SAMPLE, 500 PPB IN SLUDGE FOR RECOVERY	101.0%	97.6%	100.3%	65.0%	96.3%	97.4%	98.2%	93.2%
CA058-MW-026B	SPE QC DUPLICATE SAMPLE, 500 PPB IN SLUDGE FOR RECOVERY	102.6%	99.8%	100.9%	70.5%	99.8%	98.7%	99.6%	93.0%
CA058-MW-027A	SPE QC SAMPLE, 1000 PPB IN SLUDGE FOR RECOVERY	98.1%	100.0%	97.6%	59.5%	98.7%	96.5%	95.0%	93.0%
CA058-MW-027B	SPE QC DUPLICATE SAMPLE, 1000 PPB IN SLUDGE FOR RECOVERY	97.9%	98.0%	97.5%	61.4%	96.1%	93.3%	90.1%	89.2%

Table 1. Solid phase extraction percent recovery data determined by quantitative HPLC/MS analysis of eluate 2 and eluate 3 for each sample. Each matrix spike was prepared in duplicate and are shown.

8.5 HPLC/MS Calibration and Calibration Verification.

HPLC/MS multi-component calibration standards contained PFOA, PFOSulfinate, PFOS, FOSA, N-EtFOSA, M556, N-EtFOSAA and N-EtFOSE Eight calibration standards were prepared which contained these analytes at approximately 5, 10, 20, 40, 100, 200, 500 and 1000 ng/mL, and labeled CA058-MW-014 to CA058-MW-021. For quantitation, the exact concentration values were used. Calibration standards were prepared in methanol in 10 mL amounts and stored in clear I-chem vials at 4°C in refrigerator ID 0213. Aliquots were transferred to autovials and capped for use in HPLC/MS runs. Continuing calibration verification (CCV) was used in sequence runs, and included both CA058-MW-016 (20 ng/mL) and CA058-MW-019 (200 ng/mL) for checking both low and high range curves data as described below.

Instrument calibration was performed for each analyte with all eight calibration standards injected. Analytes were quantified 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 low calibration curve data, and with rare exception, all residuals in that range were $100\% \pm 15\%$. Values reported between 100 and 1000 ng/mL were determined from high calibration curve data, and again residuals on values in that range were $100\% \pm 15\%$ with rare exception.

For samples with concentrations determined to be above 1000 ng/mL, the samples were injected again as 10 μ L injections and quantified off of calibration curves of which 50 μ L was injected, for an instrumental dilution factor of 5. Values reported were multiplied by a factor of 5 for calculation of the final concentration. Data for dilutions was obtained using high range curve values, with residuals in the range of 100 to 1000 ng/mL of 100% \pm 15% with rare exception.

Calibration check and verification standards (CCVs) were used after 15 sample injections and included both calibration standards CA058-MW-016 (20 ng/mL) and CA058-MW-019 (200 ng/mL) for verification of low and high range curves. CCVs were flanked by methanol blanks. All quantitation was conducted using Page 23 of 55

averaged calibration curves flanking 30 samples, and one set of CCVs and

accompanying methanol blanks. All calibration curves were slightly quadratic

and had R-values of at least 0.990.

All data was determined to be of good to excellent quality, with residuals on

curves and CCVs generally in the range of 100% \pm 15%. All data was collected

as external standard calibration, without the use of an internal standard as

suggested by ETS-8-136.0. Instrumental sequence runs were setup as

described in method ETS 8-136.0 and contained both calibration curves and

CCVs with accompanying methanol blanks.

8.6 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, were prepared and incubated in an

identical manner to real samples. 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

Matrix sample spikes were not employed for this study. Matrix spike recovery

information was determined using the QC matrix spiked sample results described

in section 8.1.

8.8 Surrogate Spikes

Surrogate spikes were not employed during this study.

Page 24 of 55

Aerobic Biodegradation of *N*-EtFOSE alcohol Cleston C. Lange, Ph.D.,

8.9 Sample Results

8.9.1 Results of incubations with 47 ng/mL of *N*-EtFOSE alcohol.

All data for this report are calculated assuming 100% purity of test and reference materials since purity information was not available for all compounds. All reported concentrations for samples and controls described in this section can be found in **Table 1** of **Appendix B**. Summarized data for the 47 ng/mL samples can be found in **Table 2** of **Appendix C** and summarized data for controls can be found in **Table 3** and **Table 4** of **Appendix D**. All graphs presented in this section are not "mathematically fit" curves, but are hand drawn to accent the trend of the data.

From the graph in **Figure 1**, it is obvious that biodegradation occurred rapidly and was complete by day 14. Based on **Figure 1**, the biodegradation half-life estimate at this test concentration was ≤ 2 days.

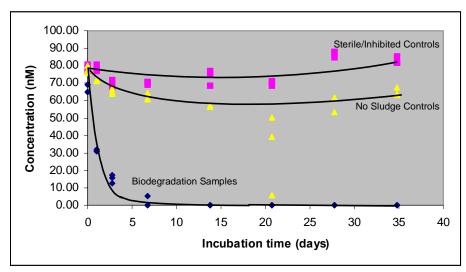


Figure 1. The 35-day biodegradation of 47 ng/mL (90 nM) *N*-EtFOSE alcohol by aerobic microorganisms present in municipal sludge, versus the no-sludge and sterile/inhibited controls, demonstrated significant biodegradation. Values from triplicate samples are shown. The two lowest values at day 21 for the no-sludge controls are from samples CA058-0801-SA-113 and CA058-0801-SA-114 that suffered a sample preparation mishap where active sludge was added inadvertently at day 7. Quantitative results from triplicate samples are shown for each time point.

The data demonstrate that *N*-EtFOSE alcohol is readily biotransformed to *N*-EtFOSAA (**Figure 2**), the expected 4-electron oxidation product of *N*-EtFOSE alcohol. During the 35 days of incubation, the *N*-EtFOSAA that formed also underwent biodegradation via a subsequent *N*-dealkylation reaction to yield M556 as shown in **Figure 3**. The M556 formed then underwent further *N*-dealkylation and oxidation reactions to yield FOSA (**Figure 4**) and PFOS (**Figure 5**). Other expected metabolites *N*-EtFOSA, PFOSulfinate, and PFOA were not detected above the analytical MQL for any of the 47 ng/mL samples.

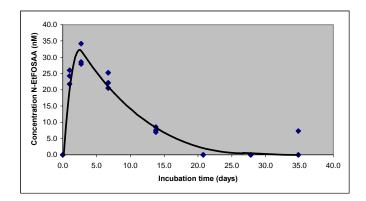


Figure 2. The production, and subsequent degradation, of *N*-EtFOSAA observed during the biodegradation of 47 ng/mL (90 nM) of *N*-EtFOSE alcohol. Quantitative results from triplicate samples are shown for each time point.

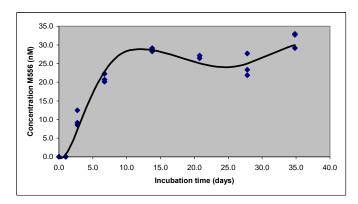


Figure 3. The production of metabolite M556 observed from the biodegradation of 47 ng/mL (90 nM) of *N*-EtFOSE alcohol. Quantitative results from triplicate samples are shown for each time point.

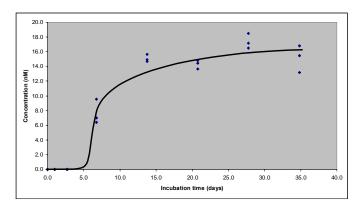


Figure 4. The production of FOSA observed from the biodegradation of 47 ng/mL (90 nM) of *N*-EtFOSE alcohol. Quantitative results from triplicate samples are shown for each time point.

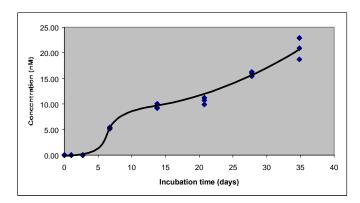


Figure5. The production of PFOS observed from the biodegradation of 47 $\,$ ng/mL (90 nM) of *N*-EtFOSE alcohol. Quantitative results from triplicate samples are shown for each time point.

8.9.2 The Results of incubation with 2,380 ng/mL of *N*-EtFOSE alcohol.

All data for this report are calculated assuming 100% purity of test and reference materials since purity information was not available for all compounds. All raw data collected for samples and controls described in this section can be found in **Table 1** of **Appendix B**. Summarized data for samples can be found in **Table 1** of **Appendix C** and summarized data for controls can be found in **Table 1** and **Table 2** of **Appendix D**. All graphs, in this section, except for **Figure 14**, are not "mathematically fit" curves, but are hand drawn to accent the trend of the data.

The 35-day incubation of 2,380 ng/mL (4.168 μ M) *N*-EtFOSE alcohol with aerated sludge provided further insight into the metabolic pathway of biodegradation. As shown in **Figure 6**, the biodegradation of *N*-EtFOSE alcohol is rapid, with a majority of the degradation occurring in 21 days. By visual inspection of **Figure 6**, the half-life of *N*-EtFOSE alcohol in this test system was approximately 2 to 3 days. Interestingly, after 21 days of incubation, the degradation of *N*-EtFOSE alcohol nearly ceased with approximately 90% of the parent material degraded, shown in **Figure 7** and **Appendix E, Table 1**. However, production of several products continued after the biodegradation of *N*-EtFOSE alcohol had ceased.

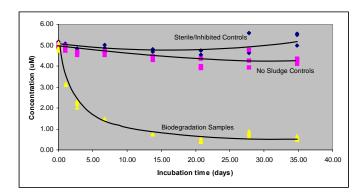


Figure 6. The biodegradation of 2,380 ng/mL *N*-EtFOSE alcohol by aerobic microorganisms present in municipal sludge over 35 days (triangles). No sludge controls (squares), and sterile/inhibited controls (circles), are also shown. Quantitative results from triplicate samples are shown for each time point.

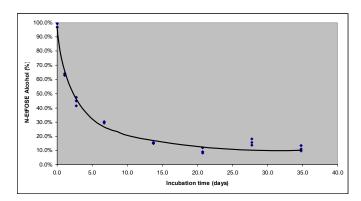


Figure 7. The percent biodegradation of *N*-EtFOSE alcohol during the 35 day incubation. The percentage value is based on the average analytically determined molar value from the day 0 triplicate samples (4.921 μ M \pm 0.176 μ M). By day 35, only 11.4% \pm 1.9% of the starting *N*-EtFOSE alcohol remained. Quantitative results from triplicate samples are shown for each time point.

In this 35-day study, heat-sterilized/chloramphenicol-inhibited sludge showed very little to no biodegradation activity of *N*-EtFOSE alcohol (**Figure 1**). This observation conclusively demonstrates that degradation was due to microbiological activity present in the active sludge.

The no-sludge control samples, which contained unsterilized mineral salts medium and *N*-EtFOSE alcohol, began to produce a small amount of *N*-EtFOSAA near the end of the study, indicating microbial contamination. This may be expected since these controls were not heat sterilized, and no steps were taken with these samples to control microbial growth. Turbidity measurements commonly used to monitor for microbial growth in solution, was not part of the study protocol. Because the sample preparation analyst was unaware of making turbidity observations, such information was not recorded.

The biodegradation of *N*-EtFOSE alcohol appeared to be second-order, as evidenced by the plot of 1/(concentration) versus time resulting in a linear relationship (**Figure 8**).

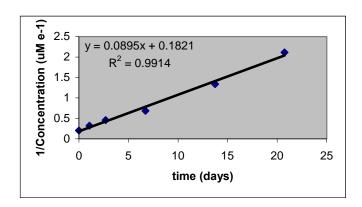


Figure 8. The determination of the second order degradation of *N*-EtFOSE alcohol as evidenced by the linear plot of [1/ (average concentration)] (μ M⁻¹) versus time (days) for the first 21 days of the biodegradation of 4.168 μ M *N*-EtFOSE alcohol. The Degradation of *N*-EtFOSE alcohol at that test concentration ceased after 21 days of incubation.

Mass balance calculations throughout the study at the test concentration of 2,380 ng/mL averaged 100.6% \pm 16.3% for all biodegradation samples, 118.6% \pm 7.9% for all sterile/inhibited controls, and 110.9% \pm 8.3% for all of the no-sludge controls (**Table 1** of **Appendix F**).

The major metabolites identified from test cultures were *N*-EtFOSAA and M556, which together accounted for nearly 83.8% of the total products observed at day 35, with *N*-EtFOSAA accounting for 34.9% of the total products and M556 accounting for 48.9%. The biodegradation dynamics for *N*-EtFOSAA, **Figure 9**, was irregular and probably represents the simultaneous production and degradation of *N*-EtFOSAA by a mixed microbial population.

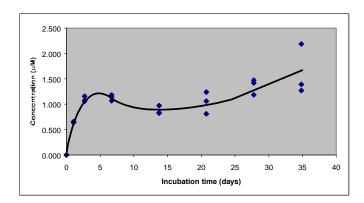


Figure 9. The production of the metabolite *N*-EtFOSAA observed during the biodegradation of 2,380 ng/mL (4.168 μ M) *N*-EtFOSE Alcohol. Quantitative results from triplicate samples are shown for each time point.

The concentration increase of M556, **Figure 10**, and was postulated to be the net result of an *N*-dealkylation reaction which removed the acetate moiety to produce *N*-EtFOSA. However, *N*-EtFOSA was not detected at significantly high levels in degradation samples, and may have formed as a rapidly degraded transient species. The *N*-EtFOSA likely underwent monooxygenation of the alkyl group to yield an alcohol, which could then undergo subsequent oxidations to yield an aldehyde and then the acid M556.

An alternative explanation may be that *N*-EtFOSAA was monooxygenated prior to dealkylation, which undergoes oxidation to produce a dicarboxylic acid. A dicarboxylic species could undergo one or two *N*-dealkylations to yield M556 or FOSA, respectively. While **Figure 15** suggests the former reaction route, the alternative mechanism is equally likely.

