

BIODEGRADATION STUDY REPORT

**The 18-Day Aerobic Biodegradation Study of
Perfluorooctanesulfonyl-Based Chemistries**

PROJECT NUMBER

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1.0 Project Personnel

1.1	Sponsor	3M Company
1.2	Sponsor Representative	Dr. James K. Lundberg
1.3	Contract Facility Personnel:	
1.3.1	Study Director	Dr. Cleston C. Lange
1.3.2	Laboratory Management:	Mr. Gabe J. Lebrun
1.3.3	Sample Preparation Analysts	Ms. Angela L. Schuler
1.2.4	HPLC/MS Analyst	Mr. Anthony E. Scales
1.2.5	Sludge Characterization Report	Mr. Daryl K. Peterson
1.2.6	Sample Custodian:	Dr. Cleston C. Lange
1.2.7	Quality Assurance Manager	Mr. Dirk Hoogenboom
1.2.8	Report Author	Dr. Cleston C. Lange

2.0 Data Requirements

Conversations between the study director and the sponsor representative, concerning details of the biodegradation study for *N*-EtFOSE alcohol (Project CA058), were held prior to September 5, 2000. Those conversations resulted in a decision that a two sample-point biodegradation study, approximately three weeks duration, should be conducted for each of the perfluorooctanesulfonyl-based chemistries targeted in that study to confirm the degradation pathways proposed in the CA058 final report ¹. The approval to proceed with the study was granted via a documented phone conversation held on September 5, 2000 between the study director and sponsor representative. The samples for this study were subsequently prepared, incubated for eighteen days, solid phase extracted (SPE), and the SPE eluates analyzed by quantitative LC/MS. This study was not conducted under the GLP regulations.

3.0 Project Objective

The objective of the study was to determine the products generated for each of the targeted perfluorooctanesulfonyl-based chemistries when incubated as test substrates with aerobic municipal wastewater treatment plant sludge. The substrates tested were

the perfluorooctanesulfonyl products of *N*-EtFOSE alcohol biodegradation, detected during the course of project CA058. In this study, the perfluorooctanesulfonyl-based substrates were individually incubated with aerobic sludge for eighteen days at a concentration of approximately 2.5 µg/mL. The results from this study helped to establish the possible environmental fate of the following test materials: *N*-EtFOSE alcohol, *N*-EtFOSAA, *N*-EtFOSA, M556, FOSA, PFOSulfinate, PFOS and PFOA. The proposed aerobic degradation pathway from this study supports the stepwise biodegradation of *N*-EtFOSE alcohol proposed in the Study CA058 report. Several of the tested substrates presented special difficulties that may necessitate further testing.

4.0 Test and Reference Articles

All test and reference articles for this study were received from the 3M Environmental Laboratory on June 8, 2000. Approximately 1 gram of each of the neat materials, except M556, was received by the study director at Pace Science Solutions (PSS), Contract Analytical Group (CAG) Laboratory, with an accompanying chain of custody (COC # 14681). Pertinent material and safety data sheets (MSDS) and the available purity information were also included for those materials. M556 was provided without a COC, MSDS or purity information. Upon receipt at PSS, each substance was given a Pace test, control, & reference (TCR) number.

All test and reference articles were stored at $-80^{\circ}\text{C} \pm 15.0^{\circ}\text{C}$ during the study. Because the purity of the test and reference materials had not been adequately established, all final data in data tables for this report were calculated assuming a purity of 100%. A lack of purity information did not negatively impact the study.

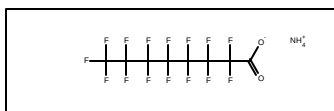
Test & reference articles are described below with their corresponding percent purity estimation, 3M identification number, and the Pace identification number.

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

5.1. Perfluorooctanoate Ammonium Salt (PFOA)

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

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

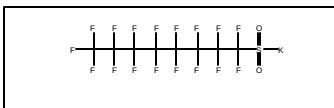


5.2. Perfluorooctanesulfinate Potassium Salt (PFOSulfinate)

Purity: **NC**

3M#: SD-007

Pace #: CA-TCR02-002

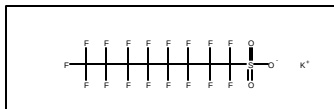


5.3. Perfluorooctanesulfonate Potassium Salt (PFOS)

Purity: **86.4%**

3M#: SD-009

Pace #: CA-TCR02-003

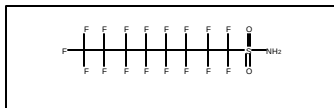


5.4. Perfluorooctanesulfonamide (FOSA)

Purity: **NC**

3M#: SD-029

Pace #: CA-TCR02-004

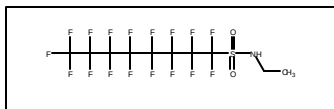


5.5. N-Ethyl perfluorooctanesulfonamide (N-EtFOSA)

Purity: **NC**

3M#: SD-012

Pace #: CA-TCR02-005

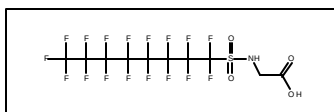


5.6. [2-(Perfluorooctanesulfonamido) acetic acid] (M556)

Purity: **NC**

3M#: 00001-5-24

Pace#: CA-TCR02-006



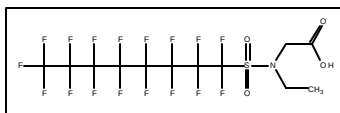
5.7. [2-(N-Ethyl-perfluorooctanesulfonamido) acetic acid]

(N-EtFOSAA) and was provided labeled as FC-129

Purity: **NC**

3M#: SE-038

Pace #: CA-TCR02-007

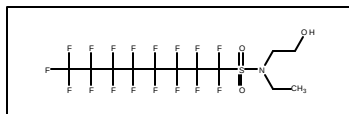


5.8. [2-(N-Ethyl-perfluorooctanesulfonamido) ethyl alcohol] (N-EtFOSE alcohol)

Purity: **99.9%**

3M#: SE-035

Pace #: CA-TCR02-008



5.0 Receipt/Generation of Samples

All samples for this study were generated at Pace. Sixty-eight test cultures and control cultures were prepared for this 18-day, two-point study. All sixty-eight cultures were extracted by solid phase extraction (SPE) to generate 204 analytical samples referred to as SPE eluates 1, 2 and 3 for each culture. Of the three sample eluates, only eluates 2 and 3 were analyzed by quantitative LC/MS. The data obtained previously from project CA058 ¹ provided ample evidence to suggest that mass balance was obtained by analysis of only eluates 2 and 3. It is assumed that no analytes were contained in the aqueous eluate 1.

6.0 Methods

6.1 Sample Preparation

6.1.1. Collection of Sludge.

To prepare test cultures, sludge was obtained from the primary municipal waste treatment facility for the Twin Cities metropolitan area.

Arrangements were made for Pace personnel to retrieve fresh mixed liquor suspended solids (MLSS) from the aeration units at the Twin Cities Metro Wastewater Treatment Facility (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 bottles of MLSS with a corresponding chain of custody (Pace C.O.C. # 465254). Upon receipt, each bottle was labeled bottles #1 through #6, and stored at 4°C. Bottle #1 was immediately 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.

The MLSS used for this study was from bottle #3 and had been stored at 4°C for approximately 5 weeks prior to being used for this study. The settled sludge constituted approximately 20% of the volume, or approximately 200 mL of settled sludge from a 1-liter bottle, based on visual inspection.

6.1.2. Culture Preparation.

All cultures were prepared on September 8, 2000 and the final sample collection occurred on September 26, 2000. This study was set up using slight modifications to the original proposed methods included with Pace proposal 000511.1. Changes were made with regard to culture volume

(changed from 20 mL to 25 mL), types of controls prepared, number of concentrations tested, and the number of sample points employed.

All substrates for this study were tested for biodegradability at a final concentration of approximately 2.5 µg/mL (part per million; ppm). The culture preparation procedure described below was documented as standard operating procedure (SOP) CAG-SP-03³.

Sample cultures were prepared using a mineral salts medium defined in EPA Guideline OPPTS 835.3200. The medium pH was 7.4 and contained per liter, 0.334 g Na₂HPO₄-2H₂O, 0.005 g NH₄Cl, 0.2175 g K₂HPO₄, and 0.085 g KH₂PO₄, 0.0275 g CaCl₂-anhydrous, 0.0225 g MgSO₄-7H₂O, and 0.00025 g FeCl₃-6H₂O. Per one liter of mineral salts medium prepared, 1 mL of methanol was added. The mineral salts medium containing sludge was prepared fresh on September 7, 2000 by adding 50 mL of settled sludge to 950 mL of mineral salts medium (reagent ID CA-R00-100). This mineral medium plus sludge solution was then used to prepare test cultures. Mineral salts medium without sludge was prepared on August 10, 2000 and was used for the preparation of the no-sludge abiotic controls (reagent ID CA-R00-085).

Cultures were prepared by transferring 25 mL of appropriate culture solution into labeled, sterile 125 mL polycarbonate Nalgene culture flasks. Appropriate amounts of the test substrate stock solutions were added to culture flasks containing culture solution. Blank controls did not receive test substrate.

In total 68 cultures were prepared, and included duplicate sets of test cultures, control cultures, and appropriate blanks for day zero sampling and day 18 sampling for each analyte.

The targeted final concentration of the test substrate in each culture was 2.5 µg/mL, or approximately 5 µM. The actual tested concentrations for each were as follows: the *N*-EtFOSE alcohol was tested at 2.856 µg/mL (5.002 µM), and prepared by adding 6 µL of a 11,900 µg/mL stock

solution CA058-SS0001 to a 25 mL culture solution; *N*-EtFOSAA was tested at 2.539 µg/mL (4.341 µM), prepared by adding 6 µL of a 10,580 µg/mL stock solution CA058-SS-009 to a 25 mL culture; the M556 was tested at 2.595 µg/mL (4.659 µM), prepared by adding 65 µL of a 998 µg/mL stock solution CA-TCR02-006 to a 25 mL culture; *N*-EtFOSA was tested at 2.825 µg/mL (5.360 µM), prepared by adding 6 µL of a 11,770 µg/mL stock solution CA058-SS-008 to a 25 mL culture; FOSA was tested at 2.566 µg/mL (5.141 µM), prepared by adding 6 µL of a 10,690 µg/mL stock solution CA058-SS-007 to a 25 mL culture; PFOS was tested at 2.455 µg/mL (4.564 µM), prepared by adding 6 µL of a 10,230 µg/mL stock solution CA058-SS-002 to a 25 mL culture; PFOSulfinate was tested at 2.647 µg/mL (5.071 µM), prepared by adding 6 µL of a 11,030 µg/mL stock solution CA058-SS-006 to a 25 mL culture; PFOA was tested at 2.645 µg/mL (6.136 µM), prepared by adding 6 µL of a 11,020 µg/mL stock solution CA058-SS-003 to a 25 mL culture.

The day zero samples were immediately placed in a freezer and maintained at –20°C until final culture preparation. All of the other test cultures and control cultures were placed in a temperature controlled shaking incubator maintained at 25°C ± 3°C, and shaken at 200 rpm for aeration. Samples were incubated for 18 days. After 18 days, the cultures were removed from the incubator and frozen until final sample preparation by SPE.

All culture preparation information, including times, analyte additions, etc. were recorded into sample preparation worksheets and signed and dated by the sample preparation analyst. All original data sheets and exact copies of incubator and freezer logs were kept in a project specific binder and labeled as project CA097.

