

**TOXICOLOGY ASSESSMENT AND COMPLIANCE ASSURANCE GROUP
3M COMPANY**

**13-430: ACUTE INHALATION TOXICITY
STUDY OF MTDID 37312 IN MALE RATS**

FINAL REPORT

ToxDocs Study Number	13-430
Test Material	MTDID 37312
Lot Number(s)	Lot 30107 (per GID 103988)
Strategic Tox Lab Study Number	ST-460
Other Identification Number(s)	FC-10
19F/1H-NMR Analytical Report LIMS Number	LIMS ## M13-1896 (see Appendix 1: Certificate of Analysis)
Study Director	Jill Hart, AAS, LATg Senior Laboratory Technologist
Study Monitor	Sue Chang, Ph.D. Senior Toxicology Specialist
Testing Facility	3M Strategic Toxicology Laboratory 3M Center, Bldg 270, room SB324 Saint Paul, MN 55144
In-Life Start Date	December 9, 2013
In-Life Termination Date	December 30, 2013

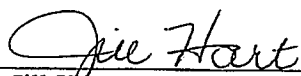
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STUDY OF MTDID 37312 IN MALE RATS**

Compliance Statement and Signature Page

This study was conducted for research and development purposes and does not conform to regulatory guidelines.

This final report has been reviewed and approved by:



Jill Hart, AAS, LATg
Senior Laboratory Technologist
Study Director

19 JAN. 2015
Date



Sue Chang, Ph.D.
Senior Toxicology Specialist
Study Monitor

19 Jan, 2015
Date

1 Study Summary

This study was to evaluate the serum uptake and metabolism of MTDID 37312 (white solid, 99.9%pure, *N*-ethyl-heptadecafluoro-*N*-(2-hydroxyethyl)-1-octanesulfonamide, N-EtFOSE) upon inhalation exposure in male Sprague Dawley rats.

Using a flow-through setup, male rats (N=4) were exposed, whole body, to approximately 7 ppb (v/v) of N-EtFOSE for 6 hours. Observations were made continuously throughout the exposure. All animals appeared normal during the entire study period and all animals survived until the scheduled necropsy. There were no significant findings or gross lesions observed during the necropsy.

Serum samples collected before and after exposure were analyzed for N-EtFOSE concentrations as well as the concentrations of its metabolite, perfluorooctane sulfonate (PFOS), by LC-MS/MS.

While three of the four animals had quantifiable N-EtFOSE in the serum immediately after the exposure, only one animal had quantifiable serum PFOS at the same time. Eighteen hours after exposure, while only one animal still had quantifiable serum N-EtFOSE, all of them had PFOS present in the serum. The level of serum PFOS continued to increase up to Days 8 – 14 after exposure before it declined on Day 21.

Within the limit of the study design, data obtained here suggested N-EtFOSE can be absorbed into blood and metabolized via inhalation exposure. Percent of inhaled N-EtFOSE dose converted to PFOS-equivalent dose ranged from $12.21 \pm 7.22\%$ to $21.59 \pm 9.52\%$ in male Sprague Dawley rats.

2 Study Objective

This study was to evaluate the serum uptake and metabolism of MTDID 37312 (white solid, 99.9%pure, *N*-ethyl-heptadecafluoro-*N*-(2-hydroxyethyl)-1-octanesulfonamide, N-EtFOSE) upon inhalation exposure in male Sprague Dawley rats.

3 Test Material Information

3.1 Test Substance

Test Material ID	MTDID 37312
Lot	Lot 30107 (per GID 103988)
Other Identification	N-EtFOSE, N-EtFOSE Alcohol, FC-10
Purity	99.9%
Physical State of Test Material	Vapor
Chemical Name	<i>N</i> -ethyl-heptadecafluoro- <i>N</i> -(2-hydroxyethyl)-1-octanesulfonamide
Chemical Structure	$\text{CF}_3(\text{CF}_2)_x\text{-SO}_2\text{-N}(\text{CH}_2\text{CH}_3)\text{-CH}_2\text{CH}_2\text{-OH}$

Molecular Weight	571 g/mol
Density	N/A
Vapor Pressure	~17 ppb @ 23° C (estimated)

3.2 Dose Preparation

Dose Preparation	Due to low vapor pressure at room temperature, the test material was coated onto micro beads and the beads were packaged in a sealed stainless steel generator cartridge by the 3M Environmental Lab. The test atmosphere was generated at room temperature when air flow was introduced through the generator cartridge containing N-EtFOSE at a constant flow rate (2 L/min) and into the 40 L chamber.
Physical State of administered material	Vapor
Route of Administration	Inhalation (whole body exposure)

4.1 Test System Information

Species	Rat
Strain	Sprague-Dawley
Source	Harlan
Age at Initiation of Treatment	6-8 weeks
Weight at Initiation of Treatment	253-275 g
Number and Sex	4 males
Identification	Unique tail mark in indelible ink
IACUC Animal Usage Application Number	2013-0001

4.2 Animal Husbandry

Housing	All rats were group housed in solid bottom cages throughout the study with the exception of the exposure period.
Diet/Water	Harlan Teklad Rat/Mouse 2018 Diet (Harlan Teklad, Madison, WI) and tap water were available <i>ad libitum</i> throughout the study except during the exposures.
Housing Environment	Temperature Range 72 ± 3°F Humidity Range 30 – 70% Minimum of 10 exchanges of room air per hour 12 hour light/dark cycle
Test Chamber Environment	Temperature Range 69 – 72°F Humidity Range 37 – 93%

5.1 Study Design

Animals were exposed, whole body, to N-EtFOSE at approximately 7 ppb (v/v) for 6 hours. Control animals were not utilized in this study. The study design is presented in the following table:

Dose Group	N-EtFOSE Concentration (ppb)	Exposure Time	N (males)	Interim tail vein bleeding (for sera)	Euthanasia
1	7 ppb (v/v) <i>(see Section 9.5 as well as Appendix 2)</i>	6 hours	4	Pre-exposure; also immediately, 18 h, 24 h, 48 h, 72 h, Day 8, and Day 14 post exposure	21 days post exposure

6 Parameters Evaluated

6.1 Clinical Observations

Each animal was observed throughout the study for clinical signs of toxicity and mortality.

6.2 Body Weight

Body weights were recorded for all animals prior to exposure and on Days 1-4, 7-11, 14, 17-18, and 21 post-exposure.

6.4 Serum Collection

Prior to exposure and throughout study period, interim blood samples (approximately 200 uL) were collected via tail vein to labelled collection tubes without anticoagulant at designated time as outlined in Study Design. On Day 21 post-dose, all animals were euthanized via CO₂ asphyxiation and for each animal, blood was collected ($\approx$ 6 mL) via the abdominal aorta and transferred to labeled collection tubes without anticoagulant.

The blood samples were allowed to clot for 15 to 30 minutes at room temperature and then centrifuged at 2000 x *g* for 15 minutes. The resulting supernatant (serum) was transferred to labelled polypropylene microcentrifuge tubes. Serum samples were stored frozen at -70°C pending LC-MS/MS analyses.

6.5 Gross Necropsy, Organ Weights and Tissue Collection

Gross necropsies were performed on all animals on Day 21 post-exposure. The livers were harvested from each animal, weighed, and stored frozen at -80 degrees C. There were no other tissues collected.

6.6 Analytical

LC-MS/MS was used to determine the test chamber air samples by using Occupational Safety and Health Administration (OSHA) versatile sampler (OVS) tubes to trap air immediately before the inlet of the exposure chamber and also immediately at the outlet of the chamber followed. The tube absorbent materials were then extracted and analyzed for N-EtFOSE by LC-MS/MS (work performed by 3M Environmental Laboratory).

Separately, LC-MS/MS was performed by 3M Medical Department Bioanalytical Lab to determine concentrations of N-EtFOSE and PFOS in the serum samples collected from animals.

7 Statistical Analysis

Microsoft Excel software was used to calculate mean and standard deviation.

8 Raw Data

The raw data for clinical observations and body weights are recorded in 3M Notebook # 163984, pages 107 – 111.

9 Results

9.1 Clinical Observations

Clinical observations were normal throughout the study. All animals survived until the scheduled necropsy.

9.2 Body Weights/Liver Weights

Body weights and liver weights are shown below.

