3M

Study Title

INHERENT AEROBIC AQUATIC BIODEGRADABILITY OF FLUOROALIPHATIC POLYMERIC ESTER OF[ ]

Data Requirement

40 CFR 792

Authors

[ ]

Study Completion Date

Date of signing

Performing Laboratory

3M Environmental Technology and Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Project Identification

E02-0913

Total Number of Pages
142

This page has been reserved for specific country requirements.[1]

GLP Compliance Statement

Study Title: Inherent Aerobic Aquatic Biodegradability of Fluoroaliphatic Polymeric Ester of [ ]

Study Identification Number: E02-0913

This study was conducted in compliance with Toxic Substances Control Act (TSCA) Good Laboratory Practice (GLP) Standards, 40 CFR 792, with the exceptions listed below:

Exceptions to GLP compliance:

- 40 CFR 792.130(e): The authenticated hardcopy printouts are considered the original raw data.
- 40 CFR 792.105: The purity for [ ] have not been determined.
- 40 CFR 792.105 (b): The stability of the reference substances was not determined prior to study initiation.

[ ]

[ ] 03/10/03
Date

03/10/03
Date

QUALITY ASSURANCE STATEMENT

Study Title: Inherent Aerobic Aquatic Biodegradability of Fluoroaliphatic Polymeric Ester of [ ]

Study Identification Number: E02-0913

This study was audited by the 3M Environmental Laboratory Quality Assurance Unit (QAU), as indicated in the following table. The findings were reported to the study director and laboratory management.

Inspection Dates	Phase	Date Reported to	
		Management	Study Director
10/18/02	Protocol	10/18/02	10/18/02
10/24/02	In-Phase (dosing)	10/28/02	10/28/02
11/25/02	In-Phase (SPE)	11/25/02	11/25/02
1/13/03-1/28/03	Data/Report	1/28/03	1/28/03

[_____] _____ 3-10-03

QAU Representative Date

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STUDY INFORMATION

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Study Location

Testing Facility

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Study Personnel

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Study Dates

Study Initiation: 10/24/02

Experimental Initiation: 10/24/02

Experimental Completion: 3/5/03

Study Completion: Date of signing

Location of Archives

All original raw data, protocol, and analytical report have been archived at the 3M Environmental Laboratory according to 40 CFR Part 792. The test substance and analytical reference standard reserve samples are archived at the 3M Environmental Laboratory according to 40 CFR Part 792.

SUMMARY

This study was undertaken to determine the aerobic, aquatic biodegradation potential of the test substance (a complex mixture of fluoroaliphatic polymeric esters) when exposed to municipal wastewater treatment sludge. This was accomplished by utilizing aspects from the following guidelines: USEPA Zahn-Wellens/EMPA Test (OPPTS 835.3200) and USEPA Modified SCAS (OPPTS 835.3210). The test substance was suspended into three mediums: Medium A (mineral salts medium and sludge), Medium B (mineral salts medium, sludge, and antimicrobial agent) and Medium C (mineral salts medium and antimicrobial agent). After incubation for varying intervals, study samples of polymer were prepared using solid phase extraction (SPE) and analyzed using High Performance Liquid Chromatography / Mass Spectrometry (HPLC/MS). The target analytes are the predicted degradation products

perfluorobutane sulfonate (PFBS), and
The target analytes are based on the biodegradation study of
, as reported in 3M
Environmental Laboratory study #
by the Microbial Activity Present in Municipal Wastewater Treatment Sludge" Samples were semi-quantitatively analyzed for using two different techniques. Initially, was quantified using the response factor of . Subsequently, a standard of was synthesized and characterized. The sample extracts of Medium A were reanalyzed and quantified versus a calibration curve of Due to the polymeric nature of , the predicted degradation products were measured rather than direct measurement of the loss of the starting material.

The analytical results demonstrate that under the conditions of the study, the test substance is biodegraded (OPPTS 835.3210 "Modified SCAS Test" defines a greater than 70% loss of starting material as ultimate biodegradability). The major metabolites identified from the test cultures were (representing degradation of the initial concentration of on day 0) and M (representing 114% degradation of the initial concentration of on day 4). Observed minor metabolites include and PFBS. Complete mass balance between parent material and measured degradation products appears to have been achieved on day 4. See Table 1.

Table 1: Medium A Exposed to Uninhibited Sludge) Percent Degradation Results[1]

Analyte	Day 0	Day 4	Day 14	Day 28
	%	1.0%	0.40%	<0.15%
	0.84%	114%	58%	63%
	<3.5%	1.2%	0.47%	0.26%
	<0.92%	1.4%	1.9%	2.4%
	<1.0%	<0.20%	0.12%	0.37%
PFBS[3]	<0.33%	0.28%	0.97%	2.3%

[1] The percent degradation is based on the theoretical amount of fluorine available in the initial dose of The percentages reported in Table 1 were determined by dividing the average amount of analyte found in each test vessel by the theoretical amount if otally biotransformed into that specific analyte. as calculated using standard.
[3] The analytical accuracy for all analytes is ±20% or better.

INTRODUCTION

The primary objective of this investigation is to identify the inherent aerobic aquatic biodegradation potential of the fluoropolymer when exposed to the microbial populations present in wastewater treatment sludge. This was accomplished by utilizing aspects from the following guidelines: USEPA Zahn-Wellens/EMPA Test (OPPTS 835.3200) and USEPA Modified SCAS (OPPTS 835.3210).

The test substance, is a complex mixture of fluoroaliphatic polymeric esters rather than discreet monomeric material. The present investigation was conducted using the noted EPA methods as guidelines and incorporating portions of these guidelines to accommodate the specific testing requirements, such as individual samples per sampling event and specific target analysis instead of COD, DOC or CO_2. The present study is designed to utilize municipal wastewater treatment sludge as the inoculum. The focus is to determine the ability of viable microbial populations to degrade or transform into predicted fluorochemical breakdown products based on the biological degradation study of . as reported in 3M Environmental Laboratory study # " Biodegradation of by the Microbial Activity Present in Municipal Wastewater Treatment Sludge." The proposed biodegradative pathway is illustrated in Figure 1.

Figure 1: The Proposed) Biodegradative Pathway

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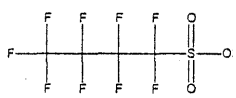

PFBS

TEST SUBSTANCE

Table 2. Test Substances

Test Substance	[redacted]
IUPAC Name	
Chemical Formula	
Identifier	
Source	3M Specialty Chemicals
Expiration Date	07/27/06
Storage Conditions	Room Temperature
Chemical Lot Number	1
TCR Number	
Physical Description	Yellow Viscous Oil
Purity	97.5%
Solubility**	. in ASTM type I water

*Based on NMR data, **From 3M Environmental Laboratory study

REFERENCE SUBSTANCES

Table 3. Reference Substances

Reference Substance		PFBS	
IUPAC Name		Potassium perfluoro-butanesulfonate	
Chemical Formula		$C_4F_9SO_3K$	
Identifier		29420-49-3*	
Source	Aldrich Chemical	3M Specialty Chemicals	3M Specialty Chemicals
Expiration Date	5/1/2010	12/4/2006	Not Provided
Storage Conditions	Frozen	Frozen	Frozen
Chemical Lot Number			
TCR Number			
Physical Description	Clear liquid	White powder	White powder
Purity	99%	96.7%	Not determined

Reference Substance		
IUPAC Name		
Chemical Formula		
Identifier		
Source	3M Specialty Chemicals	3M Specialty Chemicals
Expiration Date	01/30/07	01/30/07
Storage Conditions	Frozen	Ambient
Chemical Lot Number		
TCR Number		
Physical Description	White crystals	White crystals
Purity	97.25%	95.55%

Reference Substance			
IUPAC Name			
Chemical Formula			
Identifier			
Source	Pace Analytical Services	3M Specialty Chemicals	3M Specialty Chemicals
Expiration Date	2/4/2013	12/1/2010	11/29/2005
Storage Conditions	Frozen	Frozen	Frozen
Chemical Lot Number			
TCR Number			
Physical Description	White Powder	Light yellow powder	Off White powder
Purity	50.1%	Not Determined	98.63%

*CAS Number, **3M Identifier Code. The location of the documentation of the method(s) of synthesis of the test, control, and reference items are the same as the source of the compound.
concentrations.

In order to quantify the predicted degradation product was initially used. , was chosen to quantify because it is the to . The molecular formula for is Since both compounds are similar with exception of carbon chain lengths, it was assumed that they have similar response factors. Subsequent to analysis, was synthesized and the response factors of similar calibration standards were compared under the same conditions to provide a better estimate of the concentrations in the original analysis. It should be noted that the purity for used for this study is not thoroughly established and therefore the results for should be treated as semi-quantitative only. calibration curve used was compared to a characterized standard, providing a correction factor of %. The semi-quantitative results reported here are corrected using this purity information.

CONTROL SUBSTANCES

Table 4. Control Substances

Control Substances			Sodium Lauryl Sulfate
Formula			$C_{13}H_{25}OSO_3Na$
IUPAC Name			Sodium Lauryl Sulfate
Use	Surrogate Standard	Internal Standard for LC/MS analysis	Toxicity and Reference Control
Source	3M Specialty Chemicals	3M Specialty Chemicals	Mallinckrodt
Expiration Date	8/31/2006	10/18/2006	2/26/2007
Storage Conditions	Frozen	Frozen	Room Temperature
Chemical Lot Number			7718 V16603
TCR Number			TN-A-6021
Physical Description	White powder	White powder	White powder
Purity	86.9%	98.6%	99%

TEST SYSTEM

Mixed liquor suspended solids (MLSS) were obtained from the aeration units at the Metro Wastewater Treatment Plant, St. Paul, MN. The suspended sludge was allowed to settle for 24 hours at room temperature. The approximate percentage of sludge per volume of container was 19%.

Mediums A, B, and C were then prepared. The mineral salts medium used was based on the USEPA Zahn-Wellens/EMPA Test (OPPTS 835.3200). After the sludge settled for 24 hours, Medium A was prepared by adding 200 mL of the settled sludge to 4.0 L of mineral salts medium. The total suspended solids for this medium was determined to be 0.747 g/L which is within the specification of 0.2 to 1.0 g dry matter/L as given by OPPTS 835.3200. Medium A was then used to create Medium B. Medium B consisted of a portion of Medium A and 130 µg/mL chloramphenicol. Medium C consisted of mineral salts medium and 126 µg/mL chloramphenicol.

Individual culture vessels were prepared by dispensing 25 mL of the appropriate medium into 125 mL glass Erlenmeyer flasks. Then each flask was directly spiked with the test or control substance (or nothing in the case of the blanks) as appropriate. Sample flasks were spiked as indicated in table 4. Day 0 samples were frozen and the rest of the samples were placed in incubators at 24 ± 3°C. All samples except for the day 28 samples maintained this temperature range. For the first 8 days of the study the temperature of the day 28 samples ranged from 20.8 to 28.0°C. After day 8 these samples were maintained at the specified 24 ± 3°C. Since the temperature was well within an acceptable range for supporting viable bacteria, the samples associated with day 28 were not rejected. Exposure to slightly elevated temperatures is not expected to adversely affect the quality of the data.

Table 5: Test System Preparation

Sample Description	# of Replicates	Test/Control Substance added*	Reference Substances added**	Medium Added	Analysis to be conducted
Blank Controls	2	None	No	A	Reference Substances
Blank Controls Inhibited	2	None	No	B	Reference Substances
Abiotic Controls	2	None	No	C	Reference Substances
Test Substance	3	Test	No	A	Reference Substances
Test Substance Matrix spike	2	Test	Yes	A	Reference Substances
Test Substance Inhibited	3	Test	No	B	Reference Substances
Test Substance Inhibited Matrix spike	2	Test	Yes	B	Reference Substances
Abiotic Test Substance	3	Test	No	C	Reference Substances
Abiotic Test Substance Matrix spike	2	Test	Yes	C	Reference Substances
Toxicity Control	3	Test and SLS	No	A	SLS
Control Substance (SLS)	2	SLS	No	A	SLS
	2		No	C	
	2		No	B	

*The test substance was at a concentration of 36 mg/L, equivalent to 20mg Carbon / L per EPA recommended guideline. SLS was spiked at a concentration of 40 mg/L and was spiked at a concentration of mg/L.
**Reference substances were added as post extraction matrix spikes at a nominal concentration of 500 ng/mL.

METHOD SUMMARIES

Preparatory Methods

Samples were prepared using ETS-8-39.0 "Solid Phase Extraction of Soils, Sediments and Sludges." In summary, samples were allowed to thaw after removal from the freezer. The sample was then vortex mixed for approximately 15 seconds and 0.25 mL of glacial acetic acid was added to the sample (to reach a nominal concentration of 1% acetic acid). A plug of glass wool was placed in the solid phase extraction (SPE) cartridge to prevent suspended solids from plugging the cartridge. The SPE cartridges were conditioned with at least two 5 mL washes of methanol and at least two 5 mL washes with 1% acetic acid, taking care not to run the column to dryness. The selected sample was then decanted into the SPE cartridge and collected as eluent one. The column was allowed to run to dryness. Ten mL of tetrahydrofuran (THF) was then added to the Erlenmeyer flask that originally contained the sample, swirled, and decanted into the SPE cartridge and collected as eluent two. The column was allowed to run to dryness. Eluent 2 was analyzed via LC/MS.

Analytical Method

Samples were analyzed via ETS-8-155.1 "Analysis of Waste Stream, Water Extracts or Other Systems Using HPLC-Electrospray/Mass Spectrometry." After the samples were prepared they were diluted (if appropriate) and aliquoted into autovials for analysis via LC/MS. The following parameters were used.

Analytical Equipment

Liquid Chromatograph: Hewlett-Packard® Series 1100 Liquid Chromatograph system
Analytical column: Keystone® Betasil™ C_{18} 2x50mm, 5µm particle size
Column temperature: 30 °C
Stop Time: 9.0 minutes
Flow rate: 300 µL/min
Injection volume: 2 or 5 µL
Mobile phase components:
Solvent A: 2.0 mM ammonium acetate in ASTM Type I water
Solvent B: HPLC Grade Methanol
Solvent Gradient:

Time	%B
0.00	15%
0.50	15%
3.00	100%
5.50	100%
6.00	15%
9.00	15%

Mass Spectrometer: Hewlett-Packard® Series 1100 API/Mass Spectrometer Detector
Software: Agilent ChemStation™ A.08.03
Capillary Voltage: 4000 V
Gain = 1.0 EMV
Mode: Electrospray Negative
Gas Temperature: 350 °C
Drying Gas: 8.0 L /min.
Nebulizer Pressure: 30 psig
Analysis Type: Single Ion Monitoring (SIM)

Compound	Ion (m/z)	Fragmentor (volts)
SLS	265	80
		120
PFBS	299	80
		100
		90
		100
		120
		70
		140
		80

ANALYTICAL RESULTS

For a detailed listing of analytical results, refer to Attachment B: Data Tables. The analytical results consist of data taken from 10 analytical sequences.

- **Regressions.** Quadratic curve fits were applied to calibration standards and sample data to improve quantitation over the concentration range appropriate to the data. All calibration curves had least-square fits of 0.990 or greater.
- **Calibration Standards.** Standards ranging in concentration from approximately 2.5 to 750 ng analyte per mL tetrahydrofuran were used for the calibration curves. Calibration curves were originally prepared in mineral salts medium and extracted using SPE cartridges. High/low calibration standards that were not within the 80%-120% (70%-120% for the lower limit of quantitation) criteria were deactivated with the exception of [ ] on 11/19/02 and [ ] on 12/05/02. For [ ] the 25.23 ng/mL standard (the lowest standard in the curve) had a recovery of 60%. This standard was kept in order to maintain a five point calibration curve. Since the 25.23 ng/mL [ ] standard had an area count of 10866 and the highest sample area count for this analysis was 3475, the 25.23 ng/mL standard was accepted as the lower limit of quantification for this analysis. For [ ] the 10 ng/mL standard was kept in order to keep the 25 ng/mL standard within the 70%-120% criteria for the LLOQ. The LLOQ for this analysis was raised to 25 ng/mL. There should be no adverse affect on the data.