6.1.3 Solid Phase Extraction of Cultures

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 a plug of quartz glass wool to deter plugging of the SPE cartridge 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. The wash solution eluates were discarded to waste. All of the SPE eluates for samples were collected in clear I-chem vials with labels that identified them as eluate 1, 2 or 3, as defined below.

Samples that had been frozen were thawed at room temperature before extraction. Following the thawing, and prior to the 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 the sample contents drawn by vacuum through the appropriately labeled 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 $4^{\circ}\text{C} \pm 3^{\circ}\text{C}$. All data was recorded on appropriated culture preparation worksheets. The SPE procedure was documented as method CAG-SP-04².

6.2 Instrumental Analysis

Quantitative LC/MS analysis was conducted on an HP1100 high performance liquid chromatography with mass spectrometer detector (HPLC/MSD) system. The HPLC/MSD was set up with two columns (4.6 x 150 mm Betasil C8 column and a 4 x 35 mm NG1 guard column) and a pressure relief valve (Alltech Part No. 39025) that served as a flow through splitter. This operating method used was documented as method CAG-ORG-23⁴. The MSD was operated in electrospray ionization-negative mode with selected ion monitoring (SIM). Ions monitored included: *m/z* 413 (PFOA); *m/z* 483 (PFOSulfinate); *m/z* 498 (FOSA); *m/z* 499 (PFOS); *m/z* 526 (N-EtFOSA); *m/z* 556 (M556); *m/z* 584 (N-EtFOSAA); and *m/z* 630 (N-EtFOSE Alcohol-acetate adduct). All analysis was quantitative, with 8 calibration standards prepared spanning approximately 5 to 1000 ng/mL (ppb) and containing each of the target analytes. Typical injection volumes for samples and calibration standards were 50 µL, except were otherwise noted for instrumental dilutions in section 7.5 below.

MSD Settings:

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

HPLC Settings:

Flow: 1 mL/min, splitter used, near 10:1 split

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

6.3 Data Transformations and Calculations

6.3.1 Molar Calculations:

Because all data was reported from the analysis as ng/mL (part per billion, ppb), the transformation of ng/mL to molar concentrations had to be conducted to obtain mass balance information. The mole conversion values used for each of the analytes were as follows:

PFOA-NH₄⁺ salt = 431 nanogram (ng) per nanomole (nmole)

PFOSulfinate-K⁺ salt = 522 ng/nmole

PFOS-K⁺ salt = 538 ng/nmole

FOSA = 499 ng/nmole

N-EtFOSA = 527 ng/nmole

M556 acid = 557 ng/nmole

N-EtFOSE acid = 585 ng/nmole

N-EtFOSE alcohol = 571 ng/nmole

6.3.2 Conversion of ng/mL to micromolar (μM).

(Working Examples):

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

50 ng/mL PFOS- K⁺ = (50 ng/mL) * (0.1 nmole/522 ng) = 0.0958 nmole/mL = 0.0958 μmole/L = 0.0958 μM = 95.8 nM

6.3.3 Mass Balance Calculations:

All ng/mL values were converted to their corresponding micromolar concentrations (μM) (see section 7.3.2, above). Then, the sum of the quantified molar values (μM) for all analytes in eluate 2 and eluate 3 were calculated for each culture. The sum value was then divided by the known test substrate concentration added, and that end value reported as a percentage of the known starting concentration.

(Working Example):

Starting EtFOSE-OH was at 1800 μM

After incubation, the following were determined:

PFOA detected at 50 μM

PFOS detected at 100 μM

N-ETFOSE ACID detected at 500 μM

EtFOSE-OH detected at 1000 μM

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

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

6.4 Software Versions

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

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

ChemSketch v. 4.55 was used for preparation of chemical drawings & figures

HP Chemstation for HPLC/MSD Rev. A.07.01 was used for analysis.

7.0 Results

7.1 Incubator, Refrigerator, and Freezer Temperature Log Data.

During this study, one reciprocal shaker incubator was used. The Pace ID number for the incubator-shaker was 0270. The Shaker Speed on the incubator was set to 200 rpm, but was not regularly verified.

One -20 °C freezer, a Norlake freezer, was used during the study for the storage of cultures until they could be prepared by solid phase extraction. The freezer ID was 0050 and was stored at location SL-R6 at Pace.

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

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

The following average temperatures recorded $\pm$ their standard deviation for each during the timeframe of the study are shown below. The average temperature and standard deviation are based on the current reading, low reading, and high reading recorded in the instrument logs each day for the study, excluding weekends and holidays.

Incubator-shaker, ID 0270, from dates 9/8/00 to 10/6/00: **25.8°C $\pm$ 0.9°C**

Norlake Freezer, ID 0050, from dates 9/8/00 to 10/6/00: **-16.2°C $\pm$ 4.5° C**

IsoTemp Freezer, ID 0241, from dates 9/8/00 to 10/6/00: **-80.5°C $\pm$ 3.6°C**

Carroll walk-in cooler, ID 0140, from dates 9/8/00 to 10/6/00: **3.7°C $\pm$ 1.7°C**

True refrigerator, ID 0213, from dates 9/8/00 to 10/6/00: **3.9°C $\pm$ 1.1°C**

7.2 Mishaps recorded during the Study.

Originally, the study was intended for 21 days of incubation. However, for convenience the samples were harvested after 18 days of incubation. Hence, samples and controls labeled for 21-days of incubation were actually 18-day incubated samples.

7.3 Mixed Liquor Suspended Solids Characterization Report

The results of the chemical analysis of the mixed liquor suspended solids (MLSS) used for this study were as follows: The date of collection of the MLSS was August 1, 2000 and the digestion date was August 3, 2000. The Pace project number for the MLSS characterization report was 1035161. The pH, 5-Day BOD, and total sulfur content of the sludge were not determined, however, for future studies it is recommended that these parameters be measured.

7.3.1 Metals:

Boron:	600 µg/L
Cadmium:	12.9 µg/L
Calcium:	124,000 µg/L
Chromium:	273 µg/L
Cobalt:	15.6 µg/L
Copper:	1,450 µg/L
Iron:	31,000 µg/L
Lead:	140 µg/L
Magnesium:	29,700 µg/L
Manganese:	12,200 µg/L
Molybdenum:	60 µg/L
Nickel:	123 µg/L
Selenium:	28 µg/L
Potassium:	30,500 µg/L
Zinc:	1,320 µg/L

7.3.2. Wet Chemistry:

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
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

7.4 Quality Control/Sample Matrix Spike Results.

Analyte recoveries from sludge-containing sample matrices by the methods employed for this study have been determined twice previously for several analyte concentrations, and in both instances recoveries were near 100%, with some exceptions for M556. Those recoveries were described in the two reports submitted to 3M concerning project CA058. Analyte recoveries from similar controls was deemed unnecessary for this study.

7.5 HPLC/MS Calibration and Calibration Verification.

The LC/MS calibration standards were multi-component and contained PFOA, PFOSulfinate, PFOS, FOSA, *N*-EtFOSA, M556, *N*-EtFOSE acid and *N*-EtFOSE alcohol. Eight calibration standards were prepared in methanol at approximately 5, 10, 20, 40, 100, 200, 500 and 1000 ng/mL, and were labeled as CA058-MW-014 to CA058-MW-021. The exact concentration values for these standards were used for quantitation of the analytes in the samples. Aliquots of calibration standards (prepared as 10 mL solutions) were transferred to autovials and capped for use in LC/MS runs. Continuing calibration verification (CCV) was included in sequence runs. The CCVs used were aliquots of standards CA058-MW-016 (20 ng/mL) and CA058-MW-019 (200 ng/mL). The CCV results were used for checking both low and high range curve data integrity and reliability of reported concentrations during a sequence run.

Instrument calibration was performed for each analyte with 8 calibration standards injected. Compounds 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). Values below 100 ng/mL were reported based on the values reported from low calibration curve data, and all residuals in that range were 100% $\pm$ 25%. Values reported between 100 and 1000 ng/mL were from high calibration curve data, and again residuals on values in that range were 100% $\pm$ 25%. Concentrations reported above 1000 ng/mL were determined from instrument runs in which 10 μ L of sample was injected and quantified off of calibration curves generated from 50 μ L of calibration standards being injected. Data from instrumental dilutions were obtained using high range calibrations, with residuals

of 100% $\pm$ 25%. The instrumental dilutions resulted in a dilution factor of 5 being used to back calculate the true analytical concentration in the sample.

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 averaged calibration curves flanking 30 samples, one set of CCVs, and the pertinent methanol blanks. All calibration curves were slightly quadratic and had R-values of at least 0.990.

All data was of good to excellent quality, with residuals on curves and CCVs in the acceptable range of 100% $\pm$ 25%. Data was collected from external standard calibration, and not internal standard as suggested by ETS-8-136.0.

7.6 Blanks

Methanol blanks were employed during the HPLC/MS analysis and consisted of pure methanol injected onto the HPLC/MS columns for analysis. Methanol blanks were analyzed to determine the background level of analytes in the methanol solvent used during sample preparation. The results of methanol blank injections verified that there were no detectable target analytes in the methanol used for this study.

Sample blanks, consisting of mineral medium containing sludge and no analyte, were incubated and prepared the same as regular samples. Results obtained were used to determine whether the sample matrix previously contained any of the analytes of interest. Analysis of the sample blanks verified that there were no detectable fluorochemical analytes of interest in sample blanks during this study.

7.7 Sample Results

The term biodegradation can be used to describe biotransformation or biomineralization (complete breakdown to inorganic components). The biodegradation described below may be more appropriately termed

biotransformation. Biodegradability was arbitrarily classified depending on how much test substrate remained after 18 days incubation, and were as follows: 1) Biodegraded well ($\leq 25\%$ parent remaining); 2) Moderately biodegraded (25-75% of parent remaining); 3) Poorly biodegraded (75-95% parent remaining); and 4) Not biodegraded (95-110% of parent remaining).

7.7.1 *N*-EtFOSE Alcohol

The *N*-EtFOSE alcohol was biodegraded well in the 18-day incubation experiments (**Figure 1**) with only 15.9% of the original *N*-EtFOSE alcohol remaining after 18 days incubation with sludge (**Appendix D, Table 1**). The biodegradation resulted in *N*-EtFOSAA as the major biotransformation product. Minor products were M556, *N*-EtFOSA, FOSA, PFOS, PFOSulfinate and PFOA. The results were in good agreement with those obtained from previous biodegradation experiments (see separate reports for project CA058). The *N*-EtFOSE alcohol cultures served as a positive control for this study and supported the fact that the biological activity of the MLSS used for this study was sufficient for biodegradation to occur. Mass balance for *N*-EtFOSE alcohol cultures ranged from 88% to 107%.

