Study Title

Characterization Study EtFOSE-OH, Test Control Reference #SE-035

PHASE: SOLUBILITY OF ETFOSE-OH IN WATER, METHANOL, AND ACETONE

Data Requirement

40 CFR 160.105(b)

Author

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Phase Completion Date

Date of signing

Performing Laboratory

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Project Identification

3M Environmental Laboratory Study # FACT-TCR-004 (LIMS #E00-1715)

Centre Analytical Laboratories Study # 023-023

Total Number of Pages

258

GLP COMPLIANCE STATEMENT

Study Title: Characterization Study EtFOSE-OH, Test Control Reference #SE-035, Phase: Solubility of EtFOSE-OH in Water, Acetone, and Methanol.

Study Identification Number: FACT-TCR004, Centre Analytical Laboratories Study # 023-023

This phase of the study was conducted in compliance with Environmental Protection Agency (EPA) Good Laboratory Practice (GLP) Standards 40 CFR 160 with the exceptions in the bulleted list below.

Exceptions to GLP compliance:

- The electronic data systems in use have not been validated and there is not an electronic audit trail of corrections currently available (40 CFR 160.130 (e)). Authenticated hardcopies of chromatograms and associated documents will be considered as the original raw data.
- The test substance used for solubility testing was depleted and another lot was used for solubility in water. The purity of this second lot was verified only after its use in the study.
- The labels on containers of test substances initially did not show expiration date or storage conditions.

Mark E. Ellefson, Principal Investigator — Date: 05/30/01

William K. Reagen, Testing Facility Management — Date: 05/30/01

QUALITY ASSURANCE STATEMENT

Study Title: Characterization Study ETFOSE-OH, Test Control Reference #SE-035
PHASE: Solubility of EtFOSE-OH in Water, Methanol, and Acetone

Study Identification Number: FACT-TCR004, Centre Analytical Laboratories Study # 023-023

This phase of the study has been inspected 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
09/08/00	Solubility Screen Test	9/20/00	9/20/00
02/05-27/01	Data	02/28/01	02/28/01
05/17-18/01	Draft Phase Report	05/23/01	05/23/01

Tonya K Rudd
QAU Representative

5/30/01
Date

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STUDY INFORMATION

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Dr. William K. Reagen

Experimental Dates

Experimental Start Date: 11 September 2000
Experimental Termination Date: 09 January 2001

Archives

Reserve samples of the reference standards will be maintained by the 3M Environmental Laboratory. All study data for this phase and a copy of the phase report are archived by the 3M Environmental Laboratory.

SUMMARY

Solubility of EtFOSE-OH in water is a significant parameter because the spatial and temporal movement (mobility) of a substance is largely determined by its solubility in water. Water soluble substances readily gain access to humans and other living organisms; furthermore the knowledge of the solubility in water is a prerequisite for testing biological degradation, bioaccumulation, and other tests. Solubility of EtFOSE-OH in methanol and acetone is a significant parameter for general laboratory knowledge in the preparation and analysis of EtFOSE-OH. The approximate solubility of EtFOSE-OH in water was determined by performing a solubility screen test (ETS-8-170.0), followed by a quantitative solubility determination via the Column Elution Method (ETS-8-171.0) and HPLC/MS analysis (ETS-8-155.0). The approximate solubility of EtFOSE-OH in acetone and methanol was determined by performing a solubility screen test.

Results are presented in table 1a and 1b:

Table 1a. Summary Table of Solubility of ETFOSE-OH in Water.

SAMPLING EVENT	AVERAGE ETFOSE-OH CONCENTRATION IN WATER (NG/ML, CORRECTED FOR PURITY)	STANDARD DEVIATION, %RSD	REPLICATES REPORTED
22 rpm study	159	10, 6%	5 timepoints
11 rpm study	142	6, 4%	5 timepoints
Final Value	**151**	**11 % RPD**	**2 consecutive runs**

The solubility of ETFOSE-OH TCR-00017-98 in water was determined to be 151 ng/mL (corrected for purity) at 19.5-21 °C.

Table 1b. Summary Table of Solubility of EtFOSE-OH in Methanol and Acetone.

SOLVENT	ETFOSE-OH SOLUBILITY (MASS/VOLUME)
Methanol	> 10 %
Acetone	> 10%

The solubility of EtFOSE-OH was determined to be greater than 10% (mass/volume) in methanol and acetone at 22.5 °C.

PURPOSE

The purpose of this phase of the study was to determine the solubility of EtFOSE-OH in water, methanol, and acetone as part of the characterization of this test, control, and reference substance.

TEST SUBSTANCE

Table 2. Characterization of the Test Substance and Analytical Reference Substance

TEST SUBSTANCE	ETFOSE-OH		
3M Laboratory Identification Number	SE-031	SE-035	TCR-00017-98
Source	Wildlife International	Purified from SE-031 at Pace, Tier II	Purified from SE-031 at Tier 2 Facility
Expiration Date	11-26-2001	08-17-2001	Not Available
Storage Conditions	Frozen	Frozen	Frozen
Physical Description	Yellow solid (sandy)	White Solid	White powder
Purity	**97.7%**	**99.9%**	**99.9%** (unofficially)

EtFOSE-OH TCR-0017-98 was used for the column elution method instead of SE-035 because there was limited supply of SE-035 required to complete the entire study. Since EtFOSE-OH TCR-0017-98 was purified from the same source (SE-031) and method as SE-035, it was used as a suitable replacement for the quantitative determination of the EtFOSE-OH solubility. The purity of the reference substance, SE-035, has been determined to be 99.9%. All reported values have been corrected for purity.