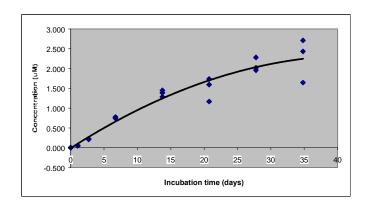


Figure 10. The production of the metabolite M556 during the biodegradation of 2,380 ng/mL (4.168 μ M) *N*-EtFOSE Alcohol. Quantitative results from triplicate samples are shown for each time point.

FOSA, of which the production is shown in **Figure 11**, may have been formed from M556 as its direct precursor, as previously discussed. The FOSA intermediate accounted for 5.0% of the total measured product at day 35.

Interestingly, PFOSulfinate production (**Figure 12**) occurred in an analogous manner to the irregular formation of *N*-EtFOSAA. This may suggest that PFOSulfinate was formed directly from *N*-EtFOSAA as a precursor or that the same microbial populations were responsible for degrading both compounds. This was not confirmed in the present study design and thus this possible route is not shown in the pathway presented in **Figure 15**. PFOSulfinate accounted for 3.5% of the total fluorochemical products detected at day 35.

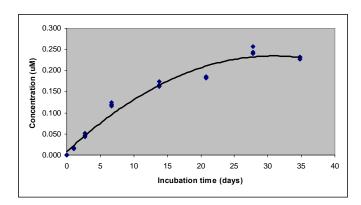


Figure 11. The production of FOSA observed during the biodegradation of 2,380 ng/mL (4.168 μ M) *N*-EtFOSE Alcohol. Quantitative results from triplicate samples are shown for each time point.

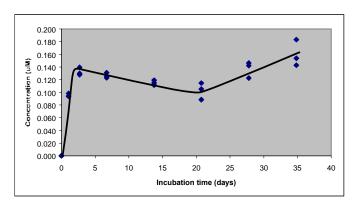


Figure 12. The production of PFOSulfinate observed during the biodegradation of 2,380 ng/mL (4.168 μ M) *N*-EtFOSE alcohol. Quantitative results from triplicate samples are shown for each time point.

Finally, the formation of PFOS (**Figure 13**) and PFOA (**Figure 14**) was observed. PFOS and PFOA appeared to be the ultimate degradation products. PFOS was probably formed from a microbial mediated sulfur oxidation mechanism that converted PFOSulfinate to PFOS. PFOA may have been formed from PFOSulfinate by an abiotic mechanism, as previously described in the literature (**Hu et.al. 1990**, **J. Fluorine Chemistry**). PFOS accounted for 7.0% of the final product measured at day 35 and PFOA accounted for 0.6% of the final product measured at day 35.

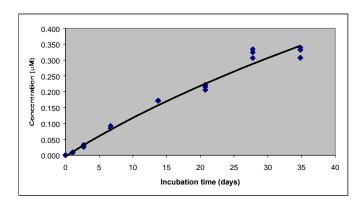


Figure 13. The production of PFOS observed during the biodegradation of 2,380 ng/mL (4.168 μ M) *N*-EtFOSE alcohol. Quantitative results from triplicate samples are shown for each time point.

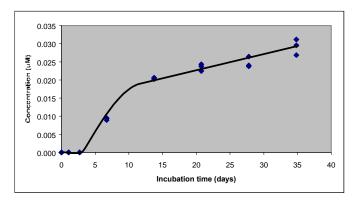


Figure 14. The production of PFOA observed after a 3-day lag period during the biodegradation of 2,380 ng/mL (4.168 μ M) *N*-EtFOSE alcohol. Quantitative results from triplicate samples are shown for each time point.

9.0 **Conclusions**

This study demonstrated that *N*-EtFOSE alcohol is rapidly biodegraded by the inherent microbial populations of municipal waste treatment facilities sludge, with 100% degradation of a 47 ng/mL (90 nM) aqueous solution of *N*-EtFOSE alcohol occurring within 14 days. Tests performed at a concentration of 2.38 μ g/mL (4.168 μ M) of *N*-EtFOSE alcohol showed that nearly 90% of the original starting material had degraded in 35 days. The data indicated that one of the primary metabolites of *N*-EtFOSE alcohol is *N*-EtFOSAA. An alcohol dehydrogenase enzyme likely catalyzes this transformation,

Page 34 of 55

anticipated to be the first step in a series of enzymatic reactions affecting the hydrocarbon tail of the molecule. Indeed, the subsequent disappearance of *N*-EtFOSAA and the continued generation of other products support this hypothesis. The second major metabolite, and most predominant product formed in 35 days, was M556. Together, *N*-EtFOSAA and M556 accounted for 83.8% of the total products at day 35 for the 2,380 ng/mL cultures (48.9% M556, 34.9% *N*-EtFOSAA). Other degradation products observed at the culmination of this study, and presented as a percentage of the total products formed at day 35, included: *N*-EtFOSA (0.1%), FOSA (5.0%), PFOSulfinate (3.5%), PFOS (7.0%) and PFOA (0.6%) (**Table 1, Appendix E**). Further elucidation of the sequence of events yielding these products is the subject of a subsequent study. A possible pathway, based on observed products in this study, is presented in **Figure 15**.

The fact that the study was conducted at concentrations near and above the suspected solubility of *N*-EtFOSE alcohol did not seem to confer any major observable differences on the ability of microorganisms to degrade the starting material. One notable difference was the complete biodegradation of the *N*-EtFOSAA formed at the low concentration, while *N*-EtFOSAA degraded slowly and accumulated significantly during incubations with a high concentration of *N*-EtFOSE alcohol.

The pathway in **Figure 15** implies that PFOS and PFOA are the ultimate end-products. This is not substantiated since smaller molecular weight products were not targeted for analysis. However, mass balance was achieved between the parent material and products implying that all significant degradation products were identified and that PFOS and/or PFOA are endpoints in the biodegradation.

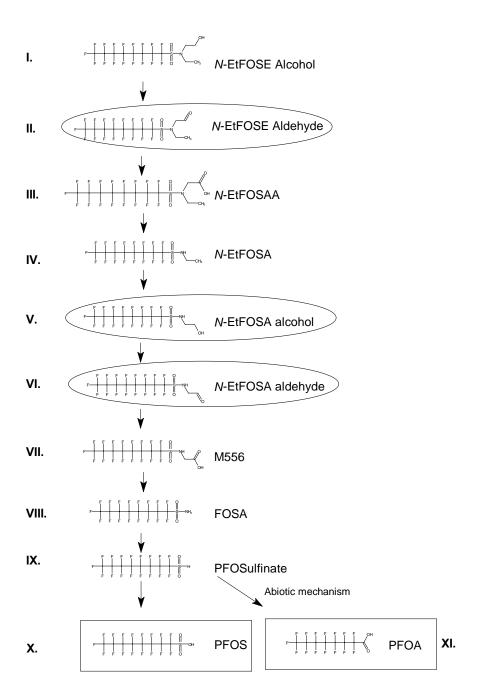


Figure 15. The metabolism of *N*-EtFOSE alcohol as mediated by the microbial activity of aerobic sludge. The pathway shows PFOS and PFOA as end-products.

10.0 **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.

Page 37 of 55

APPENDIX A

SIGNATURES OF PROJECT PERSONNEL

5-Week N-EtFOSE Alcohol Aerobic Biodegradation Study

Client Project ID: LIMS E00-2252 Contract Analytical Project Number: CA058 The following individuals participated in the conduct of this project: Study Director: Cleston C. Lange Signature Date Laboratory Management: Mark T. McCann 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 Final Report Reviewed by: Dirk W. Hoogenboom Signature Date

Page 38 of 55

Project Title:

Appendix B

TABLE 1. The quantitative results of sample analysis, presented as nanogram per milliliter, which is the same as parts per billion (ng/mL; ppb).

					С	oncentra	tion (ng/ml	_)		
					PFOS		N-		<u>N-</u> EtFOSE	N-
Sample ID	Description	Eluate	PFOA	PFOS	sulfinate	M556	EtFOSAA	FOSA	alcohol	EtFOSA
CA058-0801-SA-001	Day 0 Biodeg Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	43.3	0.0
CA058-0801-SA-002	Day 0 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	39.5	0.0
CA058-0801-SA-003	Day 0 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	37.1	0.0
CA058-0801-SA-004	Day 0 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	45.9	0.0
CA058-0801-SA-005	Day 0 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	44.6	0.0
CA058-0801-SA-006	Day 0 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	44.0	0.0
CA058-0801-SA-007	Day 0 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	43.3	0.0
CA058-0801-SA-008	Day 0 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	45.6	0.0
CA058-0801-SA-009	Day 0 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	44.4	0.0
CA058-0801-SA-010	Day 0 Sludge Blank, NO EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-011	0801-SA-011 Day 0 Sludge Blank Duplicate, NO EtFOSE Alcohol		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-012	Day 0 Sludge Blank Triplicate, No EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-013	Day 0 Biodeg Sample (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2835.8	0.0
CA058-0801-SA-014	Day 0 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2742.8	0.0
CA058-0801-SA-015	Day 0 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2668.8	0.0
CA058-0801-SA-016	Day 0 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2897.0	0.0
CA058-0801-SA-017	Day 0 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2752.9	0.0
CA058-0801-SA-018	Day 0 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2753.0	0.0
CA058-0801-SA-019	Day 0 No Sludge Control (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2684.0	0.0
CA058-0801-SA-020	Day 0 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2712.2	0.0
CA058-0801-SA-021	Day 0 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2788.4	0.0
CA058-0801-SA-001	Day 0 Biodeg Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-002	Day 0 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-003	Day 0 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-004	Day 0 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-005	Day 0 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-006	Day 0 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-007	Day 0 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-008	Day 0 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-009	Day 0 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-010	Day 0 Sludge Blank, NO EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-011	Day 0 Sludge Blank Duplicate, NO EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-012	Day 0 Sludge Blank Triplicate, No EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-013	Day 0 Biodeg Sample (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	81.3	0.0
CA058-0801-SA-014	Day 0 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	51.7	0.0
CA058-0801-SA-015	Day 0 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	49.7	0.0
CA058-0801-SA-016	Day 0 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	48.7	0.0
CA058-0801-SA-017	Day 0 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	49.8	0.0
CA058-0801-SA-018	Day 0 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	32.7	0.0
CA058-0801-SA-019	Day 0 No Sludge Control (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	104.9	0.0
CA058-0801-SA-020	Day 0 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	113.4	0.0
CA058-0801-SA-021	Day 0 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	84.1	0.0

		Concentration (ng/mL)								
Sample ID	Sample Description	Eluate	PFOA	PFOS	PFOS sulfinate	M556	<u>N-</u> EtFOSAA	FOSA	<u>N-</u> EtFOSE alcohol	<u>N-</u> EtFOSA
CA058-0801-SA-022	Day 1 Biodeg Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	15.2	0.0	17.6	0.0
CA058-0801-SA-023	Day 1 Biodeg Sample Que ppb Ett GGE Alcohol)	2	0.0	0.0	0.0	0.0	14.2	0.0	17.6	0.0
CA058-0801-SA-024	Day 1 Biodeg Sample Triplicate (50 ppb Etr OSE Alcohol)	2	0.0	0.0	0.0	0.0	12.7	0.0	18.4	0.0
CA058-0801-SA-025	Day 1 Sterile Sludge Control Sample (50 ppb Etr OSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	43.9	0.0
CA058-0801-SA-026	Day 1 Sterile Sludge Control Duplicate (50 ppb EtrOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	44.4	0.0
CA058-0801-SA-027	Day 1 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	45.9	0.0
CA058-0801-SA-027	Day 1 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	40.7	0.0
CA058-0801-SA-029	Day 1 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	41.0	0.0
	, , , , , , , , , , , , , , , , , , , ,	2			0.0	0.0				
CA058-0801-SA-030	Day 1 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0			0.0	0.0	41.0	0.0
CA058-0801-SA-031	Day 1 Sludge Blank, NO EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-032	Day 1 Sludge Blank Duplicate, NO EtFOSE Alcohol		0.0	0.0			0.0	0.0	0.0	0.0
CA058-0801-SA-033	Day 1 Sludge Blank Triplicate, No EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-034	Day 1 Biodeg Sample (2380 ppb EtFOSE Alcohol)	2	0.0	4.8	44.5	27.6	329.1	8.2	1604.9	11.2
CA058-0801-SA-035	Day 1 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	4.2	43.7	26.7	324.4	7.8	1681.3	10.7
CA058-0801-SA-036	Day 1 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	_	0.0	4.6	48.1	29.7	350.2	8.5	1704.9	11.6
CA058-0801-SA-037	Day 1 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2820.3	0.0
CA058-0801-SA-038	Day 1 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2711.9	0.0
CA058-0801-SA-039	Day 1 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2605.4	0.0
CA058-0801-SA-040	Day 1 No Sludge Control (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2719.8	0.0
CA058-0801-SA-041	Day 1 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2673.8	0.0
CA058-0801-SA-042	Day 1 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2593.2	0.0
CA058-0801-SA-022	Day 1 Biodeg Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-023	Day 1 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-024	Day 1 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-025	Day 1 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-026	Day 1 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-027	Day 1 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-028	Day 1 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-029	Day 1 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-030	Day 1 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-031	Day 1 Sludge Blank, NO EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-032	Day 1 Sludge Blank Duplicate, NO EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-033	Day 1 Sludge Blank Triplicate, No EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-034	Day 1 Biodeg Sample (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	4.4	0.0	51.9	0.0	165.5	0.0
CA058-0801-SA-035	Day 1 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	5.6	0.0	48.4	0.0	122.9	0.0
CA058-0801-SA-036	Day 1 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	3.3	0.0	35.9	0.0	76.1	0.0
CA058-0801-SA-037	Day 1 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	80.5	0.0
CA058-0801-SA-038	Day 1 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	95.9	0.0
CA058-0801-SA-039	Day 1 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	203.2	0.0
CA058-0801-SA-040	Day 1 No Sludge Control (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	131.8	0.0
CA058-0801-SA-041	Day 1 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	128.1	0.0
CA058-0801-SA-042	Day 1 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	124.4	0.0