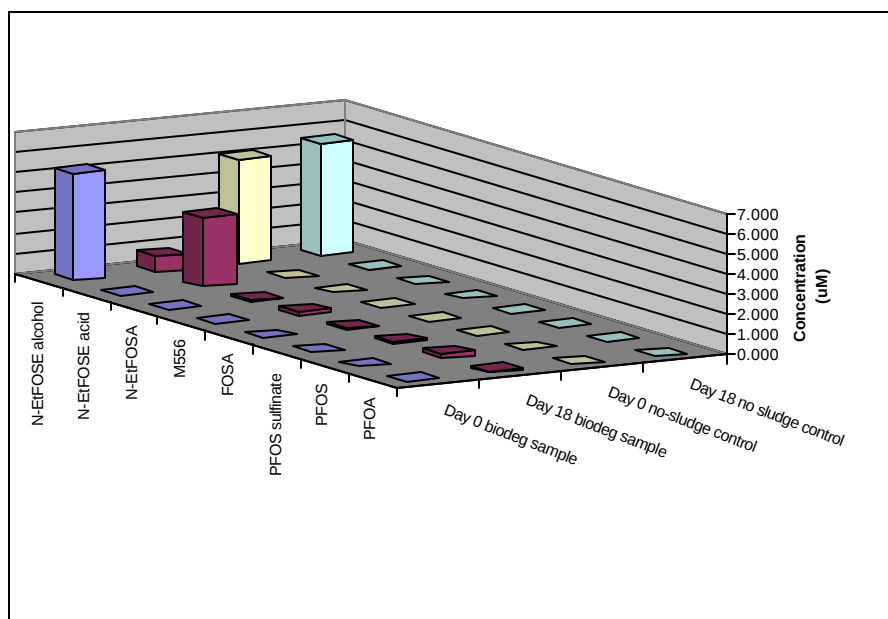


Figure 1. The biodegradation of *N*-EtFOSE alcohol, showing *N*-EtFOSE alcohol biodegraded well to yield *N*-EtFOSAA, M556 and PFOS.

7.7.2 N-EtFOSAA

The *N*-EtFOSAA was poorly degraded in 18-day incubation experiments (**Figure 2**). This was an unexpected result, since degradation of *N*-EtFOSAA had been observed in study CA058, and this compound was expected to be a good substrate. Only 9.3 % of the *N*-EtFOSAA was degraded, and yielded product M556, FOSA, PFOS and PFOA (see Appendix D, Table 1 for final results). Some of the observed products may be attributed to degradation of the impurities present in the test material, which included: *N*-EtFOSA, PFOSulfinate, and PFOA. Both *N*-EtFOSA and PFOSulfinate were present at day 0 but could not be detected by day 18, and could account for some, but not all, of the products observed. The low level of biodegradability detected could be explained if *N*-EtFOSAA is toxic to microorganisms in sludge. Alternatively, the lack of biodegradation may have been due to a reduced bioavailability of the substrate caused by a higher molecular polarity of *N*-EtFOSAA (an acid), which would result in lowered membrane permeability. Another possible explanation for the observed poor biodegradability may have been that the substrate was unable to induce the appropriate genes and enzymes required for biodegradation to occur.

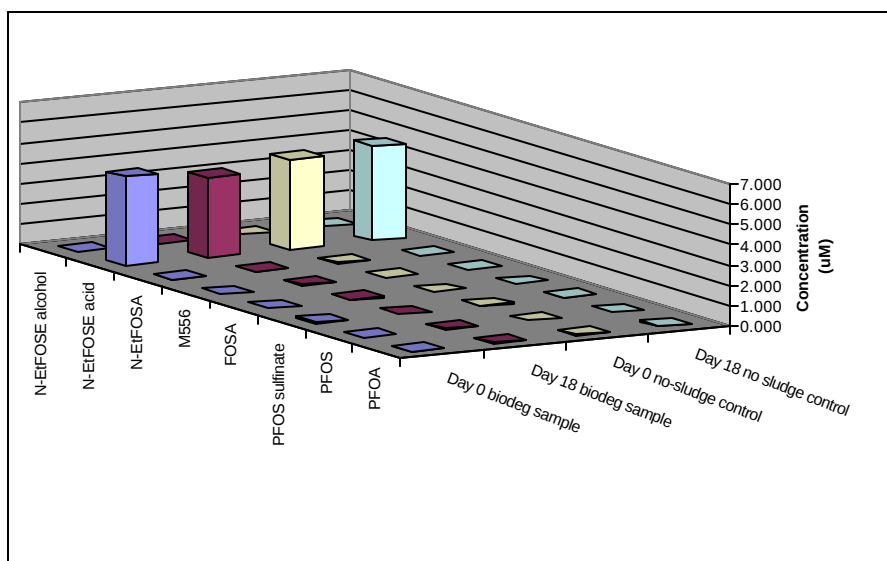


Figure 2. The biodegradation chart for *N*-EtFOSAA, showing poor biodegradability and low levels of products formed.

7.7.3 *N*-EtFOSA

After the 18-day incubation, 13.7% of the parent *N*-EtFOSA substrate remained, and the biotransformation products M556, FOSA, PFOSulfinate, PFOS and PFOA were formed (**Figure 3**). The *N*-EtFOSA was termed moderately biodegraded because only 31% of the original *N*-EtFOSA added to the no-sludge controls remained after 18 days of incubation, and no biotransformation products were detected, see **Appendix D, Table 1**. After 18 days, mass balance for *N*-EtFOSA was the poorest of all analytes tested, at approximately 30-35% recovery. These results may indicate loss of the test material by volatilization to the air, since the vapor pressure of *N*-EtFOSA, while currently unknown, may be significant, and cultures were incubated with test flasks loosely capped to allow for the exchange of air into the flasks.

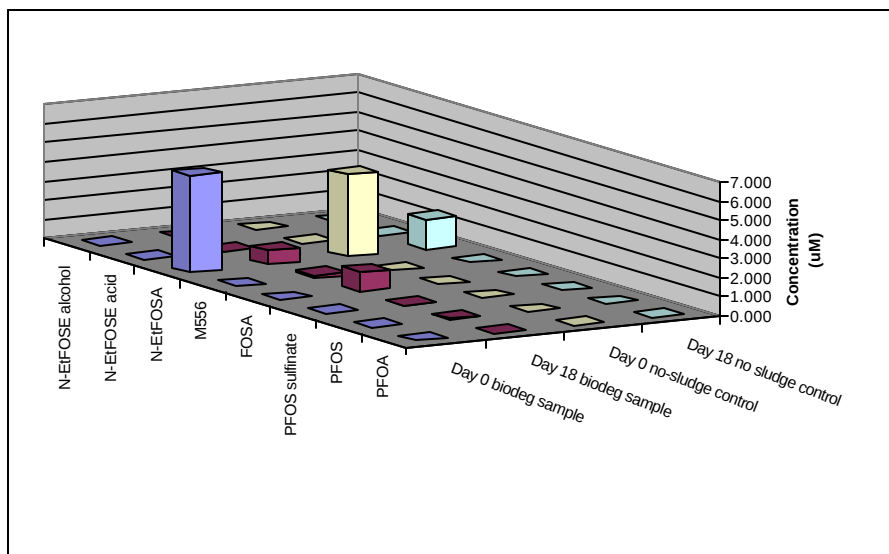


Figure 3. The biodegradation chart for *N*-EtFOSA, showing the biotransformation of *N*-EtFOSA to yield the products M556 and FOSA as the major products. Losses of *N*-EtFOSA in both the test cultures and the no-sludge control cultures may be attributed to volatilization, accounting for the poor mass balance values.

7.7.4 M556

The test substrate M556 was moderately biodegraded after the 18-day incubation (**Figure 4**), with 74.4% of the parent material remaining in cultures that contained sludge (see **Appendix D, Table 1**). The M556 did not degrade, and no quantifiable products were detected in the no-sludge controls following 18 days of incubation. Mass balance for M556 was excellent with recoveries between 95 and 112%. The results indicate that M556 was biotransformed to FOSA (major product), PFOSulfinate, PFOS and PFOA.

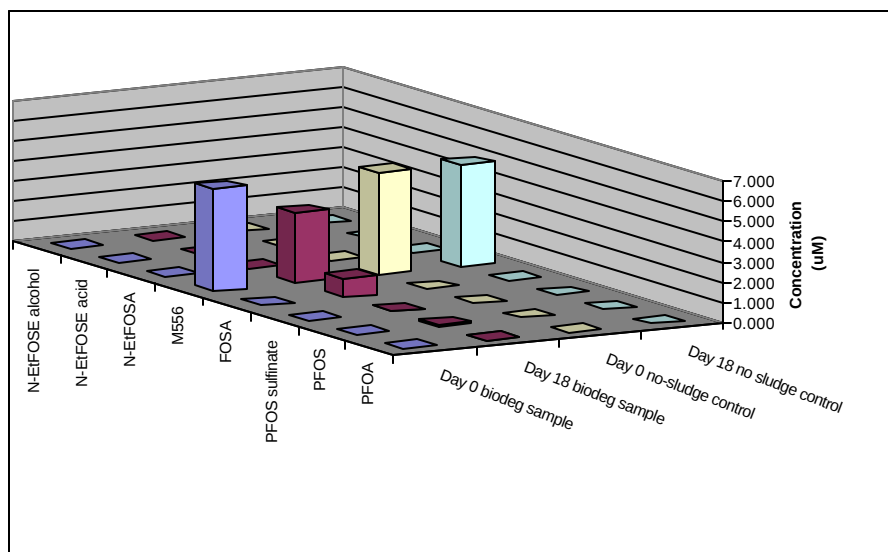


Figure 4. The biotransformation chart for M556, showing the moderate biodegradation of M556 with FOSA as the major product.

7.7.5 FOSA

The test substrate FOSA was poorly biodegraded in 18-day incubation experiments, with 89.8% of the parent material remaining in test cultures after incubation (see **Appendix D, Table 1** and **Figure 5**). No degradation of FOSA was observed in the no-sludge abiotic controls. Mass balance for FOSA was excellent with recoveries between 94% and 101%. The results indicate that FOSA was biotransformed to PFOSulfinate, PFOS and PFOA.

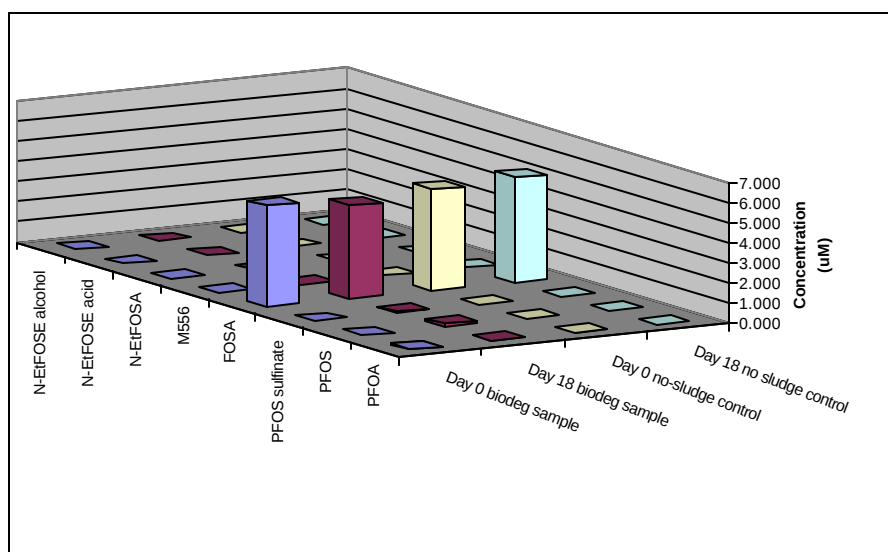


Figure 5. The biotransformation chart for FOSA, showing the biodegradation of FOSA to yield PFOS as the major product.