Animal #	Tail Mark	Body Weight (g)															Liver Weight (g)
		Day 0 (pre-exposure)	Day 1	Day 2	Day 3	Day 4	Day 7	Day 8	Day 9	Day 10	Day 11	Day 14	Day 17	Day 18	Day 21	Day 21	
3R674	Blue I	267	271	270	272	278	290	294	296	300	304	318	323	321	334	13.20	
3R675	Blue II	275	274	275	279	282	296	302	304	310	311	328	338	338	350	13.80	
3R676	Blue III	253	253	252	259	259	279	284	291	294	297	314	323	329	335	14.76	
3R677	Blue IV	274	277	273	281	282	298	303	310	314	310	335	343	347	354	14.51	

9.4 Gross Necropsy

Gross necropsies were performed on all animals on Day 21 post exposure. There were no abnormal findings nor gross lesions noted.

9.5 Analytical Results

The test atmosphere concentrations of N-EtFOSE done with OVS sampling indicated that N-EtFOSE concentrations inside the inhalation chamber varied between 9.43 ± 0.33 ppb v/v (mean concentration at inlet) and 5.31 ± 1.34 ppb v/v (mean concentration at outlet) (see Appendix 2: 3M Environmental Laboratory Final Analytical Report E13-0880). The difference in concentrations between inlet and outlet could be attributed, in part, by the adhesion property of N-EtFOSE onto the furs of the rats as well as inhalation chamber surfaces. Therefore, for the study results reported herein, the approximated overall mean of 7 ppb (7.37 ± 2.35 ppb, rounded to 7 ppb) obtained at both inlet and outlet was used to represent test chamber concentration for N-EtFOSE.

Data for serum concentrations of N-EtFOSE and PFOS are shown in the table below:

Animal #	Serum N-EtFOSE concentrations before and after 6-hour exposure to N-EtFOSE, ng/mL								
	Before	After							
	Pre-exposure	Immediately	18 hrs	24 hrs	48 hrs	72 hrs	Day 8	Day 14	Day 21
3R674	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
3R675	< LLOQ	3.1	2.2	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
3R676	< LLOQ	1.3	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
3R677	< LLOQ	1.3	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
Animal #	Serum PFOS concentrations before and after 6-hour exposure to N-EtFOSE, ng/mL								
	Before	After							
	Pre-exposure	Immediately	18 hrs	24 hrs	48 hrs	72 hrs	Day 8	Day 14	Day 21
3R674	< LLOQ	< LLOQ	6.2	6.7	7.1	7.7	11.6	11.2	7.4
3R675	< LLOQ	3.3	14.3	13.6	16.0	17.7	19.4	22.3	18.9
3R676	< LLOQ	< LLOQ	5.5	5.0	7.0	7.9	10.2	10.9	4.9
3R677	< LLOQ	< LLOQ	5.0	7.1	5.9	7.5	9.4	10.6	5.7

LLOQ = lower limit of quantitation (0.5 ng/mL for N-EtFOSE and PFOS)

9.6 Theoretical N-EtFOSE Dose Inhaled

Theoretical resting ventilation volume for Sprague Dawley Rats = 0.102 L/min

Based on experimental determination from a group of 16 Sprague Dawley rats with a mean body weight of 364 grams and mean ventilation volume of 28 mL/min/100g BW; Strohl et al., *J Appl Physiol* (1985). 1997 Jan;82(1):317-323.

Mean chamber [N-EtFOSE] = 7 ppb v/v = 0.16 ug/L

$[7 \times 10^{-9} \times (571 \text{ g/mol})] / (24.45 \text{ L/mol}) = 0.00000016 \text{ g/L} = 0.16 \text{ ug/L}$

Theoretical amount of N-EtFOSE inhaled in 6 hours (360 minutes) = 5.88 ug
 $0.102 \text{ L/min} \times 360 \text{ min} \times 0.16 \text{ ug/L} = 5.88 \text{ ug}$

9.7 Theoretical PFOS-equivalent Dose derived from Serum PFOS Concentration

Animal #	Serum PFOS concentration after N-EtFOSE inhalation exposure, ng/mL								PFOS-equivalent dose, ug/kg							
									Assuming each time point is C _{max}							
									Dose = C _{max} x V _d ; V _d = 300 mL/kg							
	Immediately	18 hrs	24 hrs	48 hrs	72 hrs	Day 8	Day 14	Day 21	Immediately	18 hrs	24 hrs	48 hrs	72 hrs	Day 8	Day 14	Day 21
3R674	< LLOQ	6.2	6.7	7.1	7.7	11.6	11.2	7.4		1.9	2.0	2.1	2.3	3.5	3.4	2.2
3R675	3.3	14.3	13.6	16.0	17.7	19.4	22.3	18.9	1.0	4.3	4.1	4.8	5.3	5.8	6.7	5.7
3R676	< LLOQ	5.5	5.0	7.0	7.9	10.2	10.9	4.9		1.7	1.5	2.1	2.4	3.1	3.3	1.5
3R677	< LLOQ	5.0	7.1	5.9	7.5	9.4	10.6	5.7		1.5	2.1	1.8	2.2	2.8	3.2	1.7

9.8 Percent Dose Converted from Inhaled N-EtFOSE Dose to Serum PFOS Dose

Animal #	BW during exposure (g)	Theoretical inhaled N-EtFOSE Dose (umol/kg)	PFOS-equivalent dose relative to inhaled N-EtFOSE dose, %							
			Immediately	18 hrs	24 hrs	48 hrs	72 hrs	Day 8	Day 14	Day 21
3R674	267	0.0386	--	9.66	10.42	11.01	11.95	18.07	17.44	11.53
3R675	275	0.0374	0.00	22.99	21.86	25.72	28.45	31.19	35.85	30.38
3R676	253	0.0407	--	8.15	7.42	10.27	11.65	15.07	16.10	7.18
3R677	274	0.0376	--	8.04	11.32	9.43	11.91	15.09	16.95	9.03
Mean	267.3	0.0386	0.00	12.21	12.75	14.11	15.99	19.85	21.59	14.53
SD	10.14	0.0015		7.22	6.30	7.77	8.31	7.68	9.52	10.72

10 Conclusions

While three of the four animals had quantifiable N-EtFOSE in the serum immediately after the exposure, only one animal had quantifiable serum PFOS at the same time. Eighteen hours after exposure, while only one animal still had quantifiable serum N-EtFOSE, all of them had PFOS present in the serum. The level of serum PFOS continued to increase up to Days 8 – 14 after exposure before it declined on Day 21.

Within the limit of the study design, data obtained here suggested N-EtFOSE can be absorbed into blood and metabolized via inhalation exposure. Percent of inhaled N-EtFOSE dose converted to PFOS-equivalent dose ranged from $12.21 \pm 7.22\%$ to $21.59 \pm 9.52\%$ in male Sprague Dawley rats.

Appendix 1:
Certificate of Analysis

3M MATERIALS RESOURCE DIVISION ANALYTICAL LABORATORYLIMS Request No. M13-1896

To: Sue Chang – (733-9073) – Toxicology Assessment and Compliance Assurance – 220-6W-08
 Dave Ehresman – (733-5070) - Toxicology Assessment and Compliance Assurance 236-1B-22
 Kent Lindstrom - (733-9882) – 3M Environmental Lab- 260-5N-17

From: Tom Kestner - (733-5633) – 3M MRD Analytical Laboratory - 236-2B-11

Subject: **¹H/¹⁹F-NMR Characterization of TCR-1085: Purified N-Et FOSE Alcohol for Tox Study**

Date: November 24, 2013

SAMPLE DESCRIPTION:

- TCR-1085 is purified N-Et FOSE alcohol.
 The nominal product is C₈F₁₇-SO₂N(CH₂CH₃)CH₂CH₂OH (white powder).

Summary of NMR Spectra Acquired for TCR-1085

NMR Spectra #'s	Experiments Descriptions
H131896.401	400 MHz ¹ H-NMR spectrum for a portion of the sample dissolved in acetone-d ₆ and spiked with small amounts of CFCl ₃ and p-HFX internal standard.
F131896.401	376.3 MHz ¹⁹ F-NMR spectrum for a portion of the sample dissolved in acetone-d ₆ and spiked with small amounts of CFCl ₃ and p-HFX internal standard.

OBJECTIVE:

The sample of TCR-1085 was subjected to ¹H-NMR and ¹⁹F-NMR spectral analyses to determine the purity of the nominal product and to characterize as many impurity components as possible. Joel Miller also performed an LCMS analysis to assist in the characterization of impurity components and his results were reported previously. In addition, you requested that I prepare and issue a certificate of analysis as a separate document in support of an upcoming 3M toxicology study.