- **Continuing Calibration Verification (CCV).** At least one calibration check was analyzed at least every ten samples to monitor instrumental drift. All continuing calibration verification checks were within 70%-120% with the exception of the analysis on 11/19/02. For all analytes the 10 ng/mL and 50 ng/mL standards were preceded by a matrix spike, which had at least a nominal concentration of 500 ng/mL. For and PFBS, , due to this high concentration, analyte carryover occurred which caused the 10 ng/mL CCV to be >120% and for just (2 out of 3) and (1 out of 3) the 50 ng/mL CCV was >120%. However, the next CCV run immediately after the >120% CCVs was within the limits as specified by the protocol. The 10 ng/mL CCV for was below the lower limit of quantitation (25.23 ng/mL) for this analysis. Since all of the samples were either below the lower limit of quantitation, diluted and reanalyzed, or had acceptable precision (≤20%), the results were accepted. There should be no adverse affect on the data.

Table 6: CCV Summary from 11/19/02 analysis

	Continuing Calibration Verification Standards (ng/mL)								
Analyte	10	50	250	10	50	250	10	50	250
	126%	117%	94%	124%	116%	92%	138%	116%	91%
	193%	107%	107%	169%	106%	104%	166%	105%	103%
	258%	125%	91%	223%	123%	91%	213%	120%	89%
	182%	116%	105%	266%	124%	107%	238%	118%	104%
	NR*	107%	101%	NR*	119%	97%	NR*	104%	97%

*NR: Not reported for this analyte. The CCV concentration is below the LOQ for this data set.

- **Lower Limit of Quantitation (LLOQ).** The LLOQ was equal to the lowest standard in the calibration curve, with a level of accuracy within 70%-120% with the exception of on 11/19/02 as noted above. The level of analyte in the LLOQ was also greater than two times the response of analyte in the blank samples.
- **System Suitability.** Five system-suitability standards were analyzed before the initial calibration curve. The criterion of <5% relative standard deviation (RSD) for the mean repeatability was achieved except for (8.12%) on 11/19/02 and (5.2%) on 3/5/03. For the samples were accepted since all of the samples were either less than the LLOQ or the samples had to be diluted and reanalyzed because they were above the upper limit of quantitation. For the samples were accepted since all of the accepted samples had acceptable precision and the CCVs for the analysis passed. The criterion of <2% RSD for the retention time repeatability was achieved for each analyte.

- **Duplicate Frequency / Acceptable Precision.** Precision was determined for each set of duplicate or triplicate samples. Out of 160 sets of samples, 152 of the sample sets had precisions of ≤ 20%. Eight out of the 160 sample sets had precisions >20% and are described in Table 9. The >20% precision results for PFBS, and appear to follow the trends of the other time points and are not suspected to have an adverse affect on the data. For the sample precision was 132%. It is suspected that there was some sample contamination in the two samples used for the precision determination since three out of the five samples for this time point were below the lower limit of quantitation. Refer to attachment B for individual precision results.

Table 7: Out of specification precision results

Analyte	Medium	Day	Relative Standard Deviation
	A	14	24.6%
	A	14	37.8%
	B	14	38.3%
	B	14	26.7%
	B	28	22.2%
	B	28	34.5%
	C	28	25.7%
	C	28	132%

- **Matrix Spikes.** Samples were spiked post-extraction at a nominal concentration of 500 ng/mL for all reference compounds except , which was not available at the time. Percent recovery was determined for duplicate pairs of matrix spikes. Out of 60 pairs of matrix spikes, 47 were within 70%-120%. Thirteen of the 60 pairs of matrix spikes were not within 70%-120% are indicated in table 8.

Table 8: Out of Specification Matrix Spikes

Analyte	Medium	Day	Percent Recovery
PFBS	A	4	132%
	A	0	154%
	B	0	148%
	C	0	141%
	A	14	150%
	B	14	138%
	C	14	149%
	A	28	136%
	B	28	129%
	C	28	134%
	A	0	168%
	C	14	126%
	C	28	187%

The high spike recoveries for may have occurred due to high concentrations of the analyte in the samples or signal enhancement due to matrix effect. The one high spike recovery for PFBS may be an outlier (using the Grubbs outlier test at the 99% confidence limit). For , it is unknown why the matrix spike recoveries were so high (ranging from 111%-154% with an average of 135%). Since the Laboratory Control Spikes had very good recoveries (ranging from 94.1% - 105%) it is not expected that these high matrix spike recoveries will have an adverse affect on the data. Refer to attachment B for individual matrix spike results. No matrix spikes were run since no reference material was available at the time the samples were extracted.

- **Laboratory Control Spikes.** All laboratory control spikes were within 70%-120% recovery. Refer to Attachment B for individual laboratory control spike results. No laboratory control spikes were run since no reference material was available at the time the samples were extracted.
- **Solvent Blanks.** All solvent blanks were less than ½ the lower limit of quantitation.
- **Matrix Blanks. All matrix blanks were less than ½ the lower limit of quantitation.**

DATA SUMMARY

The following tables are summaries of the percent of theoretical degradation of each analyte in each medium. The percent theoretical degradation is based on the amount of fluorine available in
The percentages reported in Tables 9, 10, and 11 are from the average amount of analyte found in the test vessel divided by the theoretical amount if totally biotransformed into that specific analyte. For a summary of individual results, refer to attachment B. The lower limit of quantitation (LLOQ) for each time point varied depending on the dilution required to measure the major analyte for that time point.

Table 9: Medium A (Exposed to Uninhibited Sludge) Percent Degradation Results[1]

Analyte	Day 0	Day 4	Day 14	Day 28
		1.0%	0.40%	<0.15%
	0.84%	114%	58%	63%
	<3.5%	1.2%	0.47%	0.26%
	<0.92%	1.4%	1.9%	2.4%
	<1.0%	<0.20%	0.12%	0.37%
	<0.33%	0.28%	0.97%	2.3%

[1] The percent degradation is based on the theoretical amount of fluorine available in the initial dose of The percentages reported in Table 1 were determined by dividing the average amount of analyte found in each test vessel by the theoretical amount if totally biotransformed into that specific analyte. as calculated using standard.
[3]The analytical accuracy for all analytes is ±20% or better.

Table 10: Medium B Results

Analyte	Day 0	Day 4	Day 14	Day 28
		5.7%	1.3%	0.63%
	<4.0%	N/A[2]	35%	32%
	<3.5%	1.3%	0.64%	0.35%
	<0.92%	0.60%	1.3%	1.3%
	<1.0%	<0.20%	<0.081%	<0.081%
	<0.33%	0.045%	0.092%	0.10%

as calculated using
[2]N/A: Not analyzed [3]The analytical accuracy for all analytes is ±20% or better.

Table 11: Medium C Results

Analyte	Day 0	Day 4	Day 14	Day 28
	1.4%	3.8%	6.1%	14%
	<4.0%	N/A[2]	<4.0%	3.5%
	<3.5%	0.67%	<3.5%	0.27%
	<0.92%	0.069%	<0.92%	<0.018%
	<1.0%	<0.20%	<1.0%	<0.081%
PFBS[3]	<0.33%	<0.041%	<0.33%	<0.074%

[2]N/A: Not analyzed [3]The analytical accuracy for all analytes is ±20% or better.

The aerobic, aquatic biodegradation of the test substance was observed beginning in the Day 0 sample set. In medium A, by day 0, the test substance degraded to form % of the theoretical value). After Day 0, decreases in concentration as other reference substances start to appear. Medium B shows the same patterns as medium A, however at a slower rate. It appears that the antimicrobial agent only partially inhibited the activity of the microbial population, allowing biodegradation to occur in Medium B at a reduced rate. Medium C showed a different degradation route, which is consistent with the hydrolytic degradation route as shown in a previous study.

The control substance sodium lauryl sulfate (SLS) demonstrated the required degradation in the toxicity control and control substance samples of 70 percent degradation after 14 days. At day 14, SLS had degraded to <5.8% (>94.2%) in the presence of and <8.2% in its' absence, showing that the sludge had good activity and was not inhibited by the test substance.

The control substance showed some disappearance during the coarse of the study. However, it is believed that this can be attributed to retaining on the glass walls of the Erlenmeyer flasks and adsorption onto the sludge as seen from a previous study, " Soil Adsorption/Desorption of

The results of duplicate laboratory control spikes in each of the three media were used to define the accuracy of results. Each laboratory control sample consisted of reference substance spiked in control media with subsequent extraction using SPE.

Table 12: Accuracy of Analytical Results

Analyte	Accuracy
PFBS	±6%
	±16%
	±6%
	±2%
	±6%
	±4%[1]

[1]Because the analytical standard was not available when the lab control spikes were prepared, the analytical accuracy was derived from results obtained from lab control spikes from study , "Inherent Aerobic Aquatic Biodegradability

Table 11: Medium C Results

Analyte	Day 0	Day 4	Day 14	Day 28
	1.4%	3.8%	6.1%	14%
	<4.0%	N/A[2]	<4.0%	3.5%
	<3.5%	0.67%	<3.5%	0.27%
	<0.92%	0.069%	<0.92%	<0.018%
	<1.0%	<0.20%	<1.0%	<0.081%
PFBS[3]	<0.33%	<0.041%	<0.33%	<0.074%

[2]N/A: Not analyzed [3]The analytical accuracy for all analytes is ±20% or better.

The aerobic, aquatic biodegradation of the test substance was observed beginning in the Day 0 sample set. In medium A, by day 0, the test substance degraded to form % of the theoretical value). After Day 0, decreases in concentration as other reference substances start to appear. Medium B shows the same patterns as medium A, however at a slower rate. It appears that the antimicrobial agent only partially inhibited the activity of the microbial population, allowing biodegradation to occur in Medium B at a reduced rate. Medium C showed a different degradation route, which is consistent with the hydrolytic degradation route as shown in a previous study.

The control substance sodium lauryl sulfate (SLS) demonstrated the required degradation in the toxicity control and control substance samples of 70 percent degradation after 14 days. At day 14, SLS had degraded to <5.8% (>94.2%) in the presence of and <8.2% (>91.8%) in its' absence, showing that the sludge had good activity and was not inhibited by the test substance.

The control substance showed some disappearance during the coarse of the study. However, it is believed that this can be attributed to retaining on the glass walls of the Erlenmeyer flasks and adsorption onto the sludge as seen from a previous study, " Soil Adsorption/Desorption of

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Table 12: Accuracy of Analytical Results

Analyte	Accuracy
PFBS	±6%
	±16%
	±6%
	±2%
	±6%
	±4%[1]

[1]Because the analytical standard was not available when the lab control spikes were prepared, the analytical accuracy was derived from results obtained from lab control spikes from study , "Inherent Aerobic Aquatic Biodegradability

STATISTICAL METHODS AND CALCULATIONS

The standard curve is subjected to a quadratic regression calculation. Additional statistical methods were limited to calculating means, standard deviations and relative standard deviations.

Theoretical Percent Degradation

The theoretical percent degradation was calculated based on the total amount of fluorine available from the test substance that could biotransform completely to a specific analyte. This calculation was as follows:

Total available fluorine was determined by:

$$\text{Total Available Fluorine } (\mu g) = C \times V \times F$$

where:

C = Concentration of in test vessel (μg/mL)

V = Volume of Sample (mL)

F = Percent Fluorine Composition of

Next, the percentage of fluorine was determined from the molecular weight from a reference substance:

$$\text{Percent Fluorine} = \frac{\text{Number of fluorine atoms} \times 18.998 \frac{g}{mol}}{\text{molecular weight of compound} \frac{g}{mol}}$$

The total amount of the reference compound that could be formed from the available amount of fluorine was determined by:

$$\text{Theoretical Amount} \frac{\mu g}{mL} = \frac{\text{Total Available Fluorine } (\mu g)}{\text{Percent Fluorine}} \times \frac{1}{\text{Extraction volume (ml)}}$$

$$\text{Percent Degradation} = \frac{\text{Amount of Analyte in Sample} \frac{ng}{mL}}{\text{Theoretical Amount} \frac{ng}{mL}}$$

For example using [ ]

[mol

Theorectical Amount]

So, for Day zero, medium A the percent degradation would be:

$$\text{Percent Degradation} = \frac{[\ \ \frac{ng}{mL}}{[\ \ \frac{ng}{mL}} = \quad \%$$

STATEMENT OF CONCLUSION

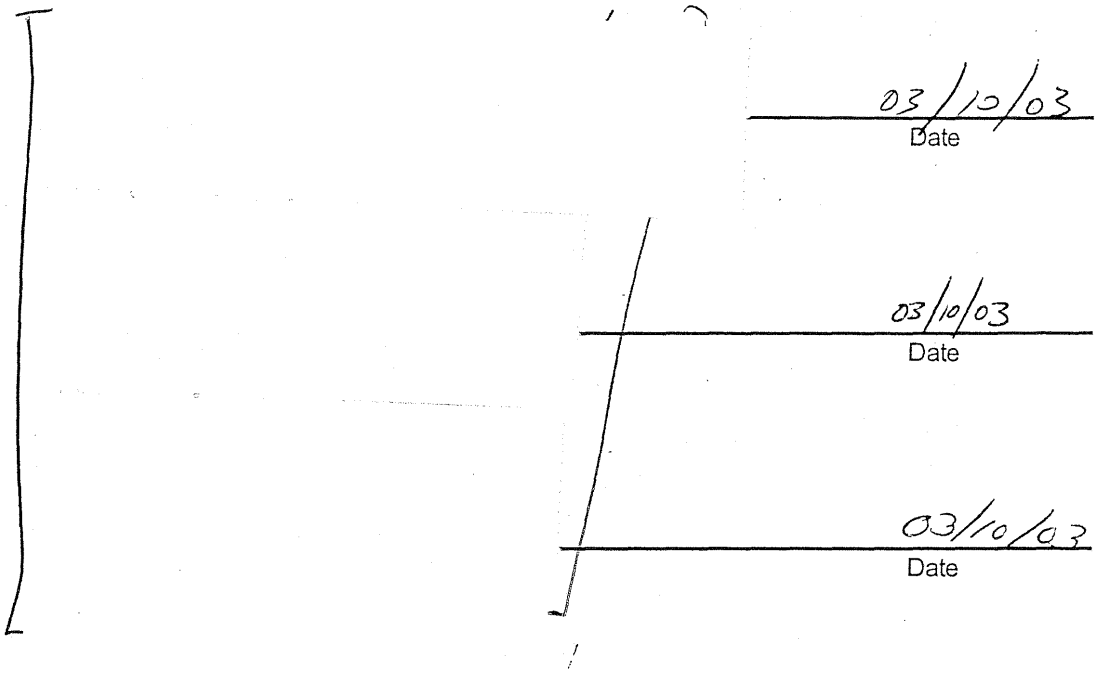
The analytical results demonstrate that under the conditions of the study, the test substance [ ] is biodegraded (OPPTS 835.3210 "Modified SCAS Test" defines a greater than 70% loss of starting material as ultimate biodegradability). The major metabolites identified from the test cultures were [ ] (representing 26% degradation of the initial concentration of [ ] on day 0) and [ ] (representing 114% degradation of the initial concentration of [ ] on day 4). Observed minor metabolites include [ ] and PFBS. Complete mass balance between parent material and measured degradation products appears to have been achieved on day 4.

LIST OF ATTACHMENTS

- **Attachment A: Extraction and Analytical Methods**
- **Attachment B: Data Tables**
- **Attachment C: Sample Chromatograms**
- **Attachment D: Test Substance Information**
- **Attachment E: Protocol, Protocol Amendments and Deviations**

SIGNATURE PAGE

We certify that this report is a true and complete representation of the data for this study:



ATTACHMENT A: EXTRACTION AND ANALYTICAL METHODS

3M Environmental Laboratory

Method

Solid Phase Extraction of Soils, Sediments and Sludges

Method Number: ETS-8-39.0

Adoption Date: Upon Signing

Effective Date: Upon Signing

Approved By:

11/18/02
Date

1 Scope and Application

This is a performance-based method that describes the extraction of target analytes from soil, sediment, sludge, or solutions thereof, using solid phase extraction (SPE) and either extracted matrix or unextracted matrix (solvent) calibrations. This method may also be extended to other matrices provided that the data quality objectives are met.

2 Method Summary

An amount of soil, sediment or sludge, wet or dry, is prepared in an aqueous 1% solution of acetic acid. The sample is capped, mixed, and put on the centrifuge to clarify the supernatant, if needed. The supernatant is passed through a pre-conditioned C_{18} SPE column, at which time the analytes are adsorbed onto the stationary phase. Finally, the analytes of interest are eluted from the SPE cartridge and analyzed using LC/MS or LC/MS/MS.

3 Definitions

3.1 SPE cartridge

A column containing an open solvent reservoir at one end and packed with bonded silica or polymer sorbents at the other end. It is designed to retain the compounds of interest under some solvent conditions and elute them under others. A separation is thus achieved; compounds can be removed from difficult matrices and introduced into appropriate solvents for analysis.

3.2 Reagent grade water

Water with no detectable target analyte.