7.7.6 PFOSulfinate

PFOSulfinate was observed to degrade in both the test cultures and the no-sludge abiotic control cultures. However, the products formed were dependent on whether sludge was present or not, as demonstrated in **Figure 6** and presented in the data from **Appendix D, Table 1**.

The formation of PFOA was observed as the major product from PFOSulfinate in the abiotic controls and was likely caused by an abiotic mechanism described previously by **Hu et al. (1990)**⁵. This reaction occurs rapidly, as approximately 15% of the PFOSulfinate transformed in the day zero abiotic controls. After 18 days, approximately 60% of the PFOSulfinate was transformed to yield PFOA (major product) and PFOS (minor product) at a 10:1 mole ratio.

In sharp contrast to the products observed in the abiotic controls, sample cultures containing sludge resulted in 84% of the PFOSulfinate being biotransformed to PFOS (major product) and PFOA (minor product) at an 8:1 mole ratio, a complete reversal of the behavior observed in abiotic controls.

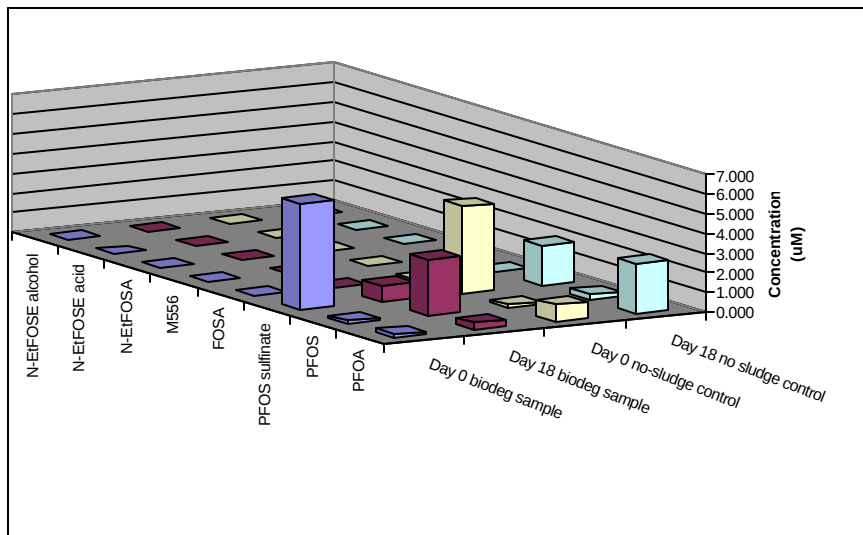


Figure 6. The transformation chart for PFOSulfinate, showing the biotransformation of PFOSulfinate to PFOS as the major product, and the chemical transformation of PFOSulfinate in abiotic controls to yield PFOA as the major product.

7.7.7 PFOS

As shown in **Figure 7** and presented in the data of **Appendix D, Table 1**, PFOS was not measurably biodegraded in the 18-day incubation experiments in either the test cultures or abiotic controls. This could be because it cannot be biodegraded under the test conditions, or because it is toxic at the tested concentrations. It is also possible that a lack of bioavailability could explain the lack of biodegradation.

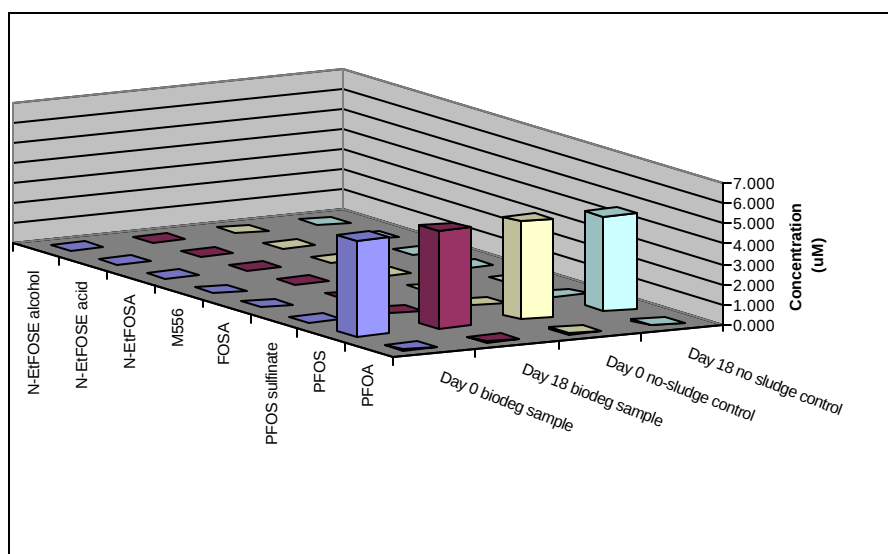


Figure 7. The transformation chart for PFOS, showing no degradation with PFOS as a test substrate.

7.7.8 PFOA

The PFOA was not measurably biodegraded in the 18-day incubation experiments, as shown in **Figure 8** and presented in the data of **Appendix D, Table 1**. As with PFOS, the lack of activity may be because it cannot be biodegraded under the test conditions, it may be toxic at the tested concentrations, or it is simply not bioavailable. PFOA was also not degraded in controls, suggesting it is stable under all the conditions tested.

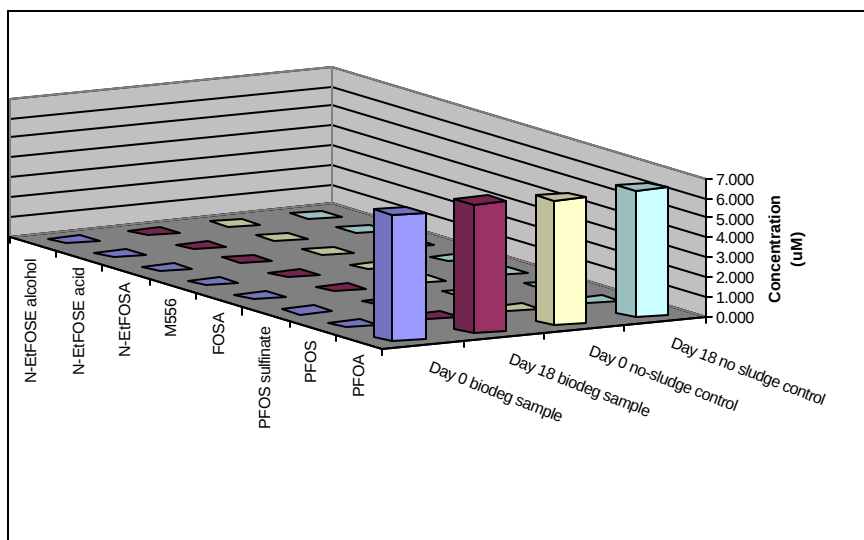


Figure 8. The transformation chart for PFOA, showing no degradation with PFOA as a substrate.

9.0 Conclusions

The 18-day incubations of the targeted fluorochemicals resulted in several interesting and unanticipated discoveries. First, the formation of PFOA from PFOSulfinate was found to occur predominantly in the no-sludge abiotic controls. This observed formation of PFOA from PFOSulfinate in the abiotic controls is supported by a 1990 publication that described a 1-electron elimination process for conversion of perfluorinated sulfinic acids to perfluorinated carboxylic acids⁴. In contrast, samples of PFOSulfinate that contained MLSS sludge formed PFOS as the major product. This was postulated to be due to a biological sulfur oxidation mechanism. Further transformations of PFOS and PFOA were

not observed. This was supported by the observation that no changes occurred in the sample cultures and controls for the study of PFOS and PFOA after 18 days of incubation. PFOS and PFOA are probably endpoints of the biodegradation of *N*-EtFOSE alcohol in municipal waste treatment facilities. The degradation pathway deduced from this work is represented in **Figure 9** below.

A second significant discovery was the observation that several of the metabolites studied which were expected to be easily biotransformed, were not significantly biotransformed at the concentrations tested. This may be explained by several possible reasons. First, the test substance may be toxic to the microorganisms responsible for the biodegradation activity at the analyte concentration tested. Second, the test substance may be too polar to cross the biological cell membranes, or may adsorb to sludge or test containers, thus limiting the bioavailability of the analyte to the interior of the cell where the reactions may take place. Third, the test substrate may be unable to successfully cause the induction of appropriate genes and enzyme required for biodegradation activity. Compounds that were expected to be highly biodegradable, but exhibited low biodegradability, were: *N*-EtFOSAA, *N*-EtFOSA, and FOSA.

As expected, PFOS and PFOA did not biodegrade during 18 days of incubation with aerobic MLSS sludge, and both are likely the endpoint products for perfluorooctanesulfonyl-based chemistries when they enter into biological waste treatment facilities.

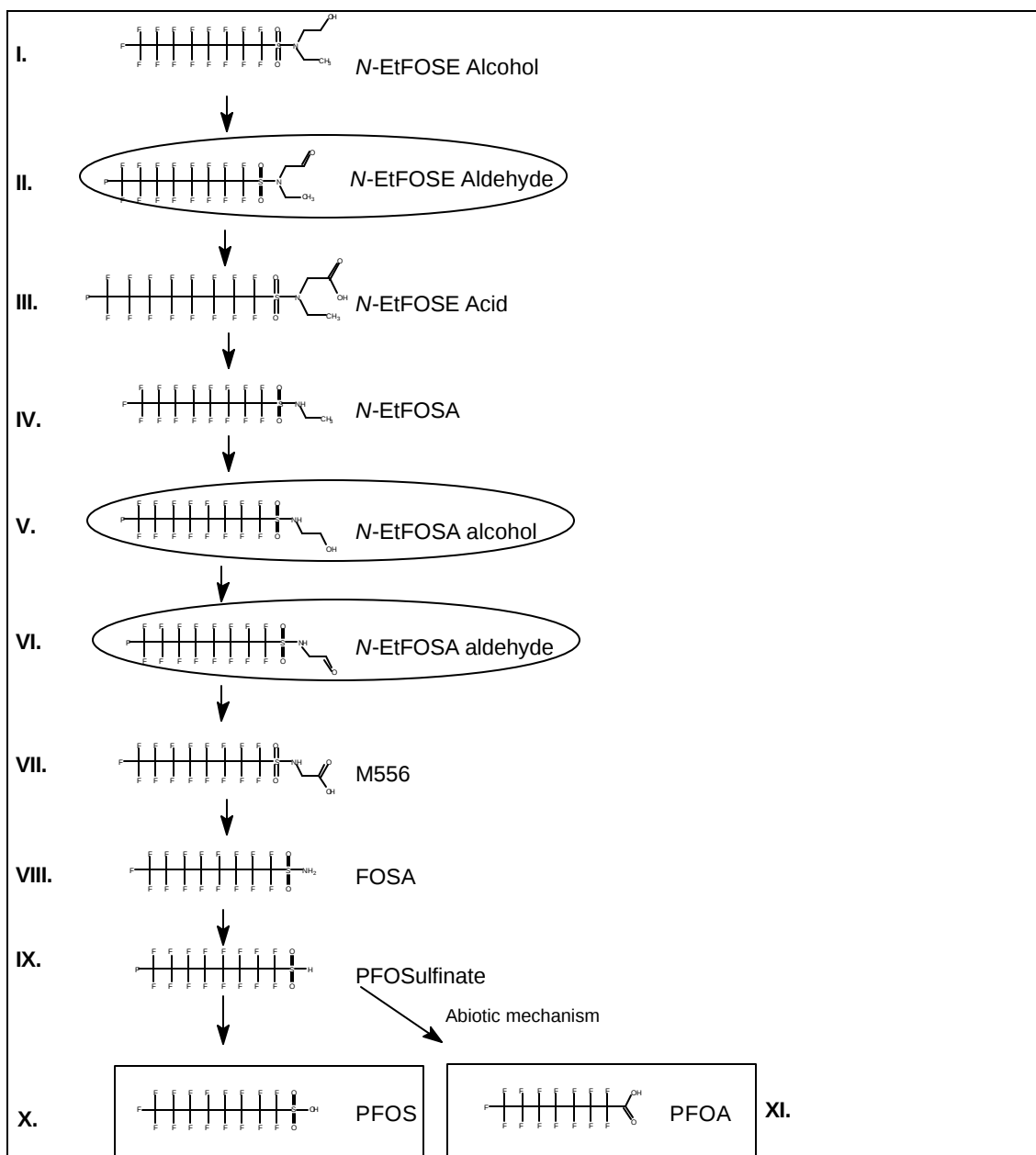


Figure 9. The proposed biodegradation pathway from project CA058 for *N*-EtFOSE alcohol as mediated by the microbial activity of aerobic municipal sludge. The pathway was confirmed by the results of this study by incubating each analyte with aerobic sludge for 18 days. PFOS and PFOA, shown in rectangles, did not degrade over the course of the study and are probably dead-end metabolites. Intermediates circled were not detected in this study, nor project CA058, but were proposed intermediates based on the expected 2-electron oxidation mechanisms of several degradative enzymes.