		Concentration (ng/mL)								
Sample ID	Description	Eluate	PFOA	PFOS	PFOS sulfinate	M556	<u>N-</u> EtFOSAA	FOSA	N- EtFOSE alcohol	<u>N-</u> EtFOSA
CA058-0801-SA-043	Day 3 Biodeg Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	6.9	13.7	0.0	7.2	0.0
CA058-0801-SA-044	Day 3 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	5.1	11.8	0.0	9.8	0.0
CA058-0801-SA-045	Day 3 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.9	4.8	11.1	0.0	9.0	0.0
CA058-0801-SA-046	Day 3 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.8	0.0	0.0	0.0	40.0	0.0
CA058-0801-SA-047	Day 3 Sterile Sludge Control Duplicate (50 ppb Etr GGE Alcohol)	2	0.0	0.0	0.8	0.0	0.0	0.0	38.6	0.0
CA058-0801-SA-048	Day 3 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	41.0	0.0
CA058-0801-SA-049	Day 3 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	36.6	0.0
CA058-0801-SA-050	Day 3 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	38.2	0.0
CA058-0801-SA-051	Day 3 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	37.1	0.0
CA058-0801-SA-052	Day 3 Sludge Blank, NO EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-053	Day 3 Sludge Blank Duplicate, NO EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-054	Day 3 Sludge Blank Triplicate, No EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-055	Day 3 Biodeg Sample (2380 ppb EtFOSE Alcohol)	2	0.0	16.3	60.2	112.6	556.4	23.4	1180.2	15.6
CA058-0801-SA-056	Day 3 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	13.9	60.7	104.1	529.1	21.1	1227.6	14.9
CA058-0801-SA-057	Day 3 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	2	0.0	17.5	66.1	113.3	595.1	25.3	1084.7	16.2
CA058-0801-SA-058	Day 3 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2721.4	0.0
CA058-0801-SA-059	Day 3 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2644.5	0.0
CA058-0801-SA-060	Day 3 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2622.4	0.0
CA058-0801-SA-061	Day 3 No Sludge Control (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2483.8	0.0
CA058-0801-SA-062	Day 3 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2523.4	0.0
CA058-0801-SA-063	Day 3 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2549.3	0.0
CA058-0801-SA-043	Day 3 Biodeg Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	6.3	0.0	0.0	0.0
CA058-0801-SA-044	Day 3 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	4.9	0.0	0.0	0.0
CA058-0801-SA-045	Day 3 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.9	0.0	5.2	0.0	0.0	0.0
CA058-0801-SA-046	Day 3 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-047	Day 3 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-048	Day 3 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-049	Day 3 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-050	Day 3 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-051	Day 3 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-052	Day 3 Sludge Blank, NO EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-053	Day 3 Sludge Blank Duplicate, NO EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-054	Day 3 Sludge Blank Triplicate, No EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-055	Day 3 Biodeg Sample (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	6.5	11.4	70.1	0.0	79.0	0.0
CA058-0801-SA-056	Day 3 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	7.1	12.8	89.3	0.0	104.1	0.0
CA058-0801-SA-057	Day 3 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	6.7	12.4	81.7	0.0	75.5	0.0
CA058-0801-SA-058	Day 3 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	37.4	0.0
CA058-0801-SA-059	Day 3 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	59.0	0.0
CA058-0801-SA-060	Day 3 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	59.5	0.0
CA058-0801-SA-061	Day 3 No Sludge Control (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	113.6	0.0
CA058-0801-SA-062	Day 3 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	145.2	0.0
CA058-0801-SA-063	Day 3 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	115.6	0.0

					С	oncentra	tion (ng/ml	_)		
					PFOS		<u>N-</u>		<u>N-</u> EtFOSE	N-
Sample ID	<u>Description</u>	Eluate	<u>PFOA</u>	PFOS	sulfinate	M556	EtFOSAA	FOSA	alcohol	EtFOSA
CA058-0801-SA-064	Day 7 Biodeg Sample (50 ppb EtFOSE Alcohol)	2	0.0	2.9	0.0	11.5	13.0	4.8	3.0	0.0
CA058-0801-SA-065	Day 7 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	2.9	0.0	11.2	11.9	3.2	0.0	0.0
CA058-0801-SA-066	Day 7 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	2.8	0.0	12.4	12.0	3.5	0.0	0.0
CA058-0801-SA-067	Day 7 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	40.4	0.0
CA058-0801-SA-068	Day 7 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	40.3	0.0
CA058-0801-SA-069	Day 7 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	39.4	0.0
CA058-0801-SA-070	Day 7 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	34.7	0.0
CA058-0801-SA-071	Day 7 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	34.8	0.0
CA058-0801-SA-072	Day 7 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	36.7	0.0
CA058-0801-SA-073	Day 7 Sludge Blank, NO EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-074	Day 7 Sludge Blank Duplicate, NO EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-075	Day 7 Sludge Blank Triplicate, No EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-076	Day 7 Biodeg Sample (2380 ppb EtFOSE Alcohol)	2	4.1	44.1	57.0	366.0	559.2	58.1	756.7	16.7
CA058-0801-SA-077	Day 7 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	2	3.8	40.9	58.3	363.4	531.1	54.2	771.9	16.3
CA058-0801-SA-078	Day 7 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	2	4.0	40.0	59.6	340.4	566.2	54.4	757.0	15.2
CA058-0801-SA-079	Day 7 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2667.8	0.0
CA058-0801-SA-080	Day 7 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2811.4	0.0
CA058-0801-SA-081	Day 7 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2728.0	0.0
CA058-0801-SA-082	Day 7 No Sludge Control (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2449.1	0.0
CA058-0801-SA-083	Day 7 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2562.5	0.0
CA058-0801-SA-084	Day 7 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2556.0	0.0
CA058-0801-SA-064	Day 7 Biodeg Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-065	Day 7 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	2.9	0.0	0.0	0.0
CA058-0801-SA-066	Day 7 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-067	Day 7 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-068	Day 7 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-069	Day 7 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-070	Day 7 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-071	Day 7 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-072	Day 7 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-073	Day 7 Sludge Blank, NO EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-074	Day 7 Sludge Blank Duplicate, NO EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-075	Day 7 Sludge Blank Triplicate, No EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-076	Day 7 Biodeg Sample (2380 ppb EtFOSE Alcohol)	3	0.0	5.6	7.2	67.0	107.0	4.2	62.2	0.0
CA058-0801-SA-077	Day 7 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	4.7	6.9	59.4	93.3	3.6	64.8	0.0
CA058-0801-SA-078	Day 7 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	5.9	8.8	70.8	126.2	5.1	87.8	0.0
CA058-0801-SA-079	Day 7 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	58.3	0.0
CA058-0801-SA-080	Day 7 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	49.3	0.0
CA058-0801-SA-081	Day 7 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	49.6	0.0
CA058-0801-SA-082	Day 7 No Sludge Control (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	143.0	0.0
CA058-0801-SA-083	Day 7 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	117.7	0.0
CA058-0801-SA-084	Day 7 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	137.9	0.0

		Concentration (ng/mL)								
			2504	2500	PFOS		<u>N-</u>		N- EtFOSE	<u>N-</u>
Sample ID	Description	Eluate	PFOA	PFOS	sulfinate	M556	EtFOSAA	FOSA	alcohol	EtFOSA
CA058-0801-SA-085	Day 14 Biodeg Sample (50 ppb EtFOSE Alcohol)	2	0.0	5.4	0.0	16.2	4.4	7.8	0.0	0.0
CA058-0801-SA-086	Day 14 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	4.9	0.0	16.0	4.1	7.3	0.0	0.0
CA058-0801-SA-087	Day 14 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	5.0	0.0	15.7	5.0	7.5	0.0	0.0
CA058-0801-SA-088	Day 14 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	43.9	0.0
CA058-0801-SA-089	Day 14 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	39.1	0.0
CA058-0801-SA-090	Day 14 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	43.2	0.0
CA058-0801-SA-091	Day 14 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	32.2	0.0
CA058-0801-SA-092	Day 14 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	32.5	0.0
CA058-0801-SA-093	Day 14 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	32.2	0.0
CA058-0801-SA-094	Day 14 Sludge Blank, NO EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-095	Day 14 Sludge Blank Duplicate, NO EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-096	Day 14 Sludge Blank Triplicate, No EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-097	Day 14 Biodeg Sample (2380 ppb EtFOSE Alcohol)	2	8.9	85.2	57.1	632.1	488.4	76.6	417.2	11.4
CA058-0801-SA-098	Day 14 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	2	8.7	87.8	56.6	705.1	429.1	79.3	402.7	11.2
CA058-0801-SA-099	Day 14 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	2	8.7	81.9	51.1	668.6	395.9	78.3	386.4	10.6
CA058-0801-SA-100	Day 14 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	3.2	0.0	2702.3	0.0
CA058-0801-SA-101	Day 14 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2686.7	0.0
CA058-0801-SA-102	Day 14 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2602.5	0.0
CA058-0801-SA-103	Day 14 No Sludge Control (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	5.9	0.0	2331.5	0.0
CA058-0801-SA-104	Day 14 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	17.0	0.0	2436.4	0.0
CA058-0801-SA-105	Day 14 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	4.2	0.0	2454.1	0.0
CA058-0801-SA-085	Day 14 Biodeg Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-086	Day 14 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-087	Day 14 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-088	Day 14 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-089	Day 14 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-090	Day 14 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-091	Day 14 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-092	Day 14 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-093	Day 14 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-094	Day 14 Sludge Blank, NO EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-095	Day 14 Sludge Blank Duplicate, NO EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-096	Day 14 Sludge Blank Triplicate, No EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-097	Day 14 Biodeg Sample (2380 ppb EtFOSE Alcohol)	3	0.0	7.1	5.1	84.3	80.3	4.8	20.8	0.0
CA058-0801-SA-098	Day 14 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	4.9	3.4	68.0	53.3	3.6	15.3	0.0
CA058-0801-SA-099	Day 14 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	10.7	7.1	138.3	91.9	8.2	36.8	0.0
CA058-0801-SA-100	Day 14 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	33.6	0.0
CA058-0801-SA-101	Day 14 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	42.4	0.0
CA058-0801-SA-102	Day 14 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	77.2	0.0
CA058-0801-SA-103	Day 14 No Sludge Control (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	130.4	0.0
CA058-0801-SA-104	Day 14 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	122.6	0.0
CA058-0801-SA-105	Day 14 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	93.8	0.0

					C	oncentra	tion (ng/ml	_)		
					PFOS				<u>N-</u> EtFOSE	Α/
Sample ID	<u>Description</u>	Eluate	PFOA	PFOS	sulfinate	M556	<u>N-</u> EtFOSAA	FOSA	alcohol	<u>N-</u> EtFOSA
CA058-0801-SA-106	Day 21 Biodeg Sample (50 ppb EtFOSE Alcohol)	2	0.0	6.0	0.0	15.1	0.0	7.2	0.0	0.0
CA058-0801-SA-107	Day 21 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	5.8	0.0	14.7	0.0	7.4	0.0	0.0
CA058-0801-SA-108	Day 21 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	5.3	0.0	15.1	0.0	6.8	0.0	0.0
CA058-0801-SA-109	Day 21 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	39.9	0.0
CA058-0801-SA-110	Day 21 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	39.2	0.0
CA058-0801-SA-111	Day 21 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	40.7	0.0
CA058-0801-SA-112	Day 21 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	28.6	0.0
CA058-0801-SA-113	Day 21 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	3.1	0.0
CA058-0801-SA-114	Day 21 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	22.5	0.0
CA058-0801-SA-115	Day 21 Sludge Blank, NO EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-116	Day 21 Sludge Blank Duplicate, NO EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-117	Day 21 Sludge Blank Triplicate, No EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-118	Day 21 Biodeg Sample (2380 ppb EtFOSE Alcohol)	2	10.5	116.1	46.2	610.4	679.8	92.3	324.1	6.8
CA058-0801-SA-119	Day 21 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	2	10.2	105.6	51.9	811.0	560.6	88.4	221.4	6.3
CA058-0801-SA-120	Day 21 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	2	9.7	111.3	57.0	888.5	429.8	86.8	247.2	6.1
CA058-0801-SA-121	Day 21 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	2.9	0.0	2658.7	0.0
CA058-0801-SA-122	Day 21 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2548.1	0.0
CA058-0801-SA-123	Day 21 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	2455.2	0.0
CA058-0801-SA-124	Day 21 No Sludge Control (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	16.3	0.0	2119.0	0.0
CA058-0801-SA-125	Day 21 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	6.8	6.8	12.2	0.0	2148.2	0.0
CA058-0801-SA-126	Day 21 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	5.7	0.0	2347.1	0.0
CA058-0801-SA-106	Day 21 Biodeg Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-107	Day 21 Biodeg Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-108	Day 21 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-109	Day 21 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-110	Day 21 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-111	Day 21 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-112	Day 21 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-113	Day 21 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-114	Day 21 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-115	Day 21 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-116	Day 21 Sludge Blank, NO EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-117	Day 21 Sludge Blank Duplicate, NO EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-118	Day 21 Sludge Blank Triplicate, No EtFOSE Alcohol	3	0.0	3.5	0.0	39.3	45.6	0.0	4.9	0.0
CA058-0801-SA-119	Day 21 Biodeg Sample (2380 ppb EtFOSE Alcohol)	3	0.0	5.3	3.0	77.1	59.3	3.0	5.6	0.0
CA058-0801-SA-120	Day 21 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	4.9	2.9	77.9	45.4	3.9	8.2	0.0
CA058-0801-SA-121	Day 21 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	50.4	0.0
CA058-0801-SA-122	Day 21 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	45.8	0.0
CA058-0801-SA-123	Day 21 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	59.2	0.0
CA058-0801-SA-124	Day 21 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	115.2	0.0
CA058-0801-SA-125	Day 21 No Sludge Control (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	144.0	0.0
CA058-0801-SA-126	Day 21 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	136.3	0.0