REFERENCE SUBSTANCE

Table 3. Characterization of the Analytical Reference Substance

REFERENCE SUBSTANCE	THPFOS	ETFOSE-OH
Source	SynQuest Labs	SE-035
Expiration Date	Not Available	Purified from SE-031 at Pace, Tier II
Storage Conditions	Frozen	08-17-2001
3M Laboratory Identification Number	TCR-00017-047 Lot #Q75-30	Frozen
Physical Description	White Powder	White Solid
Purity	*	**99.9%**

** The purity of THPFOS (currently being determined) will not affect any analytical findings.*

TEST SYSTEM

Table 4a. Description of test systems used in Solubility Screen Test

	WATER	METHANOL	ACETONE
Source	Millipore System	EM Science	EM Science
Expiration Date	Not applicable	2005	2005
Storage Conditions	Ambient	Ambient	Ambient
3M Laboratory Identification Number	Not applicable	TN-A: 4604	TN-A: 4616
Purity/Grade	ASTM Type I	HPLC Grade	HPLC Grade

Table 4b. Description of test system used in Solubility Test, Column Elution Method

	GENERATOR COLUMN	WATER	SUPPORT MATERIAL
Source	Dionex	Millipore System	Not available
Storage Conditions	Ambient	Ambient	Ambient
Description	4X35 mm Hollow Column Body, Frits	None	Glass Wool
Purity/ Grade	PEEK material	ASTM Type I	Neat
...CONTINUED	PERISTALTIC PUMP	PUMP CASSETTES	TUBING
Source	Cole-Parmer	Cole-Parmer	Solva
Storage Conditions	Ambient	Ambient	Ambient
Description	MasterFlex L/S Pump Equipped with 7 casettes	MasterFlex MicroCassette equipped tubing	Peristaltic Pump Tubes, Inner diameter= 1.14 mm
Purity/ Grade	Model No. 7524-40	Model No. 7519-65	P.V.C (Yellow)

METHOD SUMMARIES

The solubility determination of EtFOSE-OH in water, methanol, and acetone was performed according to United States Environmental Protection Agency OPPTS 830.7840, "Water Solubility: Column Elution Method; Shake Flask Method" and OECD 105, "Water Solubility" Guidelines, as implemented by 3M Laboratory in methods ETS-8-170.0 "Solubility Screen Test: Approximate Solubility Determination of a Test Substance in Various Solvents; and " ETS-8-171.0 "Column Elution Method; Solubility Determination of a Test Substance in Various Solvents". All analyses were performed according to the method ETS-8-155.0 "Analysis of Perfluorooctane Sulfonate or Other Fluorochemicals in Waste Stream or Water Extracts Using HPLC-Electrospray/Mass Spectrometry".

The Solubility Screen Test Method was used to determine the solubility range of EtFOSE-OH in water. The screen test indicated a solubility of EtFOSE-OH in water of less than 10 µg/mL at 22.5 °C. Therefore the Column Elution Method was used for the quantitative determination of EtFOSE-OH solubility. Detailed descriptions of the Solubility Screen Test Method and Column Elution Method used in this study are located in Attachment A.

PREPARATORY METHODS

- **ETS-8-170.0 "Solubility Screen Test: Approximate Solubility Determination of a Test Substance in Various Solvents."** This method is a prerequisite to the shake flask and column elution methods and gives an estimate of the solubility point of the test substance in the solvent of choice.
    - **EtFOSE-OH SE-035 in water:** After preparing solutions of water and EtFOSE-OH up to a concentration level that was approximately 5,000 µg/mL, there were still particulates visible. Therefore 12.2 mg of EtFOSE-OH were added to 100 mL of water for a concentration of approximately 100 µg/mL. There was still particulate present, therefore 10.1 mg was added to 1 L of water for a theoretical concentration of 10 µg/mL EtFOSE-OH. There was still particulate remaining in the flask after vortex mixing, sonicating, and allowing to sit for an extended amount of time. This concentration level indicated that the column elution method should be used to determine the solubility of EtFOSE-OH.
    - **EtFOSE-OH SE-035 in methanol:** Solvent was added stepwise to a total volume of 1 mL when it was observed that the particulates noticed in the solution may be dust particles. Due to the small volume it was difficult to tell with any degree of accuracy what the particulates were, therefore a larger volume was prepared for ease of observations. Approximately 170 mg EtFOSE-OH was added to a 4 mL screw top vial and 1.7 mL methanol was added to it. This provided a final concentration of ≥ 10%. Observations made it clear that the white powder of EtFOSE-OH had gone into solution and was no longer observed. Therefore it was concluded that the solubility of EtFOSE-OH was estimated to be greater than 10% (mass/volume) in methanol.
    - **EtFOSE-OH SE-035 in acetone:** Following addition of 0.1 mL to 10.08 mg EtFOSE-OH, it was observed that the EtFOSE-OH had dissolved. The dissolution observation marked the end of the solubility screen test and it was concluded that the estimated solubility of EtFOSE-OH in acetone was greater than 10% (mass/volume) in acetone.

Table 5. Summary of Screen Test method Sample Preparation.

STEP	SAMPLE PREPARATION	DATE(S) PERFORMED
1	Approximately 10 mg test substance was weighed into tared glass screw-capped vial.	9/11/00
2	0.1 mL of test solvent (water, methanol or acetone) was added to glass screw-capped vial.	9/11/00
3	Following the addition of test solvent, the vial was capped, shaken vortexed and then sonicated.	9/11/00

STEP	SAMPLE PREPARATION	DATE(S) PERFORMED
4	Following sonication, the sample was checked visually for undissolved particles of the test substance. In acetone, all of the test substance appeared to be dissolved, and the solubility was then greater than 10% (mass/volume) - no additional solubility testing required. 170 mg EtFOSE-OH was dissolved in 1.7 mL methanol, vortex-mixed, sonicated and it appeared all substance had dissolved-solubility is greater than 10% at and no further testing is required. A portion of the test substance remained undissolved in water, therefore further dilutions were necessary.	9/11/00
5	Up to 2 mL additional water were added and the vial was vortexed and sonicated. Undissolved particles were still observed at this dilution level, therefore 12.24 mg were added to 100 mL, vortex-mixed, sonicated. Undissolved particles still remained, therefore 10.10 mg were added to 1 L water, and after vortex-mixing and sonicating the flask, there were still particles after 24 hours. Solubilty is< 10 µg/mL and must be determined via the column elution method.	9/11/00