4 Warnings and Cautions

4.1 Health and Safety Warnings

Always wear appropriate gloves, eyewear, and clothing when working with solvents, samples and/or equipment.

4.2 Cautions

Take care not to allow the SPE column to run to dryness after the methanol and water pre-conditioning steps. After the SPE column is conditioned, add sample and then allow the column to run to dryness.

5 Interferences

Contaminants in solvents, reagents, glassware and other sample processing or other analysis hardware may cause interference. The routine analysis of laboratory method blanks must be used to demonstrate that there is no interference under the conditions of the analysis.

6 Instrumentation, Supplies, and Materials

6.1 Instrumentation

Vortex mixer

Vacuum Pump

SPE Extraction Manifold

Centrifuge

Shaker

Balance (+/- 0.1000 g)

Solvent trap

6.2 Supplies and Materials

Disposable pipettes, plastic or glass

Volumetric flasks, glass, type A

Vials, various sizes and materials, as appropriate

Centrifuge tubes, various sizes and materials as appropriate

Labels

Syringes, graduated

Bottle-Top Dispenser

SPE extraction cartridge, 1 g, Sep-Pak 6 cc tri-functional C_{18} (Waters), or equivalent

Other SPE extraction cartridges, characteristics to be determined by analyst

Crimp cap glass autovials and caps

Crimpers

7 Reagents and Standards

Reagent grade water

Acetonitrile, HPLC grade or equivalent

Methanol, HPLC grade or equivalent

Tetrahydrofuran, HPLC grade or equivalent

Acetic Acid, glacial, ACS grade or equivalent

8 Sample Handling

Samples should be stored according to instructions from the study director, manufacturer, chain of custody form, or as determined by the analyst. Storage conditions will be documented.

Allow samples to equilibrate to room temperature prior to extraction.

Typically fresh matrix standards are prepared with each study. Extracted standards and samples are stored in capped autovials until analysis.

If analysis will be delayed, extracted standards and samples may be refrigerated at approximately 4°C for up to 6 months, or may be stored at room temperature. If the samples are not analyzed immediately, post-extraction control samples may be evaluated to demonstrate extract stability.

9 Method Performance Data Quality Objectives

Application of the method allows for usage of either an extracted matrix calibration or an unextracted matrix calibration (external solvent calibration). The following method quality control performance criteria will be met in the application of the method:

9.1 Extracted Matrix Calibration

An extracted calibration curve will be prepared from extracted matrix standards, in the same matrix as the samples, and analyzed before each analytical set. It will consist of a minimum of nine (9) levels and may include a blank.

The equation of the calibration curve will be determined by regression analysis using the peak areas of the analyte. The accuracy of each level will be verified. Any level outside ±25% deviation from nominal must be deactivated and the regression recalculated, except the LLOQ which must be within ±30% of nominal. All levels must show a response greater than twice that of the blank.

9.2 Unextracted Solvent Calibration (external calibration)

A calibration curve of a minimum of nine (9) levels from unextracted solvent standards will be prepared, and may include a blank.

The standard curve equation will be determined by regression analysis using the peak areas of the analyte. The accuracy at each level will be verified. Any level outside ±25% deviation from nominal must be deactivated and the regression recalculated, except the LLOQ which must be within ±30% of nominal. All levels must show a response greater than twice that of the blank.

9.3 Limits of Quantitation (LOQ)

The lower level of quantitation (LLOQ) for each target analyte is determined by the lowest calibration level that shows a recovery of ±30% for the target analyte and a response greater than twice that of the blank. Should the LLOQ level calibration point be deactivated in a particular set the practical limit of quantitation for this set will be raised to the next acceptable level.

9.4 Continuing Calibration Verification (CCV)

For both extracted and unextracted calibration curves, the continued accuracy of the calibration will be verified. This will be accomplished by the re-injection of one of the original curve points, preferably one in the mid-range of the curve. A maximum of ten (10) samples may be injected before the injection of a CCV, and then more samples may be analyzed. A CCV is then analyzed again at the end of the sample set. The CCV's must show a recovery within ±25% to be considered acceptable. Samples will then be bracketed by the calibration curve and acceptable CCV's.

9.5 Blanks

9.5.1 Solvent Blank

An aliquot of methanol, or other appropriate solvent, is used as a solvent blank. Solvent blanks are not extracted.

9.5.2 Method Blank

A 1.0 mL aliquot of water, or other appropriate amount, is used as a method blank. Method blanks are extracted and analyzed with each set following this procedure.

9.5.3 Matrix Blank

An aliquot of matrix (wet or dry, as appropriate) is extracted and analyzed to determine the endogenous level of target analyte(s) in the matrix, if any. The frequency and exact makeup of the matrix blanks will depend on the scope of the study and the availability of additional matrix. Specific requirements, if any, will be addressed in the study protocol and/or the raw data.

9.6 Sample Replicate

Samples replicates are prepared according to each study protocol or will be documented in the raw data.

9.7 Surrogate standard

If surrogate standard is a component of the study, all samples are spiked with surrogate standard prior to extraction, with the exception of blank samples.

9.8 Internal standard

If internal standard is a component of the study, all samples are spiked with internal standard after extraction to obtain a concentration in the mid-range of the calibration curve.

9.9 Pre-Extraction Matrix Spike

Pre-extraction matrix spikes consist of matrix spiked with a known amount of target analyte and extracted in the same manner as the study samples. The amount of target analyte present in the matrix prior to the addition of the spike must be known. This may be accomplished by the concurrent extraction of an additional aliquot of the same matrix. Analysis of these samples provides a measure of extraction efficiency. Recoveries of target analytes should be within 30% of the theoretical concentration. If the samples fail to fall in this range the data for the study samples should be rejected. If the samples are to be accepted sufficient justification for their use should be provided in the report. The number and frequency of pre-extraction matrix spikes will be addressed either by the protocol or the project lead.

9.10 Post-Extraction Control Sample

Post-extraction control samples may be prepared for each set of extracted samples (if applicable to the study) and analyzed to determine extraction efficiency. The samples are prepared by adding additional target analyte to the final extract, and after any dilution.

Post-extraction control sample duplicates may be prepared periodically to measure the precision associated with the analysis. These samples should be analyzed in the same run as the unspiked sample.

Post-extraction control sample concentrations should fall in the mid-range of the initial calibration curve or should be prepared at 1.5-5 times the endogenous concentration of the analyte. Spike concentrations should fall in the low-range of the initial calibration curve if extremely low levels are expected.

9.11 Sample Dilution

Any sample extract with an area greater than that of the highest acceptable calibration standard will need to be diluted into the range of the calibration curve. All dilutions of the extract will be documented in the raw data.

10 Procedures

10.1 Soil Sample Preparation and Extraction

Weigh approximately 1g of sample, or other appropriate amount, into a tared sample vessel. Record this weight on the appropriate data form. Return the unused portion of the sample to storage. Spike target analyte(s) into the soil as appropriate.

Add 25mL of 1% acetic acid in reagent water, or other appropriate amount, to the sample vial. Record the volume of solution added to the sample on the appropriate data form.

Cap and mix the contents of the vessel thoroughly. Centrifuge the vessel until the solution becomes clear, if appropriate. Solutions containing less than 5% soil may not require the centrifugation step.

Attach the reservoir to the SPE cartridge and attach this reservoir/cartridge unit to a vacuum manifold.

Condition the SPE cartridge by washing twice with at least 5mL of methanol, or other appropriate solvent, followed by at least two 5mL aliquots of aqueous 1% acetic acid, **taking care not to allow the column to run to dryness after each wash.**

NOTE: Depending on the nature of the target compounds, the original sample filtrate may need to be collected and analyzed. If this is the case, place collection vessels under each cartridge at this point, prior to the addition of the sample.

After conditioning is complete, decant the aqueous portion of the sample into the reservoir/cartridge unit and **allow all of the liquid to pass through the column to dryness**. Take care not to pour the wet soil into the cartridge, it will hamper the progress of liquids through the column.

Run the vacuum on high for at least 2 minutes to adequately dry each SPE cartridge.

Add 5mL, or other appropriate amount, of methanol, or other appropriate solvent, to the original sample vessel (where the soil remains), recording the volume on the appropriate data sheet. Mix contents thoroughly and centrifuge until the contents become clear. Place collection vessels under each cartridge and decant the solvent into the cartridge as above, again allowing the column to go to dryness. Repeat this solvent elution step with fresh collection vessels if needed.

If an internal standard is desired, **transfer a known amount** of eluate into an appropriate sample vial, then add the appropriate amount of internal standard.

Transfer remaining samples into the appropriate vials for the type of analysis being carried out, if needed.

10.2 Fluid Sample (Suspended Sludge) Preparation

Fluid samples should be at approximately room temperature for preparation.

Vortex mix the fluid sample for approximately 15 seconds. Determine the volume of the original sample to extract. Record this volume on the appropriate data form. Return the unused portion of the sample to storage. If additional dilution of the sample is desired at this stage, add water and record the volume on the data form. Spike target analyte(s) into the aqueous solution as appropriate.

Add glacial acetic acid to the sample vial to reach a nominal concentration of 1%. Record the volume of acid added to the sample on the appropriate data form.

Attach the reservoir to the SPE cartridge and attach this reservoir/cartridge unit to a vacuum manifold.

Condition the SPE cartridge by washing twice with at least 5mL of methanol, or appropriate solvent, followed by at least two 5mL aliquots of aqueous 1% acetic acid, **taking care not to allow the column to run to dryness after each wash.**

NOTE: Depending on the nature of the target compounds, the original sample filtrate may need to be collected and analyzed. If this is the case, place collection vessels under each cartridge at this point, prior to the addition of the sample.

After conditioning is complete, decant the sample into the reservoir/cartridge unit and **allow all of the liquid to pass through the column to dryness**. Samples containing a high percentage of sludge may necessitate the addition of a plug of glass wool on top of the SPE packing material to prevent the plugging of the column.

Run the vacuum on high for at least 2 minutes to adequately dry each SPE cartridge.

Add 5mL, or other appropriate amount, of methanol, or other appropriate solvent, to the original sample vessel, recording the volume on the appropriate data sheet. Mix contents thoroughly and centrifuge until the contents become clear. Place collection vessels under each cartridge and decant the solvent into the cartridge as above, again allowing the column to go to dryness. Repeat this solvent elution step with fresh collection vessels if needed.

If an internal standard is desired, **transfer a known amount** of eluate into an appropriate sample vial, then add the appropriate amount of internal standard.

Transfer remaining samples into the appropriate vials for the type of analysis being carried out, if needed.

11 Data Analysis and Calculations

11.1 Calculations

If other calculations are used than those listed, they will be documented in the raw data.

Calculate the total sample Dilution Factor:

Dilution Factor (DF) = {(OW + DW)/OW} x (EV/SV) x any additional dilution of final eluate

Calculate theoretical concentrations of analyte in final eluate:

Concentration = (Concentration of Analytical Standard x Volume of standard added) / EV

Convert observed result to original sample result:

Original sample result = Observed result x DF

Calculate spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result - Matrix Blank}}{\text{Theoretical Concentration}} \times 100$$

OW = Original sample weight

DW = Diluent weight, assume density of water = 1

EV = Eluate volume (volume of final extract)

SV = Sample volume removed for extraction

DF = Dilution factorCalculate relative standard deviation using the following equation:

$$\text{Relative Standard Deviation} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

Calculate percent deviation using the following equation:

$$\% \text{ Deviation} = \frac{\text{Theoretical Conc. - Measured Conc.}}{\text{Theoretical Conc.}} \times 100$$

12 Pollution Prevention and Waste Management

Sample waste is disposed of in low BTU containers.

Flammable solvent waste is disposed of in high BTU containers.

Glass pipette waste is disposed of in broken glass containers located in the laboratory.

13 Records

Complete the extraction worksheet attached to this method, or other applicable worksheet, and store with the study raw data.

Summarize data using suitable software and store in the study folder.

14 Attachments

Attachment A: Samples of Extraction Worksheets

15 References

None

16 Affected Documents

None

17 Revisions

Revision Number	Revision Description	Revision Date

Attachment A: Samples of Extraction Worksheets

Soil Extraction Worksheet, Method ETS-8-xxx.0

Study Number _____ Type of Column Used _____

Sample ID	Sample Description	Sample Weight, g	Spike Added, uL	Volume of 1% Acetic Acid Added, mL	Cap and Mix Vessel	Centrifuge? Y/N	Precondition Extraction Column	Pass Sample through cartridge. Collect Aqueous portion? Y/N	Volume of Methanol added to Original Soil Sample, mL	Cap and Mix Vessel	Centrifuge? Y/N	Pass sample through cartridge, collect eluate	Repeat Solvent Extraction (Columns 10-13)? Y/N	Additional Dilution, if Needed	Addition of Internal standard, if needed, uL
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Exx-xxxx-xxxxxx															
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Exx-xxxx-xxxxxx															

Col 3: Balance ID
T/D/I_____

Col 4: Standard ID
Standard Concentration:_____
Amount Standard Added:_____
T/D/I_____

Col 5: Standard ID:
Volume Added:_____
T/D/I_____

Col 6: T/D/I

Col 7: Centrifuge ID:
Speed:_____rcf/rpm
Duration:_____
T/D/I

Col 8: T/D/I

Col 9: T/D/I

Col 10: MeOH ID:
Volume added:_____
T/D/I_____

Col 11: T/D/I

Col 12: Centrifuge ID:
Speed: rcf/rpm
Duration:_____
T/D/I

Col 13: T/D/I

Col 14: T/D/I

Sample Extract Storage

Samples stored in refrigerator/freezer ID_____ at _____degrees C until analysis. T/D/I_____

Attachment A: Samples of Extraction Worksheets

Fluid Sample Extraction Worksheet, Method ETS-8-39.0

Study Number _____ Type of Column Used _____

Sample ID	Sample Description	Sample Volume, mL	Additional Water Added, mL, if desired	Spike Added, uL	Volume of Glacial Acetic Acid Added, mL	Cap and Mix Vessel	Precondition Extraction Column	Pass Sample through cartridge. Collect Aqueous portion? Y/N	Elute sample with ____ mL of MeOH	Elute sample a second time? No, Yes-with ____ mL of MeOH	Additional Dilution, If Needed	Addition of Internal standard, If needed, uL	Comments
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Exx-xxxx-xxxxxx													
Exx-xxxx-xxxxxx													
Exx-xxxx-xxxxxx													
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Col 3: T/D/I

Col 4: Water ID
Amount Water Added:_____
T/D/I_____

Col 5: Standard ID:
Volume Added:_____
T/D/I_____

Col 6: Standard ID:
Volume Added:_____
T/D/I_____

Col 7: T/D/I

Col 8: T/D/I

Col 9: T/D/I

Col 10: MeOH ID:
Volume added:_____
T/D/I_____

Col 11: MeOH ID:
Volume added:_____
T/D/I_____

Col 12: Diluter ID:
Volume of sample added:_____
Volume of Diluent Added:
Diluent ID_____
Final Dilution Factor:
T/D/I_____

Col 13: Internal Standard ID
Internal standard Conc._____
Amount Added:_____
Volume of sample:
IS Concentration_____
T/D/I_____

Sample Extract Storage
Samples stored in refrigerator/freezer ID_____ at _____degrees C until analysis. T/D/I_____

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF WASTE STREAM, WATER EXTRACTS OR OTHER SYSTEMS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-155.1

Adoption Date: 11/10/00

Revision Date: 5/30/01

Author:

Approved By:

5/30/01
Date

5/30/01
Date

1.0 SCOPE AND APPLICATION

1.1 **Scope:** This method describes the analysis of waste stream, water samples or other systems using HPLC-electrospray/mass spectrometry.

1.1.1 Specific analytes, ions, matrices, solvents, solutions, quality controls, internal standard(s) and other parameters will be defined in the protocol or the sample preparation worksheet(s).

1.2 **Applicable Compounds:** Electrospray ionizable compounds.

1.3 **Matrices:**

1.3.1 Waste Streams and other systems may consist of aqueous and/or organic solvent systems or as designated in the protocol or sample preparation worksheet(s).

1.3.2 Water Samples include tap water, ground water, wastewater and other aqueous solutions. The matrix will be defined by the protocol or sample preparation worksheet(s).

2.0 SUMMARY OF METHOD

2.1 This method describes the analysis of electrospray ionizable compounds, using HPLC-electrospray mass spectrometry (HPLC-ES/MS). The analysis is performed by the mass selection of a single ion characteristic of a particular compound.