		Concentration (ng/mL)									
Sample ID	Description	Eluate	PFOA	PFOS	PFOS sulfinate	M556	<u>N-</u> EtFOSAA	FOSA	N- EtFOSE alcohol	<u>N-</u> EtFOSA	
CA058-0801-SA-127	Day 28 Biodeg Sample (50 ppb EtFOSE Alcohol)	2	0.0	6.9	0.0	10.1	0.0	7.4	0.0	0.0	
CA058-0801-SA-128	Day 28 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	6.7	0.0	9.4	0.0	8.0	0.0	0.0	
CA058-0801-SA-129	Day 28 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	7.0	0.0	11.9	0.0	7.1	0.0	0.0	
CA058-0801-SA-130	Day 28 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	37.5	0.0	
CA058-0801-SA-131	Day 28 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	36.6	0.0	
CA058-0801-SA-132	Day 28 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	37.9	0.0	
CA058-0801-SA-133	Day 28 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	26.7	0.0	
CA058-0801-SA-134	Day 28 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	26.4	0.0	
CA058-0801-SA-135	Day 28 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	23.0	0.0	
CA058-0801-SA-136	Day 28 Sludge Blank, NO EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
CA058-0801-SA-137	Day 28 Sludge Blank Duplicate, NO EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
CA058-0801-SA-138	Day 28 Sludge Blank Triplicate, No EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
CA058-0801-SA-139			11.4	125.1	57.7	786.0	581.7	100.4	324.0	3.2	
CA058-0801-SA-140	Day 28 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	2	10.2	133.1	49.7	768.0	559.5	108.0	373.0	4.2	
CA058-0801-SA-141	Day 28 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	2	10.4	138.1	59.7	893.3	470.2	102.3	281.5	3.9	
CA058-0801-SA-142	Day 28 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	3.0	0.0	0.0	0.0	2369.0	0.0	
CA058-0801-SA-143	Day 28 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	2.9	0.0	0.0	0.0	2343.4	0.0	
CA058-0801-SA-144	Day 28 Sterile Sludge Control Triplicate (2380 ppb Etr Oct Alcohol)	2	0.0	0.0	4.8	0.0	5.2	0.0	1923.6	0.0	
CA058-0801-SA-145	Day 28 No Sludge Control (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	5.9	0.0	26.2	0.0	1636.3	0.0	
CA058-0801-SA-146	Day 28 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	3.6	0.0	16.6	0.0	1736.7	0.0	
CA058-0801-SA-147	Day 28 No Sludge Control Triplicate (2380 ppb Et GOE Alcohol)	2	0.0	0.0	7.1	0.0	49.0	0.0	1950.3	0.0	
CA058-0801-SA-127	Day 28 Biodeg Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
CA058-0801-SA-128	Day 28 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
CA058-0801-SA-129	Day 28 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
CA058-0801-SA-130	Day 28 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
CA058-0801-SA-131	Day 28 Sterile Sludge Control Duplicate (50 ppb Etr GGE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
CA058-0801-SA-132	Day 28 Sterile Sludge Control Triplicate (50 ppb EtrOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
CA058-0801-SA-133	Day 28 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
CA058-0801-SA-134	Day 28 No Sludge Control Duplicate (50 ppb Et OSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
CA058-0801-SA-135	Day 28 No Sludge Control Triplicate (50 ppb Etr OSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
CA058-0801-SA-136	Day 28 Sludge Blank, NO EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
CA058-0801-SA-137	Day 28 Sludge Blank, NO Ett OSE Alcohol Day 28 Sludge Blank Duplicate, NO EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
CA058-0801-SA-138	, , , , , , , , , , , , , , , , , , , ,	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
CA058-0801-SA-139	Day 28 Sludge Blank Triplicate, No EtFOSE Alcohol Day 28 Biodeg Sample (2380 ppb EtFOSE Alcohol)	3	0.0	7.0	3.6	84.2	52.5	2.9	9.6	0.0	
CA058-0801-SA-139			0.0						9.6	0.0	
CA058-0801-SA-140	Day 28 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol) Day 28 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	6.9	3.1	75.7 89.8	52.1 41.3	2.6	7.5	0.0	
CA058-0801-SA-142	Day 28 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	39.3	0.0	
CA058-0801-SA-143	Day 28 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	57.1	0.0	
CA058-0801-SA-144	Day 28 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	66.5	0.0	
CA058-0801-SA-145	Day 28 No Sludge Control (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	61.0	0.0	
CA058-0801-SA-146	Day 28 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	100.4	0.0	
CA058-0801-SA-147	Day 28 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	108.4	0.0	

	Concentration (ng/mL)									
Sample ID	Description	Eluate	PFOA	PFOS	PFOS sulfinate	M556	<u>N-</u> EtFOSAA	FOSA	<u>N-</u> EtFOSE alcohol	<u>N-</u> EtFOSA
CA058-0801-SA-148	Day 35 Biodeg Sample (50 ppb EtFOSE Alcohol)	2	0.0	9.0	0.0	14.2	0.0	7.2	0.0	0.0
CA058-0801-SA-149	Day 35 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	2	0.0	9.9	0.0	14.1	0.0	5.7	0.0	0.0
CA058-0801-SA-150	Day 35 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	2	0.0	8.1	0.0	12.6	3.2	6.7	0.0	0.0
CA058-0801-SA-151	Day 35 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	36.8	0.0
CA058-0801-SA-152	Day 35 Sterile Sludge Control Duplicate (50 ppb Etr OSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	35.3	0.0
CA058-0801-SA-153	Day 35 Sterile Sludge Control Triplicate (50 ppb Etr OSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	35.1	0.0
CA058-0801-SA-154	Day 35 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	29.1	0.0
CA058-0801-SA-155	Day 35 No Sludge Control Duplicate (50 ppb Etr GSE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	27.4	0.0
CA058-0801-SA-156	Day 35 No Sludge Control Triplicate (50 ppb Etr GGE Alcohol)	2	0.0	0.0	0.0	0.0	0.0	0.0	27.4	0.0
CA058-0801-SA-157	Day 35 Sludge Blank, NO EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-158	Day 35 Sludge Blank, NO Etr OSE Alcohol Day 35 Sludge Blank Duplicate, NO EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-159	Day 35 Sludge Blank Duplicate, No Etr OSE Alcohol Day 35 Sludge Blank Triplicate, No EtFOSE Alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-160	Day 35 Biodeg Sample (2380 ppb EtFOSE Alcohol)	2	12.7	127.1	62.8	651.1	872.1	96.1	274.4	3.2
CA058-0801-SA-161	Day 35 Biodeg Sample (2360 ppb Etr OSE Alcohol) Day 35 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	2	11.6	137.6	75.6	1091.4	506.9	94.1	198.1	2.8
CA058-0801-SA-161	Day 35 Biodeg Sample Duplicate (2380 ppb Eti CSE Alcohol)	2	13.4	140.3	58.6	977.2	555.1	96.7	224.1	2.3
CA058-0801-SA-163	Day 35 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	3.3	0.0	0.0	0.0	2330.8	0.0
CA058-0801-SA-164	Day 35 Sterile Sludge Control Ouplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	3.5	0.0	0.0	0.0	2332.4	0.0
CA058-0801-SA-165	Day 35 Sterile Studge Control Duplicate (2380 ppb EtPOSE Alcohol)	2	0.0	0.0	11.7	0.0	0.0	0.0	2049.8	2.6
CA058-0801-SA-166	Day 35 No Sludge Control (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	19.0	0.0	321.4	0.0	1729.5	0.0
CA058-0801-SA-167	Day 35 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	18.1	0.0	89.2	0.0	1676.3	0.0
CA058-0801-SA-168	Day 35 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	2	0.0	0.0	16.4	0.0	329.6	0.0	1777.8	0.0
CA058-0801-SA-148	Day 35 Biodeg Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-149	Day 35 Biodeg Sample (30 ppb Ett OSE Alcohol) Day 35 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-150	Day 35 Biodeg Sample Duplicate (50 ppb Etr OSE Alcohol) Day 35 Biodeg Sample Triplicate (50 ppb EtrOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-151		3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-151	Day 35 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol) Day 35 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-153		3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-153	Day 35 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol) Day 35 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-155	Day 35 No Studge Control Sample (50 ppb EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-156	Day 35 No Sludge Control Triplicate (50 ppb Etr OSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-157	Day 35 Studge Blank, NO EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-158	Day 35 Studge Blank Duplicate, NO EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-159	Day 35 Studge Blank Triplicate, No EtFOSE Alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-0801-SA-159	Day 35 Biodeg Sample (2300 EtFOSE Alcohol)	3	0.0	5.4	3.4	57.4	70.6	3.8	12.0	0.0
		3							9.1	0.0
CA058-0801-SA-161 CA058-0801-SA-162	Day 35 Biodeg Sample Duplicate (2300 EtFOSE Alcohol) Day 35 Biodeg Sample Triplicate (2300 EtFOSE Alcohol)	3	0.0	5.6 5.9	3.4	77.5 72.0	40.8 44.9	3.4	8.9	0.0
CA058-0801-SA-163	Day 35 Sterile Sludge Control (2300 EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	42.9	0.0
CA058-0801-SA-164	Day 35 Sterile Sludge Control Duplicate (2300 EtFOSE Alcohol)		0.0	0.0	0.0	0.0	0.0	0.0	63.4	0.0
CA058-0801-SA-165	Day 35 Sterile Sludge Control Triplicate (2300 EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	0.0	0.0	102.1	0.0
CA058-0801-SA-166	Day 35 No Sludge Control (2300 EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	16.5	0.0	108.8	0.0
CA058-0801-SA-167	Day 35 No Sludge Control Duplicate (2300 EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	8.2	0.0	96.7	0.0
CA058-0801-SA-168	Day 35 No Sludge Control Triplicate (2300 EtFOSE Alcohol)	3	0.0	0.0	0.0	0.0	17.8	0.0	95.8	0.0

					С	oncentra	tion (ng/ml	_)		
Sample ID	<u>Description</u>	Eluate	PFOA	PFOS	PFOS sulfinate	M556	<u>N-</u> EtFOSAA	FOSA	<u>N-</u> EtFOSE alcohol	<u>N-</u> EtFOSA
CA058-MW-023A	SPE QC SAMPLE, 10 PPB IN SLUDGE FOR RECOVERY	2	10.0	9.7	10.1	0.0	9.6	10.1	11.6	10.3
CA058-MW-023B	SPE QC DUPLICATE SAMPLE, 10 PPB IN SLUDGE FOR RECOVERY	2	11.1	9.7	10.3	0.0	9.8	10.2	10.9	10.1
CA058-MW-024A	SPE QC SAMPLE, 50 PPB IN SLUDGE FOR RECOVERY	2	58.9	53.7	56.0	4.9	54.5	55.9	61.3	56.6
CA058-MW-024B	SPE QC DUPLICATE SAMPLE, 50 PPB IN SLUDGE FOR RECOVERY	2	58.0	52.5	53.2	4.5	53.0	55.1	60.1	56.7
CA058-MW-025A	SPE QC SAMPLE, 200 PPB IN SLUDGE FOR RECOVERY	2	220.5	198.8	210.7	13.8	199.0	206.3	230.5	216.6
CA058-MW-025B	SPE QC DUPLICATE SAMPLE, 200 PPB IN SLUDGE FOR RECOVERY	2	235.3	205.5	216.5	14.4	200.6	207.4	232.0	215.8
CA058-MW-026A	SPE QC SAMPLE, 500 PPB IN SLUDGE FOR RECOVERY	2	482.2	412.5	461.5	29.3	419.9	444.3	503.5	472.6
CA058-MW-026B	SPE QC DUPLICATE SAMPLE, 500 PPB IN SLUDGE FOR RECOVERY	2	491.9	429.1	468.8	31.7	439.9	454.2	514.4	474.5
CA058-MW-027A	SPE QC SAMPLE, 1000 PPB IN SLUDGE FOR RECOVERY	2	943.5	866.7	911.5	53.5	889.1	895.1	989.6	958.4
CA058-MW-027B	SPE QC DUPLICATE SAMPLE, 1000 PPB IN SLUDGE FOR RECOVERY	2	948.6	864.9	928.5	55.3	874.0	874.6	944.2	923.7
CA058-MW-023A	SPE QC SAMPLE, 10 PPB IN SLUDGE FOR RECOVERY	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-MW-023B	SPE QC DUPLICATE SAMPLE, 10 PPB IN SLUDGE FOR RECOVERY	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA058-MW-024A	SPE QC SAMPLE, 50 PPB IN SLUDGE FOR RECOVERY	3	0.0	2.9	3.3	0.0	4.9	2.5	0.0	2.9
CA058-MW-024B	SPE QC DUPLICATE SAMPLE, 50 PPB IN SLUDGE FOR RECOVERY	3	0.0	5.6	5.9	0.0	6.7	3.9	4.8	4.2
CA058-MW-025A	SPE QC SAMPLE, 200 PPB IN SLUDGE FOR RECOVERY	3	0.0	20.4	20.3	0.0	22.0	14.7	15.4	14.4
CA058-MW-025B	SPE QC DUPLICATE SAMPLE, 200 PPB IN SLUDGE FOR RECOVERY	3	0.0	15.9	16.3	0.0	21.2	11.1	11.3	10.7
CA058-MW-026A	SPE QC SAMPLE, 500 PPB IN SLUDGE FOR RECOVERY	3	18.7	36.3	35.9	0.0	38.6	24.3	22.9	21.6
CA058-MW-026B	SPE QC DUPLICATE SAMPLE, 500 PPB IN SLUDGE FOR RECOVERY	3	16.8	29.9	31.6	0.0	35.1	20.7	19.2	18.4
CA058-MW-027A	SPE QC SAMPLE, 1000 PPB IN SLUDGE FOR RECOVERY	3	30.1	54.4	57.6	0.0	51.6	33.5	28.3	26.8
CA058-MW-027B	SPE QC DUPLICATE SAMPLE, 1000 PPB IN SLUDGE FOR RECOVERY	3	22.2	37.4	40.1	0.0	41.6	23.1	21.1	20.6

Appendix B, continued

Table 2. Biodegradation tables presented as micromolar concentrations. Results of the analysis of eluate 2 and eluate 3 for each sample are combined to create the final values presented.