9.0 Sample and Data Retention

Original facility records and a copy of all data and the final report will be retained in the Pace Analytical-Tier 2 data archives for a period of 5 years after completion of the project. At the end of 5 years, the data will be disposed of following standard laboratory procedures defined in the document PSS-ARC-02.

The following will be provided to 3M personnel:

- The original signed report and any later amendments to the report.
- Copies of all original raw data in the form of chromatograms, reduced data, and written records concerning this project.
- Copies of all instrumental raw data will be archived onto CD and a copy provided to 3M

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 2 years after completion of the project at 4°C in the Carroll walk-in cooler (Pace ID 0140) located in the Pace Analytical-Tier 2 facility.

10.0 References

1. Final reports for Project CA058: "2-week *N*-EtFOSE Alcohol Biodegradation Screen Study Report" and "Aerobic Biodegradation of *N*-EtFOSE Alcohol Study Report", 3M LIMS Project # E00-2252.
2. Pace Science Solutions method CAG-SP-04 "C₁₈ Solid Phase Extraction Procedure for Fluorochemicals Recovery from Aqueous/Sludge Matrices."
3. Pace Science Solutions method CAG-SP-03 "Culture Preparation for Assessment of Aerobic Biodegradability of Fluorochemicals Using Municipal or Industrial Sludge as Microbial Inoculum"
4. Pace Science Solutions method CAG-ORG-23 "Quantitative Analysis of Fluorochemicals by High Performance Liquid Chromatography with Mass Spectrometric Detection".
5. Hu, Chang-Ming, Ze-Qi Xu, and Wei-Yuan Huang. 1990. "Reaction of Perhalofluoroalkyl Sulfinates with One-Electron Transfer Oxidants. A Facile Method for the Synthesis of Perhalofluorocarboxylic Acids". *J. Fluorine Chemistry*. **49**:433-437.

APPENDIX A

SIGNATURES OF PROJECT PERSONNEL

Project Title: 18-Day Aerobic Biodegradation Study for Perfluorooctanesulfonate Chemistries.

Client Project ID: Not Available

Contract Analytical Project Number: CA097

The following individuals participated in the conduct of this project:

Study Director	_____	_____
	Cleston C. Lange, Ph.D.	Date

Laboratory Management:	_____	_____
	Bruce E. Warden	Date

Analysts/Technicians:	_____	_____
	Angela L. Schuler	Date

_____	_____
Anthony E. Scales	Date

Sample Custodian:	_____	_____
	Cleston C. Lange, Ph.D.	Date

Report Reviewed by:	_____	_____
	Dirk W. Hoogenboom	Date

Report Reviewed by:	_____	_____
	Kuruppu N. Dharmasiri, Ph.D.	Date

Appendix B

TABLE 1. The quantitative LC/MS analysis results for eluate 2 (first SPE methanol elution). A value of zero implies the compound was not detected or was detected at a level less than 50% of the MQL of 5 ng/mL.

Sample ID	Description	Concentration (ng/mL)								
		Eluate	PFOA	PFOS	PFOS Sulfinate	M556	N-EtFOSE acid	FOSA	N-EtFOSE-alcohol	N-EtFOSA
CA097-0801-SA-200	DAY 0 BIODEG FOR PFOA	2	2758	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-201	DAY 0 BIODEG FOR PFOA DUPLICATE	2	2673	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-202	DAY 0 BIODEG FOR PFOS	2	14.1	2385	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-203	DAY 0 BIODEG FOR PFOS DUPLICATE	2	13.8	2451	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-204	DAY 0 BIODEG FOR PFOSulfinate	2	56.7	78.9	2634	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-205	DAY 0 BIODEG FOR PFOSulfinate DUPLICATE	2	84.7	79.1	2660	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-206	DAY 0 BIODEG FOR FOSA	2	3.9	0.0	0.0	0.0	0.0	2518	0.0	0.0
CA097-0801-SA-207	DAY 0 BIODEG FOR FOSA DUPLICATE	2	3.3	0.0	0.0	0.0	0.0	2330	0.0	0.0
CA097-0801-SA-208	DAY 0 BIODEG FOR N-EtFOSA	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2521
CA097-0801-SA-209	DAY 0 BIODEG FOR N-EtFOSA DUPLICATE	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2562
CA097-0801-SA-210	DAY 0 BIODEG FOR M556	2	0.0	0.0	0.0	2617	0.0	4.8	0.0	0.0
CA097-0801-SA-211	DAY 0 BIODEG FOR M556 DUPLICATE	2	0.0	0.0	0.0	2572	0.0	4.5	0.0	0.0
CA097-0801-SA-212	DAY 0 BIODEG FOR N-EtFOSE acid	2	9.2	0.0	11.2	0.0	2377	0.0	0.0	18.9
CA097-0801-SA-213	DAY 0 BIODEG FOR N-EtFOSE acid DUPLICATE	2	9.1	0.0	11.1	0.0	2349	0.0	0.0	21.8
CA097-0801-SA-214	DAY 0 BIODEG FOR N-EtFOSE alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	2810	0.0
CA097-0801-SA-215	DAY 0 BIODEG FOR N-EtFOSE alcohol DUPLICATE	2	0.0	0.0	0.0	0.0	0.0	0.0	2945	0.0
CA097-0801-SA-216	DAY 0 NO SLUDGE CONTROL FOR PFOA	2	2569	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-217	DAY 0 NO SLUDGE CONTROL FOR PFOA DUPLICATE	2	2700	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-218	DAY 0 NO SLUDGE CONTROL FOR PFOS	2	13.7	2462	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-219	DAY 0 NO SLUDGE CONTROL FOR PFOS DUPLICATE	2	12.6	2534	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-220	DAY 0 NO SLUDGE CONTROL FOR PFOSulfinate	2	333.2	72.5	2239	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-221	DAY 0 NO SLUDGE CONTROL FOR PFOSulfinate DUPLICATE	2	384.8	79.4	2158	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-222	DAY 0 NO SLUDGE CONTROL FOR FOSA	2	3.3	0.0	0.0	0.0	0.0	2475	0.0	0.0
CA097-0801-SA-223	DAY 0 NO SLUDGE CONTROL FOR FOSA DUPLICATE	2	3.6	0.0	0.0	0.0	0.0	2416	0.0	0.0
CA097-0801-SA-224	DAY 0 NO SLUDGE CONTROL FOR N-EtFOSA	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2142
CA097-0801-SA-225	DAY 0 NO SLUDGE CONTROL FOR N-EtFOSA DUPLICATE	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2071
CA097-0801-SA-226	DAY 0 NO SLUDGE CONTROL FOR M556	2	0.0	0.0	0.0	2514	0.0	0.0	0.0	0.0
CA097-0801-SA-227	DAY 0 NO SLUDGE CONTROL FOR M556 DUPLICATE	2	0.0	0.0	0.0	2638	0.0	0.0	0.0	0.0
CA097-0801-SA-228	DAY 0 NO SLUDGE CONTROL FOR N-EtFOSE acid	2	8.5	0.0	11.1	4.6	2207	0.0	0.0	17.0
CA097-0801-SA-229	DAY 0 NO SLUDGE CONTROL FOR N-EtFOSE acid DUPLICATE	2	8.5	0.0	11.2	0.0	1979	0.0	0.0	17.5
CA097-0801-SA-230	DAY 0 NO SLUDGE CONTROL FOR N-EtFOSE alcohol	2	0.0	0.0	0.0	0.0	0.0	0.0	2820	0.0
CA097-0801-SA-231	DAY 0 NO SLUDGE CONTROL FOR N-EtFOSE alcohol DUPLICATE	2	0.0	0.0	0.0	0.0	0.0	0.0	2823	0.0
CA097-0801-SA-232	DAY 0 SLUDGE BLANK	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-233	DAY 0 SLUDGE BLANK DUPLICATE	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-234	DAY 18 BIODEG FOR PFOA	2	2821	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-235	DAY 18 BIODEG FOR PFOA DUPLICATE	2	2618	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-236	DAY 18 BIODEG FOR PFOS	2	13.6	2484	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-237	DAY 18 BIODEG FOR PFOS DUPLICATE	2	13.1	2447	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-238	DAY 18 BIODEG FOR PFOSulfinate	2	149.9	1444	369.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-239	DAY 18 BIODEG FOR PFOSulfinate DUPLICATE	2	149.6	1404	409.6	0.0	0.0	0.0	0.0	0.0