3.0 DEFINITIONS

3.1 **Atmospheric Pressure Ionization (API):** Commercially available HPLC-ES/MS single quadrupole systems allow for various methods of ionization by utilizing a variety of sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APcI), Thermospray, etc. The ionization in these processes occurs at atmospheric pressure (i.e., not under a vacuum).

3.2 **Electrospray Ionization (ES, ESI):** A method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These droplets are produced by the application of a strong electrical field.

3.3 **Mass Spectrometer (MS):** Commercially manufactured MS systems are equipped with single quadrupole mass selective detectors. Ions are selected on the basis of mass to charge ratio (m/z) and subsequently detected.

3.4 **Micromass MassLynx / HP ChemStation Software:** System software designed for the specific operation of an HPLC-ES/MS. Please refer to the manual for the specific instrument software.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

4.1.1 Use caution with the voltage cables for the electrospray probe. When engaged, the probe employs a voltage of approximately 5000 Volts.

4.1.2 When handling samples or solvents wear appropriate protective clothing, gloves, and eyewear.

4.2 Cautions:

4.2.1 Operate solvent pumps below a backpressure of 400 bar (5800 psi). If the backpressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.

4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

5.1 To minimize interferences when analyzing samples, Teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6.0 EQUIPMENT

6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.

6.1.1 Micromass Platform LCZ Mass Spectrometer (or equivalent) equipped with an electrospray ionization source.

6.1.2 HP1100 low pulse solvent pump, solvent degasser, column compartment, and autosampler or equivalent HPLC system.

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

7.1.1 High purity grade nitrogen gas regulated to approximately 100 psi (or house air system.).

7.1.2 HPLC analytical column, such as a Betasil C18 column (50x2mm, 5 µm particle size) or equivalent.

7.1.3 Capped autovials or capped 15 mL centrifuge tubes.

8.0 REAGENTS AND STANDARDS

8.1 Reagents:

8.1.1 Methanol, HPLC grade or equivalent.

8.1.2 Milli-Q™ water (ASTM type I), all water used in this method should be Milli-Q™ water or equivalent, and may be provided by a Milli-Q TOC Plus system or another vendor.

8.1.3 Ammonium acetate, reagent grade or equivalent.

8.1.3.1 When preparing different amounts than those listed, adjust accordingly.

8.1.3.2 2.0 mM ammonium acetate solution: Weigh approximately 0.300 g ammonium acetate. Pour into a 2000 L volumetric flask, add the appropriate volume of Milli-Q water, mix until all solids are dissolved. Store at room temperature.

8.1.4 Other solvents and solutions may be used as stated in the protocol or the sample preparation worksheet.

8.2 Calibration Standards:

8.2.1 Typically two method blanks (Milli-Q water), two matrix blanks, and a minimum of 5 solvent standards are analyzed with each group of samples.

9.0 SAMPLE HANDLING

9.1 Standards and prepared samples may be stored in capped autovials, capped 15 mL centrifuge tubes or other suitable containers until analysis.

9.2 If analysis will be delayed, standards and prepared samples may be refrigerated at approximately 4° C until analyses can be performed (refrigerator temperatures may have a detrimental affect on the solubility of saturated solutions).

10.0 QUALITY CONTROL

10.1 Blanks:

10.1.1 Solvent blanks, method blanks, and matrix blanks are prepared and analyzed with each sample set to determine contamination or carryover.

10.1.1.1 When the study matrices consist of highly purified solvents such as Type 1 water or HPLC grade organic solvents, the method and matrix blanks may be represented by a single solvent blank.

10.1.2 Solvent blanks should be analyzed prior to each calibration curve. Method blanks and matrix blanks should be analyzed after the initial calibration curve but prior to the study samples. If carryover is a problem, consecutive solvent blanks may be necessary.

10.2 Matrix Spikes:

10.2.1 Matrix spikes may be prepared for each set of extracted samples (if applicable to the study) and analyzed to determine extraction efficiency.

10.2.2 Matrix spike duplicates may be prepared periodically to measure the precision associated with the analysis.

10.2.3 Analyze the matrix spike and matrix spike duplicate (if prepared) in the same run as the original sample.

10.2.4 Matrix spike and matrix spike duplicate concentrations should fall in the mid-range of the initial calibration curve or should be prepared at 1.5-5 times the endogenous concentration of the analyte. Spike concentrations should fall in the low-range of the initial calibration curve if extremely low levels are expected.

10.3 Continuing Calibration Verifications:

10.3.1 Continuing calibration verifications (CCV) are analyzed to verify the continued accuracy of the calibration curve.

10.3.2 Analyze a mid-range calibration standard after every tenth sample, with a minimum of one per sample set.

10.4 Internal Standard/Surrogate Standard:

10.4.1 An internal standard (IS) may be used to quantify the target analytes by establishing a relationship between the ratio of analyte response to IS response and a known concentration of the analyte of interest. The IS should be spiked at an amount that will fall within the mid-range of the calibration curve. The IS should be added after the extraction process and before analysis.

10.4.2 A surrogate standard may be used for quality control. The surrogate is used to quantitatively evaluate the entire analytical procedure including sample preparation and analysis. The surrogate should be spiked at the beginning of the sample preparation and should fall within the low to mid-range of the calibration curve.

11.0 CALIBRATION AND STANDARDIZATION

11.1 Analyze the standard curves prior to and following each set of samples. The average of two standard curves may be plotted by linear regression ($y = mx + b$) weighted 1/x, or quadratic fit ($y = ax^2 + bx + c$) using MassLynx or other suitable software. The calibration curves should not be forced through zero.

11.1.1 The closing calibration curve may be excluded if the CCV's meet acceptable criteria. If only the first curve is used, the calibration and standardization parameters are the same.

11.2 If the initial calibration curve does not meet acceptance criteria perform routine maintenance or prepare a new standard curve (if necessary) and reanalyze.

11.3 For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range. Example: when

attempting to quantitate approximately 10 ppb of analyte, generate a calibration curve consisting of the standards from 5 ppb to 100 ppb rather than the full range of the curve (5 ppb to 1000 ppb). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

11.4 High and/or low points may be excluded from the calibration curves to provide a better fit over the linear range appropriate to the data or because they did not meet the pre-determined acceptance criteria. Low-level curve points should also be excluded if their area counts are not at least twice that of the method blanks. Justification for exclusion of calibration curve points will be noted in the raw data.

11.4.1 A minimum of 5 points will be used to construct the calibration curve.

12.0 PROCEDURES

12.1 Acquisition set-up – please reference the SOP that pertains to the specific instrument. Actual parameters will be recorded on the instrument printouts.

12.1.1 Set up the sample list.

12.1.1.1 Assign a sample list filename using the first letter of the name of the instrument (T for Tucker), the year (00 for 2000), the month (04 for April), and the day (T001012 for October 12, 2000 on Tucker). If more than one list is made on the same day, use increasing letters of the alphabet starting with A at the end of the list.

12.1.1.2 Assign a method (MS) file.

12.1.1.3 Assign an HPLC program (Inlet file).

12.1.1.4 Type in sample descriptions and vial position numbers.

12.1.2 To create a method, click on method in the Acquisition control panel then mass spectrometer headings and select SIR. Set ionization mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected in addition to the SIRs. Save acquisition method. See the Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information.

12.1.3 Typically the analytical batch run sequence begins and ends with a set of solvent standards.

12.1.4 Samples are analyzed with a continuing calibration verification (CCV) injected after every tenth sample. Solvent blanks should be analyzed periodically to monitor for possible analyte carryover.

12.2 Using the Autosampler/Column Heater

12.2.1 Place sample vials into the sample tray according to the sample list prepared in Section **12.1.1**.

12.2.2 Attach the proper analytical column in the column heater compartment. If using the switching valve, ensure that the tubing is run to the appropriate ports.

12.3 Using the Inlet Editor

12.3.1 Set-up the HP1100 using the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:

12.3.1.1 Sample size = 10 µL injection

12.3.1.2 Flow rate = 300 µL/min.

12.3.1.3 Cycle time = 10.0 minutes

12.3.1.4 Mobile phase components:
Solvent A: 2.0 mM Ammonium Acetate in Water
Solvent B: Methanol (MeOH)
Solvent Gradient:

Time (min.)	% B
0.00	5.00 %
1.00	5.00 %
4.50	95.0 %
8.00	95.0 %
8.50	5.00 %
10.0	Stop

12.4 Instrument Set-up

12.4.1 Refer to the Platform LCZ User's Guide, the MassLynx NT User's Guide, the **ETS-9-36,** "Operation and Maintenance of the Micromass Platform LCZ Electrospray/Mass Spectrometer", or the SOP that pertains to the specific instrument.

12.4.2 Check the solvent level in reservoirs and refill if necessary.

12.4.3 Check the tip of the stainless steel capillary at the end of the probe with an eyepiece. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.

12.4.4 Turn on the nebulizing gas.

12.4.5 Open the tune page. Click on 'Operate' to initiate the desolvation heaters.

12.4.6 Open the Inlet Editor.

12.4.6.1 Set HPLC pump to "On".

12.4.6.2 Set the solvent flow to the desired flow rate.

12.4.6.3 Observe droplets coming out of the tip of the probe. A fine mist should be expelled with no nebulizing gas leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed.

12.4.6.4 Allow to equilibrate for at least 10 minutes.

12.4.7 The instrument uses these parameters at the following settings. These settings may change in order to optimize the response. Actual parameters will be recorded on the instrument printouts.

12.4.7.1 Drying gas 250-425 liters/hour

12.4.7.2 ESI nebulizing gas 10-15 liters/hour

12.4.7.3 HPLC constant flow mode, flow rate 10 – 500 µL/min

12.4.7.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the HPLC is operating correctly.)

12.4.7.5 Source Block temperature 150°.

12.4.7.6 Desolvation temperature 250°.

12.4.8 Print the tune page with its parameters, the Inlet page, sample list, mass spectrometer information, and all other applicable information and store it in the study binder with copies taped into the instrument run logbook.

12.4.8.1 All copies must be initialed and dated.

12.4.9 Click on start button on the MassLynx toolbar. Ensure beginning and ending sample numbers encompass all samples to be analyzed.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations (including, but not limited to):

13.1.1 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result - Background Result}}{\text{Expected Result}} \times 100$$

13.1.2 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc. - Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

13.1.3 Calculate actual concentration of analyte in matrix (µg/mL):

On-Column Concentration (µg/mL) x Dilution Factors = Calculated Concentration

14.0 METHOD PERFORMANCE

14.1 The Limit of Quantitation (LOQ) is method, analyte, and matrix specific. For many analytes, the LOQ concentration is selected as the lowest acceptable non-zero standard in the calibration curve.

14.2 Solvent and method blank area counts must be < ½ that of the lowest standard used in the calibration curve.

14.3 The coefficient of determination (r^2) value for the calibration curve must be greater than or equal to 0.980.

14.4 Continuing Calibration Verification (CCV) percent recoveries must be ± 30% of the standard concentration.

14.5 Internal Standard recoveries should be within ± 50% of the spiked concentration.

14.6 If criteria listed in this method performance section are not met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. Document all actions in the raw data.

14.7 If data is to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

15.1 Sample extract waste and flammable solvent is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

16.1 Each page generated for a study must have the following information included either in the header or hand written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.

16.2 Print the tune page, sample list, acquisition method and all other applicable information to include in the appropriate study folder. Copy these pages and tape into the instrument run logbook.

16.3 Plot the calibration curve then print these graphs and store in the study folder.

16.4 Print data integration summary, integration method, and chromatograms and file in the study binder.

16.5 Summarize data using suitable software and store in the study binder.

16.6 Back-up electronic data to appropriate medium. Record the file names and location of backed-up electronic data in the study binder.

16.7 Documentation of analyte(s) and all reference substances will include trace numbers, lot #'s, purity, expiration, and storage conditions.

17.0 ATTACHMENTS

17.1 None

18.0 REFERENCES

18.1 Platform LCZ User's Guide, Micromass UK Limited, Tudor Road, Altrincham, WA14 5RZ; or Floats Road, Wythenshawe M23 9LZ; United Kingdom.

18.2 MassLynx NT User's Guide, Micromass UK Limited, Tudor Road, Altrincham, WA14 5RZ; or Floats Road, Wythenshawe M23 9LZ; United Kingdom.

18.3 MassLynx NT Guide To Data Acquisition, Micromass UK Limited, Tudor Road, Altrincham, WA14 5RZ; or Floats Road, Wythenshawe M23 9LZ; United Kingdom.

18.4 ETS-9-34.0, "Operation and Maintenance of Hewlett Packard HPLC 1100 System Equipped with a Mass Spectrometer Detector and/or a Photo Diode Array Detector".

18.5 ETS-9-36.0, "Operation and Maintenance of the Micromass Platform LCZ Electrospray/Mass Spectrometer".

19.0 AFFECTED DOCUMENTS

19.1 None

20.0 REVISIONS

Revision Number	Reason For Revision	Revision Date
1	Title Change Section 1: Enlarged the scope of the method by including additional matrices. Also added comments about specific parameters will be defined in the protocol or prep sheets. Sections 1 and 2: Removed references to specific compounds. Section 3: Removed paragraph on Conventional vs. Z-Spray probe interface. Section 3: Changed to allow different systems and software. Section 8: Added paragraph allowing for the use of alternative solvents and solutions. Section 9: Broadened the types of storage containers and conditions. Section 10: Added comments to allow for flexibility in quality controls for different types of studies and applicability. Section 11: Added a paragraph allowing for the excluding of the second curve. Section 11: Added a paragraph (11.4) allowing for the dropping of curve points and minimum number of curve points needed. Section 12: Added comments to reference the specific SOP's for set-up. Section 12: Corrected numbering. Section 16: Added paragraph on records of analytes and substances. Section 18: Added reference to the method for the Hewlett-Packard LC/MS.	09 May 01

ATTACHMENT B: DATA TABLES

Medium A

PFBS

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Average (ng/mL) anion*	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation**
E02-0913-013	Day 0	<50.06	<50.06	<44.28	N/A	N/A	<0.33%
E02-0913-014	Day 0	<50.06					
E02-0913-015	Day 0	<50.06					
E02-0913-043	Day 4	45.09	42.17	37.30	3.398	8.06%	0.28%
E02-0913-044	Day 4	38.44					
E02-0913-045	Day 4	42.98					
E02-0913-103	Day 14	152.4	148.2	131.1	4.790	3.23%	0.97%
E02-0913-104	Day 14	149.3					
E02-0913-105	Day 14	143.0					
E02-0913-163	Day 28	389.4	345.0	305.2	38.58	11.2%	2.3%
E02-0913-164	Day 28	325.6					
E02-0913-165	Day 28	320.0					

* Average was multiplied by a factor of 0.8846 to correct to anion

** Based on the total amount of fluorine in PFBS, F

N/A = Not applicable

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation*
E02-0913-013	Day 0	<490.8	<490.8	N/A	N/A	<3.5%
E02-0913-014	Day 0	<490.8				
E02-0913-015	Day 0	<490.8				
E02-0913-043	Day 4	167.5	165.4	4.406	2.66%	1.2%
E02-0913-044	Day 4	168.3				
E02-0913-045	Day 4	160.3				
E02-0913-103	Day 14	75.95	66.66	8.046	12.1%	0.47%
E02-0913-104	Day 14	61.90				
E02-0913-105	Day 14	62.13				
E02-0913-163	Day 28	29.20	36.90	6.675	18.1%	0.26%
E02-0913-164	Day 28	41.11				
E02-0913-165	Day 28	40.38				

* Based on the total amount of fluorine in

N/A = Not applicable

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation*
E02-0913-013	Day 0	<126.1	<126.1	N/A	N/A	<1.0%
E02-0913-014	Day 0	<126.1				
E02-0913-015	Day 0	<126.1				
E02-0913-043	Day 4	<25.23	<25.23	N/A	N/A	<0.20%
E02-0913-044	Day 4	<25.23				
E02-0913-045	Day 4	<25.23				
E02-0913-103	Day 14	16.57	15.49	0.9347	6.03%	0.12%
E02-0913-104	Day 14	14.89				
E02-0913-105	Day 14	15.02				
E02-0913-163	Day 28	46.24	46.24	1.965	4.25%	0.37%
E02-0913-164	Day 28	44.27				
E02-0913-165	Day 28	48.20				