PEGS					Concent	ration (µM)			
The Filtering Stampter City (Let ETCOSE Activation)									
200 10000 20000									
20 Part Stanger Central Stanger (Dept STOSE) Accord 0.000									
The Object Stands Courted Stangles (Closed ENTOSE Activates)									
200 200									
20		0.000	0.000	0.000	0.000	0.000	0.000	0.078	0.000
Day 10 No Belage Courted Capitation (6) page EFFOSE Activate)	Day 0 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)								
20									
Description									
Description									
Description									
Degree Degree Degree Degree EFOSE Accorded Degree Degree Degree Triplatione 2390 pp. EFOSE Accorded Degree De		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Day O Starte Stanter Trejlocate (2009 gpb EFFOSE Alcohol) 0.0000 0.0000 0.0000 0.000 0.000 0.000 0.0000 0.0	Day 0 Biodeg Sample (2380 ppb EtFOSE Alcohol)								
Day O Statell Studger Control (2309) pcb EFFOSE Alcohol) Day O Statell Studger Control Optimized (2509) pcb EFFOSE Alcohol) Day O Statell Studger Control Tripiciser (2309) pcb EFFOSE Alcohol) Day O Statell Studger Control Tripiciser (2309) pcb EFFOSE Alcohol) Day O Studger Studger Control Tripiciser (2309) pcb EFFOSE Alcohol) Day O Studger Control Tripiciser (2309) pcb EFFOSE Alcohol) Dosorrian (2509)									
Dept									
Description									
Description									
Day 1 Not Studge Control Duplicate (2380 pp. EFFOSE Alcohol)									
Day On No Studge Control Triplicatine (2380 ppb EIFOSE Alcohol) D.000 M-EIFOSE									
Description					0.000	0.000	0.000	5.031	
Description					Concent	ration (μM)			
Day 1 Bloded Sample Duplicates (50 pbb EFFOSE Alcohol)	<u>Description</u>	PFOA	PFOS		<u>M556</u>	N-EtFOSAA	<u>FOSA</u>		N-EtFOSA
Day 1 Blocker Sample Traplicites (SD pob EFFOSE Alcohol)	, , , , , , , , , , , , , , , , , , , ,								
Day 1 Stellies Studge Comrot Aampleis (50 ppb. EFFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 0.0077 0.000 Day 1 Stellies Studge Control Deplication (50 pb. EFFOSE Alcohol) 0.000 0.									
Day 1 Streine Studge Corntrol Duplicates (50 ppt EFFOSE Alcohor) 1 O.000 0.0000 0.00									
Day 1 Sieriei Studge Control Triplicate (50 pp. EFFOSE Alcohot) 0.0000 0.000									
Day 1 No Studge Corntrol Spannies (50 pol EFFOSE Atchod)	, , , , , , , , , , , , , , , , , , , ,								
Day 1 No Sludge Control Duplicate (50 ppb EFOSE Alcohol)									
Day 1 Studge Blank, NO EFFOSE Alcohol 0.000 0.00		0.000	0.000	0.000	0.000	0.000	0.000		0.000
Day 1 Studge Blank Cupilicate, NO EIFOSE Alcohol 0.000 0	Day 1 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)								
Day 1 Studge Blank Triplicate, No EIFOSE Alcohol)									
Day 1 Biodag Sample (2280 ppb EIFOSE Alcohol) 0.000 0.009 0.094 0.049 0.651 0.016 3.100 0.021									
Day 1 Biodes Sample Duplicate (2380 ppb EIFOSE Alcohol) 0.000									
Day 1 Biodeg Sample Triplicate (2380 ppb EIFOSE Alcohol) 0.000 0									
Day 1 Sterile Studge Control (2380 ppb EFFOSE Alcohol) 0.000 0.									
Day 1 Sterile Studge Control Triplicate (2380 ppb EIFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 0.000 4.994 0.000		0.000	0.000	0.000	0.000	0.000	0.000	5.080	0.000
Day 1 No Sludge Control (2380 ppb EIFOSE Alcohol) 0.000 0.001 0.000 0.001 0.000 0.001 0.000 0.001 0.000 0.001 0.000 0.001 0.000 0.001 0.000 0.001 0.000 0.001 0.000 0.001 0.000 0.001 0.000 0.001 0.000 0.001 0.000 0.001 0.000									
Day 1 No Sludge Control Duplicate (2380 ppb EIFOSE Alcohol) 0.000 0.001 0.000 0.001 0.000									
Day 1 No Sludge Control Triplicate (2380 ppb EIFOSE Alcohol) D.000									
PFOS PFOS Sulfinate M556 N-EtFOSA FOSA Alcohol N-EtFOSE Alcohol N-EtFOSA Sulfinate M556 N-EtFOSA FOSA Alcohol N-EtFOSA Alcohol N-EtFOSA Alcohol N-EtFOSA Alcohol N-EtFOSA Alcohol N-EtFOSA N-EtFOSA Alcohol N-EtFOSA N-EtFOSA Alcohol N-EtFOSA N-ETFOS									
Description PFOA PFOS Sulfinate M556 N-EtFOSA FOSA alcohol N-EtFOSA 0.000 0.0	Day 1 No Sidage Control Triplicate (2300 ppb Eti CSE Alcohor)	0.000	0.000	0.000			0.000	4.700	0.000
Day 3 Biodeg Sample (50 ppb EiFOSE Alcohol) 0.000 0.000 0.000 0.012 0.034 0.000 0.013 0.000 Day 3 Biodeg Sample Duplicate (50 ppb EiFOSE Alcohol) 0.000 0.000 0.000 0.009 0.029 0.000 0.017 0.000 Day 3 Biodeg Sample Triplicate (50 ppb EiFOSE Alcohol) 0.000 0.000 0.004 0.009 0.028 0.000 0.017 0.000 Day 3 Sterile Sludge Control Sample (50 ppb EiFOSE Alcohol) 0.000<				PFOS				N-EtFOSE	
Day 3 Biodeg Sample Duplicate (50 ppb EIFOSE Alcohol) 0.000 0.000 0.000 0.009 0.029 0.000 0.017 0.000 Day 3 Biodeg Sample Triplicate (50 ppb EIFOSE Alcohol) 0.000 0.000 0.004 0.009 0.028 0.000 0.016 0.000 Day 3 Sterile Sludge Control Sample (50 ppb EIFOSE Alcohol) 0.000									
Day 3 Biodeg Sample Triplicate (50 ppb EIFOSE Alcohol) 0.000 0.000 0.004 0.009 0.028 0.000 0.016 0.000 Day 3 Sterile Sludge Control Sample (50 ppb EIFOSE Alcohol) 0.000									
Day 3 Sterile Sludge Control Sample (50 ppb EIFOSE Alcohol) 0.000 0.000 0.003 0.000	, , , , , , , , , , , , , , , , , , , ,								
Day 3 Sterile Sludge Control Duplicate (50 ppb EIFOSE Alcohol) 0.000 0.000 0.003 0.000									
Day 3 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol) 0.000									
Day 3 No Sludge Control Sample (50 ppb EIFOSE Alcohol) 0.000				_					
Day 3 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol) 0.000									
Day 3 Sludge Blank, NO EtFOSE Alcohol 0.000	Day 3 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)								
Day 3 Sludge Blank Duplicate, NO EtFOSE Alcohol 0.000 0.0									
Day 3 Sludge Blank Triplicate, No EtFOSE Alcohol 0.000 0.0223 1.071 0.047 2.205 0.030 Day 3 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol) 0.000 0.026 0.130 0.210 1.057 0.042 2.332 0.028 Day 3 Sterile Sludge Sample Triplicate (2380 ppb EtFOSE Alcohol) 0.000 0.033 0.139 0.226 1.157 0.051 2.032 0.031 Day 3 Sterile Sludge Control (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 4.831 0.000 Day 3 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000<									
Day 3 Biodeg Sample (2380 ppb EtFOSE Alcohol) 0.000 0.030 0.128 0.223 1.071 0.047 2.205 0.030 Day 3 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol) 0.000 0.026 0.130 0.210 1.057 0.042 2.332 0.028 Day 3 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol) 0.000 0.033 0.139 0.226 1.157 0.051 2.032 0.031 Day 3 Sterile Sludge Control (2380 ppb EtFOSE Alcohol) 0.000									
Day 3 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol) 0.000 0.026 0.130 0.210 1.057 0.042 2.332 0.028 Day 3 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol) 0.000 0.033 0.139 0.226 1.157 0.051 2.032 0.031 Day 3 Sterile Sludge Control (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 4.735 0.000 Day 3 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 0.000 4.697 0.000 Day 3 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 4.697 0.000 Day 3 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 4.674 0.000									
Day 3 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol) 0.000 0.033 0.139 0.226 1.157 0.051 2.032 0.031 Day 3 Sterile Sludge Control (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 4.831 0.000 Day 3 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 4.735 0.000 Day 3 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 4.697 0.000 Day 3 No Sludge Control (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 4.674 0.000									
Day 3 Sterile Sludge Control (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 4.831 0.000 Day 3 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 4.735 0.000 Day 3 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 4.697 0.000 Day 3 No Sludge Control (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 0.000 4.674 0.000 Day 3 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol) 0.000 0.									
Day 3 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 4.697 0.000 Day 3 No Sludge Control (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 0.000 4.549 0.000 Day 3 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 4.674 0.000	Day 3 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000		0.000
Day 3 No Sludge Control (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 4.549 0.000 Day 3 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 0.000 4.674 0.000									
Day 3 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol) 0.000 0.000 0.000 0.000 0.000 4.674 0.000									
	, , , , , , , , , , , , , , , , , , , ,								
	Day 3 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol) Day 3 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	4.674	0.000

Page 48 of 55

				Concent	ration (μM)			
<u>Description</u>	PFOA	<u>PFOS</u>	PFOS sulfinate	M556	N-EtFOSAA	FOSA	N-EtFOSE alcohol	N-EtFOSA
Day 7 Biodeg Sample (50 ppb EtFOSE Alcohol)	0.000	0.005	0.000	0.021	0.022	0.010	0.005	0.000
Day 7 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	0.000	0.005	0.000	0.020	0.025	0.006	0.000	0.000
Day 7 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	0.000	0.005	0.000	0.022	0.021	0.007	0.000	0.000
Day 7 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000
Day 7 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000
Day 7 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.069 0.061	0.000
Day 7 No Sludge Control Sample (50 ppb EtFOSE Alcohol) Day 7 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.061	0.000
Day 7 No Sludge Control Duplicate (50 ppb Etr GSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.064	0.000
Day 7 Sludge Blank, NO EtFOSE Alcohol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Day 7 Sludge Blank Duplicate, NO EtFOSE Alcohol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Day 7 Sludge Blank Triplicate, No EtFOSE Alcohol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Day 7 Biodeg Sample (2380 ppb EtFOSE Alcohol)	0.009	0.092	0.123	0.777	1.139	0.125	1.434	0.032
Day 7 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	0.009	0.085	0.125	0.759	1.067	0.116	1.465	0.031
Day 7 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	0.009	0.085	0.131	0.738	1.184	0.119	1.479	0.029
Day 7 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	4.774	0.000
Day 7 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	5.010	0.000
Day 7 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	4.864	0.000
Day 7 No Sludge Control (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	4.540 4.694	0.000
Day 7 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	4.718	0.000
Day 7 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.000		ration (µM)	0.000	4.710	0.000
Description	PFOA	PFOS	PFOS sulfinate	M556	N-EtFOSAA	FOSA	N-EtFOSE alcohol	N-EtFOSA
Day 14 Biodeg Sample (50 ppb EtFOSE Alcohol)	0.000	0.010	0.000	0.029	0.008	0.016	0.000	0.000
Day 14 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	0.000	0.009	0.000	0.029	0.007	0.015	0.000	0.000
Day 14 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	0.000	0.009	0.000	0.028	0.009	0.015	0.000	0.000
Day 14 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.000
Day 14 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.068	0.000
Day 14 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.076	0.000
Day 14 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.000
Day 14 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.057	0.000
Day 14 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.000
Day 14 Sludge Blank, NO EtFOSE Alcohol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Day 14 Sludge Blank Duplicate, NO EtFOSE Alcohol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Day 14 Sludge Blank Triplicate, No EtFOSE Alcohol Day 14 Biodeg Sample (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.119	1.286	0.972	0.163	0.767	0.000
Day 14 Biodeg Sample (2380 ppb EtFOSE Alcohol) Day 14 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	0.021	0.172	0.115	1.388	0.825	0.166	0.732	0.021
Day 14 Biodeg Sample Diplicate (2380 ppb EtFOSE Alcohol)	0.020	0.172	0.111	1.449	0.834	0.173	0.741	0.020
Day 14 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.005	0.000	4.791	0.000
Day 14 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	4.780	0.000
Day 14 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	4.693	0.000
Day 14 No Sludge Control (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.010	0.000	4.312	0.000
Day 14 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.029	0.000	4.482	0.000
Day 14 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.007	0.000	4.462	0.000
		1	2500	Concent	ration (μM)			1
Description	BEOA	DEOS	PFOS culfinate	MEEC	A/ E4EOS A A	EOSA	N-EtFOSE	AL E+EOSA
Description Day 21 Biodea Sample (50 pph EtEOSE Alcohol)	9FOA 0.000	0.011	sulfinate 0.000	M556 0.027	<u>N-EtFOSAA</u> 0.000	FOSA 0.014	<u>alcohol</u> 0.000	0.000
Day 21 Biodeg Sample (50 ppb EtFOSE Alcohol) Day 21 Biodeg Sample (50 ppb EtFOSE Alcohol)	0.000	0.011	0.000	0.027	0.000	0.014	0.000	0.000
Day 21 Biodeg Sample (50 ppb EtFOSE Alcohol) Day 21 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	0.000	0.011	0.000	0.020	0.000	0.013	0.000	0.000
Day 21 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.070	0.000
Day 21 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.069	0.000
Day 21 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000
Day 21 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000
Day 21 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000
Day 21 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.039	0.000
Day 21 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Day 21 Sludge Blank, NO EtFOSE Alcohol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Day 21 Sludge Blank Duplicate, NO EtFOSE Alcohol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Day 21 Sludge Blank Triplicate, No EtFOSE Alcohol	0.024 0.024	0.222	0.089 0.105	1.166	1.240 1.060	0.185	0.576	0.013 0.012
Day 21 Biodeg Sample (2380 ppb EtFOSE Alcohol)	0.024	0.206 0.216	0.105	1.594 1.735	0.812	0.183 0.182	0.398 0.447	0.012
Day 21 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol) Day 21 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	0.022	0.216	0.115	0.000	0.812	0.182	4.745	0.000
Day 21 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	4.543	0.000
Day 21 Sterile Studge Control (2380 ppb EtFOSE Alcohol) Day 21 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	4.404	0.000
Day 21 Sterile Studge Control Deplicate (2380 ppb Etr GSE Alcohol)	0.000	0.000	0.000	0.000	0.028	0.000	3.913	0.000
Day 21 No Sludge Control (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.013	0.012	0.021	0.000	4.014	0.000