- **ETS-8-171.0 "Column Elution Method: Solubility Determination of a Test Substance in Various Solvents."** Approximately 100 mg glass wool was weighed into a tared 15 mL plastic centrifuge tube. EtFOSE-OH TCR-00017-98 was weighed into a tared weigh boat for a final amount of approximately 100 mg. The EtFOSE-OH was then added to the centrifuge tube containing the glass wool by tapping the weigh boat and scraping particulate into the centrifuge tube. Acetone was then added to the tubes so that the glass wool was completely submerged (approximately 10 mL acetone). Five centrifuge tubes were prepared with EtFOSE-OH, and two additional solvent blank tubes were prepared with glass wool only. The centrifuge tubes were then placed on a nitrogen blow-down apparatus to evaporate the acetone. The tubes were checked periodically to check for the progression of the evaporating solvent, and the glass wool was continually pushed below the surface of the solvent so that it was always submerged during the evaporation process. Following the evaporation of solvent, the glass wool was then removed from the centrifuge tube and weighed in a tared weigh boat. For glass wool with EtFOSE-OH absorbed onto it, the weight of the EtFOSE-OH was determined by difference of the glass wool weight before and after the absorption process. The glass wool was then put into the 4 mm X 35 mm PEEK column bodies which were taped vertically to a 1L Nalgene bottle for support. Tubing was affixed to the bottom of the column which was fed through the peristaltic pump and into the bottom of a 15 mL centrifuge tube. The tubing affixed to the bottom of the column was the "inlet" end of the column, where water from the centrifuge tube could flow in. The 15 mL centrifuge tube was filled with approximately 13 mL Milli-Q™ water. The cap to the centrifuge tube had two small holes drilled into it so as to allow passage of the "inlet" and "outlet" tubing to pass in and out of the centrifuge tube. The pump was turned on for each column just enough to fill the barrel with water. After each column was filled with water, the columns were capped and allowed to sit for approximately 2 hours. After allowing the columns to set and equilibrate, the capped top end was replaced with "outlet" tubing which fed into the top of a second EMPTY 15 mL centrifuge tube. The pump was turned on and the columns were flushed for approximately 10 minutes, in which time approximately 8 mL were diverted to secondary centrifuge tubes. The primary centrifuge tube which held the water supply was replenished to a total volume of approximately 13 mL, and the "outlet" tubing was placed into the centrifuge tube just above the surface of the water. The columns with test subtance and method blank columns were placed in a temperature-controlled incubator set at approximately 20 °C. The peristaltic pump was then turned on to allow for recirculation of the water in the centrifuge tubes. The pump

flow rate was set and "Time 0" commenced at the time of the peristaltic pump turn on. See Table 6 documenting the column preparation:

Table 6. Column Elution Method Preparation for Study (FACT-TCR004), ETFOSE-OH TCR-00017-98 at 22 rpm and 11 rpm.

STEP	GENERATOR COLUMN PREPARATION	QTY	DATE(S) PERFORMED	
			22 RPM	11 RPM
1	Approximately 100 mg glass wool was weighed into a tared centrifuge tube.	7	10-05-00	11-07-00
2	Sample Preparation- Approximately 100 mg was weighed into a tared weigh boat and then transferred to the centrifuge tube containing glass wool.	5	10-05-00	11-07-00
3	Method Blank Preparation- started at step number one skipped step number 2 to step number 4.	2	10-05-00	11-07-00
4	Approximately 10 mL acetone was added to the centrifuge tubes and any unsubmerged glass wool was forced below the surface of the solvent.	7	10-05-00	11-07-00/ 11-13-00
5	The centrifuge tubes were placed on a nitrogen blow-down apparatus to evaporate the solvent. The tubes were monitored and as solvent evaporated, the glass wool was continually pushed below the surface of the acetone to allow for maximal contact with the solution.	7	10-05-00	11-07-00/ 11-13-00
6	Removed glass wool with care and weighed in a tared weigh boat. Calculated weight difference from step1 and 6.	7	10-05-00	11-07-00/ 11-13-00
7	Filled centrifuge tubes with approximately 13 mL Milli-Q™ water.	7	10-05-00	11-13-00
8	Placed glass wool into the emptied column bodies.	7	10-05-00	11-13-00
9	Fitted tubing to the columns, through the pump and into the centrifuge tubes. Filled columns with water, capped, and allowed to equilibrate for approximately 2 hours.	7	10-06-00	11-13-00
10	Hooked columns up with tubing, put together with the water reservoir (i.e. centrifuge tube) and pump. Flushed columns, replenished water reservoir and set up the system to recirculate.	7	10-06-00	11-13-00
11a	Put the entire set up in the incubator set to 20 °C. The peristaltic pump was set to 740 µL/min (22 rpm) to commence flow.	7	12:00 p.m., 10-06-00 ("Time 0")	Not applicable
11b	Put the entire set up in the incubator set to 20 °C. The peristaltic pump was set to 175 µL/min (11 rpm) to commence flow.	7	Not applicable	3:30 p.m., 11-13-00 ("Time 0")

SAMPLE COLLECTION AND ANALYSIS

At each sampling interval, 100 µL sample was aliquoted in triplicate from the recirculating solution of each of the seven columns into labeled glass autovials containing 500 µL methanol. Vials were capped and shaken to thoroughly mix sample. Autovials were stored at 3-6 °C until analysis in cooler R-4. On the dates designated in tables 7a and 7b internal standard was added to the autovial prior to analysis via HPLC/MS according to analytical method ETS-8-155.0.