* Based on the total amount of fluorine in

N/A = Not applicable

Medium A

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation*
E02-0913-013	Day 0	<125.2	<125.2	N/A	N/A	<0.92%
E02-0913-014	Day 0	<125.2				
E02-0913-015	Day 0	<125.2				
E02-0913-043	Day 4	205.2	194.0	10.11	5.21%	1.4%
E02-0913-044	Day 4	185.5				
E02-0913-045	Day 4	191.4				
E02-0913-103	Day 14	257.8	261.2	12.10	4.63%	1.9%
E02-0913-104	Day 14	274.6				
E02-0913-105	Day 14	251.1				
E02-0913-163	Day 28	365.4	320.8	40.72	12.7%	2.4%
E02-0913-164	Day 28	311.6				
E02-0913-165	Day 28	285.5				

* Based on the total amount of fluorine in

N/A = Not applicable

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation*
E02-0913-013	Day 0	4004	4129	254.3	6.16%	26%
E02-0913-014	Day 0	3961				
E02-0913-015	Day 0	4422				
E02-0913-043	Day 4	181.1	161.4	17.17	10.6%	1.0%
E02-0913-044	Day 4	149.6				
E02-0913-045	Day 4	153.5				
E02-0913-103	Day 14	82.02	64.89	15.94	24.6%	0.40%
E02-0913-104	Day 14	50.50				
E02-0913-105	Day 14	62.15				
E02-0913-163	Day 28	<25.09	<25.09	N/A	N/A	<0.15%
E02-0913-164	Day 28	<25.09				
E02-0913-165	Day 28	<25.09				

* Based on the total amount of fluorine in

N/A = Not applicable

Sample	Timepoint	Result (ng/mL)	Times Correction Factor (ng/mL) **	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation*
E02-0913-013	Day 0	<500.0	<665.0	<665.0	N/A	N/A	<4.0%
E02-0913-014	Day 0	<500.0	<665.0				
E02-0913-015	Day 0	<500.0	<665.0				
E02-0913-016	Day 0	<500.0	<665.0				
E02-0913-017	Day 0	<500.0	<665.0				
E02-0913-103	Day 14	6247	8309	10105	3824	37.8%	60%
E02-0913-104	Day 14	12680	16865				
E02-0913-105	Day 14	6508	8656				
E02-0913-106	Day 14	5670	7542				
E02-0913-107	Day 14	6883	9155				
E02-0913-163	Day 28	4717	6274	6221	489.4	7.87%	37%
E02-0913-164	Day 28	4874	6483				
E02-0913-165	Day 28	4051	5388				
E02-0913-166	Day 28	4744	6309				
E02-0913-167	Day 28	5001	6652				

* Based on the total amount of fluorine ir

** A correction factor was calculated to be
and '

N/A = Not applicable

Medium A

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation*
E02-0913-013	Day 0	157.8	141.9	15.78	11.1%	0.84%
E02-0913-014	Day 0	141.5				
E02-0913-015	Day 0	126.3				
E02-0913-043	Day 4	19494	19243	218.2	1.13%	114%
E02-0913-044	Day 4	19133				
E02-0913-045	Day 4	19101				
E02-0913-103	Day 14	9572	9666	133.4	1.38%	58%
E02-0913-104	Day 14	9761				
E02-0913-163	Day 28	10336	10599	1084	10.2%	63%
E02-0913-164	Day 28	11791				
E02-0913-165	Day 28	9670				

* Based on the total amount of fluorine in

N/A = Not applicable

Medium B

PFBS

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Average (ng/mL) anion**	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation ***
E02-0913-018	Day 0	<50.06	<50.06	<44.28	N/A	N/A	<0.33%
E02-0913-019	Day 0	<50.06					
E02-0913-020	Day 0	<50.06					
E02-0913-048	Day 4	8.188	6.938	6.138	1.196	17.2%	0.045%
E02-0913-049	Day 4	5.804					
E02-0913-050	Day 4	6.823					
E02-0913-108	Day 14	*	14.07	12.44	5.381	38.3%	0.092%
E02-0913-109	Day 14	10.26					
E02-0913-110	Day 14	17.87					
E02-0913-168	Day 28	14.27	15.16	13.41	0.968	6.38%	0.10%
E02-0913-169	Day 28	16.19					
E02-0913-170	Day 28	15.02					

* SPE clogged, unable to extract

** Average was multiplied by a factor of 0.8846 to correct to anion

*** Based on the total amount of fluorine ir PFBS.

N/A = Not applicable

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation **
E02-0913-018	Day 0	<490.8	<490.8	N/A	N/A	<3.5%
E02-0913-019	Day 0	<490.8				
E02-0913-020	Day 0	<490.8				
E02-0913-048	Day 4	192.5	180.6	16.73	9.26%	1.3%
E02-0913-049	Day 4	187.9				
E02-0913-050	Day 4	161.5				
E02-0913-108	Day 14	*	90.46	5.982	6.61%	0.64%
E02-0913-109	Day 14	94.69				
E02-0913-110	Day 14	86.23				
E02-0913-168	Day 28	44.25	50.19	11.14	22.2%	0.35%
E02-0913-169	Day 28	43.29				
E02-0913-170	Day 28	63.04				

* SPE clogged, unable to extract

** Based on the total amount of fluorine in

N/A = Not applicable

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation **
E02-0913-018	Day 0	<126.1	<126.1	N/A	N/A	<1.0%
E02-0913-019	Day 0	<126.1				
E02-0913-020	Day 0	<126.1				
E02-0913-048	Day 4	<25.23	<25.23	N/A	N/A	<0.20%
E02-0913-049	Day 4	<25.23				
E02-0913-050	Day 4	<25.23				
E02-0913-108	Day 14	*	<10.09	N/A	N/A	<0.081
E02-0913-109	Day 14	<10.09				
E02-0913-110	Day 14	<10.09				
E02-0913-168	Day 28	<10.09	<10.09	N/A	N/A	<0.081
E02-0913-169	Day 28	<10.09				
E02-0913-170	Day 28	<10.09				

* SPE clogged, unable to extract

** Based on the total amount of fluorine in PFBA.

N/A = Not applicable

Medium B

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation **
E02-0913-018	Day 0	<125.2	<125.2	N/A	N/A	<0.92%
E02-0913-019	Day 0	<125.2				
E02-0913-020	Day 0	<125.2				
E02-0913-048	Day 4	79.17	81.12	6.199	7.64%	0.60%
E02-0913-049	Day 4	76.13				
E02-0913-050	Day 4	88.06				
E02-0913-108	Day 14	*	175.4	17.68	10.1%	1.3%
E02-0913-109	Day 14	162.9				
E02-0913-110	Day 14	187.9				
E02-0913-168	Day 28	163.3	176.5	29.15	16.5%	1.3%
E02-0913-169	Day 28	156.4				
E02-0913-170	Day 28	209.9				

* SPE clogged, unable to extract

* Based on the total amount of fluorine in

N/A = Not applicable

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation **
E02-0913-018	Day 0	3815	3990	181.8	4.56%	25%
E02-0913-019	Day 0	3978				
E02-0913-020	Day 0	4178				
E02-0913-048	Day 4	851.2	917.7	109.4	11.9%	5.7%
E02-0913-049	Day 4	857.9				
E02-0913-050	Day 4	1044				
E02-0913-108	Day 14	*	214.0	57.05	26.7%	1.3%
E02-0913-109	Day 14	173.7				
E02-0913-110	Day 14	254.38				
E02-0913-168	Day 28	116.2	101.1	34.92	34.5%	0.63%
E02-0913-169	Day 28	61.21				
E02-0913-170	Day 28	126.0				

* SPE clogged, unable to extract

** Based on the total amount of fluorine in

Sample	Timepoint	Result (ng/mL)	Times Correction Factor (ng/mL) ***	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation **
E02-0913-018	Day 0	<500.0	<665.0	<665.0	N/A	N/A	<4.0%
E02-0913-019	Day 0	<500.0	<665.0				
E02-0913-020	Day 0	<500.0	<665.0				
E02-0913-021	Day 0	<500.0	<665.0				
E02-0913-022	Day 0	<500.0	<665.0				
E02-0913-108	Day 14	*	*	5915	746	12.6%	35%
E02-0913-109	Day 14	5090	6770				
E02-0913-110	Day 14	4201	5587				
E02-0913-111	Day 14	*	*				
E02-0913-112	Day 14	4052	5389				
E02-0913-168	Day 28	3796	5049	5416	314.8	5.81%	32%
E02-0913-169	Day 28	3914	5206				
E02-0913-170	Day 28	4054	5392				
E02-0913-171	Day 28	4399	5850				
E02-0913-172	Day 28	4198	5584				

* SPE clogged, unable to extract

** Based on the total amount of fluorine in

*** A correction factor was calculated to be 1

for the differences in response factor, and

N/A = Not applicable

Medium C

PFBS

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Average (ng/mL) anion*	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation **
E02-0913-023	Day 0	<50.06	<50.06	<44.28	N/A	N/A	<0.33%
E02-0913-024	Day 0	<50.06					
E02-0913-025	Day 0	<50.06					
E02-0913-053	Day 4	<6.258	<6.258	<5.536	N/A	N/A	<0.041%
E02-0913-054	Day 4	<6.258					
E02-0913-055	Day 4	<6.258					
E02-0913-113	Day 14	<50.06	<50.06	<44.28	N/A	N/A	<0.33%
E02-0913-114	Day 14	<50.06					
E02-0913-115	Day 14	<50.06					
E02-0913-173	Day 28	<10.01	<10.01	<8.855	N/A	N/A	<0.074%
E02-0913-174	Day 28	<10.01					
E02-0913-175	Day 28	<10.01					

* Average was multiplied by a factor

** Based on the total amount of fluorine in PFBS,

N/A = Not applicable

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation*
E02-0913-023	Day 0	<490.8	<490.8	N/A	N/A	<3.5%
E02-0913-024	Day 0	<490.8				
E02-0913-025	Day 0	<490.8				
E02-0913-053	Day 4	96.23	94.28	7.585	8.05%	0.67%
E02-0913-054	Day 4	85.91				
E02-0913-055	Day 4	100.7				
E02-0913-113	Day 14	<490.8	<490.8	N/A	N/A	<3.5%
E02-0913-114	Day 14	<490.8				
E02-0913-115	Day 14	<490.8				
E02-0913-173	Day 28	47.23	38.73	7.491	19.3%	0.27%
E02-0913-174	Day 28	33.10				
E02-0913-175	Day 28	35.85				

* Based on the total amount of fluorine in

N/A = Not applicable

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation*
E02-0913-023	Day 0	<126.1	<126.1	N/A	N/A	<1.0%
E02-0913-024	Day 0	<126.1				
E02-0913-025	Day 0	<126.1				
E02-0913-053	Day 4	<25.23	<25.23	N/A	N/A	<0.20%
E02-0913-054	Day 4	<25.23				
E02-0913-055	Day 4	<25.23				
E02-0913-113	Day 14	<126.1	<126.1	N/A	N/A	<1.0%
E02-0913-114	Day 14	<126.1				
E02-0913-115	Day 14	<126.1				
E02-0913-173	Day 28	<10.09	<10.09	N/A	N/A	<0.081%
E02-0913-174	Day 28	<10.09				
E02-0913-175	Day 28	<10.09				

* Based on the total amount of fluorine in

N/A = Not applicable

Medium C

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation*
E02-0913-023	Day 0	<125.2	<125.2	N/A	N/A	<0.92%
E02-0913-024	Day 0	<125.2				
E02-0913-025	Day 0	<125.2				
E02-0913-053	Day 4	9.331	9.331	N/A	N/A	0.069%
E02-0913-054	Day 4	<2.504				
E02-0913-055	Day 4	<2.504				
E02-0913-113	Day 14	<125.2	<125.2	N/A	N/A	<0.92%
E02-0913-114	Day 14	<125.2				
E02-0913-115	Day 14	<125.2				
E02-0913-173	Day 28	<2.504	<2.504	N/A	N/A	<0.018%
E02-0913-174	Day 28	<2.504				
E02-0913-175	Day 28	<2.504				

* Based on the total amount of fluorine in

N/A = Not applicable

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation*
E02-0913-023	Day 0	228.0	222.5	6.009	2.70%	1.4%
E02-0913-024	Day 0	223.5				
E02-0913-025	Day 0	216.1				
E02-0913-053	Day 4	605.3	610.9	12.02	1.97%	3.8%
E02-0913-054	Day 4	624.7				
E02-0913-055	Day 4	602.7				
E02-0913-113	Day 14	996.7	985.8	9.692	0.983%	6.1%
E02-0913-114	Day 14	978.3				
E02-0913-115	Day 14	982.3				
E02-0913-173	Day 28	1997	2327	597.5	25.7%	14%
E02-0913-174	Day 28	3016				
E02-0913-175	Day 28	1966				

* Based on the total amount of fluorine in

Sample	Timepoint	Result (ng/mL)	Times Correction Factor (ng/mL) **	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent Degradation*
E02-0913-023	Day 0	<500.0	<665.0	<665.0	N/A	N/A	<4.0%
E02-0913-024	Day 0	<500.0	<665.0				
E02-0913-025	Day 0	<500.0	<665.0				
E02-0913-026	Day 0	<500.0	<665.0				
E02-0913-027	Day 0	<500.0	<665.0				
E02-0913-113	Day 14	<500.0	<665.0	<665.0	N/A	N/A	<4.0%
E02-0913-114	Day 14	<500.0	<665.0				
E02-0913-115	Day 14	<500.0	<665.0				
E02-0913-116	Day 14	<500.0	<665.0				
E02-0913-117	Day 14	<500.0	<665.0				
E02-0913-173	Day 28	858.2	1141.4	590.8	778.7	132%	3.5%
E02-0913-174	Day 28	30.21	40.2				
E02-0913-175	Day 28	<10.00	<19.88				
E02-0913-176	Day 28	<10.00	<19.88				
E02-0913-177	Day 28	<10.00	<19.88				

* Based on the total amount of fluorine in

*** A correction factor was calculated to be
or the differences in response factor,

N/A = Not applicable

Matrix Spikes

PFBS

Sample	Timepoint	Medium	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Average Sample Result (ng/mL)	True Value (ng/mL)	Percent Recovery
E02-0913-016	Day 0	A	490.75	491.2	0.5698	0.12%	<50.06	500.6	98.1%
E02-0913-017	Day 0	A	491.55						
E02-0913-021	Day 0	B	429.05	431.6	3.609	0.84%	<50.06	500.6	86.2%
E02-0913-022	Day 0	B	434.15						
E02-0913-026	Day 0	C	430.18	429.4	1.156	0.27%	<50.06	500.6	85.8%
E02-0913-027	Day 0	C	428.55						
E02-0913-046	Day 4	A	712.25	705.1	10.07	1.4%	42.17	500.6	132%
E02-0913-047	Day 4	A	698.01						
E02-0913-051	Day 4	B	504.95	497.7	10.30	2.1%	6.938	500.6	98.0%
E02-0913-052	Day 4	B	490.38						
E02-0913-056	Day 4	C	506.81	508.1	1.846	0.36%	<6.258	500.6	102%
E02-0913-057	Day 4	C	509.42						
E02-0913-106	Day 14	A	596.05	615.2	27.05	4.4%	148.2	500.6	93.3%
E02-0913-107	Day 14	A	634.31						
E02-0913-111	Day 14	B	*	456.8	N/A	N/A	14.07	500.6	88.4%
E02-0913-112	Day 14	B	456.75						
E02-0913-116	Day 14	C	439.70	434.4	7.535	1.7%	<50.06	500.6	86.8%
E02-0913-117	Day 14	C	429.04						
E02-0913-166	Day 28	A	877.21	816.5	85.80	10.5%	345.0	500.6	94.2%
E02-0913-167	Day 28	A	755.87						
E02-0913-171	Day 28	B	460.10	460.1	0.02121	0.0046%	15.16	500.6	88.9%
E02-0913-172	Day 28	B	460.13						
E02-0913-176	Day 28	C	416.77	407.4	13.31	3.3%	<10.01	500.6	81.4%
E02-0913-177	Day 28	C	397.95						
								AVERAGE	94.6%