Appendix B,

TABLE 1 CONTINUED

Sample ID	Sample Description	Eluate	Concentration (ng/mL)							
			PFOA	PFOS	PFOS Sulfinate	M556	N-EtFOSE acid	FOSA	N-EtFOSE-alcohol	N-EtFOSA
CA097-0801-SA-240	DAY 18 BIODEG FOR FOSA	2	12.2	73.9	22.1	0.0	0.0	1947	0.0	0.0
CA097-0801-SA-241	DAY 18 BIODEG FOR FOSA DUPLICATE	2	13.1	76.2	19.2	0.0	0.0	2300	0.0	0.0
CA097-0801-SA-242	DAY 18 BIODEG FOR N-EtFOSA	2	0.0	16.9	6.6	72.4	0.0	497.4	0.0	362.5
CA097-0801-SA-243	DAY 18 BIODEG FOR N-EtFOSA DUPLICATE	2	0.0	16.2	5.7	72.5	0.0	478.3	0.0	332.2
CA097-0801-SA-244	DAY 18 BIODEG FOR M556	2	4.7	21.5	6.1	1539	0.0	399.5	0.0	0.0
CA097-0801-SA-245	DAY 18 BIODEG FOR M556 DUPLICATE	2	4.9	21.3	7.5	1813	0.0	441.0	0.0	0.0
CA097-0801-SA-246	DAY 18 BIODEG FOR N-EtFOSE acid	2	13.7	12.0	4.6	31.2	2250	23.4	0.0	7.6
CA097-0801-SA-247	DAY 18 BIODEG FOR N-EtFOSE acid DUPLICATE	2	12.2	10.6	4.7	27.7	1929	25.8	0.0	10.3
CA097-0801-SA-248	DAY 18 BIODEG FOR N-EtFOSE alcohol	2	20.2	116.9	57.3	73.5	1779	23.4	411.6	9.5
CA097-0801-SA-249	DAY 18 BIODEG FOR N-EtFOSE alcohol DUPLICATE	2	18.4	91.5	25.2	71.8	1727	26.2	430.9	15.0
CA097-0801-SA-250	DAY 18 NO SLUDGE CONTROL FOR PFOA	2	2733	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-251	DAY 18 NO SLUDGE CONTROL FOR PFOA DUPLICATE	2	2755	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-252	DAY 18 NO SLUDGE CONTROL FOR PFOS	2	14.1	2366	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-253	DAY 18 NO SLUDGE CONTROL FOR PFOS DUPLICATE	2	14.2	2529	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-254	DAY 18 NO SLUDGE CONTROL FOR PFOSulfinate	2	1255	129.9	787.5	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-255	DAY 18 NO SLUDGE CONTROL FOR PFOSulfinate DUPLICATE	2	913.9	128.7	1226	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-256	DAY 18 NO SLUDGE CONTROL FOR FOSA	2	3.7	0.0	0.0	0.0	0.0	2482	0.0	0.0
CA097-0801-SA-257	DAY 18 NO SLUDGE CONTROL FOR FOSA DUPLICATE	2	4.0	0.0	0.0	0.0	0.0	2557	0.0	0.0
CA097-0801-SA-258	DAY 18 NO SLUDGE CONTROL FOR N-EtFOSA	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1112
CA097-0801-SA-259	DAY 18 NO SLUDGE CONTROL FOR N-EtFOSA DUPLICATE	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	397.6
CA097-0801-SA-260	DAY 18 NO SLUDGE CONTROL FOR M556	2	0.0	0.0	0.0	2645	0.0	0.0	0.0	0.0
CA097-0801-SA-261	DAY 18 NO SLUDGE CONTROL FOR M556 DUPLICATE	2	0.0	0.0	0.0	2492	0.0	0.0	0.0	0.0
CA097-0801-SA-262	DAY 18 NO SLUDGE CONTROL FOR N-EtFOSE acid	2	9.7	0.0	10.8	5.1	2421	0.0	0.0	0.0
CA097-0801-SA-263	DAY 18 NO SLUDGE CONTROL FOR N-EtFOSE acid DUPLICATE	2	9.7	0.0	11.2	0.0	2514	0.0	0.0	0.0
CA097-0801-SA-264	DAY 18 NO SLUDGE CONTROL FOR N-EtFOSE alcohol	2	0.0	0.0	3.7	0.0	7.1	0.0	3398	0.0
CA097-0801-SA-265	DAY 18 NO SLUDGE CONTROL FOR N-EtFOSE alcohol DUPLICATE	2	0.0	0.0	0.0	0.0	15.0	0.0	2630	0.0
CA097-0801-SA-266	DAY 18 SLUDGE BLANK	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-267	DAY 18 SLUDGE BLANK DUPLICATE	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix B, Table 1 continued.

TABLE 2. The quantitative LC/MS analysis results for eluate 3 (second SPE methanol elution). A value of zero implies the compound was not detected or was detected at a level less than 50% of the MQL of 5 ng/mL.

Sample ID	Description	Eluate	Concentration (ng/mL)							
			PFOA	PFOS	PFOS Sulfinate	M556	N-EtFOSE acid	FOSA	N-EtFOSE- alcohol	N-EtFOSA
CA097-0801-SA-200	DAY 0 BIODEG FOR PFOA	3	59.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-201	DAY 0 BIODEG FOR PFOA DUPLICATE	3	54.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-202	DAY 0 BIODEG FOR PFOS	3	0.0	161.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-203	DAY 0 BIODEG FOR PFOS DUPLICATE	3	0.0	127.7	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-204	DAY 0 BIODEG FOR PFOSulfinate	3	0.0	6.7	170.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-205	DAY 0 BIODEG FOR PFOSulfinate DUPLICATE	3	0.0	7.1	174.2	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-206	DAY 0 BIODEG FOR FOSA	3	0.0	0.0	0.0	0.0	0.0	70.3	0.0	0.0
CA097-0801-SA-207	DAY 0 BIODEG FOR FOSA DUPLICATE	3	0.0	0.0	0.0	0.0	0.0	93.8	0.0	0.0
CA097-0801-SA-208	DAY 0 BIODEG FOR N-EtFOSA	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	124.1
CA097-0801-SA-209	DAY 0 BIODEG FOR N-EtFOSA DUPLICATE	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	130.0
CA097-0801-SA-210	DAY 0 BIODEG FOR M556	3	0.0	0.0	0.0	233.0	0.0	0.0	0.0	0.0
CA097-0801-SA-211	DAY 0 BIODEG FOR M556 DUPLICATE	3	0.0	0.0	0.0	240.4	0.0	0.0	0.0	0.0
CA097-0801-SA-212	DAY 0 BIODEG FOR N-EtFOSE acid	3	0.0	0.0	0.0	0.0	231.8	0.0	0.0	0.0
CA097-0801-SA-213	DAY 0 BIODEG FOR N-EtFOSE acid DUPLICATE	3	0.0	0.0	0.0	0.0	223.9	0.0	0.0	0.0
CA097-0801-SA-214	DAY 0 BIODEG FOR N-EtFOSE alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	196.2	0.0
CA097-0801-SA-215	DAY 0 BIODEG FOR N-EtFOSE alcohol DUPLICATE	3	0.0	0.0	0.0	0.0	0.0	0.0	137.3	0.0
CA097-0801-SA-216	DAY 0 NO SLUDGE CONTROL FOR PFOA	3	180.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-217	DAY 0 NO SLUDGE CONTROL FOR PFOA DUPLICATE	3	8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-218	DAY 0 NO SLUDGE CONTROL FOR PFOS	3	0.0	104.7	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-219	DAY 0 NO SLUDGE CONTROL FOR PFOS DUPLICATE	3	0.0	146.6	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-220	DAY 0 NO SLUDGE CONTROL FOR PFOSulfinate	3	14.1	4.2	151.7	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-221	DAY 0 NO SLUDGE CONTROL FOR PFOSulfinate DUPLICATE	3	8.6	0.0	71.1	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-222	DAY 0 NO SLUDGE CONTROL FOR FOSA	3	0.0	0.0	0.0	0.0	0.0	59.6	0.0	0.0
CA097-0801-SA-223	DAY 0 NO SLUDGE CONTROL FOR FOSA DUPLICATE	3	0.0	0.0	0.0	0.0	0.0	56.1	0.0	0.0
CA097-0801-SA-224	DAY 0 NO SLUDGE CONTROL FOR N-EtFOSA	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	172.9
CA097-0801-SA-225	DAY 0 NO SLUDGE CONTROL FOR N-EtFOSA DUPLICATE	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	157.7
CA097-0801-SA-226	DAY 0 NO SLUDGE CONTROL FOR M556	3	0.0	0.0	0.0	241.2	0.0	0.0	0.0	0.0
CA097-0801-SA-227	DAY 0 NO SLUDGE CONTROL FOR M556 DUPLICATE	3	0.0	0.0	0.0	271.4	0.0	0.0	0.0	0.0
CA097-0801-SA-228	DAY 0 NO SLUDGE CONTROL FOR N-EtFOSE acid	3	0.0	0.0	0.0	0.0	453.7	0.0	0.0	0.0
CA097-0801-SA-229	DAY 0 NO SLUDGE CONTROL FOR N-EtFOSE acid DUPLICATE	3	0.0	0.0	0.0	0.0	561.8	0.0	0.0	0.0
CA097-0801-SA-230	DAY 0 NO SLUDGE CONTROL FOR N-EtFOSE alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	169.3	0.0
CA097-0801-SA-231	DAY 0 NO SLUDGE CONTROL FOR N-EtFOSE alcohol DUPLICATE	3	0.0	0.0	0.0	0.0	0.0	0.0	148.6	0.0
CA097-0801-SA-232	DAY 0 SLUDGE BLANK	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-233	DAY 0 SLUDGE BLANK DUPLICATE	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-234	DAY 18 BIODEG FOR PFOA	3	67.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-235	DAY 18 BIODEG FOR PFOA DUPLICATE	3	150.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-236	DAY 18 BIODEG FOR PFOS	3	0.0	104.6	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-237	DAY 18 BIODEG FOR PFOS DUPLICATE	3	0.0	143.3	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-238	DAY 18 BIODEG FOR PFOSulfinate	3	5.7	130.2	35.3	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-239	DAY 18 BIODEG FOR PFOSulfinate DUPLICATE	3	6.1	121.0	36.4	0.0	0.0	0.0	0.0	0.0

Appendix B

TABLE 2, CONTINUED

Sample ID	Description	Eluate	Concentration (ng/mL)							
			PFOA	PFOS	PFOS Sulfinate	M556	N-EtFOSE acid	FOSA	N-EtFOSE-alcohol	N-EtFOSA
CA097-0801-SA-240	DAY 18 BIODEG FOR FOSA	3	0.0	11.5	4.1	0.0	0.0	233.4	0.0	0.0
CA097-0801-SA-241	DAY 18 BIODEG FOR FOSA DUPLICATE	3	0.0	7.4	0.0	0.0	0.0	146.7	0.0	0.0
CA097-0801-SA-242	DAY 18 BIODEG FOR N-EtFOSA	3	0.0	0.0	0.0	13.1	0.0	34.7	0.0	51.6
CA097-0801-SA-243	DAY 18 BIODEG FOR N-EtFOSA DUPLICATE	3	0.0	0.0	0.0	11.5	0.0	30.7	0.0	31.3
CA097-0801-SA-244	DAY 18 BIODEG FOR M556	3	0.0	5.2	0.0	386.8	0.0	75.0	0.0	0.0
CA097-0801-SA-245	DAY 18 BIODEG FOR M556 DUPLICATE	3	0.0	0.0	0.0	139.0	0.0	14.7	0.0	0.0
CA097-0801-SA-246	DAY 18 BIODEG FOR N-EtFOSE acid	3	0.0	0.0	0.0	5.1	191.2	0.0	0.0	0.0
CA097-0801-SA-247	DAY 18 BIODEG FOR N-EtFOSE acid DUPLICATE	3	0.0	0.0	0.0	6.8	299.9	0.0	0.0	0.0
CA097-0801-SA-248	DAY 18 BIODEG FOR N-EtFOSE alcohol	3	0.0	15.2	7.3	13.8	260.1	0.0	30.1	0.0
CA097-0801-SA-249	DAY 18 BIODEG FOR N-EtFOSE alcohol DUPLICATE	3	0.0	14.1	4.8	15.9	288.2	0.0	33.2	0.0
CA097-0801-SA-250	DAY 18 NO SLUDGE CONTROL FOR PFOA	3	36.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-251	DAY 18 NO SLUDGE CONTROL FOR PFOA DUPLICATE	3	31.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-252	DAY 18 NO SLUDGE CONTROL FOR PFOS	3	0.0	100.7	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-253	DAY 18 NO SLUDGE CONTROL FOR PFOS DUPLICATE	3	0.0	75.7	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-254	DAY 18 NO SLUDGE CONTROL FOR PFOSulfinate	3	12.9	9.3	43.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-255	DAY 18 NO SLUDGE CONTROL FOR PFOSulfinate DUPLICATE	3	9.7	8.8	66.5	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-256	DAY 18 NO SLUDGE CONTROL FOR FOSA	3	0.0	0.0	0.0	0.0	0.0	74.4	0.0	0.0
CA097-0801-SA-257	DAY 18 NO SLUDGE CONTROL FOR FOSA DUPLICATE	3	0.0	0.0	0.0	0.0	0.0	65.5	0.0	0.0
CA097-0801-SA-258	DAY 18 NO SLUDGE CONTROL FOR N-EtFOSA	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	94.3
CA097-0801-SA-259	DAY 18 NO SLUDGE CONTROL FOR N-EtFOSA DUPLICATE	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.8
CA097-0801-SA-260	DAY 18 NO SLUDGE CONTROL FOR M556	3	0.0	0.0	0.0	229.5	0.0	0.0	0.0	0.0
CA097-0801-SA-261	DAY 18 NO SLUDGE CONTROL FOR M556 DUPLICATE	3	0.0	0.0	0.0	237.5	0.0	0.0	0.0	0.0
CA097-0801-SA-262	DAY 18 NO SLUDGE CONTROL FOR N-EtFOSE acid	3	0.0	0.0	0.0	0.0	248.8	0.0	0.0	0.0
CA097-0801-SA-263	DAY 18 NO SLUDGE CONTROL FOR N-EtFOSE acid DUPLICATE	3	0.0	0.0	0.0	0.0	305.9	0.0	0.0	0.0
CA097-0801-SA-264	DAY 18 NO SLUDGE CONTROL FOR N-EtFOSE alcohol	3	0.0	0.0	0.0	0.0	0.0	0.0	173.1	0.0
CA097-0801-SA-265	DAY 18 NO SLUDGE CONTROL FOR N-EtFOSE alcohol DUPLICATE	3	0.0	0.0	0.0	0.0	4.4	0.0	206.2	0.0
CA097-0801-SA-266	DAY 18 SLUDGE BLANK	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CA097-0801-SA-267	DAY 18 SLUDGE BLANK DUPLICATE	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix C