Note: Samples were named according to the time of sample collection and preparation. However, some data points were incorrectly calcuated and the sample ID does not accurately reflect the actual time that the sample was collected. The following table shows the incorrect timepoints as read on the sample preparation worksheets, summaries, and chromatograms, and the "corrected timepoint" is the actual time of collection and preparation (Note not all timepoints were calculated incorrectly. The table only includes the incorrect timepoints.):

22 rpm Study:	
"Old Timepoint"	***Corrected***
Day 3, 72 hours	*Day 3, 74 hours*
Day 4, 96 hours	*Day 4, 97 hours*
Day 12, 288 hours	*Day 12, 290 hours*
Day 13, 302 hours	*Day 13, 314 hours*
Day 14, 326 hours	*Day 14, 338 hours*
Day 27, 649 hours	*Day 27, 651 hours*

11rpm Study:	
"Old Timepoint"	***Corrected***
Day 2, 39 hours	*Day 2, 41 hours*
Day 3, 63 hours	*Day 3, 65 hours*
Day 4, 87.5 hours	*Day 4, 89.5 hours*
Day 4, 88.5 hours	*Day 4, 90.5 hours*
Day 4, 89.5 hours	*Day 4, 91.5 hours*
Day 4, 90.5 hours	*Day 4, 92.5 hours*
Day 4, 91.5 hours	*Day 4, 93.5 hours*
Day 4, 92.5 hours	*Day 4, 94.75 hours*
Day 4, 93 hours	*Day 4, 95 hours*
Day 5, 116 hours	*Day 5, 117.5 hours*
Day 6, 140 hours	*Day 6, 142 hours*
Day 7, 157 hours	*Day 7, 161 hours*
Day 8, 181 hours	*Day 8, 185.25 hours*
Day 9, 205 hours	*Day 9, 209 hours*

The following table describes the sample collection and preparation regimen:

Table 7a. Sample Collection, Preparation, and Analysis for Study FACT-TCR004 EtFOSE-OH 22 rpm flow rate

STEP	PROCEDURE	REPLICATES	DATE(S) PERFORMED
1	At time 0 hours samples were placed in a temperature controlled incubator ID# I-16 set to 20 °C.	7 columns set up to the peristaltic pump, 2 blanks and 5 containing test substance.	10-06-00
2	100 µL aliquots of the column's supernatant (water containing dissolved ETFOSE-OH) were collected and dispensed into autovials containing 500 µL methanol.	Day 1, 28 hours	10-07-00
		Day 2, 49 hours	10-08-00
		Day 3, 72 hours	10-09-00
		Day 4, 96 hours	10-10-00
		Day 5, 119 hours	10-11-00
		Day 6, 146 hours	10-12-00
		Day 7, 165 hours	10-13-00
		Day 7, 166 hours	
		Day 7, 167 hours	
		Day 7, 168 hours	
	3 replicates per test system, per day:	Day 7, 169 hours	
		Day 10, 238 hours	10-16-00
		Day 11, 264 hours	10-17-00
		Day 12, 288 hours	10-18-00
		Day 13, 314 hours	10-19-00
		Day 14, 338 hours	10-20-00
		Day 27, 649 hours	11-02-00
	*a) 1 mL aliquot of supernatant was collected into a 2 mL centrifuge tube and centrifuged. 100 µL supernatant was then dispensed into an autovial containing 500 µL methanol.	*a) Day 27, 649 hours	

3	Internal Standard was added to the 600 µL of sample in the autovial. IS solution information: Solution "a" = THPFOS 00001-28-01, 16 µL, 10 µg/mL; and PFBS 00001-30-03, 16 µL, 15 µg/mL added Solution "b"= THPFOS 00001-21-01 (22.2 µg/mL), 11 µL added	Day 1, 28 hours, solution "a"	10-11-00
		Day 2, 49 hours, solution "a"	
		Day 3, 72 hours, solution "a"	10-12-00
		Day 4, 96 hours, solution "a"	10-13-00
		Day 5, 119 hours, solution "a"	
		Day 6, 146 hours, solution "a"	
		Day 7, 165 hours, solution "a"	10-16-00
		Day 7, 166 hours, solution "a"	
		Day 7, 167 hours, solution "a"	
		Day 7, 168 hours, solution "a"	
		Day 7, 169 hours, solution "a"	
		Day 10, 238 hours, solution "a"	
		Day 11, 264 hours, solution "a"	10-27-00
		Day 12, 288 hours, solution "a"	
		Day 13, 302 hours, solution "a"	
		Day 14, 326 hours, solution "a"	
		Day 27, 649 hours, solution "b"	01-02-01
		Day27,649 hours,solution "b" (centrifuged sample)	
5	Samples were analyzed via HPLC/ES/MS on HP 1100 LC/MSD "Hillary."	Day 1, 28 hours	10-17-00, sequence h001017.s
		Day 2, 49 hours	10-17-00, sequence h001017.s
		Day 3, 72 hours	10-19-00, sequence h001019.s
		Day 4, 96 hours	10-19-00, sequence h001019.s
		Day 5, 119 hours	10-22-00, sequence h001022.s
		Day 6, 146 hours	10-22-00, sequence h001022.s
		Day 7, 165 hours	10-24-00, sequence h001024.s
		Day 7, 166 hours	10-24-00, sequence h001024.s
		Day 7, 167 hours	10-24-00, sequence h001024.s
		Day 7, 168 hours	10-24-00, sequence h001024.s
		Day 7, 169 hours	10-24-00, sequence h001024.s
		Day 10, 238 hours	10-24-00, sequence h001024.s
		Day 11, 264 hours	10-27-00, sequence h001027.s
		Day 12, 288 hours	10-28-00, sequence h001028.s
		Day 13, 314 hours	10-28-00, sequence h001028.s
		Day 14, 338 hours	10-28-00, sequence h001028.s
		Day 27, 649 hours	01-02-00, sequence h010102.s
		Day 27, 649 hours (centrifuged)	01-02-00, sequence h010102.s

Table 7b. Sample Collection, Preparation, and Analysis for Study FACT-TCR004 EtFOSE-OH 11 rpm flow rate