* SPE clogged, unable to extract

N/A = Not applicable

Sample	Timepoint	Medium	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Average Sample Result (ng/mL)	True Value (ng/mL)	Percent Recovery
E02-0913-016	Day 0	A	551.63	550.2	2.045	0.37%	<490.8	490.8	112%
E02-0913-017	Day 0	A	548.74						
E02-0913-021	Day 0	B	533.57	532.4	1.705	0.32%	<490.8	490.8	108%
E02-0913-022	Day 0	B	531.15						
E02-0913-026	Day 0	C	556.73	541.5	21.50	4.0%	<490.8	490.8	110%
E02-0913-027	Day 0	C	526.33						
E02-0913-046	Day 4	A	760.03	698.7	86.77	12%	165.4	490.8	109%
E02-0913-047	Day 4	A	637.31						
E02-0913-051	Day 4	B	651.36	670.1	26.55	4.0%	180.6	490.8	99.7%
E02-0913-052	Day 4	B	688.90						
E02-0913-056	Day 4	C	598.84	598.6	0.375	0.06%	94.28	490.8	103%
E02-0913-057	Day 4	C	598.31						
E02-0913-106	Day 14	A	504.58	530.6	36.74	6.9%	66.66	490.8	94.5%
E02-0913-107	Day 14	A	556.54						
E02-0913-111	Day 14	B	*	504.1	N/A	N/A	90.46	490.8	84.3%
E02-0913-112	Day 14	B	504.07						
E02-0913-116	Day 14	C	547.88	539.1	12.36	2.3%	<490.8	490.8	110%
E02-0913-117	Day 14	C	530.41						
E02-0913-166	Day 28	A	567.33	573.3	8.43	1.5%	36.90	490.8	109.3%
E02-0913-167	Day 28	A	579.25						
E02-0913-171	Day 28	B	570.29	575.7	7.679	1.3%	50.19	490.8	107.1%
E02-0913-172	Day 28	B	581.15						
E02-0913-176	Day 28	C	495.79	478.4	24.61	5.1%	38.73	490.8	89.6%
E02-0913-177	Day 28	C	460.98						
								AVERAGE	103.1%

* SPE clogged, unable to extract

N/A = Not applicable

Matrix Spikes

Sample	Timepoint	Medium	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Average Sample Result (ng/mL)	True Value (ng/mL)	Percent Recovery
E02-0913-016	Day 0	A	768.4	774.5	8.675	1.12%	<126.1	504.5	154%
E02-0913-017	Day 0	A	780.7						
E02-0913-021	Day 0	B	740.4	745.3	6.830	0.916%	<126.1	504.5	148%
E02-0913-022	Day 0	B	750.1						
E02-0913-026	Day 0	C	699.7	709.1	13.29	1.87%	<126.1	504.5	141%
E02-0913-027	Day 0	C	718.5						
E02-0913-046	Day 4	A	625.9	592.1	47.74	8.06%	<25.23	504.5	117%
E02-0913-047	Day 4	A	558.4						
E02-0913-051	Day 4	B	597.6	558.8	54.85	9.82%	<25.23	504.5	111%
E02-0913-052	Day 4	B	520.0						
E02-0913-056	Day 4	C	608.5	586.3	31.39	5.35%	<25.23	504.5	116%
E02-0913-057	Day 4	C	564.1						
E02-0913-106	Day 14	A	750.3	771.0	29.33	3.80%	15.49	504.5	150%
E02-0913-107	Day 14	A	791.7						
E02-0913-111	Day 14	B	*	696.6	N/A	N/A	<10.09	504.5	138%
E02-0913-112	Day 14	B	696.6						
E02-0913-116	Day 14	C	739.7	751.4	16.50	2.20%	<126.1	504.5	149%
E02-0913-117	Day 14	C	763.0						
E02-0913-166	Day 28	A	716.4	732.4	22.65	3.09%	46.24	504.5	136%
E02-0913-167	Day 28	A	748.5						
E02-0913-171	Day 28	B	691.2	649.1	59.56	9.18%	<10.09	504.5	129%
E02-0913-172	Day 28	B	607.0						
E02-0913-176	Day 28	C	663.0	678.0	21.23	3.13%	<10.09	504.5	134%
E02-0913-177	Day 28	C	693.0						
								AVERAGE	135%

* SPE clogged, unable to extract

N/A = Not applicable

Sample	Timepoint	Medium	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Average Sample Result (ng/mL)	True Value (ng/mL)	Percent Recovery
E02-0913-016	Day 0	A	486.6	487.4	1.047	0.215%	<125.2	500.7	97.3%
E02-0913-017	Day 0	A	488.1						
E02-0913-021	Day 0	B	471.4	472.3	1.259	0.266%	<125.2	500.7	94.3%
E02-0913-022	Day 0	B	473.2						
E02-0913-026	Day 0	C	468.1	470.1	2.843	0.605%	<125.2	500.7	93.9%
E02-0913-027	Day 0	C	472.1						
E02-0913-046	Day 4	A	711.5	683.6	39.50	5.78%	194.0	500.7	97.8%
E02-0913-047	Day 4	A	655.7						
E02-0913-051	Day 4	B	547.7	557.8	14.36	2.57%	81.12	500.7	95.2%
E02-0913-052	Day 4	B	568.0						
E02-0913-056	Day 4	C	503.2	500.9	3.175	0.634%	9.331	500.7	98.2%
E02-0913-057	Day 4	C	498.7						
E02-0913-106	Day 14	A	717.8	735.2	24.64	3.35%	261.2	500.7	94.7%
E02-0913-107	Day 14	A	752.7						
E02-0913-111	Day 14	B	*	603.7	N/A	N/A	175.4	500.7	85.5%
E02-0913-112	Day 14	B	603.7						
E02-0913-116	Day 14	C	477.9	474.4	4.929	1.04%	<125.2	500.7	94.8%
E02-0913-117	Day 14	C	471.0						
E02-0913-166	Day 28	A	757.6	776.1	26.18	3.37%	320.8	500.7	90.9%
E02-0913-167	Day 28	A	794.7						
E02-0913-171	Day 28	B	626.6	623.3	4.660	0.748%	176.5	500.7	89.2%
E02-0913-172	Day 28	B	620.0						
E02-0913-176	Day 28	C	437.2	431.2	8.535	1.98%	<2.504	500.7	86.1%
E02-0913-177	Day 28	C	425.1						
								AVERAGE	93.2%

* SPE clogged, unable to extract

N/A = Not applicable

Matrix Spikes

Sample	Timepoint	Medium	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Average Sample Result (ng/mL)	True Value (ng/mL)	Percent Recovery
E02-0913-016	Day 0	A	4997	4970	39.25	0.790%	4129	501.8	168%
E02-0913-017	Day 0	A	4942						
E02-0913-021	Day 0	B	4604	4563	57.23	1.25%	3990	501.8	114%
E02-0913-022	Day 0	B	4523						
E02-0913-026	Day 0	C	737.3	739.4	2.991	0.405%	222.5	501.8	103%
E02-0913-027	Day 0	C	741.5						
E02-0913-046	Day 4	A	759.4	739.8	27.68	3.74%	161.4	501.8	115%
E02-0913-047	Day 4	A	720.2						
E02-0913-051	Day 4	B	1453	1449	5.862	0.405%	917.7	501.8	106%
E02-0913-052	Day 4	B	1445						
E02-0913-056	Day 4	C	1218	1209	12.59	1.04%	610.9	501.8	119%
E02-0913-057	Day 4	C	1200						
E02-0913-106	Day 14	A	591.4	618.9	38.83	6.27%	64.89	501.8	110%
E02-0913-107	Day 14	A	646.4						
E02-0913-111	Day 14	B	*	620.0	N/A	N/A	214.0	501.8	80.9%
E02-0913-112	Day 14	B	620.0						
E02-0913-116	Day 14	C	1530	1616	122.6	7.59%	985.8	501.8	126%
E02-0913-117	Day 14	C	1703						
E02-0913-166	Day 28	A	554.7	544.7	14.21	2.61%	<25.09	501.8	109%
E02-0913-167	Day 28	A	534.6						
E02-0913-171	Day 28	B	617.4	609.5	11.17	1.83%	101.1	501.8	101%
E02-0913-172	Day 28	B	601.6						
E02-0913-176	Day 28	C	3384	3264	170.4	5.22%	2327	501.8	187%
E02-0913-177	Day 28	C	3143						
								AVERAGE	120%

* SPE clogged, unable to extract

N/A = Not applicable

Laboratory Control Spikes

PFBS

Sample	Medium	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	True Value (ng/mL)	Percent Recovery
E02-0913-003	A	1292	1323	43.54	3.29%	1251	106%
E02-0913-004	A	1353					
E02-0913-005	B	1278	1263	21.12	1.67%	1251	101%
E02-0913-006	B	1248					
E02-0913-007	C	1285	1290	6.147	0.477%	1251	103%
E02-0913-008	C	1294					

Sample	Medium	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	True Value (ng/mL)	Percent Recovery
E02-0913-003	A	1025	1037	16.24	1.57%	1227	84.5%
E02-0913-004	A	1048					
E02-0913-005	B	1050	1032	25.52	2.47%	1227	84.1%
E02-0913-006	B	1014					
E02-0913-007	C	1046	1046	0.142	0.0136%	1227	85.3%
E02-0913-008	C	1046					

Sample	Medium	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	True Value (ng/mL)	Percent Recovery
E02-0913-003	A	1307	1319	15.98	1.21%	1261	105%
E02-0913-004	A	1330					
E02-0913-005	B	1319	1288	43.08	3.34%	1261	102%
E02-0913-006	B	1258					
E02-0913-007	C	1105	1186	114.4	9.64%	1261	94.1%
E02-0913-008	C	1267					

Laboratory Control Spikes

Sample	Medium	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	True Value (ng/mL)	Percent Recovery
E02-0913-003	A	1198	1230	44.80	3.64%	1252	98.2%
E02-0913-004	A	1261					
E02-0913-005	B	1256	1233	32.43	2.63%	1252	98.5%
E02-0913-006	B	1210					
E02-0913-007	C	1234	1239	6.674	0.539%	1252	98.9%
E02-0913-008	C	1243					

Sample	Medium	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	True Value (ng/mL)	Percent Recovery
E02-0913-003	A	1257	1286	41.04	3.19%	1254	103%
E02-0913-004	A	1315					
E02-0913-005	B	1331	1309	30.58	2.34%	1254	104%
E02-0913-006	B	1287					
E02-0913-007	C	1331	1333	3.451	0.259%	1254	106%
E02-0913-008	C	1336					

Sodium Lauryl Sulfate

Toxicity Control

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent from Day 0
E02-0913-028	Day 0	61167	57300	4470	7.80%	100%
E02-0913-029	Day 0	52407				
E02-0913-030	Day 0	58327				
E02-0913-058	Day 4	<3330	3388	N/A	N/A	5.9%
E02-0913-059	Day 4	3388				
E02-0913-060	Day 4	<3330				
E02-0913-118	Day 14	<3330	<3330	N/A	N/A	<5.8%
E02-0913-119	Day 14	<3330				
E02-0913-120	Day 14	<3330				
E02-0913-178	Day 28	<111	<111	N/A	N/A	<0.19%
E02-0913-179	Day 28	<111				
E02-0913-180	Day 28	<111				

Control Substance

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent from Day 0
E02-0913-031	Day 0	43921	40373	5018	12.4%	100%
E02-0913-032	Day 0	36825				
E02-0913-061	Day 4	<3330	<3330	N/A	N/A	<8.2%
E02-0913-062	Day 4	<3330				
E02-0913-121	Day 14	<3330	<3330	N/A	N/A	<8.2%
E02-0913-122	Day 14	<3330				
E02-0913-181	Day 28	<111	<111	N/A	N/A	<0.27%
E02-0913-182	Day 28	<111				

Abiotic

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent from Day 0
E02-0913-033	Day 0	266488	265882	855.9	0.322%	100%
E02-0913-034	Day 0	265277				
E02-0913-063	Day 4	266328	257820	12032	4.67%	97%
E02-0913-064	Day 4	249312				
E02-0913-123	Day 14	255471	260936	7728	2.96%	98%
E02-0913-124	Day 14	266400				
E02-0913-183	Day 28	179799	193741	19716	10.2%	73%
E02-0913-184	Day 28	207682				

Inhibited

Sample	Timepoint	Result (ng/mL)	Average (ng/mL)	Standard Deviation (ng/mL)	Relative Standard Deviation	Percent from Day 0
E02-0913-035	Day 0	198883	213328	20429	9.58%	100%
E02-0913-036	Day 0	227774				
E02-0913-065	Day 4	142445	159194	23687	14.9%	75%
E02-0913-066	Day 4	175943				
E02-0913-125	Day 14	152035	153401	1933	1.26%	72%
E02-0913-126	Day 14	154768				
E02-0913-185	Day 28	101296	101296	N/A	N/A	47%
E02-0913-186	Day 28	*				

* SPE clogged, unable to extract

ATTACHMENT C: SAMPLE CHROMATOGRAMS

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P-00-1085

Report E02-0913

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ATTACHMENT D: TEST SUBSTANCE INFORMATION

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CERTIFICATE OF ANALYSIS

Exygen Research COA Reference #:

3M Product:
Lot/Batch Number: 1
Test Control Reference #:
Purity: 97.5%

Test Name	Specifications	Result
Purity[1]	**Report value**	**97.5%**
Appearance	Yellow viscous oil	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium 4. Potassium 5. Nickel 6. Iron 7. Manganese		 1. 0.005 wt./wt.% 2. 0.002 wt./wt.% 3. 0.050 wt./wt.% 4. <0.001 wt./wt.% 5. <0.001 wt./wt.% 6. <0.001 wt./wt.% 7. <0.001 wt./wt.%
Total % Impurity (NMR)[2]		2.4 wt./wt.%
Total % Impurity (LC/MS)		None Detected
Total % Impurity (GC/MS)		None Quantified
Related Compounds – POAA		<0.01 wt./wt.%
Residual Solvents (TGA)		None Detected
Inorganic Anions (IC) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate		 1. <0.013 wt./wt.% 2. <0.004 wt./wt.% 3. <0.035 wt./wt.% 4. <0.008 wt./wt.% 5. <0.005 wt./wt.% 6. <0.006 wt./wt.% 7. 0.089 wt./wt.%
Organic Acids [3] (IC)		 1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. <0.1 wt./wt.% 4. <0.2 wt./wt.%
Elemental Analysis[4]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine		

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Exygen Research COA Reference #

Date of Last Analysis: 07/27/00

Expiration Date: 07/27/01

Storage Conditions: Room Temperature

Re-assessment Date: 07/27/06

[1]Purity = 100% - (sum of metal impurities, 0.057% +NMR impurities, 2.4%+Inorganic Sulfate, 0.089)

Total impurity from all tests = 2.55%
Purity = 100% - 2.55% = 97.5%

[2]NMR:

3.

[4]Theoretical value calculations based on the empirical formula, obtained from the NMR analysis.