EXPERIMENTAL:

A 62 mg portion of the TCR-1085 sample was totally dissolved in ~ 0.7 mL of deuterated acetone (acetone-d₆) and then the solution was spiked with small amounts of CFCl₃ and 1,4-bis(trifluoromethyl)benzene (p-HFX) for NMR analyses. A 400 MHz ¹H-NMR spectrum and a 376.3 MHz ¹⁹F-NMR spectrum were acquired using an Agilent VNMRs 400 FT-NMR spectrometer that was operating with a 5mm inverse-detection gradient probe at an analysis temperature of 22-23 °C.

RESULTS:

The combined ¹⁹F-NMR and ¹H-NMR spectral data indicate the sample of TCR-1085 consists of ~ **99.9 relative wt.%** of the nominal product, C_nF_{2n+1}-SO₂-N(CH₂CH₃)-CH₂CH₂OH, in at least six isomeric forms, where **n** is mainly 8. Trace amounts of two impurities were also assigned. The qualitative and quantitative compositional results that were derived from a single trial ¹⁹F/¹H-NMR cross integration spectral analysis are summarized in **TABLE-1**. The relative weight percent concentrations shown in TABLE-1 should be very close to their respective absolute wt.% values. Trace amounts of unassigned components are also detected in the NMR spectra but additional work would be needed in an effort to assign or quantify the other impurities.

Small amounts of longer or shorter chain fluorochemical homolog impurities could have easily gone undetected because the ¹⁹F-NMR technique is not particularly well suited for characterizing small amounts

of homologs unless the chains are very short (i.e., C₁-C₃). Characterization of fluorochemical **homolog** impurities was performed by Joel Miller using LCMS.

If you have any questions about these NMR results, or if any further work is needed, please let me know. .

Tom Kestner

c: Joel Miller
Scott Schmick

TABLE-1

Sample: TCR-1085, Purified N-Et FOSE alcohol

Compositional Results by ¹⁹F/¹H-NMR Cross Integration Spectral Analysis

<i>Structural Assignments</i> ¹	¹⁹ F/ ¹ H-NMR Relative Wt.% Concentrations
Sum of at least 6 desired product isomers: C _n F _{2n+1} -SO ₂ -N(CH ₂ CH ₃)-CH ₂ CH ₂ -OH where n assumed to be 8 for calculation purposes	Purity = 99.9%
CF ₃ (CF ₂) _x -SO ₂ -N(CH ₂ CH ₃)-CH ₂ CH ₂ -OH (Normal chain , where x assumed to be 7 for calculation purposes)	70.55%
CF ₃ (CF ₂) _x -CF(CF ₃)-(CF ₂) _y -SO ₂ -N(CH ₂ CH ₃)-CH ₂ CH ₂ -OH (Internal monomethyl branch , where x+y assumed to be 5, and x ≠ 0, y ≠ 0, for calculation purposes)	16.93%
(CF ₃) ₂ CF-(CF ₂) _x -SO ₂ -N(CH ₂ CH ₃)-CH ₂ CH ₂ -OH (Isopropyl branch , where x assumed to be 5 for calculation purposes)	10.36%
C _x F _{2x+1} -CF(CF ₃)-SO ₂ -N(CH ₂ CH ₃)-CH ₂ CH ₂ -OH (Alpha branch , where x assumed to be 6 for calculation purposes)	1.67%
(CF ₃) ₃ C-(CF ₂) _x -SO ₂ -N(CH ₂ CH ₃)-CH ₂ CH ₂ -OH (t-butyl branch , where x assumed to be 4 for calculation purposes)	0.24%
CF ₃ -(CF ₂) _x -C(CF ₃) ₂ -(CF ₂) _y -SO ₂ -N(CH ₂ CH ₃)-CH ₂ CH ₂ -OH (Internal gem-dimethyl branch , where x+y assumed to be 4, and x ≠ 0, for calculation purposes)	0.12%
Probable carboxamide C _m F _{2m+1} -CO-NH-CH ₂ CH ₃ where m assumed to be 7 for calculation purposes	0.12%
Probable N-Me FOSE alcohol C _n F _{2n+1} -SO ₂ -N(CH ₃)-CH ₂ CH ₂ -OH where n assumed to be 8 for calculation purposes	0.018%

1. Trace amounts of unassigned impurity components are also detected in the NMR spectra.

Appendix 2:

3M Environmental Laboratory Final Analytical Report E13-0880

Final Analytical Report

Analysis of Gas-Phase EtFOSE-OH in Air Samples Collected from an Exposure Chamber with OVS-Tubes During an Inhalation Toxicology Study with Rats

Laboratory Request Number: E13-0888

Method or Regulatory Requirement: Non-GLP

Testing Laboratory

3M Environmental Health and Safety Operations (EHS Opns)
3M Environmental Laboratory

Analytical Lead

Cleston Lange, Ph.D.
3M Environmental Health and Safety Operations
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Project Requester

Sue Chang
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1 Introduction / Summary

The objective of this study was to measure the level of gas-phase 2-[N- (ethyl)-perfluorooctanesulfonamido]-ethyl alcohol (a.k.a. EtFOSE-OH) in air that was delivered to a polyacrylate exposure chamber during a 6 hour inhalation exposure study with rats conducted by 3M-Strategic Toxicology in collaboration with the 3M EHS Opns Environmental Laboratory. Air flowing from a generator cartridge that contained glass beads coated with solid EtFOSE-OH delivered gas-phase EtFOSE-OH to the exposure chamber at a nominal flow rate of 2 L/min. Air samples were collected immediately before the inlet of the exposure chamber and also immediately at the outlet of the chamber. Samples were collected at the inlet and outlet via a split flow gas line to an OVS sampler tube with a nominal sampling flow rate of 0.1 L/min and with an ~60 minute collection time for each OVS (nominal 6 Liters total collected per OVS tube). Six inlet samples and six outlet samples were collected sequentially during the 6 hour exposure period. Herein are provided the analytical measurement result for those OVS samples provided from the EtFOSE-OH inhalation exposure study conducted in December 2013, as well as all of the associated QC samples prepared and analyzed with those samples.

For analysis of EtFOSE-OH, each OVS tube was disassembled into three fractions following the procedures described in method ETS-8-105.0. Each fraction was extracted with 10 mL acetone containing d₉-EtFOSE-OH as an internal standard (IS). The extracts were then quantitatively analyzed by LC/MS/MS against solvent calibration standards. A lower limit of quantitation (LLOQ) of 0.100 ng/mL was achieved during the analyses. The total mass quantity of EtFOSE-OH trapped on each OVS was determined based on the summed quantity of EtFOSE-OH extracted and measured for each of the three OVS tube fractions. For preparation of the quality control (QC) samples included with each analytical batch, fresh OVS tubes were fortified onto the inlet filter with 10, 100, 1000 or 10000 ng of EtFOSE-OH, without air passed through them. Once spiked, each was disassembled, extracted and analyzed the same as samples in the batch; prior method development work (study E13-0722) showed no loss of EtFOSE-OH from QCs with up to 60 Liters of air passed through them. The QC results demonstrated an overall excellent recovery and data accuracy of $99.2 \pm 5.9\%$ for all QC levels. In general, the amount of EtFOSE-OH captured at the inlet to the exposure chamber was on the order of 1500 ng, and at the outlet was on the order of 1000 ng. Recovery ($\pm$ standard deviation) for the 1000 ng QCs (n = 6) was therefore the best indicator of the data accuracy for the study and showed an accuracy of $101\% \pm 3.4\%$. Blanks prepared with samples were essentially devoid of any significant levels of EtFOSE-OH and were less than the lower limit of quantitation (LLOQ).

As listed in **Table 1** and plotted in **Figure 1**, the determined air concentrations of EtFOSE-OH at the inlet were significantly higher than at the outlet. Throughout the course of the exposure, it was apparent that equilibrium (i.e. inlet/outlet ratio of 1) was not established. The inlet/outlet air concentration ratio at the onset of the study for the 0-1 hr sampling and 1-2 hr sampling were 2.7 and 2.4, respectively. However, an inlet/outlet ratio of approximately 1.50 was achieved after the first 2 hours and was relatively stable after that, as shown in **Figure 2**. The gas-phase concentration at the chamber inlet for the 2-3 hour sampling was 222 ng/L (9.5 ppbV) and the outlet was 148 ng/L (6.3 ppbV), and for the 5-6 hour sampling the inlet/outlet ratio was 1.46 with an inlet concentration of 225 ng/L (9.6 ppbV) and outlet concentration of 155 ng/L (6.6 ppbV).