TABLE 1. The summed results for the SPE eluate 2 and eluate 3 collections from day 0 and day 18 samples for each analyte with aerobic sludge. Note that *N*-EtFOSE acid and FOSA were poorly degraded in 18 days suggesting either toxicity at the tested concentration or a lack of bioavailability to microorganisms. Reduced bioavailability may be a result of increased polarity of the test compound versus that of the more neutral *N*-EtFOSE alcohol, and thus a decreased mobility across the hydrophobic cell membrane might be expected. A value of zero implies the compound was not detected or was detected at a level less than 50% of the MQL of 5 ng/mL.

Sample Description	Sample ID	ELUATE	Concentration (ng/mL)							
			PEQA	PFOS	PFOS sulfinate	M556	<i>N</i> -EtFOSE acid	FOSA	<i>N</i> -EtFOSE alcohol	<i>N</i> -EtFOSA
DAY 0 BIODEG FOR PFOA	CA097-0801-SA-200	2+3	2818	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DAY 0 BIODEG FOR PFOA DUPLICATE	CA097-0801-SA-201	2+3	2728	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DAY 18 BIODEG FOR PFOA	CA097-0801-SA-234	2+3	2889	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DAY 18 BIODEG FOR PFOA DUPLICATE	CA097-0801-SA-235	2+3	2768	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DAY 0 NO SLUDGE CONTROL FOR PFOA	CA097-0801-SA-216	2+3	2750	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DAY 0 NO SLUDGE CONTROL FOR PFOA DUPLICATE	CA097-0801-SA-217	2+3	2708	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DAY 18 NO SLUDGE CONTROL FOR PFOA	CA097-0801-SA-250	2+3	2769	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DAY 18 NO SLUDGE CONTROL FOR PFOA DUPLICATE	CA097-0801-SA-251	2+3	2786	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DAY 0 BIODEG FOR PFOS	CA097-0801-SA-202	2+3	14.1	2546	0.0	0.0	0.0	0.0	0.0	0.0
DAY 0 BIODEG FOR PFOS DUPLICATE	CA097-0801-SA-203	2+3	13.8	2579	0.0	0.0	0.0	0.0	0.0	0.0
DAY 18 BIODEG FOR PFOS	CA097-0801-SA-236	2+3	13.6	2589	0.0	0.0	0.0	0.0	0.0	0.0
DAY 18 BIODEG FOR PFOS DUPLICATE	CA097-0801-SA-237	2+3	13.1	2590	0.0	0.0	0.0	0.0	0.0	0.0
DAY 0 NO SLUDGE CONTROL FOR PFOS	CA097-0801-SA-218	2+3	13.7	2567	0.0	0.0	0.0	0.0	0.0	0.0
DAY 0 NO SLUDGE CONTROL FOR PFOS DUPLICATE	CA097-0801-SA-219	2+3	12.6	2681	0.0	0.0	0.0	0.0	0.0	0.0
DAY 18 NO SLUDGE CONTROL FOR PFOS	CA097-0801-SA-252	2+3	14.1	2467	0.0	0.0	0.0	0.0	0.0	0.0
DAY 18 NO SLUDGE CONTROL FOR PFOS DUPLICATE	CA097-0801-SA-253	2+3	14.2	2604	0.0	0.0	0.0	0.0	0.0	0.0
DAY 0 BIODEG FOR PFOSulfinate	CA097-0801-SA-204	2+3	56.7	85.7	2804	0.0	0.0	0.0	0.0	0.0
DAY 0 BIODEG FOR PFOSulfinate DUPLICATE	CA097-0801-SA-205	2+3	84.7	86.3	2834	0.0	0.0	0.0	0.0	0.0
DAY 18 BIODEG FOR PFOSulfinate	CA097-0801-SA-238	2+3	155.6	1574	404.3	0.0	0.0	0.0	0.0	0.0
DAY 18 BIODEG FOR PFOSulfinate DUPLICATE	CA097-0801-SA-239	2+3	155.7	1525	446.0	0.0	0.0	0.0	0.0	0.0
DAY 0 NO SLUDGE CONTROL FOR PFOSulfinate	CA097-0801-SA-220	2+3	347.3	76.8	2391	0.0	0.0	0.0	0.0	0.0
DAY 0 NO SLUDGE CONTROL FOR PFOSulfinate DUPLICATE	CA097-0801-SA-221	2+3	393.4	79.4	2229	0.0	0.0	0.0	0.0	0.0
DAY 18 NO SLUDGE CONTROL FOR PFOSulfinate	CA097-0801-SA-254	2+3	1268	139.2	830.5	0.0	0.0	0.0	0.0	0.0
DAY 18 NO SLUDGE CONTROL FOR PFOSulfinate DUPLICATE	CA097-0801-SA-255	2+3	923.6	137.5	1292	0.0	0.0	0.0	0.0	0.0
DAY 0 BIODEG FOR FOSA	CA097-0801-SA-206	2+3	3.9	0.0	0.0	0.0	0.0	2589	0.0	0.0
DAY 0 BIODEG FOR FOSA DUPLICATE	CA097-0801-SA-207	2+3	3.3	0.0	0.0	0.0	0.0	2423	0.0	0.0
DAY 18 BIODEG FOR FOSA	CA097-0801-SA-240	2+3	12.2	85.4	26.2	0.0	0.0	2180	0.0	0.0
DAY 18 BIODEG FOR FOSA DUPLICATE	CA097-0801-SA-241	2+3	13.1	83.6	19.2	0.0	0.0	2446	0.0	0.0
DAY 0 NO SLUDGE CONTROL FOR FOSA	CA097-0801-SA-222	2+3	3.3	0.0	0.0	0.0	0.0	2535	0.0	0.0
DAY 0 NO SLUDGE CONTROL FOR FOSA DUPLICATE	CA097-0801-SA-223	2+3	3.6	0.0	0.0	0.0	0.0	2472	0.0	0.0
DAY 18 NO SLUDGE CONTROL FOR FOSA	CA097-0801-SA-256	2+3	3.7	0.0	0.0	0.0	0.0	2556	0.0	0.0
DAY 18 NO SLUDGE CONTROL FOR FOSA DUPLICATE	CA097-0801-SA-257	2+3	4.0	0.0	0.0	0.0	0.0	2623	0.0	0.0
DAY 0 BIODEG FOR <i>N</i> -EtFOSA	CA097-0801-SA-208	2+3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2645
DAY 0 BIODEG FOR <i>N</i> -EtFOSA DUPLICATE	CA097-0801-SA-209	2+3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2692
DAY 18 BIODEG FOR <i>N</i> -EtFOSA	CA097-0801-SA-242	2+3	0.0	16.9	6.6	85.5	0.0	532.1	0.0	414.1
DAY 18 BIODEG FOR <i>N</i> -EtFOSA DUPLICATE	CA097-0801-SA-243	2+3	0.0	16.2	5.7	84.0	0.0	509.0	0.0	363.6
DAY 0 NO SLUDGE CONTROL FOR <i>N</i> -EtFOSA	CA097-0801-SA-224	2+3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2314
DAY 0 NO SLUDGE CONTROL FOR <i>N</i> -EtFOSA DUPLICATE	CA097-0801-SA-225	2+3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2228
DAY 18 NO SLUDGE CONTROL FOR <i>N</i> -EtFOSA	CA097-0801-SA-258	2+3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1207
DAY 18 NO SLUDGE CONTROL FOR <i>N</i> -EtFOSA DUPLICATE	CA097-0801-SA-259	2+3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	431.4