				Concent	ration (µM)			
			PFOS	Concent	Lation (p.m)		N-EtFOSE	
Sample Description	PFOA	PFOS	sulfinate	M556	N-EtFOSAA	FOSA	alcohol	N-EtFOSA
Day 28 Biodeg Sample (50 ppb EtFOSE Alcohol)	0.000	0.016	0.000	0.023	0.000	0.017	0.000	0.000
Day 28 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	0.000	0.015	0.000	0.022	0.000	0.018	0.000	0.000
Day 28 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	0.000	0.016	0.000	0.028	0.000	0.017	0.000	0.000
Day 28 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.087	0.000
Day 28 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.085	0.000
Day 28 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.088	0.000
Day 28 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.062	0.000
Day 28 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.061	0.000
Day 28 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.053	0.000
Day 28 Sludge Blank, NO EtFOSE Alcohol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Day 28 Sludge Blank Duplicate, NO EtFOSE Alcohol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Day 28 Sludge Blank Triplicate, No EtFOSE Alcohol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Day 28 Biodeg Sample (2380 ppb EtFOSE Alcohol)	0.026	0.306	0.142	2.019	1.472	0.240	0.774	0.007
Day 28 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	0.024	0.325	0.123	1.958	1.419	0.256	0.887	0.010
Day 28 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	0.024	0.334	0.146	2.281	1.187	0.243	0.671	0.009
Day 28 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.007	0.000	0.000	0.000	5.588	0.000
Day 28 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.007	0.000	0.000	0.000	5.570	0.000
Day 28 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.011	0.000	0.012	0.000	4.617	0.000
Day 28 No Sludge Control (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.014	0.000	0.061	0.000	3.938	0.000
Day 28 No Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.008	0.000	0.039	0.000	4.262	0.000
Day 28 No Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.016	0.000	0.114	0.000	4.777	0.000
				Concent	ration (μM)			
			PFOS				N-EtFOSE	
Sample Description	<u>PFOA</u>	<u>PFOS</u>	<u>sulfinate</u>	M556	N-EtFOSAA	FOSA	<u>alcohol</u>	N-EtFOSA
Day 35 Biodeg Sample (50 ppb EtFOSE Alcohol)	0.000	0.021	0.000	0.033	0.000	0.017	0.000	0.000
Day 35 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	0.000	0.023	0.000	0.033	0.000	0.013	0.000	0.000
Day 35 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	0.000	0.019	0.000	0.029	0.007	0.015	0.000	0.000
Day 35 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.085	0.000
Day 35 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.082	0.000
Day 35 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.082	0.000
Day 35 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.000
Day 35 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.064	0.000
Day 35 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000
Day 35 Sludge Blank, NO EtFOSE Alcohol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Day 35 Sludge Blank Duplicate, NO EtFOSE Alcohol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Day 35 Sludge Blank Triplicate, No EtFOSE Alcohol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Day 35 Biodeg Sample (2380 ppb EtFOSE Alcohol)	0.030	0.307	0.154	1.644	2.187	0.232	0.665	0.007
Day 35 Biodeg Sample Duplicate (2380 ppb EtFOSE Alcohol)	0.027	0.332	0.183	2.712	1.271	0.226	0.481	0.007
Day 35 Biodeg Sample Triplicate (2380 ppb EtFOSE Alcohol)	0.031	0.339	0.143	2.434	1.392	0.232	0.541	0.005
Day 35 Sterile Sludge Control (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.008	0.000	0.000	0.000	5.507	0.000
Day 35 Sterile Sludge Control Duplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.008	0.000	0.000	0.000	5.559	0.000
Day 35 Sterile Sludge Control Triplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.027	0.000	0.000	0.000	4.993	0.006
In	0.000	0.000	0.044	0.000	0.784	0.000	4.265	0.000
Day 35 No Sludge Control (2380 ppb EtFOSE Alcohol)								
Day 35 No Studge Control (2380 ppb EtFOSE Alcohol) Day 35 No Studge Control Duplicate (2380 ppb EtFOSE Alcohol) Day 35 No Studge Control Triplicate (2380 ppb EtFOSE Alcohol)	0.000	0.000	0.042	0.000	0.226 0.806	0.000	4.114 4.347	0.000

Appendix C Biodegradation Samples Final Data

TABLE 1. The compiled data results from the biodegradation of 2,380 ng/ml (4.168 μ M) *N*-EtFOSE alcohol over 35 days of incubation. Approximately 90% biodegradation was observed after 35 days. All values are presented as micromolar (μ M) concentrations.

					Concentr	ation (μM)			
Sample Description	Incubation time (days)	PFOA	PFOS	PFOS sulfinate	M556	N-EtFOSAA	FOSA	N-EtFOSE alcohol	N-EtFOSA
Day 0 Biodeg Sample (2300 EtFOSE Alcohol)	0.0	0.000	0.000	0.000	0.000	0.000	0.000	5.109	0.000
Day 0 Biodeg Sample Duplicate (2300 EtFOSE Alcohol)	0.0	0.000	0.000	0.000	0.000	0.000	0.000	4.894	0.000
Day 0 Biodeg Sample Triplicate (2300 EtFOSE Alcohol)	0.0	0.000	0.000	0.000	0.000	0.000	0.000	4.761	0.000
Day 1 Biodeg Sample (2300 EtFOSE Alcohol)	1.0	0.000	0.009	0.094	0.049	0.651	0.016	3.100	0.021
Day 1 Biodeg Sample Duplicate (2300 EtFOSE Alcohol)	1.0	0.000	0.008	0.094	0.048	0.637	0.016	3.160	0.020
Day 1 Biodeg Sample Triplicate (2300 EtFOSE Alcohol)	1.0	0.000	0.009	0.098	0.053	0.660	0.017	3.119	0.022
Day 3 Biodeg Sample (2300 EtFOSE Alcohol)	2.7	0.000	0.030	0.128	0.223	1.071	0.047	2.205	0.030
Day 3 Biodeg Sample Duplicate (2300 EtFOSE Alcohol)	2.7	0.000	0.026	0.130	0.210	1.057	0.042	2.332	0.028
Day 3 Biodeg Sample Triplicate (2300 EtFOSE Alcohol)	2.7	0.000	0.033	0.139	0.226	1.157	0.051	2.032	0.031
Day 7 Biodeg Sample (2300 EtFOSE Alcohol)	6.7	0.009	0.092	0.123	0.777	1.139	0.125	1.434	0.032
Day 7 Biodeg Sample Duplicate (2300 EtFOSE Alcohol)	6.7	0.009	0.085	0.125	0.759	1.067	0.116	1.465	0.031
Day 7 Biodeg Sample Triplicate (2300 EtFOSE Alcohol)	6.7	0.009	0.085	0.131	0.738	1.184	0.119	1.479	0.029
Day 14 Biodeg Sample (2300 EtFOSE Alcohol)	13.8	0.021	0.172	0.119	1.286	0.972	0.163	0.767	0.022
Day 14 Biodeg Sample Duplicate (2300 EtFOSE Alcohol)	13.8	0.020	0.172	0.115	1.388	0.825	0.166	0.732	0.021
Day 14 Biodeg Sample Triplicate (2300 EtFOSE Alcohol)	13.8	0.020	0.172	0.111	1.449	0.834	0.173	0.741	0.020
Day 21 Biodeg Sample (2300 EtFOSE Alcohol)	20.8	0.024	0.222	0.089	1.166	1.240	0.185	0.576	0.013
Day 21 Biodeg Sample Duplicate (2300 EtFOSE Alcohol)	20.8	0.024	0.206	0.105	1.594	1.060	0.183	0.398	0.012
Day 21 Biodeg Sample Triplicate (2300 EtFOSE Alcohol)	20.8	0.022	0.216	0.115	1.735	0.812	0.182	0.447	0.011
Day 28 Biodeg Sample (2300 EtFOSE Alcohol)	27.8	0.026	0.306	0.142	2.019	1.472	0.240	0.774	0.007
Day 28 Biodeg Sample Duplicate (2300 EtFOSE Alcohol)	27.8	0.024	0.325	0.123	1.958	1.419	0.256	0.887	0.010
Day 28 Biodeg Sample Triplicate (2300 EtFOSE Alcohol)	27.8	0.024	0.334	0.146	2.281	1.187	0.243	0.671	0.009
Day 35 Biodeg Sample (2300 EtFOSE Alcohol)	34.8	0.030	0.307	0.154	1.644	2.187	0.232	0.665	0.007
Day 35 Biodeg Sample Duplicate (2300 EtFOSE Alcohol)	34.8	0.027	0.332	0.183	2.712	1.271	0.226	0.481	0.007
Day 35 Biodeg Sample Triplicate (2300 EtFOSE Alcohol)	34.8	0.031	0.339	0.143	2.434	1.392	0.232	0.541	0.005

TABLE 2. The compiled results for the biodegradation samples tested at 47 ng/ml (90 nM) *N*-EtFOSE alcohol over 35 days of incubation. Complete degradation was observed by day 7. All analytical concentrations are presented as nanomolar (nM).

					Camaamin	ation (mM)			
-			1	1	Concentr	ation (nM)			
Sample Description	Incubation time (days)	PFOA	PFOS	PFOS sulfinate	M556	N-EtFOSAA	FOSA	N-EtFOSE alcohol	N-EtFOSA
Day 0 Bide Sample (50 ppb EtFOSE Alcohol)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	75.8	0.0
Day 0 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	69.2	0.0
Day 0 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	65.0	0.0
Day 1 Biodeg Sample (50 ppb EtFOSE Alcohol)	1.0	0.0	0.0	0.0	0.0	26.0	0.0	30.9	0.0
Day 1 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	1.0	0.0	0.0	0.0	0.0	24.2	0.0	30.9	0.0
Day 1 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	1.0	0.0	0.0	0.0	0.0	21.7	0.0	32.2	0.0
Day 3 Biodeg Sample (50 ppb EtFOSE Alcohol)	2.7	0.0	0.0	0.0	12.4	34.2	0.0	12.6	0.0
Day 3 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	2.7	0.0	0.0	0.0	9.1	28.5	0.0	17.2	0.0
Day 3 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	2.7	0.0	0.0	3.6	8.6	27.9	0.0	15.8	0.0
Day 7 Biodeg Sample (50 ppb EtFOSE Alcohol)	6.7	0.0	5.4	0.0	20.7	22.2	9.6	5.2	0.0
Day 7 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	6.7	0.0	5.3	0.0	20.1	25.2	6.4	0.0	0.0
Day 7 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	6.7	0.0	5.1	0.0	22.3	20.5	7.0	0.0	0.0
Day 14 Biodeg Sample (50 ppb EtFOSE Alcohol)	13.8	0.0	10.0	0.0	29.1	7.5	15.7	0.0	0.0
Day 14 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	13.8	0.0	9.2	0.0	28.7	7.0	14.7	0.0	0.0
Day 14 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	13.8	0.0	9.3	0.0	28.3	8.6	14.9	0.0	0.0
Day 21 Biodeg Sample (50 ppb EtFOSE Alcohol)	20.8	0.0	11.2	0.0	27.1	0.0	14.5	0.0	0.0
Day 21 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	20.8	0.0	10.7	0.0	26.4	0.0	14.8	0.0	0.0
Day 21 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	20.8	0.0	9.9	0.0	27.1	0.0	13.7	0.0	0.0
Day 28 Biodeg Sample (50 ppb EtFOSE Alcohol)	27.8	0.0	15.9	0.0	23.4	0.0	17.2	0.0	0.0
Day 28 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	27.8	0.0	15.4	0.0	21.9	0.0	18.5	0.0	0.0
Day 28 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	27.8	0.0	16.3	0.0	27.7	0.0	16.5	0.0	0.0
Day 35 Biodeg Sample (50 ppb EtFOSE Alcohol)	34.8	0.0	20.9	0.0	32.9	0.0	16.8	0.0	0.0
Day 35 Biodeg Sample Duplicate (50 ppb EtFOSE Alcohol)	34.8	0.0	22.9	0.0	32.7	0.0	13.2	0.0	0.0
Day 35 Biodeg Sample Triplicate (50 ppb EtFOSE Alcohol)	34.8	0.0	18.7	0.0	29.2	7.3	15.5	0.0	0.0

Appendix D Control Samples Final Data

TABLE 1. The compiled results of the sterile/inhibited control samples at 2,380 ng/ml (4.168 mM) N-EtFOSE alcohol over 35 days of incubation. No significant abiotic degradation was observed. All concentrations are presented as micromolar (μ M).