Additionally, as part of the LC/MS/MS analysis, sufficient chromatographic resolution of linear and branched isomers was achieved to allow estimation of the percent linear isomer and percent as total-branched isomers. While the EtFOSE-OH used to construct the EtFOSE-OH generator cartridge was 70% linear isomer EtFOSE-OH (30% branched isomers) EtFOSE-OH, the EtFOSE-OH collected from gas-phase samples at room temperature in the inlet and outlet of the test

chamber showed enrichment for branched isomers. This observation was consistent with isomer enrichment observed during the previous test chamber equilibration study E13-0722. During this study, at the initiation of delivery of gas phase EtFOSE-OH from the generator tube to the exposure chamber, the EtFOSE-OH isomer composition was ~38% linear isomer at the inlet. However, a significant change in the isomer composition at the inlet occurred over the course of the 6 hour exposure period which was not observed at the outlet, and increased to 62% linear isomer by the end of the exposure. In contrast, the isomer composition appeared relatively stable at the outlet during the entire 6 hour exposure period and composed of ~32% linear isomer at the beginning to and ~34% linear isomer at the end of the exposure period (see **Figure 2**). These results indicate that deposition of branched EtFOSE-OH on the chamber walls likely occurred during the earlier conducted pre-study chamber equilibration study E13-0722, and during this study sublimation of branched EtFOSE-OH off of the walls, and possibly preferential deposition of linear isomer onto the surface from the incoming gas, contributed to a continuous level of enrichment of branched isomers in exhaust air measured at the outlet.

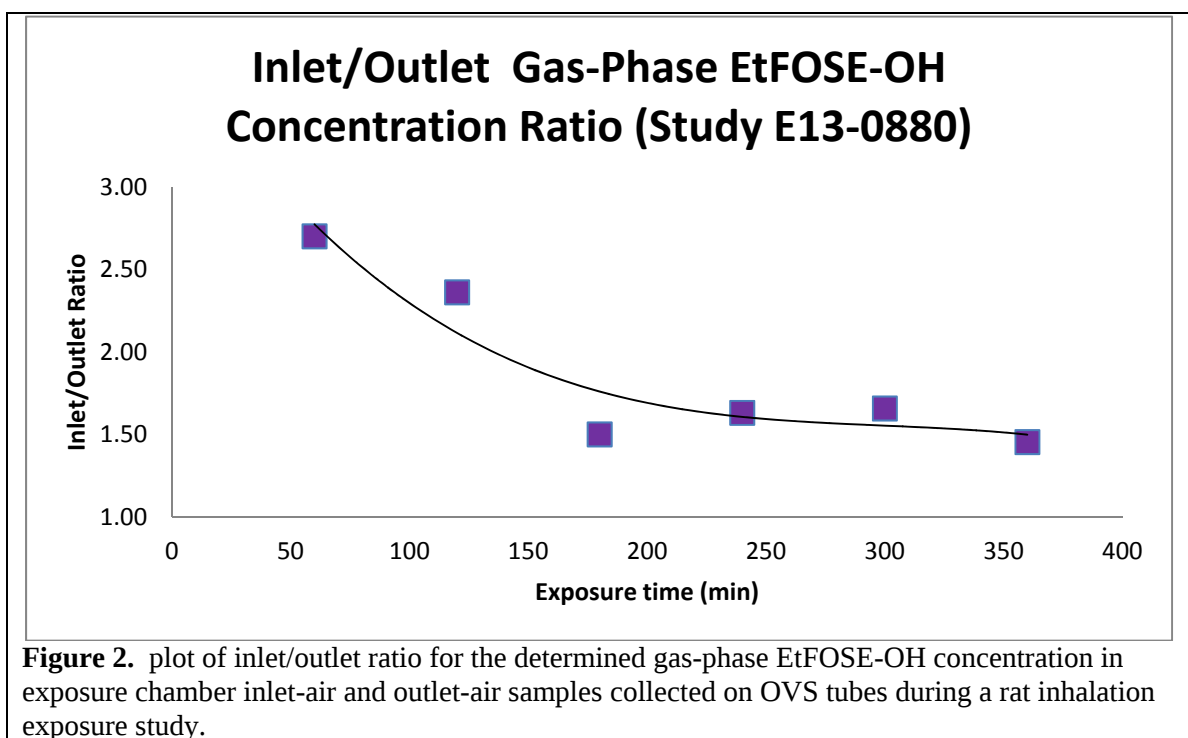
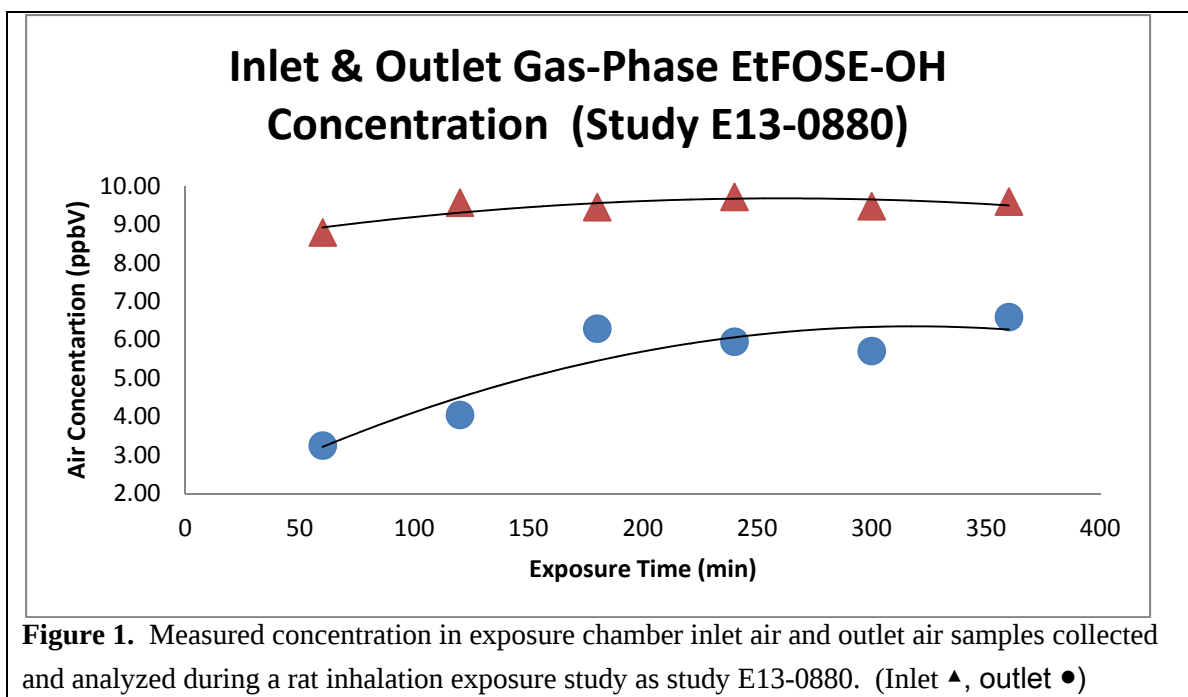
Based on these analytical results, exposure levels of gas-phase EtFOSE-OH concentrations were between ~6 and 9 ppbV during the exposure based on inlet and outlet concentration. Furthermore, it was shown that the gas-phase EtFOSE-OH was enriched with branched isomers. The inhalation-dose of highly-branched EtFOSE-OH could have a profound impact on the absorption, distribution, metabolism and excretion (ADME) properties of the EtFOSE-OH, and should be considered when comparing these inhalation-dose results to previous toxicological studies.

Table 1. Summarized Results for EtFOSE-OH Concentration and Linear Isomer Composition

Sample ID	Sample Description	EtFOSE-OH				
		Actual Air Volume Captured on OVS (L)	Total Recovered from OVS (ng)	Air Concentration (ng/L)	Air Concentration (ppbV)	Linear Isomer (Percent of Total)
E13-0880-003	Test #1 Inlet, 0.1 L/min	6.77	1397	206	8.79	38.4%
E13-0880-004	Test #1 Outlet, 0.1 L/min	6.86	524	76.4	3.25	31.5%
E13-0880-005	Test #2 Inlet, 0.1 L/min	6.77	1520	225	9.56	45.9%
E13-0880-006	Test #2 Outlet, 0.1 L/min	6.86	652	95.1	4.05	28.2%
E13-0880-007	Test #3 Inlet, 0.1 L/min	6.77	1501	222	9.45	52.4%
E13-0880-008	Test #3 Outlet, 0.1 L/min	6.86	1013	148	6.29	32.3%
E13-0880-009	Test #4 Inlet, 0.1 L/min	6.77	1544	228	9.71	55.8%
E13-0880-010	Test #4 Outlet, 0.1 L/min	6.86	958	140	5.95	32.2%
E13-0880-011	Test #5 Inlet, 0.1 L/min	6.77	1505	222	9.47	59.7%
E13-0880-012	Test #5 Outlet, 0.1 L/min	6.86	919	134	5.71	32.3%
E13-0880-013	Test #6 Inlet, 0.1 L/min	5.64	1271	225	9.59	61.5%
E13-0880-014	Test #6 Outlet, 0.1 L/min	5.71	885	155	6.59	33.6%

The analytical measurement uncertainty for these study results was estimated at $\pm 12\%$ based on QC results.