STEP	PROCEDURE	REPLICATES	DATE(S) PERFORMED
1	At time 0 hours samples were placed in a temperature controlled incubator ID# I-16 set to 20 °C.	7 columns set up to the peristaltic pump, 2 blanks and 5 containing test substance.	11-13-00
2	100 µL aliquots of the column's supernatant (water containing dissolved ETFOSE-OH) were collected and dispensed into autovials containing 500 µL methanol.	Day 2, 39 hours	11-15-00
		Day 3, 63 hours	11-16-00
		Day 4, 87.5 hours	11-17-00
		Day 4, 88.5 hours	
		Day 4, 89.5 hours	
		Day 4, 90.5 hours	
		Day 4, 91.5 hours	
		Day 4, 92.5 hours	
		Day 4, 93 hours	
	3 replicates per column, per day:	Day 5, 116 hours	11-18-00
		Day 6, 140 hours	11-19-00
		Day 7, 157 hours	11-20-00
		Day 8, 181 hours	11-21-00
		Day 9, 205 hours	11-22-00
3	Internal Standard was added to the 600 µL of sample in the autovial. IS Information: THPFOS/PFBS ID#00003-140, 16.5 µL	Day 2, 39 hours	11-17-00
		Day 3, 63 hours	11-17-00
		Day 4, 87.5 hours	11-17-00
		Day 4, 88.5 hours	11-17-00
		Day 4, 89.5 hours	11-17-00
		Day 4, 90.5 hours	11-17-00
		Day 4, 91.5 hours	11-22-00
		Day 4, 92.5 hours	11-17-00
		Day 4, 93 hours	11-22-00
		Day 5, 116 hours	11-20-00
		Day 6, 140 hours	11-20-00
		Day 7, 157 hours	11-22-00
		Day 8, 181 hours	11-22-00
		Day 9, 205 hours	11-22-00
5	Samples were analyzed via HPLC/ES/MS on HP 1100 LC/MSD "Hillary."	Day 2, 39 hours	11-22-00, h001122.s
		Day 3, 63 hours	11-22-00, h001122.s
		Day 4, 87.5 hours	11-24-00, h001124.s
		Day 4, 88.5 hours	11-24-00, h001124.s
		Day 4, 89.5 hours	11-24-00, h001124.s
		Day 4, 90.5 hours	11-26-00, h001126.s
		Day 4, 91.5 hours	11-27-00, h001127.s
		Day 4, 92.5 hours	11-27-00, h001127.s
		Day 4, 93 hours	11-27-00, h001127.s
		Day 5, 116 hours	11-29-00, h001129.s
		Day 6, 140 hours	11-29-00, h001129.s
		Day 7, 157 hours	11-29-00, h001129.s
		Day 8, 181 hours	12-01-00, h001201.s
		Day 9, 205 hours	12-01-00, h001201.s

The pH of each column as well as the temperature of the incubator was recorded at each sampling interval. The pH was consistently approximately 5.5 for both 22 and 11 rpm studies. The temperature range of the apparatus (and therefore water temperature of the sample) throughout the 22 rpm study was 19.5-21.0 °C. The temperature range of the water during the saturation process for the 11 rpm study was 20.0-21.0 °C.

ANALYTICAL METHOD

- Method presented in Attachment A.
- ETS-8-155.0 "Analysis of Perfluorooctane Sulfonate or Other Fluorochemicals in Waste Stream or Water Extracts Using HPLC-Electrospray/Mass Spectrometry". Diluted samples (i.e. diluted supernatants) were analyzed using HPLC/ES/MS in the negative ion mode. EtFOSE-OH levels were evaluated versus calibration standards ranging in concentration from 2.507-501.4 ng/mL. Internal Standard quantification was used to best fit the data. Target ions were 630 m/z (acetate adduct EtFOSE-OH-test substance), 427 m/z (deprotonated THPFOS-internal standard), and 299 m/z (deprotonated PFBS-qualitative analysis only, not used to report data).

Analytical Equipment

Liquid Chromatograph: Hewlett-Packard® Series 1100 Liquid Chromatograph system
Analytical column: Keystone® Betasil™ C_{18} 2x50mm, 5µm particle size
Column temperature: Ambient
Cycle Time: 10.0 minutes
Flow rate: 300µL/min
Injection volume: 10µL
Mobile phase components:
Solvent A: 2.0 mM ammonium acetate in water
Solvent B: Methanol
Solvent Gradient:

Time	% B
0.00	15 %
0.50	15 %
0.80	60 %
3.50	100 %
6.50	100 %
7.00	15 %
9.75	15 %
10.00	Stop

Mass Spectrometer: Hewlett-Packard® Series 1100 API/Mass Spectrometer Detector
Software: HP ChemStation™ 8.03
Fragmentor Voltage: m/z 630=55 V; m/z 427=100 V; m/z 299= 100 V
Capillary Voltage: 3500 V
Gain = 2.0 EMV
Mode: Electrospray Negative
Gas Temperature: 350 °C
Drying Gas: 10.0 L /min.

Nebulizer Pressure: 25 psig
Analysis Type: Single Ion Monitoring (SIM)

ANALYTICAL RESULTS

Data quality objectives outlined in the 3M Environmental Laboratory method were met (see Appendix A).

- **Regressions.** Quadratic curve fit was 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 (R^2 values ranged from 0.9913 - 0.9998 for 22 rpm flow, and 0.9971-0.9993 for 11 rpm flow).
- **Calibration Standards.** All calibration criteria were met. It was not necessary to use exponential or quadratic fits of the data, as the concentration range was linear. The curves were examined closely, particularly for accuracy of quantitation at the low and high ends of the curve. It was necessary to use an abbreviated portion of the curve to better fit the data range and provide better accuracy for the data range quantified. The calibration range used for the majority of samples to quantify the data was 5.01 ng/mL to 401.1 ng/mL, exceptions to this calibration range are listed below in table 8:

Table 8. Calibration curve ranges used for the quantification of EtFOSE-OH 11 and 22 rpm data.