This Work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160)

Prepared By: _____ 7/2/02
Date

Scientist, Exygen Research

Reviewed By: 7/3/02
Date

Laboratory Manager, Exygen Research

COA023-026

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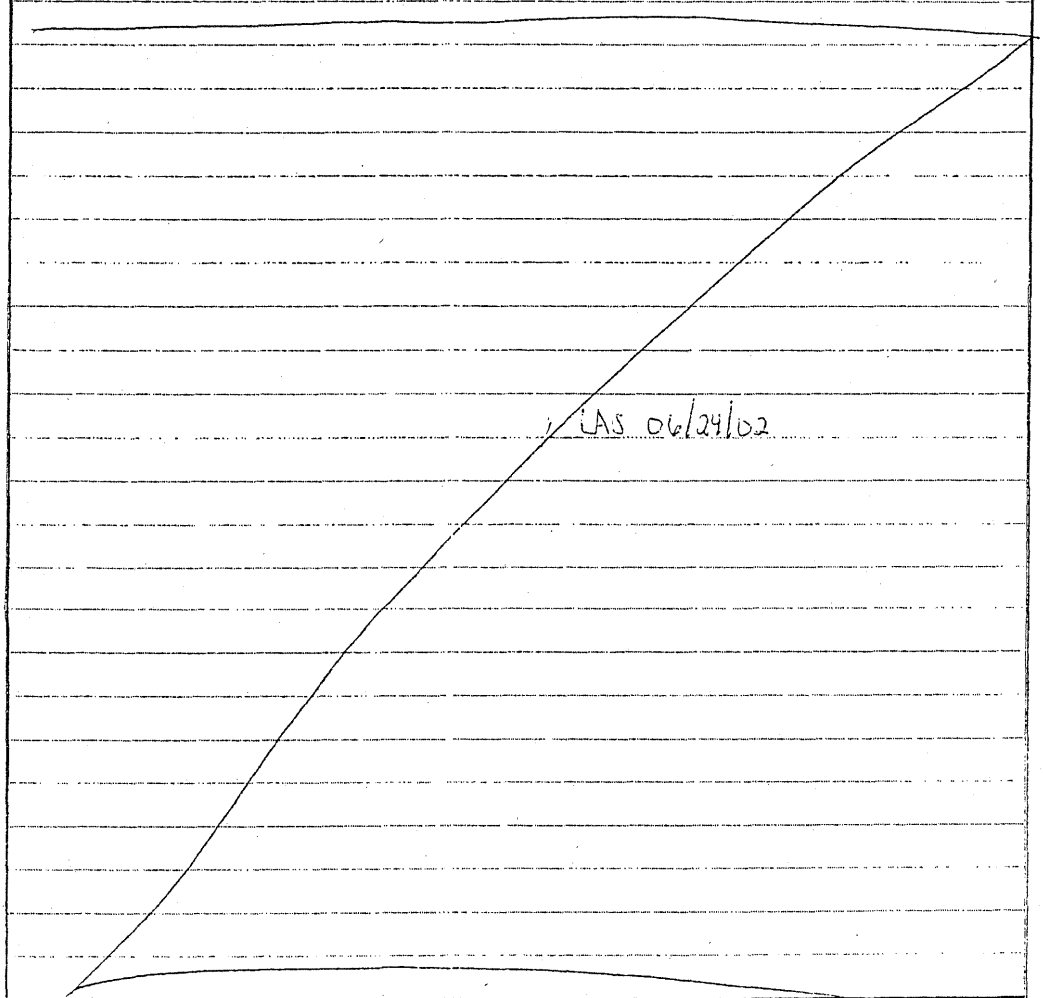
3M ENVIRONMENTAL LABORATORY

Note to File

Project or Study Number: FACT-

Associated Study Number: LIMS # ____ ____

The expiration dates for ____ may be extended 5 years (07/27/06) as stability was demonstrated by GC and Total Fluorine analyses with report number ____



Recorded By:

Date
06/24/02 06/24/02

Exact Copy of Original
LAS 06/24/02
Initial Date

Form ETS-4-15.0

ATTACHMENT E: PROTOCOL, PROTOCOL AMENDMENTS AND DEVIATIONS

3M

PROTOCOL

Inherent Aerobic Aquatic Biodegradability of Fluoroaliphatic Polymeric Ester

Data Requirement
40 CFR 792

Performing Laboratory
3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification
ET&SS E02-0913

E02-0913

Inherent Aerobic Aquatic Biodegradability of Fluoroaliphatic Polymeric Este

Test Substance

Sponsor

3M Environmental Technology and Safety Services
935 Bush Avenue, Building 2-3E-09
St. Paul, MN 55106

Study Director

3M Environmental Technology and Safety Services
935 Bush Avenue, Building 2-3E-09
St. Paul, MN 55106

Principal Investigator (PI)

3M Environmental Technology and Safety Services
935 Bush Avenue, Building 2-3E-09
St. Paul, MN 55106

Study Location(s)

Testing Facility

3M Environmental Laboratory
3M Environmental Technology and Safety Services
935 Bush Avenue, Building 2-3E-09
St. Paul, MN 55106

Proposed Study Timetable

Experimental Start Date October 24, 2002

Experimental Termination Date December 31, 2002

1. INTRODUCTION

Microbial populations in nature make up about one-half of the biomass on earth and are associated with the major biochemical cycles of elements and nutrients, besides the decomposition of organic matter. Changes in microbial populations or effects on functions of the microbial communities could result in interference with their natural degradative functions that are essential to self-purification processes in the aquatic and terrestrial environments. In order to assess the environmental fate of a compound, it is pertinent that its chemical properties, biological behavior and transport processes (sorption properties) be addressed.

2. PURPOSE

The primary objective of this investigation is to identify the inherent aerobic aquatic biodegradation potential of the fluoropolymer as mediated by the microbial activity of populations obtained from wastewater treatment sludge. This will be accomplished by utilizing aspects from the following guidelines: USEPA Zahn-Wellens/EMPA Test (OPPTS 835.3200) and USEPA Modified SCAS (OPPTS 835.3210).

The test substance, , is a complex mixture of fluoroaliphatic polymeric esters rather than a discreet monomeric material. The present investigation will obtain information on the potential for biological degradation and/or biotransformation of the polymeric material as well as yielding information on the intermediates, end products and insight into the potential for partitioning. The present investigation will be conducted using the noted EPA methods as guidelines and incorporating portions of these guidelines to accommodate the specific testing requirements, such as individual samples per sampling event and specific target analysis instead of COD, DOC or CO_2. (Refer to Section 8 for specifics on the modifications.) The present study is designed to utilize municipal wastewater treatment sludge as the inoculum. The focus is to determine the ability of viable microbial populations to degrade or transform into fluorochemical breakdown products based on the biological degradation study of . The predicted breakdown products of that are going to be monitored are the reference substances as described in section 7.

3. REGULATORY COMPLIANCE

This study will be conducted in accordance the United States Environmental Protection Agency Good Laboratory Practice Regulations for Non-clinical Laboratory Studies, 40 CFR 792.

4. QUALITY ASSURANCE

The 3M Environmental Laboratory Quality Assurance Unit will audit the study conduct, raw data, and final report to determine compliance with Good Laboratory Practice Regulations, this protocol, and 3M Environmental Laboratory Standard Operating Procedures.

5. TEST SUBSTANCE

Table 1 Test Substances

Test Substance	[redacted]
IUPAC Name	
Chemical Formula	
Identifier	
Source	3M Specialty Chemicals
Expiration Date	07/27/06
Storage Conditions	Room Temperature
Chemical Lot Number	
TCR Number	
Physical Description	Yellow Viscous Oil
Purity	97.5%

*Based on NMR data

The test substance is a complex mixture of fluoroaliphatic polymeric esters. is a viscous liquid with a distinctive odor. It has a boiling point of at standard temperature and pressure it has a vapor pressure of nm Hg, a specific gravity of 1.1, a measured pH range of and viscosity of . Lot # was obtained from 3M Specialty Chemicals, Chemical characterization data was performed by: 3M Specialty Chemicals (boiling point, viscosity, specific gravity, pH, vapor pressure, flash point, results of GC analysis, results of gel-permeation chromatography, and results of NMR analysis) and Centre Analytical (total amount of fluorine containing moieties present). The total amount of organic fluorine in was determined to by "Total Fluoride Analysis". A report of these findings is on file in the 3M Environmental Laboratory.

5.1 Sample Retention

A retention sample will be taken and stored in the dark at ambient temperature. The sample will be archived for 10 years from the study completion date, or as long as a reliable analysis can be performed.

5.2 Disposition

Should the retention sample be discarded, the final disposition will be documented in the 3M facility archive records.

5.3 Safety Precautions

When handling samples or solvents wear protective gloves, eyewear and clothing. The operator must be familiar with instrumentation and their associated hazards, such as, but limited to, high temperature, effluent venting, solvent use, and vacuum systems. All material safety data sheets or chemical hazard information should be reviewed as appropriate.

6. CONTROL SUBSTANCES

Table 2. Control Substances

Control Substances	[redacted]	[redacted]	Sodium Lauryl Sulfate
Formula			$C_{13}H_{25}OSO_3Na$
IUPAC Name			Sodium Lauryl Sulfate
Use	Surrogate Standard	Internal Standard for LC/MS analysis	Toxicity and Reference Control
Source	3M Specialty Chemicals	3M Specialty Chemicals	Mallinckrodt
Expiration Date	8/31/2006	10/18/2006	2/26/2007
Storage Conditions	Frozen	Frozen	Room Temperature
Chemical Lot Number			7718 V16603
TCR Number			TN-A-6021
Physical Description	White powder	White powder	White powder
Purity	86.9%	98.6%	99%

7. REFERENCE SUBSTANCES

Table 3. Reference Substances

Reference Substance			
IUPAC Name			
Chemical Formula			
Identifier			
Source	Aldrich Chemical	3M Specialty Chemicals	3M Specialty Chemicals
Expiration Date	5/1/2010	12/4/2006	Not Provided
Storage Conditions	Frozen	Frozen	Frozen
Chemical Lot Number			
TCR Number			
Physical Description	Clear liquid	White powder	White powder
Purity	99%	96.7%	Not determined

Reference Substance		
IUPAC Name		
Chemical Formula		
Identifier		
Source	3M Specialty Chemicals	3M Specialty Chemicals
Expiration Date	Not provided	Not Provided
Storage Conditions	Frozen	Ambient
Chemical Lot Number		
TCR Number		
Physical Description	White crystals	White crystals
Purity	97.25	95.55

*CAS Number, **3M Identifier Code. The location of the documentation of the method(s) of synthesis of the test, control, and reference items are the same as the source of the compound.

8. EXPERIMENTAL DESIGN

8.1 Preparation of the Test System

The following table describes which substances will be added to which medium. There are no contaminants expected in the materials used for preparing and dosing the test system. Information on the preparation of the medium and the concentrations required for the test, control, and reference substances are also described below.

Table 4: Test System Preparation

Sample Description	# of Replicates	Test/Control Substance added	Reference Substances added	Medium Added [1]	Analysis to be conducted
Blank Controls	2	None	No	A	Reference Substances
Blank Controls Inhibited	2	None	No	B	Reference Substances
Abiotic Controls	2	None	No	C	Reference Substances
Test Substance	3	Test	No	A	Reference Substances
Test Substance Matrix spike	2	Test	Yes	A	Reference Substances
Test Substance Inhibited	3	Test	No	B	Reference Substances
Test Substance Inhibited Matrix spike	2	Test	Yes	B	Reference Substances
Abiotic Test Substance	3	Test	No	C	Reference Substances
Abiotic Test Substance Matrix spike	2	Test	Yes	C	Reference Substances
Toxicity Control	3	Test and SLS	No	A	SLS
Control Substance (SLS)	2	SLS	No	A	SLS
	2		No	C	
	2		No	B	

[1] See definitions of mediums under section 8.1.2

8.1.1 *Sludge Inoculum.*

Arrangements will be made to have Pace Analytical Services, Field Laboratory, Minneapolis, MN personnel obtain fresh mixed liquor suspended solids (MLSS) from the aeration units at the Metro Wastewater Treatment Plant, St. Paul, MN. This sludge has been used in previous studies at 3M and Pace. Approximately six to eight liters of MLSS will be collected in either Nalgene™ polypropylene bottles or a two-gallon Cubitainer™ or equivalent.

The suspended sludge in the containers will be allowed to settle for at least 24 hours. The approximate percentage of settled sludge per volume of container will be noted. Previous studies had values that were approximately 20% (e.g. 200 mL of sludge in a 1 liter container). This value is for comparison purposes only and is not meant as a criterion for passing the collected sludge.

8.1.2 *Mineral Salts Medium*

The mineral salts medium employed will be based on the USEPA Zahn-Wellens/EMPA Test (OPPTS 835.3200).

Medium A. Prepare this solution by adding approximately 100 mL of settled sludge to 2 liters of mineral salts medium. This solution should be swirled regularly during dispensing in order to keep the mixture homogenous. The mixed liquor suspended solids (MLSS) should be determined for this medium. The final result should be between 0.2 g/L and 1.0 g/L. If the MLSS is outside this range, justification should be given in the final report.

Medium B. A portion of Medium A will be treated with 100 µg/mL of chloramphenicol as a microbial growth inhibitor. This solution should be swirled regularly during dispensing in order to keep the mixture homogenous.

Medium C. Mineral salt medium, prepared without the sludge inoculum, will be treated with 100 µg/mL of chloramphenicol as a microbial growth inhibitor. This will be labeled as Medium C and used to prepare the 25 mL abiotic culture vessels. (To assess the potential for abiotic mechanisms, e.g. hydrolysis.)

8.1.3 *Culture Vessel Setup*

The individual culture vessels will be prepared by dispensing 25 mL of the appropriate medium into125 mL glass Erlenmeyer flasks containing labels with the appropriate information. The vessels will be covered with a loose cap in order to reduce evaporation. The route of administration will be directly spiking the medium (this is the most direct route using solutions) in the culture vessel as detailed below.

8.1.4 *Test Substance Stock Solution*

The concentration of the test substance should be approximately 36 mg/L in each appropriate culture vessel (per table 4) as per the suggestion of the EPA guidelines of 20 mg Carbon/L. The concentration was calculated by the theoretical value calculations based on the empirical formula, obtained from the NMR analysis (see report). The initial stock solution should be prepared in acetone but any subsequent dilutions should be made in mineral salts medium without inoculum.

8.1.5 *Control Substances*

The concentration of the control substance, sodium lauryl sulfate, should be approximately 40 mg/L in each appropriate culture vessel (per table 4) as per the suggestion of the EPA guidelines of 20 mg Carbon/L. The biodegradation of this compound must reach at least 70 percent within 14 days.

will also be added as a control substance to mediums B and C (as per table 4). The concentration of added in the 25 mL culture vessel should be approximately 112 mg/L. There should be minimal degradation of throughout the course of the study.

will be added as the last step of the preparation process prior to analysis by LC/MS. It should be added at an approximate concentration of 250 ng/mL.

All initial stock solutions should be prepared in acetone but any subsequent dilutions should be made in mineral salts medium without inoculum.

8.1.6 *Reference Substances*

Reference substances will be added to laboratory control spikes and post extraction matrix spikes to determine recovery. These substances will be added at a nominal concentration of 500 ng/mL. All initial stock solutions should be prepared in acetone but any subsequent dilutions should be made in mineral salts medium without inoculum for the laboratory control spikes and in tetrahydrofuran for the post extraction matrix spikes.

8.1.7 *Sample Collection*

The experiment will be set up for determination of biodegradation and/or biotransformation over a six week period, with triplicate samples set up, except for the blanks, post spikes and control substances, which will be duplicated only. Samples will be collected on days 0, 4, 7, 14, 21, 28 and 42 for each medium type.

Analysis will be conducted initially on selected early time points. Based on these results, it will be determined if it is necessary to analyze the other time points. Table 4 shows the sample preparation scheme for the investigation.

On day zero, samples will be prepared and immediately extracted or placed in a freezer which is maintained at -19 ± 7°C. All other test vessels will be placed in a temperature controlled orbital shaker incubator which is maintained at 24 ± 3°C under dark conditions for up to 42 days.

During sampling events, the samples will be removed from the incubator and will be either extracted immediately or frozen.

8.2 Sample Extraction Method

ETS-8-39 will be used as the sample extraction method. In summary, an amount of sludge is prepared in an aqueous 1% solution of acetic acid. The sample is capped, mixed, and put on the centrifuge to clarify the supernatant, if needed. The supernatant is passed through a pre-conditioned C_{18} SPE column, at which time the analytes are adsorbed onto the stationary phase. Finally, the analytes of interest are eluted from the SPE cartridge and analyzed by appropriate methodology.

8.3 Analytical Methods

Samples are to be analyzed via HPLC/Electrospray MS as per ETS-8-155. The following table describes the SIM ions to use.

Table 5. SIM Ions

SIM IONS	
Compound	SIM Ion (m/z)

9. Methods for Control of Bias

Control of bias is accomplished using analytical spikes (as an indicator of sample recovery and accuracy) and matrix blanks (for evaluation of possible contamination of the matrix). Solvent blanks will also be analyzed (possible sample contamination during the dilution process). Triplicate sample results are an indicator of precision and the internal standard will be used to measure instrument variability.

10. Data Quality Objectives

10.1 Samples

Sample precision should be $\leq$ 20% relative standard deviation.

10.2 Calibration Standards.

Samples will be bracketed by a calibration curve and passing CCVs. Calibration standards used to generate an external calibration curve should be prepared in medium A and extracted in the same manner as the samples. The number of calibration standards and the concentration levels should be sufficient to encompass the expected concentrations of the study samples. The coefficient of determination (r^2) of the standard curve must be equal to or greater than 0.990. If the calibration curve residuals are greater than 20% deviation (LOQ 70%-120%) from the theoretical value, quadratic curve fitting and/or dropping low/high curve points may be required if data review shows this to be a consistent and more accurate representation of the instrument response. Deviations will be documented in the raw data with technical justification. The Study Director will be consulted for direction and for final acceptance or rejection of the data.

10.3 Continuing Calibration Verification (CCV).

Analyze a mid-range calibration standard after a maximum of every ten samples. Acceptable CCV values are 70 –120% of true value.

10.4 Solvent blank.

Solvent blanks are run before and after every calibration curve, and after every CCV. Solvents blanks may be run before and after matrix and control blanks if contamination is noted. Acceptable values for the blanks are values below 50% of the limit of quantitation (LOQ). If analyte carryover is a problem, use back-to-back solvent blanks and use the last solvent blank to evaluate carryover.

10.5 Laboratory Control Spikes

Two laboratory control spikes (LCS) will be prepared for each medium (medium A, B, and C) for the Day 0 samples. The LCS will be spiked at a level that is expected in the samples. The analyst shall accept percent spike recoveries between 70% and 120% of theoretical value. Spike recoveries outside of this range should be noted and used with other criteria to evaluate the condition of the analytical run or necessity for repeat analysis. Consult with the Study Director for direction and final acceptance or rejection of the analytical run.