[a] Value was estimated based on peak area response of the linear isomer chromatographic peak versus total peak area of linear + branched isomer peaks (see **Figure 3** for LC/MS/MS Chromatogram of EtFOSE-OH).



2 Analytical and Preparatory

2.1 Reference Substance

Table 2. Reference Substances			
Substance	EtFOSE-OH (linear + branched)	EtFOSE-OH (linear)	d ₉ -EtFOSE-OH
TCR No.	TCR-1085	TCR09-0061-1/10	TCR09-0053-2/4 TCR09-0053-3/4
Source	3M, Purified by HPLC at Pace Analytical Services	Wellington	Wellington
Use	Generator Cartridge Loading and QC fortifications	Calibration Standard Preparations	Quantitation Internal Standard
Chemical Name	2[(N-methyl)-perfluorooctanesulfonamido]-ethyl alcohol	2[(N-methyl)-perfluorooctanesulfonamido]-ethyl alcohol	Deuterated 2[(N-methyl)-perfluorooctanesulfonamido]-ethyl alcohol
Chemical Formula	C ₁₂ H ₁₀ F ₁₇ NO ₃ S	C ₁₂ H ₁₀ F ₁₇ NO ₃ S	C ₁₂ HD ₉ F ₁₇ NO ₃ S
Formula Wt.	571	571	580
Isomer Composition	Mixed Linear (70%) & Branched (30%) Isomers	Pure Linear Isomer	Pure Linear Isomer
Purity	99.9%	50 µg/mL Solution in MeOH	50 µg/mL Solution in MeOH
Expiration	7/31/2016	2/23/2014	2/18/2014

2.2 Analytical Sample and Sample Batch Preparation

Disassembly and extraction of the OVS tubes was performed following method ETS-8-105.0. Each OVS sampling tube was dismantled into three sets of components, as follows: component 1 was the 1st bed of XAD®-4 resin and 1st PUF plug; component 2 was the XAD®-4 resin from the 2nd bed and the 2nd polyurethane foam (PUF) plug; and component 3 was the glass fiber filter, HDPE retaining ring and glass tube housing. Each of the three components was separately extracted with 10 mL of acetone containing d₉-EtFOSE-OH which served as IS. This resulted in three 10 mL analytical sample extracts per OVS tube (net 10-fold dilution of each solid state component set). The LC/MS/MS analysis of the extracts was conducted using the parameters described in **Tables 3, 4 and 5**. Two analytical batches were analyzed to collect the data for reporting and were batches m131210a and m131211a.

Calibration standards were prepared for each analytical batch ranging from nominal 0.100 ng/mL to 500 ng/mL and were prepared by fortifying pure linear EtFOSE-OH at known levels into acetone containing d₉-EtFOSE-OH, same acetone mix as used to extract samples. Quantitation was by internal standard method. Calibration curve regression was performed with 1/x weighting and coefficient of determination (R²) for the calibration curve regressions were ≥ 0.995. Reinjection of calibration standards during the analysis was performed as continuing calibration verification (CCVs) during each run and all results for CCVs were within 100 ± 10% for all

analytical runs reported.

Additional new purchased OVS tubes of the same type were used for the exposure chamber experiments (ORBO™65P OVS Fluoride Tube with Glass Fiber Filter, Supelco Item 20029-U) and were used to prepare quality control (QC) samples for each analytical batch. The QC samples were fortified with reference substance TCR-1085 (70% linear, 30% branched isomers) and was the same reference substance used to make the generator cartridge. The QCs were fortified at four levels, each level in triplicate, and each level prepared by spiking 10.0 ng, 100 ng, 1000 ng or 11400 ng of EtFOSE-OH (mixed linear/branched isomer material) onto the filter in the OVS inlet side. QCs did not have air passed through them; however, previous method development work as part of study E13-0722 showed that EtFOSE-OH is retained on OVS tubes with up to 60 liters of air passed through them, a volume of air that was ~10-fold more than the volume of air collected on OVS tubes during this study.

The component-3 extract is where EtFOSE-OH is typically trapped and retained, and for the 11400 ng QC the component-3 extract was diluted 1000-fold (as a dilution QC). However, samples did not require dilution, hence, the dilution QC was determined to be unnecessary. Results of QCs were used to verify the performance of the analytical method. The QC results are summarized in the results section in **Table 8**, and individual QC OVS component results are shown in **Table 9**.

Laboratory media blanks were control OVS tubes without fortification and without air passed through them/ Results of media blanks are provided in the results section as **Table 6**.

The Air Pre-Test and Post-Test air blanks were collected by passing non-dosed tank-air through them. The air used was the same tank air as passed through the generator column and test chamber during the exposure. Results for air blanks are presented in the results section as **Table 7**.

2.3 LC/MS/MS Analysis

Table 3. Instrument Information (Study E13-0880)	
Instrument Name	ETS-MaryAnn (batches m131210a & m131211a)
Analytical Method ID	ETS-8-105.0 for Extraction
Liquid Chromatograph	Agilent 1200
Analytical column	Betasil C18 (100 x 2.1 mm; 5 µm); 30°C
Injection Volume	1.0 µL
Mass Spectrometer	API 5000 Triple Quadrupole
Ion Source	Turbo Spray
Polarity	Negative
Software	Analyst 1.6.1

Table 4. Liquid Chromatography Conditions (Study E13-0880)				
Step Number	Total Time [min]	Flow Rate [mL/min]	Percent A [2mM Aqueous Ammonium Acetate]	Percent B [2 mM Ammonium Acetate in Methanol]
0	0.00	0.500	20.0	80.0
1	0.50	0.500	20.0	80.0
2	2.50	0.500	99.0	1.0
3	3.10	0.500	99.0	1.0
4	3.11	0.500	20.0	80.0
5	4.50	0.500	20.0	80.0

Table 5. Mass Transitions (Study E13-0880)	
Analyte	Mass Transitions (MRMs) Monitored
EtFOSE-OH	630>59
C9Analog ^[a]	680>59
C7 Analog ^[a]	580>59
d9-EtFOSE-OH (IS)	639>59
[a] Mass transitions for C9 and C7 Analogs of EtFOSE-OH were included, but not used for quantitation during this study.	

2.4 Calculations

The results as part per billion by volume (ppbV) is a gas phase concentration value (nL/L), and was determined as described below:

First the volume of EtFOSE-OH in the air sample is calculated by the ideal gas law, as follows:

$$PV = nRT$$

$$\text{or, } [V = nRT/P]$$

where, $V = V_{\text{EtFOSE-OH}}$ (L) and is the volume occupied by EtFOSE-OH in the air sample from the OVS

n = mole EtFOSE-OH captured on OVS using 571 g/mole conversion value

R is the ideal gas constant at 0.08206 [(atm*mol)/(L*°K)]

P is assumed 1 atm

T is assumed 23 °C (296.15 °K).

Then, ppbV (nL per Liter of Air) was calculated where V_{Air} = volume of air sample through the OVS in liters, and $V_{\text{EtFOSE-OH}}$ is the volume of EtFOSE-H in that air sample entered in nL

$$\text{ppbV} = V_{\text{EtFOSE-OH}} (\text{nL}) / V_{\text{Air}} (\text{L})$$

All other calculations were performed per 3M EHSO Environmental Laboratory standard operating procedures (SOPs).

3 Results

3.1 Media Blanks

OVS Tube media blanks results were below the LLOQ. The results are summarized in **Table 6**. An LLOQ of 0.100 ng/mL translates to an LLOQ of 1.00 ng per OVS component, hence, 3 ng per OVS tube for the three components.

Table 6. Summarized Results for OVS Tube Media Blanks					
Sample ID	Sample Description	Nominal Air Volume Captured (L)	EtFOSE-OH		
			Total Recovered from OVS (ng)	Air Concentration (ng/L)	Air Concentration (ppbV)
E13-0880-001	OVS Media Blank 1	0	< 3.00	NA	NA
E13-0880-016	OVS Media Blank 2	0	< 3.00	NA	NA

3.2 Air Blanks

Laboratory blanks were devoid of EtFOSE-OH and all results were below the lower limit of quantitation of 0.100 ng/mL (i.e. <1.00 ng per component, < 3.00 ng per OVS). Results of laboratory blanks are summarized in **Table 7**.