BATCH	CURVE RANGE USED/ LOQ	
h1017a2.b	**2.5**-250.7 ng/mL	22 rpm study samples
h1017b1.b	**2.5**-401.1 ng/mL	
h1017c3.b	5.01-250.7 ng/mL	
h1024a2.b	**10**-401.1 ng/mL	
h1122b1.b	5.01-501.4 ng/mL	11 rpm study samples

- **Limit of Quantitation (LOQ).** The LOQ is equal to the lowest standard in the calibration curve. The LOQ for the majority of sample batches was 5.01 ng EtFOSE-OH /mL, with the exceptions to this in table 8 (in **bold** print) above.
- **Sample Replicates.** Sample replicates must meet the 30% precision criteria to be reported. Samples were excluded by one of two scenarios: 1) suspected outlier value was excluded via Q-Test (statistical analysis) or 2) the %RSD for the measurement was greater than 30%, therefore signifying that the data was too scattered to draw a conclusion. It was necessary to exclude the following samples to meet the data quality objective of 30% or less precision:
  - **22 rpm data.** Samples 100600-CEM-EtF-146.2.1, 100600-CEM-EtF-146.2.2, 100600-CEM-EtF-146.2.3 (Day 6 (146 hrs), column 2); 100600-CEM-EtF-146.4.3; 100600-CEM-EtF-167.1.1; 100600-CEM-EtF-167.3.3; 100600-CEM-EtF-167.5.3; 100600-CEM-EtF-238.3.3; 100600-CEM-EtF-238.4.2; 100600-CEM-EtF-264.2.3; and 100600-CEM-EtF-288.5.3 were excluded from the reported data using Dixon's Q-Test with greater than 80% confidence. Samples 100600-CEM-EtF-166.5.1, 100600-CEM-EtF-166.5.2, 100600-CEM-EtF-166.5.3 (Day 7 (167 hrs), Column 5); 100600-CEM-EtF-302.all (all samples for Day 13); and 100600-CEM-EtF-326.all (all samples for Day 14) were

excluded because the data was too scattered and did not meet data quality objectives for precision (<30% RSD).

- **11 rpm data.** Samples 110700-CEM-EtF-63.4.1, 110700-CEM-EtF-87.4.2, 110700-CEM-EtF-88.5.2, 110700-CEM-EtF-89.2.all (column 2, reps1,2,and 3), 110700-CEM-EtF-89.4.3, 110700-CEM-EtF-90.5.3, 110700-CEM-EtF-92.5.1, and 110700-CEM-EtF-116.5.2 were excluded from the reported data using Dixon's Q-Test with greater than 80% confidence. Samples 110700-CEM-EtF-87.all (all samples for Day 4, 87hrs); 110700-CEM-EtF-90.4.1, 110700-CEM-EtF-90.4.2, and 110700-CEM-EtF-90.4.3 (Day 4 (90.5 hrs), Column 4); were excluded because the data was too scattered and did not meet data quality objectives for precision (<30% RSD).

- **Continuing Calibration Verification.** For quantitative determinations, a mid-level matrix calibration check was analyzed every five samples to monitor instrumental drift, with a limit of ±15% of the target concentrations.
- **Blanks.**
  - Method blanks (Milli-Q water taken through the entire sample preparation, dilution, and analysis process) provided a measure of laboratory contamination. Acceptable values for the blanks were less than 50% of the limit of quantitation (LOQ).
  - Solvent blanks (aliquots of methanol) provided a measure of reagent and instrument contamination. Acceptable values for the blanks were less than 50% of the limit of quantitation (LOQ).
- **Specificity (according to OPPTS 830.7840):** The solubility determination as stated in the EPA Guidelines follows. This method should only be applied to:
  - Pure substance. The official purity is to be determined, however comparison results between SE-035 (purity of 99.9%) and TCR-0017-98 from this study indicate that within the limits of experimental error, there is no distinct difference between the two lots. Therefore, for the purposes of this study, the purity of TCR-00017-98 is 99.9%.
  - Substances that are stable in water. Hydrolytic studies have been conducted at the 3M Environmental Laboratory showing stability.

DATA SUMMARY AND DISCUSSION

Tables 9a and 9b summarizes individual sample data. Representative chromatograms are presented in Attachment C. The table displays EtFOSE-OH concentrations for individual injections for the 22 and 11 rpm studies. Also included are the average concentrations for each column triplicate, standard deviation, and % relative standard deviation. Sample calculations may be found in Attachment E.

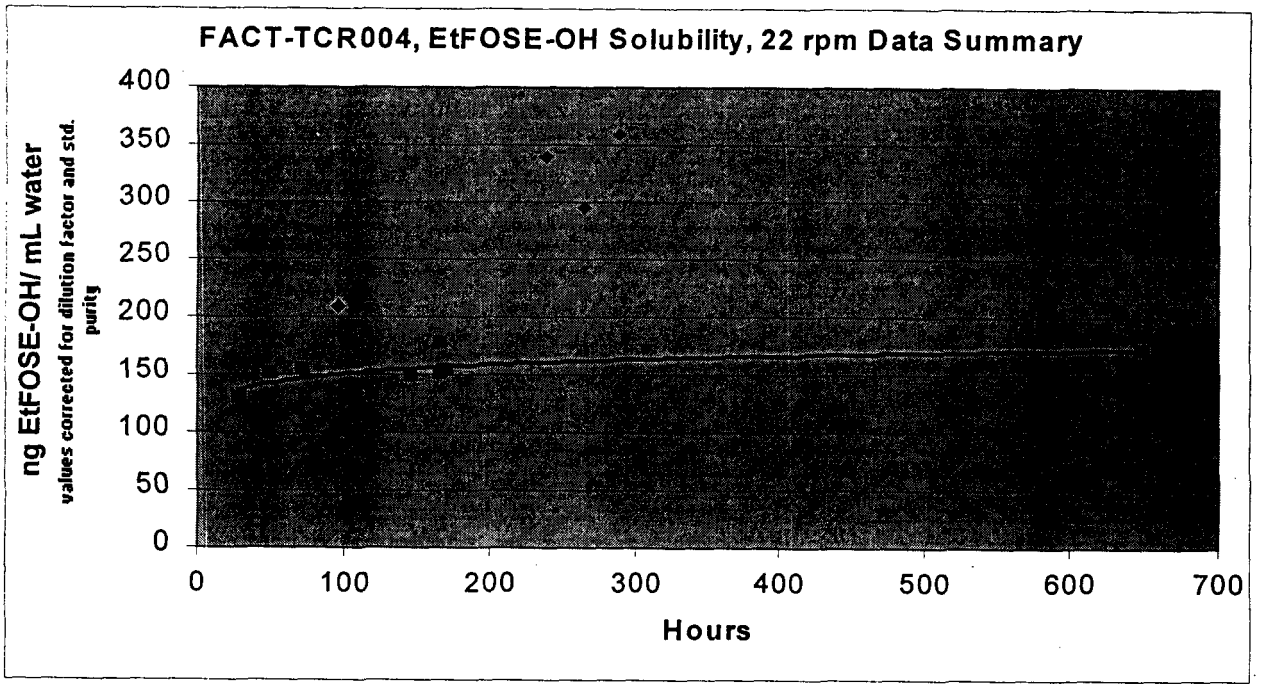
For the 22 rpm study, samples were collected once daily up through day 6. It was postulated that the solution had reached a plateau in concentration by day 6, and the multiple sampling (i.e. collection of 5 or more consecutive sample sets) was therefore performed on Day 7. Samples were continually taken once daily after Day 7 until analytical confirmation could be made that the solubility point had been reached by Day 7. By evaluating the data, results indicate that the solution became saturated by Day 2, and the 5 consecutive timepoints on day 7 confirmed that the solubility of EtFOSE-OH had been reached, and could be reported within 30% variability as required.