					Concentra	ation (μM)			
Sample Description	Incubation time (days)	PFOA	PFOS	PFOS sulfinate	M556	N-EtFOSAA	FOSA	N-EtFOSE alcohol	N-EtFOSA
Day 0 Sterile Sludge Control (2300 EtFOSE Alcohol)	0.0	0.00	0.00	0.00	0.00	0.00	0.00	5.16	0.00
Day 0 Sterile Sludge Control Duplicate (2300 EtFOSE Alcohol)	0.0	0.00	0.00	0.00	0.00	0.00	0.00	4.91	0.00
Day 0 Sterile Sludge Control Triplicate (2300 EtFOSE Alcohol)	0.0	0.00	0.00	0.00	0.00	0.00	0.00	4.88	0.00
Day 1 Sterile Sludge Control (2300 EtFOSE Alcohol)	1.0	0.00	0.00	0.00	0.00	0.00	0.00	5.08	0.00
Day 1 Sterile Sludge Control Duplicate (2300 EtFOSE Alcohol)	1.0	0.00	0.00	0.00	0.00	0.00	0.00	4.92	0.00
Day 1 Sterile Sludge Control Triplicate (2300 EtFOSE Alcohol)	1.0	0.00	0.00	0.00	0.00	0.00	0.00	4.92	0.00
Day 3 Sterile Sludge Control (2300 EtFOSE Alcohol)	2.7	0.00	0.00	0.00	0.00	0.00	0.00	4.83	0.00
Day 3 Sterile Sludge Control Duplicate (2300 EtFOSE Alcohol)	2.7	0.00	0.00	0.00	0.00	0.00	0.00	4.73	0.00
Day 3 Sterile Sludge Control Triplicate (2300 EtFOSE Alcohol)	2.7	0.00	0.00	0.00	0.00	0.00	0.00	4.70	0.00
Day 7 Sterile Sludge Control (2300 EtFOSE Alcohol)	6.7	0.00	0.00	0.00	0.00	0.00	0.00	4.77	0.00
Day 7 Sterile Sludge Control Duplicate (2300 EtFOSE Alcohol)	6.7	0.00	0.00	0.00	0.00	0.00	0.00	5.01	0.00
Day 7 Sterile Sludge Control Triplicate (2300 EtFOSE Alcohol)	6.7	0.00	0.00	0.00	0.00	0.00	0.00	4.86	0.00
Day 14 Sterile Sludge Control (2300 EtFOSE Alcohol)	13.8	0.00	0.00	0.00	0.00	0.01	0.00	4.79	0.00
Day 14 Sterile Sludge Control Duplicate (2300 EtFOSE Alcohol)	13.8	0.00	0.00	0.00	0.00	0.00	0.00	4.78	0.00
Day 14 Sterile Sludge Control Triplicate (2300 EtFOSE Alcohol)	13.8	0.00	0.00	0.00	0.00	0.00	0.00	4.69	0.00
Day 21 Sterile Sludge Control (2300 EtFOSE Alcohol)	20.8	0.00	0.00	0.00	0.00	0.00	0.00	4.74	0.00
Day 21 Sterile Sludge Control Duplicate (2300 EtFOSE Alcohol)	20.8	0.00	0.00	0.00	0.00	0.00	0.00	4.54	0.00
Day 21 Sterile Sludge Control Triplicate (2300 EtFOSE Alcohol)	20.8	0.00	0.00	0.00	0.00	0.00	0.00	4.40	0.00
Day 28 Sterile Sludge Control (2300 EtFOSE Alcohol)	27.8	0.00	0.00	0.01	0.00	0.00	0.00	5.59	0.00
Day 28 Sterile Sludge Control Duplicate (2300 EtFOSE Alcohol)	27.8	0.00	0.00	0.01	0.00	0.00	0.00	5.57	0.00
Day 28 Sterile Sludge Control Triplicate (2300 EtFOSE Alcohol)	27.8	0.00	0.00	0.01	0.00	0.01	0.00	4.62	0.00
Day 35 Sterile Sludge Control (2300 EtFOSE Alcohol)	34.8	0.00	0.00	0.01	0.00	0.00	0.00	5.51	0.00
Day 35 Sterile Sludge Control Duplicate (2300 EtFOSE Alcohol)	34.8	0.00	0.00	0.01	0.00	0.00	0.00	5.56	0.00
Day 35 Sterile Sludge Control Triplicate (2300 EtFOSE Alcohol)	34.8	0.00	0.00	0.03	0.00	0.00	0.00	4.99	0.01

TABLE 2. The compiled results of the no-sludge control samples at 2,380 ng/ml (4.168 mM) N-EtFOSE alcohol over 35 days of incubation. Some degradation due to suspected microbial contamination was observed due to un-sterilized mineral media being used to set up those controls. This suggests that the first reaction is a ubiquitous alcohol dehydrogenase reaction. All concentrations are presented as micromolar (μ M).

					Concentr	ation (µM)			1
	Incubation			PFOS	Concenti	ation (µivi)		N-EtFOSE	
Sample Description	time (days)	PFOA	PFOS	sulfinate	M556	N-EtFOSAA	FOSA	alcohol	N-EtFOSA
Day 0 No Sludge Control (2300 EtFOSE Alcohol)	0.0	0.00	0.00	0.00	0.00	0.00	0.00	4.88	0.00
Day 0 No Sludge Control Duplicate (2300 EtFOSE Alcohol)	0.0	0.00	0.00	0.00	0.00	0.00	0.00	4.95	0.00
Day 0 No Sludge Control Triplicate (2300 EtFOSE Alcohol)	0.0	0.00	0.00	0.00	0.00	0.00	0.00	5.03	0.00
Day 1 No Sludge Control (2300 EtFOSE Alcohol)	1.0	0.00	0.00	0.00	0.00	0.00	0.00	4.99	0.00
Day 1 No Sludge Control Duplicate (2300 EtFOSE Alcohol)	1.0	0.00	0.00	0.00	0.00	0.00	0.00	4.91	0.00
Day 1 No Sludge Control Triplicate (2300 EtFOSE Alcohol)	1.0	0.00	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Day 3 No Sludge Control (2300 EtFOSE Alcohol)	2.7	0.00	0.00	0.00	0.00	0.00	0.00	4.55	0.00
Day 3 No Sludge Control Duplicate (2300 EtFOSE Alcohol)	2.7	0.00	0.00	0.00	0.00	0.00	0.00	4.67	0.00
Day 3 No Sludge Control Triplicate (2300 EtFOSE Alcohol)	2.7	0.00	0.00	0.00	0.00	0.00	0.00	4.67	0.00
Day 7 No Sludge Control (2300 EtFOSE Alcohol)	6.7	0.00	0.00	0.00	0.00	0.00	0.00	4.54	0.00
Day 7 No Sludge Control Duplicate (2300 EtFOSE Alcohol)	6.7	0.00	0.00	0.00	0.00	0.00	0.00	4.69	0.00
Day 7 No Sludge Control Triplicate (2300 EtFOSE Alcohol)	6.7	0.00	0.00	0.00	0.00	0.00	0.00	4.72	0.00
Day 14 No Sludge Control (2300 EtFOSE Alcohol)	13.8	0.00	0.00	0.00	0.00	0.01	0.00	4.31	0.00
Day 14 No Sludge Control Duplicate (2300 EtFOSE Alcohol)	13.8	0.00	0.00	0.00	0.00	0.03	0.00	4.48	0.00
Day 14 No Sludge Control Triplicate (2300 EtFOSE Alcohol)	13.8	0.00	0.00	0.00	0.00	0.01	0.00	4.46	0.00
Day 21 No Sludge Control (2300 EtFOSE Alcohol)	20.8	0.00	0.00	0.00	0.00	0.03	0.00	3.91	0.00
Day 21 No Sludge Control Duplicate (2300 EtFOSE Alcohol)	20.8	0.00	0.00	0.01	0.01	0.02	0.00	4.01	0.00
Day 21 No Sludge Control Triplicate (2300 EtFOSE Alcohol)	20.8	0.00	0.00	0.00	0.00	0.01	0.00	4.35	0.00
Day 28 No Sludge Control (2300 EtFOSE Alcohol)	27.8	0.00	0.00	0.01	0.00	0.06	0.00	3.94	0.00
Day 28 No Sludge Control Duplicate (2300 EtFOSE Alcohol)	27.8	0.00	0.00	0.01	0.00	0.04	0.00	4.26	0.00
Day 28 No Sludge Control Triplicate (2300 EtFOSE Alcohol)	27.8	0.00	0.00	0.02	0.00	0.11	0.00	4.78	0.00
Day 35 No Sludge Control (2300 EtFOSE Alcohol)	34.8	0.00	0.00	0.04	0.00	0.78	0.00	4.27	0.00
Day 35 No Sludge Control Duplicate (2300 EtFOSE Alcohol)		0.00	0.00	0.04	0.00	0.23	0.00	4.11	0.00
Day 35 No Sludge Control Triplicate (2300 EtFOSE Alcohol)	34.8	0.00	0.00	0.04	0.00	0.81	0.00	4.35	0.00

Appendix D, continued

TABLE 3. The compiled results of the sterile/inhibited sludge control samples at 47 ng/ml (90 nM) *N*-EtFOSE alcohol over 35 days of incubation. Degradation was observed in day 21 sterile sludge controls because on day 7 they received fresh sludge by mistake. All concentrations are presented as nanomolar (nM).

presented as nanomolal (nivi).					Concenti	ration (nM)			
Sample Description	Incubation time (days)	PFOA	PFOS	PFOS sulfinate	M556	N-EtFOSAA	FOSA	N-EtFOSE alcohol	N-EtFOSA
Day 0 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	80.4	0.0
Day 0 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	78.2	0.0
Day 0 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	77.0	0.0
Day 1 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	1.0	0.0	0.0	0.0	0.0	0.0	0.0	76.9	0.0
Day 1 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	1.0	0.0	0.0	0.0	0.0	0.0	0.0	77.8	0.0
Day 1 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	1.0	0.0	0.0	0.0	0.0	0.0	0.0	80.4	0.0
Day 3 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	2.7	0.0	0.0	2.9	0.0	0.0	0.0	70.1	0.0
Day 3 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	2.7	0.0	0.0	2.9	0.0	0.0	0.0	67.6	0.0
Day 3 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	2.7	0.0	0.0	0.0	0.0	0.0	0.0	71.9	0.0
Day 7 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	6.7	0.0	0.0	0.0	0.0	0.0	0.0	70.8	0.0
Day 7 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	6.7	0.0	0.0	0.0	0.0	0.0	0.0	70.6	0.0
Day 7 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	6.7	0.0	0.0	0.0	0.0	0.0	0.0	68.9	0.0
Day 14 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	13.8	0.0	0.0	0.0	0.0	0.0	0.0	76.9	0.0
Day 14 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	13.8	0.0	0.0	0.0	0.0	0.0	0.0	68.4	0.0
Day 14 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	13.8	0.0	0.0	0.0	0.0	0.0	0.0	75.6	0.0
Day 21 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	20.8	0.0	0.0	0.0	0.0	0.0	0.0	69.9	0.0
Day 21 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	20.8	0.0	0.0	0.0	0.0	0.0	0.0	68.7	0.0
Day 21 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	20.8	0.0	0.0	0.0	0.0	0.0	0.0	71.2	0.0
Day 28 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	27.8	0.0	0.0	0.0	0.0	0.0	0.0	86.9	0.0
Day 28 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	27.8	0.0	0.0	0.0	0.0	0.0	0.0	84.9	0.0
Day 28 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)		0.0	0.0	0.0	0.0	0.0	0.0	88.0	0.0
Day 35 Sterile Sludge Control Sample (50 ppb EtFOSE Alcohol)	34.8	0.0	0.0	0.0	0.0	0.0	0.0	85.4	0.0
Day 35 Sterile Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	34.8	0.0	0.0	0.0	0.0	0.0	0.0	81.8	0.0
Day 35 Sterile Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	34.8	0.0	0.0	0.0	0.0	0.0	0.0	81.5	0.0

TABLE 4. The compiled results of the no-sludge control samples at 47 ng/ml (90 nM) *N*-EtFOSE alcohol over 35 days of incubation. Some degradation due to suspected microbial contamination was observed due to un-sterilized mineral media being used to set up those controls (shown shaded). This suggests that the first reaction may be catalyzed by a ubiquitous alcohol dehydrogenase reaction. All concentrations are presented as nanomolar (nM).