Table 7. Summarized Results for Air Blanks					
Sample ID	Sample Description	Nominal Air Volume Captured (L)	EtFOSE-OH		
			Total Recovered from OVS (ng)	Air Concentration (ng/L)	Air Concentration (ppbV)
E13-0880-001	Air Pre-Test Blank, nominal flow 1 L/min	6	< 3.00	< 0.500	< 0.0213
E13-0880-016	Air Post-Test Blank, nominal flow 1 L/min	7	< 3.00	< 0.429	< 0.0182

3.3 QC Results

Four levels of quality control (QC) samples were prepared, each level in triplicate, by spiking OVS tubes with 10.0 ng, 100 ng, 1000 ng and 11400 ng of EtFOSE-OH, and then extracting the same as samples. For the 11400 ng QC, the component-3 10 mL acetone extract was diluted 10-fold prior to analysis to achieve a result within the range of the calibration (total dilution of 100-fold for that OVS solid state component).

As summarized in **Table 8**, the QC results showed excellent data accuracy and precision with an overall average and standard deviation of 99.2% $\pm$ 5.9% (RSD 6.0%). EtFOSE-OH was efficiently recovered off of the OVS tubes at all levels with all QC recoveries within 100 $\pm$ 17%. The majority of EtFOSE-OH was recovered from component 3 (glass fiber filter, HDPE retaining ring and glass tube housing) with some captured from component 2 (the 1st bed of XAD®-4 resin-beads and 1st PUF plug). Component 3 (2nd bed and 2nd polyurethane foam (PUF) plug) typically did not contain much EtFOSE-OH.

The estimated analytical measurement uncertainty for this study was calculated from statistical analysis of the QC results, per 3M EHSO Environmental Laboratory SOP ETS-12-12.2.

Table 8. QC Summary Results (Study E13-0880)				
Analytical Batch ID	EtFOSE-OH Recovery from OVS QCs			
	10.0 ng QC	100 ng QC	1000 ng QC	11400 ng QC
m131210	83.3%	96.5%	96.4%	103%
	101%	94.4%	104%	104%
	95.7%	92.3%	98.9%	107%
m131211a	95.2%	87.3%	105%	99.3%
	97.4%	103%	104%	105%
	98.9%	103%	101%	107%
<i>Average Recovery</i>	95.3%	96.1%	101%	104%
<i>Std. Dev.</i>	6.2%	6.2%	3.4%	2.8%
<i>RSD</i>	6.5%	6.5%	3.3%	2.7%
<i>n</i>	6	6	6	6
Overall Average	99.2%			
Overall Standard Deviation	5.9%			
Overall RSD	6.0%			
N	24			
Estimated Analytical Measurement Uncertainty (95% CI)	$\pm$ 12%			

3.4 Individual Sample Results

Table 9. Individual QC Results (Study E13-0880)							
QC ID	Analytical Run ID	Fortification Level (ng)	OVS Tube Component	Determined Amount (ng)	Total EtFOSE-OH (ng) ^[a]	QC Recovery ^[a]	Average Recovery
KDM10-Dec2013-017	m131210a	10.0	1	<1.00	8.33	83.3%	93.3% (RSD 9.7%)
			2	<1.00			
			3	8.33			
KDM10-Dec2013-018	m131210a	10.0	1	<1.00	10.1	101%	
			2	<1.00			
			3	10.1			
KDM10-Dec2013-019	m131210a	10.0	1	<1.00	9.57	95.7%	
			2	<1.00			
			3	9.57			
KDM10-Dec2013-020	m131210a	100	1	6.91	96.5	96.5%	94.4% (RSD 2.2%)
			2	<1.00			
			3	89.6			
KDM10-Dec2013-021	m131210a	100	1	4.26	94.4	94.4%	
			2	<1.00			
			3	90.1			
KDM10-Dec2013-022	m131210a	100	1	5.53	92.3	92.3%	
			2	<1.00			
			3	86.8			
KDM10-Dec2013-023	m131210a	1000	1	110	964	96.4%	99.8% (RSD 3.9%)
			2	<1.00			
			3	854			
KDM10-Dec2013-024	m131210a	1000	1	161	1040	104%	
			2	<1.00			
			3	879			
KDM10-Dec2013-025	m131210a	1000	1	94.9	989	98.9%	
			2	<1.00			
			3	894			
KDM10-Dec2013-026	m131210a	11400	1	1580	11800	103%	105% (RSD 1.8%)
			2	2.28			
			3	10200			
KDM10-Dec2013-027	m131210a	11400	1	965	11900	104%	
			2	4.11			
			3	10900			
KDM10-Dec2013-028	m131210a	11400	1	1690	12200	107%	
			2	1.15			
			3	10500			
KDM10-Dec2013-029	m131211a	10.0	1	<1.00	9.52	95.2%	97.2% (RSD
			2	<1.00			
			3	9.52			

Table 9. Individual QC Results (Study E13-0880)

QC ID	Analytical Run ID	Fortification Level (ng)	OVS Tube Component	Determined Amount (ng)	Total EtFOSE-OH (ng) ^[a]	QC Recovery ^[a]	Average Recovery
KDM10-Dec2013-030	m131211a	10.0	1	<1.00	9.74	97.4%	1.9%)
			2	<1.00			
			3	9.74			
KDM10-Dec2013-031	m131211a	10.0	1	<1.00	9.89	98.9%	
			2	<1.00			
			3	9.89			
KDM10-Dec2013-032	m131211a	100	1	5.29	87.3	87.3%	97.8%
			2	<1.00			
			3	82.0			
KDM10-Dec2013-033	m131211a	100	1	5.92	103	103%	(RSD 9.3%)
			2	<1.00			
			3	96.7			
KDM10-Dec2013-034	m131211a	100	1	8.59	103	103%	
			2	<1.00			
			3	94.9			
KDM10-Dec2013-035	m131211a	1000	1	59.5	1050	105%	103%
			2	<1.00			
			3	991			
KDM10-Dec2013-036	m131211a	1000	1	60.4	1040	104%	(RSD 2.2%)
			2	<1.00			
			3	976			
KDM10-Dec2013-037	m131211a	1000	1	69.3	1010	101%	
			2	<1.00			
			3	937			
KDM10-Dec2013-038	m131211a	11400	1	1120	11320	99.3%	104%
			2	<1.00			
			3	10200			
KDM10-Dec2013-038	m131211a	11400	1	1130	11930	105%	(RSD 3.7%)
			2	<1.00			
			3	10800			
KDM10-Dec2013-039	m131211a	11400	1	1160	12160	107%	
			2	<1.00			
			3	11000			

All values are rounded to 3 significant figures, but precise data was used in data calculations.

[a] Values of Zero (0) were used for results below the LLOQ (<LLOQ) when calculating total EtFOSE-OH and QC recoveries.

Table 3. Individual Air Sample Results (Study E13-0880)						
Sample ID	Component Extracted	EtFOSE-OH Component Amount Measured (ng)	EtFOSE-OH Amount from OVS tube (ng) and [Percent Linear Isomer]	Air Volume Captured (L)	EtFOSE- OH Air Concentra- tion	Inlet/Out- let Ratio
Inlet Test #1	1	2.07	1397 [38.4%]	6.77	8.79	2.70
	2	14.7				
	3	1380				
Outlet Test #1	1	18.7	524 [31.5%]	6.86	3.25	
	2	2.94				
	3	502				
Inlet Test #2	1	3.11	1520 [45.9%]	6.77	9.56	2.36
	2	16.9				
	3	1500				
Outlet Test #2	1	18.8	652 [28.2%]	6.86	4.05	
	2	19.9				
	3	613				
Inlet Test #3	1	169	1501 [52.4%]	6.77	9.45	1.50
	2	22.3				
	3	1310				
Outlet Test #3	1	55.8	1013 [32.3%]	6.86	6.29	
	2	27.9				
	3	929				
Inlet Test #4	1	9.69	1544 [55.8%]	6.77	9.71	1.63
	2	24.2				
	3	1510				
Outlet Test #4	1	122	958 [32.2%]	6.86	5.95	
	2	21.2				
	3	815				
Inlet Test #5	1	413	1505 [59.7%]	6.77	9.47	1.66
	2	32.1				
	3	1060				
Outlet Test #5	1	149	919 [32.3%]	6.86	5.71	
	2	10.3				
	3	760				
Inlet Test #6	1	<1.00	1271 [61.5%]	5.64	9.59	1.46
	2	10.5				
	3	1260				
Outlet Test #6	1	89.6	885 [33.6%]	5.71	6.59	
	2	17.9				
	3	777				