10.6 Matrix spikes.

The analyst shall accept percent spike recoveries between 70% and 120% of theoretical value. Spike recoveries outside of this range should be noted and used with other criteria to evaluate the condition of the analytical run or necessity for repeat analysis. Consult with the Study Director for direction and final acceptance or rejection of the analytical run.

10.7 Limit of Quantitation (LOQ).

The lowest concentration that can be reliably measured within specified limits of accuracy during routine laboratory operating conditions. The LOQ is defined as the lowest non-zero standard (70%-120%) in the calibration curve that is greater than or equal to 2 times the level of the matrix blank. Sample LOQ are highly matrix-dependent.

10.8 Internal Standard.

Internal standard will be added to sample as the last step of the preparatory procedure. The same amount of internal standard will also be added to the standards. This will solely be used to monitor instrument performance and not used for quantitation.

10.9 Demonstration of Specificity.

The analytical technique of HPLC/MS provides for chromatographic separation of targeted materials and detection/quantitation of selected ions which are characteristic of the targeted compounds.

10.10 System Suitability

Five injections of a mid-level standard will be run prior to each calibration curve. The area counts should have a relative standard deviation <5% and the retention times should be <2%.

11. Statistical Analysis

Standard deviations will be calculated using either Microsoft Excel® (Version 8.0e or newer) or Microsoft Access®.

The built in function contains the following equation, which is based on the individual entities (n) being less than 30:

$$\sqrt{\frac{n\Sigma x^2 - (\Sigma x)^2}{n(n-1)}}$$

Sample precision will be reported as % relative standard deviation (%RSD) for three or more replicates.

Means will be calculated by adding the individual entities and dividing the resultant sum by the number of individual entities.

12. REPORT

A report of the results of the study will be prepared by 3M Environmental Laboratory. The report will include, but not be limited to, the following, when applicable:

Name and address of the facility performing the study,

Dates upon which the study was initiated and completed.

A statement of compliance by the Study Director addressing any exceptions to Good Laboratory Practice Standards.

Objectives and procedures as stated in the approved protocol, including any amendments to the original protocol.

The test substance identification by name, chemical abstracts number or code number, strength, purity, and composition or other appropriate characteristics, if provided by the Sponsor.

Stability and the solubility of the test substances under the conditions of administration, if provided by the Sponsor.

A description of the methods used to conduct the test(s).

A description of the test system.

A description of any circumstances that may have affected the quality or the integrity of the data.

The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study.

A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the analytical chemistry data, and a statement of the conclusions drawn from the analyses.

Statistical methods used to evaluate the data, if applicable.

The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.

The location where raw data and the final report are to be stored.

A statement prepared by the Quality assurance unit listing the dates that study inspections and audits were made and the dates of any findings reported to the Study Director and Management.

If it is necessary to make corrections or additions to a final report after it has been accepted, the changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended, the reasons for the amendment, and will be signed by the Study Director.

13. Location of Raw Data, Records, and Final Report

Original data or copies thereof, will be available at 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those items listed below will be retailed in the archives of 3M Environmental Laboratory for a period of 10 years following signing of the final report.

The following raw data and records will be retained in the study folder in the archives according to 3M Environmental Laboratory Standard Operating Procedures.

Approved protocol and amendments

Study correspondence

Shipping records

Raw data

Approved final report (original signed copy)

Electronic copies of data

The following supporting records will be retained separately from the study folder in the archives according to 3M Environmental Laboratory Standard Operating Procedures:

Training records

Calibration records

Instrument maintenance logs

Standard Operating Procedures, Equipment Procedures, and Methods

Appropriate specimens

14. Data / Sample Retention

Extracts will be kept for 6 months, or as long as the preparation affords evaluation. Other raw data will be kept for 10 years following the effective date of the applicable final test rule.

15. Protocol Amendments and Deviations

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes (unplanned) will be in the form of written deviations, signed by the Study Director and filed with the raw data. All changes to the protocol and the reason for the changes will be indicated in the final report.

16. SIGNATURES

10/24/02
Date

10/24/02
Date

10/24/02
Date

Study Title

Inherent Aerobic Aquatic Biodegradability of Fluoroaliphatic Polymeric Ester

PROTOCOL AMENDMENT NO. #1

Amendment Date:
December 13, 2002

Performing Laboratory
3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification
ET&SS E02-0913

This amendment modifies the following portion(s) of the protocol:

PROTOCOL READS: Section 8.1.2 "The mixed liquor suspended solids (MLSS) should be determined for this medium. The final result should be between 0.2 g/L and 1.0 g/L."

AMEND TO READ: The mixed liquor suspended solids (MLSS) should be determined for this medium. The final result should be between 0.2 g/L and 1.0 g/L. The procedure for this determination is given in attachment A.

REASON: To add the procedure for the mixed liquor suspended solids determination.

PROTOCOL READS: Section 10.2 "Calibration standards used to generate an external calibration curve should be prepared in medium A and extracted in the same manner as the samples".

AMEND TO READ: Calibration standards used to generate an external calibration curve should be prepared in mineral salts medium and extracted in the same manner as the samples

REASON: Standard curves should be prepared in mineral salts medium, not in medium A.

PROTOCOL READS: Section 7. Reference Substances are , PFBS,

AMEND TO READ:

Reference Substance	
IUPAC Name	
Chemical Formula	
Identifier	
Source	3M Specialty Chemicals
Expiration Date	12/1/2010
Storage Conditions	Frozen
Chemical Lot Number	
TCR Number	
Physical Description	Light yellow powder
Purity	Not Determined

will be used to quantify ions that are related to the possible degradation product

REASON: To add s a reference substance.

PROTOCOL READS: Section 10.1 "Sample precision should be ≤20% relative standard deviation."
AMEND TO READ: Sample precision should be ≤20% relative standard deviation. Sample precision outside of this range should be noted and used with other criteria to evaluate the condition of the analytical run or necessity for repeat analysis. Consult with the Study Director for direction and final acceptance or rejection of the analytical run.

REASON: To clarify original intent of how precisions >20% should be evaluated.

PROTOCOL READS: Section 8.3 Table 5: SIM ion for

AMEND TO READ: Section 8.3 Table 5: SIM ion

REASON: After method development, the ion had a better signal than the

Attachment A
Determination of Mixed Liquor Suspended Solids (MLSS)[1]

Equipment Needed:

1. Glass Fiber Filter Disks
2. Aluminum Weighing Tins
3. Filtering apparatus with pump
4. Desiccator
5. Drying Oven
6. Analytical Balance

The determination of Mixed Liquor Suspended Solids will be determined as follows:

1. Place a glass fiber filter disk in an aluminum weighing tin.
2. Weigh the tin and filter on an analytical balance to the nearest 0.1 mg.
3. Remove the filter from the aluminum weighing tin and place the filter paper on the filtering apparatus and wet with Milli-Q water. Turn on the pump and ensure that the filter is properly seated in the apparatus.
4. Take a 5 mL aliquot from medium A. This aliquot should be taken approximately halfway down the flask and halfway between the wall of the flask and vortex of the solution. Dispense the solution onto the filter.
5. Rinse the filter three times with Milli-Q water and allow the filter to sit for at least three minutes while the pump is still on after the last rinse.
6. Turn off the pump and remove the filter. Place the filter on the original aluminum weighing tin.
7. Place the filter and tin in a drying oven (temperature >90°C) for approximately two hours. Remove the filter and tin and place in a desiccator for at least an hour.
8. Weigh the glass fiber filter and aluminum weighing tin to nearest 0.1 mg.
9. Calculate MLSS as

$$\text{MLSS (g/L)} = \frac{\text{Final Weight (mg)} - \text{Initial weight (mg)}}{\text{Volume used (mL)}}$$

[1] This is based on a modified procedure from: APHA, AWWA, WEF "Standard Methods for the Examination of Water and Wastewater", Section 2540D "Total Suspended Solids at 103-105°C" 19th Edition.

Amendment Approval

01/13/03

entative Date

01/13/03

M Date

Study Title

Inherent Aerobic Aquatic Biodegradability of Fluoroaliphatic Polymeric Ester

PROTOCOL AMENDMENT NO. #2

Amendment Date:
February 19, 2003

Performing Laboratory
3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification
ET&SS E02-0913

This amendment modifies the following portion(s) of the protocol:

PROTOCOL READS: Section 7. Reference Substances are ' PFBS,

AMEND TO READ:

Reference Substance	
IUPAC Name	
Chemical Formula	
Identifier	Not Available
Source	Pace
Expiration Date	2/4/2013
Storage Conditions	Frozen
Chemical Lot Number	
TCR Number	
Physical Description	White Powder
Purity	50.1%

REASON: To add as a reference substance.

Amendment Approval

02/19/03

ïive Date

2/19/03

Date

Study Title

Inherent Aerobic Aquatic Biodegradability of Fluoroaliphatic Polymeric Ester

PROTOCOL AMENDMENT NO. #3

Amendment Date:
February 27, 2003

Performing Laboratory
3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification
ET&SS E02-0913

This amendment modifies the following portion(s) of the protocol:

PROTOCOL READS: Section 7. Reference Substances are PFBS and

AMEND TO READ:

Reference Substance	
IUPAC Name	
Chemical Formula	
Identifier	
Source	3M Specialty Chemicals
Expiration Date	11/29/2005
Storage Conditions	Frozen
Chemical Lot Number	
TCR Number	
Physical Description	Off white powder
Purity	98.63%

REASON: To add the additional reference substance used for this study.

Amendment Approval

2/27/03

ıtative Date

2/27/03

Date

Study Title

Inherent Aerobic Aquatic Biodegradability of Fluoroaliphatic Polymeric Ester

PROTOCOL AMENDMENT NO. #4

Amendment Date:
March 6, 2003

Performing Laboratory
3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification
ET&SS E02-0913

This amendment modifies the following portion(s) of the protocol:

PROTOCOL READS: Section 10.2 "Calibration standards used to generate an external calibration curve should be prepared in mineral salts medium and extracted in the same manner as the samples."
AMEND TO READ: "Calibration standards used to generate an external calibration curve should be prepared in mineral salts medium and extracted in the same manner as the samples. Selected timepoints will be analyzed using unextracted standards."

REASON: Due to the late edition of these compounds to the study, unextracted standards are to be used to quantitate these compounds.

Amendment Approval

03/07/03

presentative Date

3/7/03

Date

3M Confidential

Record of Deviation

I. Identification

Study / Project No.
E02-0913

Deviation type (*Check one*): SOP X Protocol | Method Other: | Equipment Procedure

Document number
E02-0913 Protocol

Date(s) of occurrence
Sequence D021119 (Dudejr 11/19/02)

II. Description

Required procedure/process:

1. Section 10.3: "Acceptable CCV values are 70-120% of true value."
2. Section 10.2: "If the calibration curve residuals are greater than 20% deviation (LOQ 70%-120%) from the theoretical value, quadratic curve fitting and/or dropping low/high curve points may be required if data review..."

Actual procedure/process:

1. For all analytes the 10 pg/µL CCV was not within the specified criteria and (2 out of 3) and out of 3) the 50 pg/µL standard was outside the specified criteria.
2. The lowest standard for was not within the specified criteria (60%).

III. Actions Taken

(such as amendment issued, SOP revision, etc.)

1. For all analytes a 10 pg/µL CCV was run after a high level spike (500 pg/µL). For PFBS the level of the matrix spike caused instrument carryover into the next injection, which resulted in a high CCV. Since the other CCVs passed (50 and 250 pg/µL) and samples repeated within 20% RSD, it is suspected that carryover was not a problem with these study samples. For the 10 pg/µL CCV was outside the calibration range used so it was discarded. For most of the associated samples (with exception of the blank controls) were well above the 50 pg/µL CCV (closer to the passing 250 pg/µL CCV). However, the day four medium C samples ranged from 86 pg/µL to 101 pg/µL for . Since these samples had repeated <20 % RSD they were accepted. If analyte carryover was a problem, the samples would not have repeated within specification and would of shown decreasing concentrations. For all analytes, the study control blanks were all below the LOQ. Given all this, it is not suspected that the out of specification CCVs will have an adverse affect on the data.

2. The 25.23 pg/µL standard was kept in order to maintain a 5 point calibration curve. Also, all of the study samples (with exception of the matrix spikes) were all well below the 25.23 pg/µL standard. Given this, it was deemed more accurate to state that the samples were below 25.23 pg/µL instead of 50.45 pg/µL.

Recorded by — Date 01/20/03

Date 1/22/03

Deviation No. 1
(assigned by Study Director or Project Lead at the end of study or project)

3M Confidential

Record of Deviation

I. Identification

Study / Project No.
E02-0913

Deviation type (Check one)	SOP	Method	Equipment Procedure
	X Protocol	Other:	

Document number	Date(s) of occurrence
E02-0913 Protocol	Sequence D021119 (Dudejr 11/19/02)

II. Description

Required procedure/process:
Section 10.10: "The area counts should have a relative standard deviation of <5%..."

Actual procedure/process:
.12%) was outside the specification.

III. Actions Taken

(such as amendment issued, SOP revision, etc.)

Since all of the samples were either diluted and reinjected at a later date (required reanalysis) or less than the LLOQ it was decided to accept the results since the repeatability really doesn't matter below the LLOQ and any of the samples that were detected were reanalyzed (required reanalysis due to being above the upper limit of quantitation).

Recorded by

Date
01/20/03

Date
1/22/03

Deviation No. 2

ed by Study Director or Project Lead at the end of study or project)

3M Confidential

Record of Deviation

I. Identification

Study / Project No.
E02-0913

Deviation type (Check one): SOP | X Protocol | Method | Other: | Equipment Procedure

Document number: E02-0913 Protocol

Date(s) of occurrence: Sequence I021231 (Itchy 12/31/02)

II. Description

Required procedure/process:
Section 10.3 "Acceptable CCV values are 70-120% of true value."

Actual procedure/process:
The 25.00 pg/µL CCV for was not within the 70-120% criteria (123%, 127%, and 128%).

III. Actions Taken

(such as amendment issued, SOP revision, etc.)

For this study is used as a surrogate to approximate another compound. In this analysis, the 25.00 pg/µL CCV did not pass the criteria as specified by the protocol. All of the samples that have values to be reported were either below the lower limit of quantitation or closer to the 250 pg/µL CCV which passed (CCV range 111%-117%, sample range 189.81 – 344.17 pg/µL).

Since the LLOQ for this analysis was 25.00pg/µL and the CCV was high, the high recovery would have shown if any samples were at or near 25.00 pg/µL. Given this and that all of the rest of the sample hits were closer to the 250 pg/µL CCV, the results were accepted. No affect on the data is expected.

Recorded by

Date 1/3/03

Date 01/13/03

Deviation No. 3

Director or Project Lead at the end of study or project)

3M Confidential

Record of Deviation

I. Identification

Study / Project No. E02-0913

Deviation type *(Check one)*	SOP	X Method	Equipment Procedure
	Protocol	Other:	

Document number
ETS-8-39.0

Date(s) of occurrence
11/18/02, 11/19/02, 11/22/02, 11/25/02, 11/26/02, and 12/19/02

II. Description

Required procedure/process:
Section 10.2 of the method states "Mix content thoroughly and centrifuge until contents become clear".

Actual procedure/process:
Samples were not centrifuged. Instead, a glass wool plug was added to the SPE apparatus and was used to catch any particulates that may be suspended in the solution used to elute the compounds of interest.

III. Actions Taken

(such as amendment issued, SOP revision, etc.)

No impact on the study.

Recorded by

Date 1/14/03

Date 1/14/03

eviation No. 4

ector or Project Lead at the end of study or project)

3M Confidential

Record of Deviation

I. Identification

Study / Project No. E02-0913			
Deviation type *(Check one)*	SOP X Protocol	Method Other:	Equipment Procedure
Document number E02-0913 Protocol		Date(s) of occurrence Sequence D030305 (Dudejr 3/5/03)	

II. Description

Required procedure/process:

Section 10.10: "The area counts should have a relative standard deviation of <5%..."

Actual procedure/process:

(5.2%) was outside the specification.

III. Actions Taken

(such as amendment issued, SOP revision, etc.)

After evaluating the data, since the calibration curves passed, the CCVs passed and the accepted sample replicates Showed repeatability within the specified criteria (<20%), the data was accepted.

Recorded by

Date 3/7/03

A

Date 3/7/03

viation No. 5

ctor or Project Lead at the end of study or project)