By evaluating the data presented in table 9a, it may be noticed that for timepoints 238, 264, 288, 302, and 326 hours the concentration is almost double that of the rest of the data. It was discovered that this disparity in concentrations was due to a sample preparation error. These timepoints were therefore plotted as diamonds to denote outliers of the saturation trend. The 96 hour timepoint is also plotted as a diamond symbol because it is also an outlier to the saturation trend. By averaging evaluating all data points prior to 238 hours plus the 649 hour timepoint, the 96 hour timepoint may be excluded via Dixon's Q-Test.

Table 9a. EtFOSE-OH Sample Concentrations by timepoint for 22 rpm study.

Timepoint	ng EtFOSE-OH/mL water (corrected for standard purity)	Std Dev, %RSD	Replicates Reported
Day 1 (28 hours)	130	15, 12%	5 columns, 3 replicates per column= 15 samples
Day 2 (49 Hours)	151	14, 9%	5 columns, 3 replicates per column= 15 samples
Day 3 (72 Hours)	155	25, 16%	5 columns, 3 replicates per column= 15 samples
Day 4 (96 Hours)	209	53, 25%	5 columns, 3 replicates per column= 15 samples
Day 5 (119 Hours)	133	11, 8%	5 columns, 3 replicates per column= 15 samples
Day 6 (146 Hours)	150	24, 16%	3 columns, 3 reps per column + 1 column, 2 reps per column=11 samples
Day 7 (165 Hours)	179	35, 20%	5 columns, 3 replicates per column= 15 samples
Day 7 (166 Hours)	152	21, 14%	4 columns, 3 replicates per column= 12 samples
Day 7 (167 Hours)	157	43, 27%	3 columns, 3 replicates per column + 2 col, 2 reps per column= 13 samples
Day 7 (168 Hours)	155	28, 18%	5 columns, 3 replicates per column= 15 samples
Day 7 (169 Hours)	154	30, 19%	5 columns, 3 replicates per column= 15 samples
Day 10 (238 Hours)	340	61, 18%	3 col, 3 replicates per column + 2 col, 2 reps per col= 13 samples
Day 11 (264 Hours)	296	74, 25%	4 columns, 3 replicates per column + 1 col, 2 reps per col= 14 samples
Day 12 (288 Hours)	359	89, 25%	3 columns, 3 replicates per column + 1 column 2 reps per col= 11 samples
Day 13 (302 Hours)	499	314, 63%	Data excluded due to poor overall precision...data too scattered.
Day 14 (326 Hours)	375	139, 37%	Data excluded due to poor overall precision...data too scattered.
Day 21 (649 Hours)	173	31, 18%	5 columns, 3 replicates per column= 15 samples

Highlighted samples denote excluded or unacceptable data.