Appendix C

TABLE 1, CONTINUED

Sample Description	Sample ID	ELUATE	Concentration (ng/mL)							
			PEQA	PEQS	PEQS sulfinate	M556	N-EtFOSE acid	EOA	N-EtFOSE alcohol	N-EtEOA
DAY 0 BIODEGR FOR M556	CA097-0801-SA-210	2+3	0.0	0.0	0.0	2850	0.0	4.8	0.0	0.0
DAY 0 BIODEGR FOR M556 DUPLICATE	CA097-0801-SA-211	2+3	0.0	0.0	0.0	2812	0.0	4.5	0.0	0.0
DAY 18 BIODEGR FOR M556	CA097-0801-SA-244	2+3	4.7	26.7	6.1	1926	0.0	474.6	0.0	0.0
DAY 18 BIODEGR FOR M556 DUPLICATE	CA097-0801-SA-245	2+3	4.9	21.3	7.5	1952	0.0	455.7	0.0	0.0
DAY 0 NO SLUDGE CONTROL FOR M556	CA097-0801-SA-226	2+3	0.0	0.0	0.0	2755	0.0	0.0	0.0	0.0
DAY 0 NO SLUDGE CONTROL FOR M556 DUPLICATE	CA097-0801-SA-227	2+3	0.0	0.0	0.0	2909	0.0	0.0	0.0	0.0
DAY 18 NO SLUDGE CONTROL FOR M556	CA097-0801-SA-260	2+3	0.0	0.0	0.0	2875	0.0	0.0	0.0	0.0
DAY 18 NO SLUDGE CONTROL FOR M556 DUPLICATE	CA097-0801-SA-260	2+3	0.0	0.0	0.0	2729	0.0	0.0	0.0	0.0
DAY 0 BIODEGR FOR N-EtFOSE acid	CA097-0801-SA-212	2+3	9.2	0.0	11.2	0.0	2609	0.0	0.0	18.9
DAY 0 BIODEGR FOR N-EtFOSE acid DUPLICATE	CA097-0801-SA-213	2+3	9.1	0.0	11.1	0.0	2573	0.0	0.0	21.8
DAY 18 BIODEGR FOR N-EtFOSE acid	CA097-0801-SA-246	2+3	13.7	12.0	4.6	36.3	2441	23.4	0.0	7.6
DAY 18 BIODEGR FOR N-EtFOSE acid DUPLICATE	CA097-0801-SA-247	2+3	12.2	10.6	4.7	34.5	2229	25.8	0.0	10.3
DAY 0 NO SLUDGE CONTROL FOR N-EtFOSE acid	CA097-0801-SA-228	2+3	8.5	0.0	11.1	4.6	2661	0.0	0.0	17.0
DAY 0 NO SLUDGE CONTROL FOR N-EtFOSE acid DUPLICATE	CA097-0801-SA-229	2+3	8.5	0.0	11.2	0.0	2540	0.0	0.0	17.5
DAY 18 NO SLUDGE CONTROL FOR N-EtFOSE acid	CA097-0801-SA-262	2+3	9.7	0.0	10.8	5.1	2670	0.0	0.0	0.0
DAY 18 NO SLUDGE CONTROL FOR N-EtFOSE acid DUPLICATE	CA097-0801-SA-263	2+3	9.7	0.0	11.2	0.0	2820	0.0	0.0	0.0
DAY 0 BIODEGR FOR N-EtFOSE alcohol	CA097-0801-SA-214	2+3	0.0	0.0	0.0	0.0	0.0	0.0	3007	0.0
DAY 0 BIODEGR FOR N-EtFOSE alcohol DUPLICATE	CA097-0801-SA-215	2+3	0.0	0.0	0.0	0.0	0.0	0.0	3082	0.0
DAY 18 BIODEGR FOR N-EtFOSE alcohol	CA097-0801-SA-248	2+3	20.2	132.1	64.6	87.4	2038.7	23.4	441.6	9.5
DAY 18 BIODEGR FOR N-EtFOSE alcohol DUPLICATE	CA097-0801-SA-249	2+3	18.4	105.6	30.0	87.8	2015.3	26.2	464.1	15.0
DAY 0 NO SLUDGE CONTROL FOR N-EtFOSE alcohol	CA097-0801-SA-230	2+3	0.0	0.0	0.0	0.0	0.0	0.0	2989	0.0
DAY 0 NO SLUDGE CONTROL FOR N-EtFOSE alcohol DUPLICATE	CA097-0801-SA-231	2+3	0.0	0.0	0.0	0.0	0.0	0.0	2972	0.0
DAY 18 NO SLUDGE CONTROL FOR N-EtFOSE alcohol	CA097-0801-SA-264	2+3	0.0	0.0	3.7	0.0	7.1	0.0	3571	0.0
DAY 18 NO SLUDGE CONTROL FOR N-EtFOSE alcohol DUPLICATE	CA097-0801-SA-265	2+3	0.0	0.0	0.0	0.0	19.4	0.0	2836	0.0
DAY 0 SLUDGE BLANK	CA097-0801-SA-232	2+3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DAY 0 SLUDGE BLANK DUPLICATE	CA097-0801-SA-233	2+3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DAY 18 SLUDGE BLANK	CA097-0801-SA-266	2+3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DAY 18 SLUDGE BLANK DUPLICATE	CA097-0801-SA-267	2+3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix D

TABLE 1. The final results table for each perfluorooctanesulfonyl-based substrate tested. Values were converted to micromolar (uM) concentrations from the data in Appendix C. The values reported below are the averaged value determined for duplicate samples. The percent of parent remaining in samples and the mass balance data are calculated from the expected concentration, and was the amount of test material added to each culture at the beginning of the experiment.

Sample Type	Concentration (mM)								Expected Conc. (uM)	% Parent Remaining	Molar Mass Balance
Sample Description	PFOA	PFOS	PFOS Sulfinate	FOSA	M556	N-EtFOSA	N-EtFOSAA	N-EtFOSE alcohol			
DAY 0 BIODEG FOR PFOA	6.434	0.000	0.000	0.000	0.000	0.000	0.000	0.000	6.136	104.9%	104.9%
DAY 18 BIODEG FOR PFOA	6.563	0.000	0.000	0.000	0.000	0.000	0.000	0.000	6.136	107.0%	107.0%
DAY 0 NO SLUDGE CONTROL FOR PFOA	6.332	0.000	0.000	0.000	0.000	0.000	0.000	0.000	6.136	103.2%	103.2%
DAY 18 NO SLUDGE CONTROL FOR PFOA	6.445	0.000	0.000	0.000	0.000	0.000	0.000	0.000	6.136	105.0%	105.0%
DAY 0 BIODEG FOR PFOS	0.032	4.763	0.000	0.000	0.000	0.000	0.000	0.000	4.564	104.4%	105.1%
DAY 18 BIODEG FOR PFOS	0.031	4.813	0.000	0.000	0.000	0.000	0.000	0.000	4.564	105.5%	106.1%
DAY 0 NO SLUDGE CONTROL FOR PFOS	0.031	4.877	0.000	0.000	0.000	0.000	0.000	0.000	4.564	106.9%	107.5%
DAY 18 NO SLUDGE CONTROL FOR PFOS	0.033	4.713	0.000	0.000	0.000	0.000	0.000	0.000	4.564	103.3%	104.0%
DAY 0 BIODEG FOR PFOSulfinate	0.164	0.160	5.400	0.000	0.000	0.000	0.000	0.000	5.071	106.5%	112.9%
DAY 18 BIODEG FOR PFOSulfinate	0.361	2.880	0.814	0.000	0.000	0.000	0.000	0.000	5.071	16.1%	80.0%
DAY 0 NO SLUDGE CONTROL FOR PFOSulfinate	0.859	0.145	4.425	0.000	0.000	0.000	0.000	0.000	5.071	87.3%	107.1%
DAY 18 NO SLUDGE CONTROL FOR PFOSulfinate	2.542	0.257	2.033	0.000	0.000	0.000	0.000	0.000	5.071	40.1%	95.3%
DAY 0 BIODEG FOR FOSA	0.008	0.000	0.000	5.022	0.000	0.000	0.000	0.000	5.141	97.7%	97.8%
DAY 18 BIODEG FOR FOSA	0.029	0.157	0.044	4.636	0.000	0.000	0.000	0.000	5.141	90.2%	94.7%
DAY 0 NO SLUDGE CONTROL FOR FOSA	0.008	0.000	0.000	5.017	0.000	0.000	0.000	0.000	5.141	97.6%	97.7%
DAY 18 NO SLUDGE CONTROL FOR FOSA	0.009	0.000	0.000	5.189	0.000	0.000	0.000	0.000	5.141	100.9%	101.1%
DAY 0 BIODEG FOR M556	0.000	0.000	0.000	0.009	5.083	0.000	0.000	0.000	4.659	109.1%	109.3%
DAY 18 BIODEG FOR M556	0.011	0.045	0.013	0.932	3.481	0.000	0.000	0.000	4.659	74.7%	96.2%
DAY 0 NO SLUDGE CONTROL FOR M556	0.000	0.000	0.000	0.000	5.084	0.000	0.000	0.000	4.659	109.1%	109.1%
DAY 18 NO SLUDGE CONTROL FOR M556	0.000	0.000	0.000	0.000	5.030	0.000	0.000	0.000	4.659	108.0%	108.0%
DAY 0 BIODEG FOR N-EtFOSA	0.000	0.000	0.000	0.000	0.000	5.063	0.000	0.000	5.360	94.5%	94.5%
DAY 18 BIODEG FOR N-EtFOSA	0.000	0.031	0.012	1.043	0.152	0.738	0.000	0.000	5.360	13.8%	36.9%
DAY 0 NO SLUDGE CONTROL FOR N-EtFOSA	0.000	0.000	0.000	0.000	0.000	4.310	0.000	0.000	5.360	80.4%	80.4%
DAY 18 NO SLUDGE CONTROL FOR N-EtFOSA	0.000	0.000	0.000	0.000	0.000	1.554	0.000	0.000	5.360	29.0%	29.0%
DAY 0 BIODEG FOR N-EtFOSAA	0.021	0.000	0.021	0.000	0.000	0.039	4.429	0.000	4.341	102.0%	103.9%
DAY 18 BIODEG FOR N-EtFOSAA	0.030	0.021	0.009	0.049	0.064	0.017	3.991	0.000	4.341	91.9%	96.3%
DAY 0 NO SLUDGE CONTROL FOR N-EtFOSAA	0.020	0.000	0.021	0.000	0.004	0.033	4.445	0.000	4.341	102.4%	104.2%
DAY 18 NO SLUDGE CONTROL FOR N-EtFOSAA	0.023	0.000	0.021	0.000	0.005	0.000	4.693	0.000	4.341	108.1%	109.2%
DAY 0 BIODEG FOR N-EtFOSE alcohol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	5.332	5.002	106.6%	106.6%
DAY 18 BIODEG FOR N-EtFOSE alcohol	0.045	0.221	0.091	0.050	0.157	0.023	3.465	0.793	5.002	15.9%	96.9%
DAY 0 NO SLUDGE CONTROL FOR N-EtFOSE alcohol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	5.219	5.002	104.3%	104.3%
DAY 18 NO SLUDGE CONTROL FOR N-EtFOSE alcohol	0.000	0.000	0.004	0.000	0.000	0.000	0.023	5.610	5.002	112.2%	112.7%

APPENDIX A

SIGNATURES OF PROJECT PERSONNEL

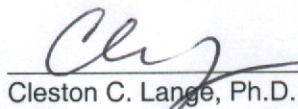
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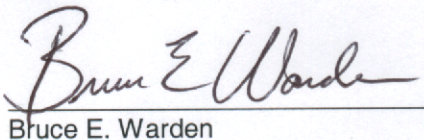
The following individuals participated in the conduct of this project:

Study Director


Cleston C. Lange, Ph.D.

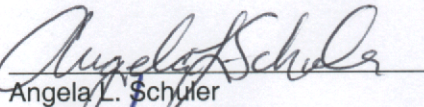
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Date

Laboratory Management:

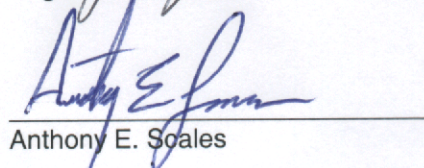

Bruce E. Warden

2/22/01
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Analysts/Technicians:

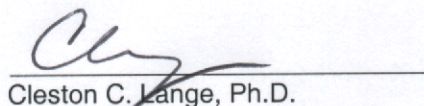

Angela L. Schuler

2/20/01
Date


Anthony E. Soales

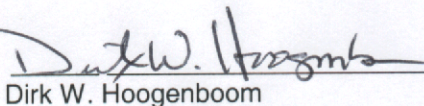
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Sample Custodian:


Cleston C. Lange, Ph.D.

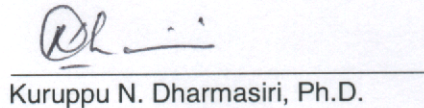
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Report Reviewed by:


Dirk W. Hoogenboom

20 Feb 2001
Date

Report Reviewed by:


Kuruppu N. Dharmasiri, Ph.D.

Feb 20, 2001
Date