					0	-41 (84)			
			1		Concentra	ation (nM)			
Sample Description	Incubation time (days)	PFOA	PFOS	PFOS sulfinate	M556	N-EtFOSAA	FOSA	N-EtFOSE alcohol	N-EtFOSA
Day 0 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	0.0	0.000	0.000	0.000	0.000	0.000	0.000	75.797	0.000
Day 0 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	0.0	0.000	0.000	0.000	0.000	0.000	0.000	79.884	0.000
Day 0 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	0.0	0.000	0.000	0.000	0.000	0.000	0.000	77.736	0.000
Day 1 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	1.0	0.000	0.000	0.000	0.000	0.000	0.000	71.322	0.000
Day 1 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	1.0	0.000	0.000	0.000	0.000	0.000	0.000	71.869	0.000
Day 1 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	1.0	0.000	0.000	0.000	0.000	0.000	0.000	71.725	0.000
Day 3 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	2.7	0.000	0.000	0.000	0.000	0.000	0.000	64.028	0.000
Day 3 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	2.7	0.000	0.000	0.000	0.000	0.000	0.000	66.874	0.000
Day 3 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	2.7	0.000	0.000	0.000	0.000	0.000	0.000	64.970	0.000
Day 7 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	6.7	0.000	0.000	0.000	0.000	0.000	0.000	60.718	0.000
Day 7 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	6.7	0.000	0.000	0.000	0.000	0.000	0.000	61.016	0.000
Day 7 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	6.7	0.000	0.000	0.000	0.000	0.000	0.000	64.331	0.000
Day 14 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	13.8	0.000	0.000	0.000	0.000	0.000	0.000	56.389	0.000
Day 14 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	13.8	0.000	0.000	0.000	0.000	0.000	0.000	56.944	0.000
Day 14 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	13.8	0.000	0.000	0.000	0.000	0.000	0.000	57.000	0.000
Day 21 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	20.8	0.000	0.000	0.000	0.000	0.000	0.000	50.068	0.000
Day 21 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	20.8	0.000	0.000	0.000	0.000	0.000	0.000	5.501	0.000
Day 21 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	20.8	0.000	0.000	0.000	0.000	0.000	0.000	39.405	0.000
Day 28 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	27.8	0.000	0.000	0.000	0.000	0.000	0.000	61.838	0.000
Day 28 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	27.8	0.000	0.000	0.000	0.000	0.000	0.000	61.144	0.000
Day 28 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	27.8	0.000	0.000	0.000	0.000	0.000	0.000	53.334	0.000
Day 35 No Sludge Control Sample (50 ppb EtFOSE Alcohol)	34.8	0.000	0.000	0.000	0.000	0.000	0.000	67.445	0.000
Day 35 No Sludge Control Duplicate (50 ppb EtFOSE Alcohol)	34.8	0.000	0.000	0.000	0.000	0.000	0.000	63.636	0.000
Day 35 No Sludge Control Triplicate (50 ppb EtFOSE Alcohol)	34.8	0.000	0.000	0.000	0.000	0.000	0.000	63.476	0.000

Page 53 of 55

Appendix E

Table 1. The percent degradation of *N*-EtFOSE alcohol and percent of products based on molar equivalents of each analyte detected through the study for the 2,380 ng/mL samples. A value of 100% is based on the average value from triplicate samples for the day 0 *N*-EtFOSE alcohol samples. The average value at day 0 was analytically determined to be 4.921 μ M. The averaged percentage of each analyte at day 35 is shown at the bottom. The sum of the analyte percentages at day 35 was equal to 105.6

				% of total	fluorochemicals N	Measured		
Dave Incohetica	DEGA	PFOS	PFOS sulfinate	MESC	A/ E4EOCA A	F00A	N-EtFOSE	AL EAEOGA
Days Incubation	PFOA	_		M556	N-EtFOSAA	FOSA	alcohol	N-EtFOSA
0.0	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	103.8%	0.0%
0.0	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	99.5%	0.0%
0.0	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	96.7%	0.0%
1.1	0.0%	0.2%	1.9%	1.0%	13.2%	0.3%	63.0%	0.4%
1.1	0.0%	0.2%	1.9%	1.0%	12.9%	0.3%	64.2%	0.4%
1.1	0.0%	0.2%	2.0%	1.1%	13.4%	0.3%	63.4%	0.4%
2.7	0.0%	0.6%	2.6%	4.5%	21.8%	1.0%	44.8%	0.6%
2.7	0.0%	0.5%	2.6%	4.3%	21.5%	0.9%	47.4%	0.6%
2.7	0.0%	0.7%	2.8%	4.6%	23.5%	1.0%	41.3%	0.6%
6.7	0.2%	1.9%	2.5%	15.8%	23.1%	2.5%	29.1%	0.6%
6.7	0.2%	1.7%	2.5%	15.4%	21.7%	2.4%	29.8%	0.6%
6.7	0.2%	1.7%	2.7%	15.0%	24.1%	2.4%	30.1%	0.6%
13.7	0.4%	3.5%	2.4%	26.1%	19.8%	3.3%	15.6%	0.4%
13.7	0.4%	3.5%	2.3%	28.2%	16.8%	3.4%	14.9%	0.4%
13.7	0.4%	3.5%	2.3%	29.4%	16.9%	3.5%	15.1%	0.4%
20.7	0.5%	4.5%	1.8%	23.7%	25.2%	3.8%	11.7%	0.3%
20.7	0.5%	4.2%	2.1%	32.4%	21.5%	3.7%	8.1%	0.2%
20.7	0.5%	4.4%	2.3%	35.3%	16.5%	3.7%	9.1%	0.2%
27.8	0.5%	6.2%	2.9%	41.0%	29.9%	4.9%	15.7%	0.2%
27.8	0.5%	6.6%	2.5%	39.8%	28.8%	5.2%	18.0%	0.2%
27.8	0.5%	6.8%	3.0%	46.4%	24.1%	4.9%	13.6%	0.2%
34.8	0.6%	6.2%	3.1%	33.4%	44.4%	4.7%	13.5%	0.2%
34.8	0.5%	6.8%	3.7%	55.1%	25.8%	4.6%	9.8%	0.1%
34.8	0.6%	6.9%	2.9%	49.5%	28.3%	4.7%	11.0%	0.1%
Average value at day 35	0.6%	6.6%	3.3%	46.0%	32.9%	4.7%	11.4%	0.1%
Standard Deviation	0.0%	0.3%	0.4%	11.3%	10.1%	0.1%	1.9%	0.0%
RSD (%)	7.5%	5.1%	13.1%	24.5%	30.8%	1.4%	16.7%	16.6%
Percent of products at day 35	0.6%	7.0%	3.5%	48.9%	34.9%	5.0%		0.1%

Appendix F

Table 1. Mass Balance data for all biodeg samples, sterile control sample, and no-sludge control samples. Note that for the day 21 no-sludge controls there was only one good value due to a sample preparation error. The % mass balance shown was calculated using expected concentration of 4.168 μ M.

			ass balance	A11 '	0	B' - d	4.168 μM ma		L. IAU
		e Sterile controls	no-sludge controls	All samples			Sterile controls	<u>10-sludge contro</u>	
Day 0	90.9%	-	-	90.9%	Day 0	122.6%	-	-	122.69
Day 0	83.0%	-	-	83.0%	Day 0	117.4%	-		117.49
Day 0	78.0%	-	-	78.0%	Day 0	114.2%	-		114.29
Day 0	-	96.4%	-	96.4%	Day 0	-	123.8%	-	123.89
Day 0	-	93.7%	-	93.7%	Day 0	-	117.8%	-	117.89
Day 0		92.3%	-	92.3%	Day 0		117.0%	-	117.09
Day 0	-	-	90.9%	90.9%	Day 0	-	-	117.2%	117.29
Day 0	-	-	95.8%	95.8%	Day 0	-	-	118.7%	118.79
Day 0		-	93.2%	93.2%	Day 0		-	120.7%	120.79
Day 1	68.2%	-	-	68.2%	Day 1	94.6%	-	-	94.6%
Day 1	66.1%	-	-	66.1%	Day 1	95.6%	-		95.6%
Day 1	64.6%	-	-	64.6%	Day 1	95.4%	-	-	95.4%
Day 1	-	92.2%	-	92.2%	Day 1		121.9%	-	121.99
Day 1	-	93.3%	-	93.3%	Day 1		118.0%	-	118.09
Day 1	-	96.4%	-	96.4%	Day 1	-	118.0%	-	118.09
Day 1	-	-	85.5%	85.5%	Day 1	-	-	119.8%	119.89
Day 1	-	-	86.2%	86.2%	Day 1	-	-	117.7%	117.79
Day 1		-	86.0%	86.0%	Day 1		-	114.2%	114.29
Day 3	71.0%	-	-	71.0%	Day 3	89.6%	-	-	89.6%
Day 3	65.8%	-	-	65.8%	Day 3	91.8%	-	-	91.8%
Day 3	67.0%	-	-	67.0%	Day 3	88.0%	-	-	88.0%
Day 3	-	87.5%	-	87.5%	Day 3	-	115.9%	-	115.99
Day 3	-	84.5%	-	84.5%	Day 3	-	113.6%	-	113.6
Day 3	-	86.2%	-	86.2%	Day 3	-	112.7%	-	112.7
Day 3	-	-	76.8%	76.8%	Day 3	-	-	109.1%	109.1
Day 3	-	-	80.2%	80.2%	Day 3	-	-	112.1%	112.19
Day 7	-	-	77.9%	77.9%	Day 7	-	-	112.0%	112.0
Day 7	75.6%	-	-	75.6%	Day 7	89.5%	-	-	89.59
Day 7	68.4%	-	-	68.4%	Day 7	87.7%	-	-	87.79
Day 7	65.9%	-	-	65.9%	Day 7	90.6%	-	-	90.6%
Day 7	-	84.9%	-	84.9%	Day 7	-	114.5%	-	114.5
Day 7	-	84.6%	-	84.6%	Day 7	-	120.2%	-	120.2
Day 7	-	82.7%	-	82.7%	Day 7	-	116.7%	-	116.7
Day 7	-	-	72.8%	72.8%	Day 7	-	-	108.9%	108.9
Day 7	-	-	73.2%	73.2%	Day 7	-	-	112.6%	112.6
Day 7	-	-	77.1%	77.1%	Day 7	-	-	113.2%	113.2
ay 14	74.8%	-	•	74.8%	Day 14	84.5%	-	-	84.59
ay 14	71.4%	-	-	71.4%	Day 14	82.5%	-	-	82.59
ay 14	73.2%	-	-	73.2%	Day 14	84.5%	-	-	84.59
ay 14	-	92.2%	-	92.2%	Day 14	-	115.1%	-	115.1
ay 14	-	82.0%	•	82.0%	Day 14	-	114.7%	-	114.7
ay 14	-	90.7%	-	90.7%	Day 14	-	112.6%	-	112.6
ay 14	-	-	67.6%	67.6%	Day 14	-	-	103.7%	103.7
ay 14	-	-	68.3%	68.3%	Day 14	-	-	108.2%	108.2
ay 14	-	-	67.5%	67.5%	Day 14	-	-	107.2%	107.2
ay 21	63.3%	-	-	63.3%	Day 21	84.3%	-	-	84.39
ay 21	62.2%	-	-	62.2%	Day 21	85.9%	-	-	85.99
ay 21	60.8%	-	-	60.8%	Day 21	85.0%	-	-	85.09
ay 21	-	83.8%		83.8%	Day 21	-	113.9%	-	113.9
ay 21	-	82.4%	-	82.4%	Day 21	-	109.0%	-	109.0
ay 21	-	85.4%	-	85.4%	Day 21	-	105.6%	-	105.6
ay 21	-	-	60.0%	60.0%	Day 21	-	-	94.5%	94.59
ay 28	67.7%		-	67.7%	Day 28	-	-	97.4%	97.49
ay 28	66.9%	-	-	66.9%	Day 28	-	-	104.6%	104.6
ay 28	72.5%	-	-	72.5%	Day 28	119.6%	-	-	119.6
ay 28	-	-	-	104.2%	Day 28	120.0%	-	-	120.0
ay 28	-		-	101.8%	Day 28	117.4%	-	-	117.4
ay 28	-	-	-	105.5%	Day 28	-	134.2%	-	134.2
ay 28	-	-	-	74.1%	Day 28	-	133.8%	-	133.8
ay 28	-	-	-	73.3%	Day 28	-	111.3%	-	111.3
ay 28	-		-	63.9%	Day 28	-		96.3%	96.39
ay 35	84.7%	-	-	84.7%	Day 35	-	-	103.4%	103.4
ay 35	82.5%	-	-	82.5%	Day 35	-	-	117.7%	117.7
ay 35	84.8%	-	-	84.8%	Day 35	125.4%	-	-	125.4
ay 35		102.4%	-	102.4%	Day 35	125.7%	-	-	125.7
ay 35		98.1%	-	98.1%	Day 35	122.8%	-	-	122.8
ay 35	-	97.7%	-	97.7%	Day 35	-	132.3%	-	132.3
ay 35	-	-	80.9%	80.9%	Day 35	-	133.6%	-	133.6
ay 35	-	-	76.3%	76.3%	Day 35	-	120.6%	-	120.6
ay 35	-	-	76.1%	76.1%	Day 35	-		122.2%	122.2
ay 35	-	-	-	-	Day 35	-	-	105.1%	105.1
ay 35	-	 -	-		Day 35	-	- 1	124.5%	124.5
erage=	72.0%	90.0%	78.5%	80.5%	Average=	100.6%	118.6%	110.9%	110.0
d Dev=	8.2%	6.1%	9.4%	11.8%	Std Dev=	16.3%	7.9%	8.3%	13.59
	11.3%	6.8%	12.0%	14.6%	RSD =	16.2%	6.6%	7.5%	12.39

APPENDIX A

SIGNATURES OF PROJECT PERSONNEL

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

Client Project ID: LIMS E00-2252

Contract Analytical Project Number: CA058

The following individuals participated in the conduct of this project:

Study Director:	1	//
Cleston C. Lange	Signature	Date
Laboratory Management:		
Mark T. McCann	Signature	Date
Report Author:		11
Cleston C. Lange	Signature	
Sample Preparation Analyst:	00 000	
Angela L. Schuler	Signature	Date
HPLC/MS Analysis Conducted by:	140	,
Anthony E. Scales	Signature	// 1/00 Date
Final Report Reviewed by:	~ -1.11	
Dirk W. Hoogenboom	Signature	Ol Mov Zoll Date

Page 38 of 55

Original