4 Conclusion

A lower limit of quantitation (LLOQ) of 0.100 ng/mL was achieved during the analyses. The total mass quantity of EtFOSE-OH trapped on each OVS was determined based on the summed quantity of EtFOSE-OH measured for each of the three OVS fractions. Quality control (QC) samples were prepared with each analytical batch by fortifying fresh OVS tubes with EtFOSE-OH at nominal 10, 100, 1000 and 10000 ng, each level prepared in triplicate, and then disassembling, extracting and analyzing them the same as samples. QC result demonstrated an overall excellent data accuracy of $99.2 \pm 5.9\%$ for all QC levels. In general, the amount of EtFOSE-OH captured at the inlet was on the order of 1500 ng per OVS, and at the outlet was on the order of 1000 ng per OVS. Recovery results for the 1000 ng QCs ($n = 6$) was therefore the best indicator of the data accuracy for the study and showed an accuracy of $101\% \pm 3.4\%$. Blanks prepared with samples essentially did not contain any significant levels of EtFOSE-OH and were less than the lower limit of quantitation (LLOQ).

Analysis results for the individual OVS components for samples and QCs showed that typically 90-100% of the EtFOSE-OH was captured in component 3 (glass fiber filter, HDPE retaining ring and glass tube housing), ~0-10% in component 1 (1st XAD bed and PUF, middle stage) and less than 1% was captured on component 2 (2nd XAD bed and 2nd PUF), the final stage of the OVS, indicating no breakthrough.

Based on these analytical results, exposure levels of gas-phase EtFOSE-OH concentrations were between ~6 and 9 ppbV during the exposure based on the measured inlet and outlet concentration. Furthermore, as shown in **Figure 3**, it was demonstrated that the gas-phase EtFOSE-OH in the exposure chamber was enriched with branched isomers.

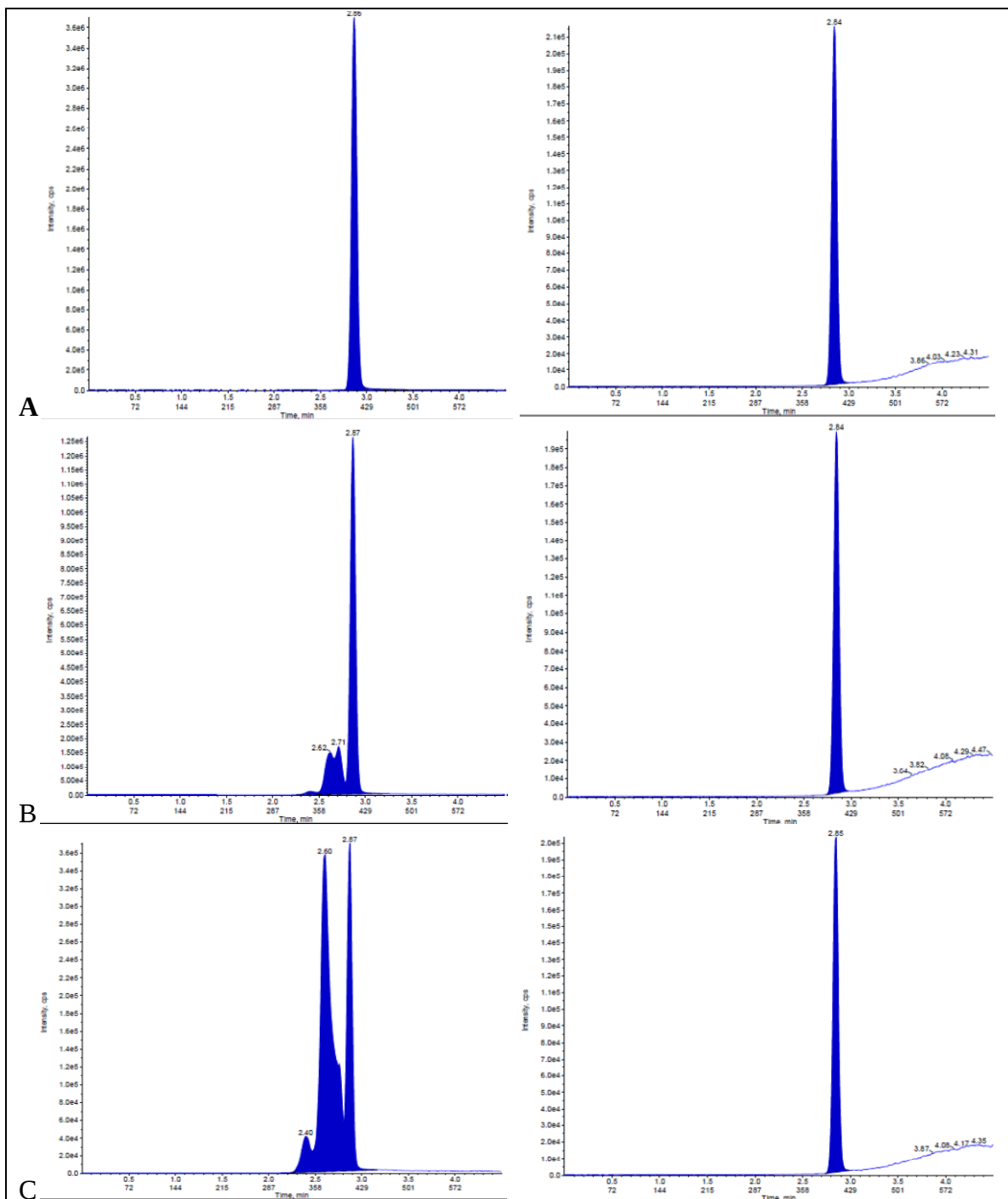


Figure 3. The LC/MS/MS chromatograms of EtFOSE-OH (left) and corresponding linear d_9 -EtFOSE-OH internal standard peak (right). Samples from top to bottom were the component-3 extracts of the following samples and analyzed in the same analytical run m131210a: **A)** 200 ng/mL calibration standard prepared with linear EtFOSE-OH obtained commercially from Wellington Laboratories; **B)** 1000 ng QC fortified with substance TCR-1085 (70% linear, 30% branched isomers) and was same substance as used to make the generator cartridge for dosing; and **C)** the isomer profile of component 3 extract of sample E13-0880-006 (1-2 hour sampling point, chamber outlet) showing the enrichment of branched isomers in gas-phase EtFOSE-OH (~28% linear isomer and ~72% branched isomers).

5 Data/Sample Retention

Samples generated during this study E13-0880 will be retained indefinitely, or until requested by the study Requestor to dispose of them, and will only be disposed of with laboratory management approval.

6 Attachments

E13-0880 Final Analytical Report, Appendix-A: "Field Summary: Ethyl-FOSE Rat Exposure Chamber Test"; Lab Request Number E13-0880 (3M EHS Opns. Environmental Laboratory-Field Services Group Report)

7 Signatures

The following 3M Environmental Laboratory personnel have reviewed and approved this report:



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Date



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The results and report were audited by Quality Associates, Inc. (QAI)



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Field Summary

Ethyl-FOSE Rat Exposure Chamber Test

Laboratory Request Number: E13-0880

Client's Department: 108022

Method or Regulatory Requirement: On-Site Modified EPA Method 320

Testing Laboratory

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3M Environmental Laboratory – On-site FTIR/OVS Tubes

Report Author: Jess Eldridge

Field Team: Jess Eldridge, Sue Chang, Jill Hart, Trina John

Field Report***Project Number: E13-0880******Date of Report: January 10, 2014*****1 Introduction / Summary**

The purpose of this testing was to support 3M Strategic Toxicology by performing a study on rats exposed to ethyl-FOSE generated from a generator cartridge. This test was a follow-up to the preliminary work performed under lab request E13-0722. Testing consisted of FTIR and OVS tube LC/MS based measurements of the target compound at the inlet and outlet of the test chamber. If other compounds were present, they would be reported by FTIR if feasible.