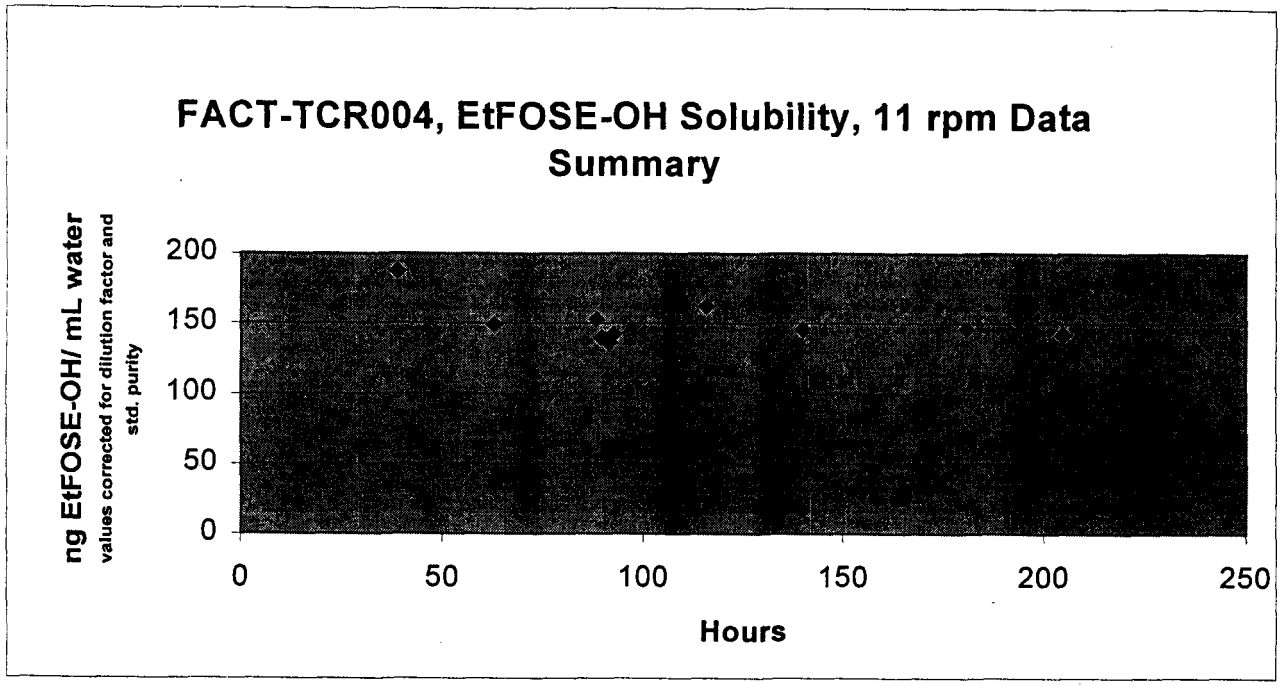


Since the saturation point of the water solution occurred after only 2 days in the 22 rpm study, the multiple sampling point for the 11 rpm study was performed at the 4 day point, to ensure that the solution had sufficient time to saturate. On the fourth day of the 11 rpm experiment, six consecutive samples were collected, and then samples were also collected for four more days after to document the behavior of the solution. As the results indicate, saturation did occur by day four for the 11 rpm study, and the final data reported for the 11 rpm solubility point was based on the average of day four's results.

Table 9b. EtFOSE-OH sample concentrations by timepoint for 11 rpm study.

TIMEPOINT	NG ETFOSE-OH/ML (CORRECTED FOR PURITY)	STD DEV, %RSD	REPLICATES REPORTED
Day 2, 39 Hours	187	38, 20%	5 columns, 3 replicates per column= 15 samples
Day 3, 63 Hours	149	25, 17%	4 columns, 3 replicates per column + 1 column, 2 reps per col= 14 samples
Day 4, 87 Hours	169	52, 31%	Data excluded due to poor overall precision...data too scattered.
Day 4, 88 Hours	153	17, 11%	4 columns, 3 replicates per column + 1 column, 2 reps per col= 14 samples
Day 4, 89 Hours	140	25, 18%	3 columns, 3 replicates per column, + 1 column, 2 reps per col= 11 samples
Day 4, 90 Hours	139	26, 19%	3 columns, 3 replicates per column, + 1 column, 2 reps per col= 11 samples
Day 4, 91 Hours	136	40, 29%	5 columns, 3 replicates per column= 15 samples
Day 4, 92 Hours	142	29, 20%	4 columns, 3 replicates per column + 1 column, 2 reps per col= 14 samples
Day 5, 116 Hours	162	27, 17%	4 columns, 3 replicates per column + 1 column, 2 reps per col= 14 samples
Day 6, 140 Hours	146	24, 16%	5 columns, 3 replicates per column= 15 samples
Day 7, 181 Hours	146	13, 9%	5 columns, 3 replicates per column= 15 samples
Day 8, 205 Hours	142	21, 15%	5 columns, 3 replicates per column= 15 samples

Highlighted samples denote excluded or unacceptable data.



The final solubility of EtFOSE-OH TCR-00017-98 in water and EtFOSE-OH SE-035 in methanol and acetone is summarized in tables 10a and 10b:

Table 10a. Summary Table of Solubility of EtFOSE-OH in Water.

Sampling Event	Average ETFOSE-OH Concentration in Water (µg/mL, corrected for purity)	Standard Deviation, %RSD	Replicates Reported
22 rpm study	159	10, 6%	5 timepoints
11 rpm study	142	6, 4%	5 timepoints
Final Value	**151**	**11% RPD**	**2 consecutive runs**

The solubility of ETFOSE-OH TCR-00017-98 in water was determined to be 151 ng/mL (corrected for purity) 19.5-21 °C.

Table 10b. Summary Table of Solubility of EtFOSE-OH SE-035 in Methanol and Acetone.

Solvent	EtFOSE-OH Solubility (mass/volume)
Methanol	> 10 %
Acetone	> 10%

The solubility of EtFOSE-OH SE-035 was determined to be greater than 10% (mass/volume) in methanol and acetone at 22.5 °C.

Attachment B contains data summary tables.

STATISTICAL METHODS

Statistical methods were limited to calculating means and standard deviations. Data that did not meet acceptance criteria as described in the OPPTS and OECD guidelines was excluded following statistical examination using Dixon's Q-test. Refer to Attachment E for applicable equations.

STATEMENT OF CONCLUSION

Under the conditions of the present study, the solubility of EtFOSE-OH TCR-00017-98 in ASTM Type I water is 151 ng/mL 19.5-21 °C. The solubility of EtFOSE-OH SE-035 was determined to be greater than 10% (mass/volume) in methanol and acetone 22.5 °C.

REFERENCES

1. Fate, Transport and Transformation Test Guidelines Office of Prevention, Pesticides and Toxic Substances (OPPTS) 830.7840 Water Solubility: Column Elution Method; Shake Flask Method. EPA 712-C-96-041, August 1996.

2. OECD 105: Water Solubility. Adopted 27July, 1995.

3. Harris, Daniel C. Statistics. Quantitative Chemical Analysis. 4th ed.; W.H. Freeman and Company: New York, 1982; p 70-71.

4. Natrella, Mary Gibbons. The Treatment of Outliers. Experimental Statistics, National Bureau of Standards Handbook 91; U.S. Government Printing Office: Washington, D.C., 1963; Pages 17-3, and T-27.

LIST OF ATTACHMENTS

- Attachment A: Extraction and Analytical Methods
- Attachment B: Data Summary Tables
- Attachment C: Sample Chromatograms
- Attachment D: Deviations from the Protocol
- Attachment E: Sample Calculations

SIGNATURE PAGE

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

Mark E. Ellefson
Principal Investigator

05/30/01
Date

William K. Reagen
Laboratory Manager

05/30/01
Date