On-site Fourier Transform Infrared (FTIR) spectroscopy testing and OSHA Versatile Sampler (OVS) tube sampling (Supelco) were conducted in Room SB 322/324 located in Building 270 for the 3M Toxicology and 3M Materials Resources Division groups. A “Breathing Air” quality compressed gas cylinder # P411310802/019928 was used to flow air through the ethyl-FOSE generator tube at a rate of 2.1 lpm into and through the 40 liter exposure chamber. Gas exiting the chamber was flowed through the FTIR cell and exhausted in the adjacent vent hood. The continuous air flow measurement was made using a calibrated rotometer supplied by the 3M Toxicology lab; SN 337973-1. The chamber inlet and outlet locations were configured each with a valved sampling port from which an OVS sample could be drawn via SKC AirChek[®] 52 pump. The pumps were set to sample at approximately 100 cc/min for each test per location. Pump # 150 was used at the inlet location and Pump # 222 was used at the outlet location for the entire testing event. A beginning and end flow rate was measured for each OVS sample via a Dry-Cal flow meter; 3M Instrument # 158675. A “dummy” OVS tube was used to measure the flows rather than the actual sampling tubes assuming the flow rates would be consistent between all tubes. Since the exposure chamber was under slight pressure, a flow rate at the inlet and outlet sampling locations were performed once while plumbed into the system. A correction factor was then determined to apply to all other flow rates measured throughout this event based upon measurements made during the preliminary test under lab request E13-0722. A detailed spreadsheet of exact flow rates, correction factors, and test times is presented in Section 7. The FTIR results are shown in Section 8. Hand written notes taken during the event including the lot numbers and expiration dates per OVS sample are shown in Section 9. The Chain of Custody Forms are shown in Section 10. The General Project Outline (GPO) is shown in Section 11.

December 9th, 2013 Summary:

Testing began on December 9th 2013 and included continuous FTIR testing and six one hour OVS tube samples collected at the inlet and outlet of the exposure chamber for the entire day.

A breathing air cylinder OVS tube sample was collected from 8:18 to 8:24 at a flow rate of 1 lpm. This test was performed as a blank to confirm the absence of ethyl-FOSE in the cylinder.

The OVS tube tests were numbered 1-6. Test 1 was from 9:12 to 10:12, Test 2 was from 10:15 to 11:15, Test 3 was from 11:18 to 12:18, and Test 4 was from 12:21 to 13:21, Test 5 was from 13:24 to 14:24, and Test 6 was from 14:27 to 15:17 (50 minutes). A blank OVS tube was submitted along with the other samples; designated time 8:22.

The exposure test was complete and the system shut down at 15:19. The system was opened and the rats were removed by Jill Hart, Trina John, and Sue Chang for the continuance of the biological phase of the exposure test.

The breathing air cylinder was connected directly to the FTIR at 15:30 flowing at 1 lpm until 15:37. This test was performed to confirm the presence of carbon monoxide, carbon dioxide, and methane as was observed in the preliminary testing. The same cylinder was used for the rat exposure test as used for the preliminary testing.

At 15:43 the generator tube exhaust was plumbed directly to the FTIR to test whether the ethyl-FOSE could be directly measured. The flow rate was 2 lpm into the FTIR. Spectral collection continued until 15:51 at which time the sampling was discontinued.

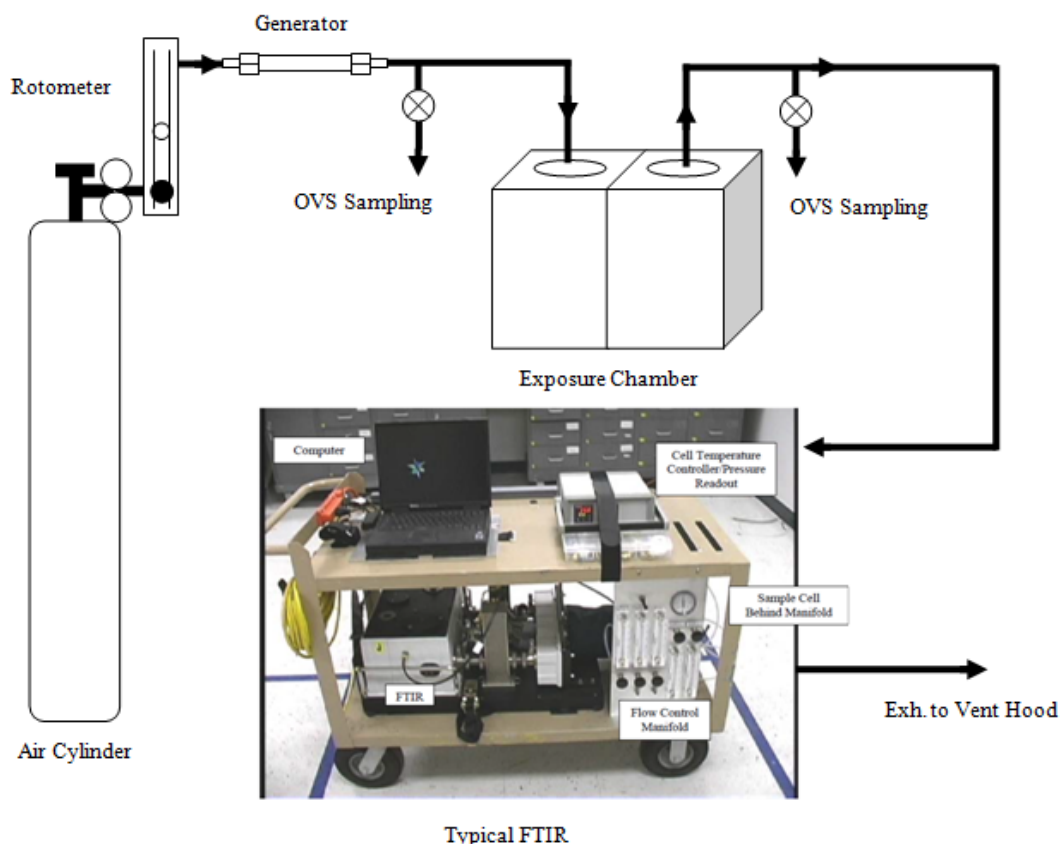
The FTIR data showed the presence of methanol, methane, carbon monoxide, carbon dioxide, and ammonia. The methanol was coming from within the chamber apparently from past cleaning activities. There was a thin white plastic sheet material on the bottom of the chamber that we suspected acted like a sponge slowly emitting the methanol. The ammonia was presumed to be from rat urine. The concentration continued to increase throughout the test.

After all the samples were collected the flow of air through the generator tube and exposure chamber was stopped and everything valved closed and plugged/capped. The test was ended at 14:09 12/9/13.

2 Methods - Analytical and Preparatory

The exposure chamber testing was performed by plumbing an air supply line from the breathing air cylinder to the rotometer then to the ethyl-FOSE generator tube. From the generator tube a T was installed with a valve to provide a sampling port for the OVS tubes. From that point the tube continued to the inlet of the exposure chamber located on one end. Exiting the chamber from the other end is another T with a valve to provide a sampling port for the outlet OVS location. After the T the line continues to the FTIR cart. The line was attached to one of the sampling inlet ports on the control panel. From the panel the line continues to the FTIR sample cell inlet where sample gas traverses the length of the cell out the exhaust line where the gases were vented into the adjacent hood. FTIR measurements were initially collected continuously then after several minutes the frequency was changed to 5 minute intervals. The overall sampling system was leak checked each day prior to testing. Cylinder air was flowed at 2 lpm into the entire system while the final exhaust was plugged. The pressure on the system was monitored by the pressure sensor in the FTIR. When the system was pressurized between 1-2 psia above ambient, the cylinder valve was closed. The system was checked for pressure change over at least a 5 minute time frame. Acceptance criteria is <4% change. All leak checks ranged between 0 and -0.51%. A schematic of the sampling system is shown in Figure 1 below.

Figure 1 – Sample System Schematic



The method used for the FTIR analyses was a modified procedure of ETS-8-31.2 “Measurement of Vapor Phase Compounds by Extractive Fourier Transform Infrared (FTIR) Spectroscopy”, which is based upon NIOSH 3800 and EPA Method 320. For these tests the exposure chamber air was extracted through a Midac I-series FTIR system. The FTIR system was equipped with a nominal 20.0 meter optical pathlength gas cell operated at ambient temperatures. The FTIR continuously collected data throughout the tests and created average sample spectra approximately every 60 seconds.

FTIR results were generated using Midac AutoQuant™ software and are reported in ppmv (parts per million by volume). The software utilizes a classical least squares (CLS) algorithm to compare a sample spectrum to a set of reference spectra. The least-squares algorithm computes what fraction of each reference spectrum must be added to reproduce the sample spectrum. The equivalent proportion of reference spectra is equal to the concentration in the sample. CLS assumes a linear relationship between absorbance and concentration.

Concentrations of analytes in representative FTIR sample spectrum were verified using manual subtraction of a reference spectrum from the sample spectrum utilizing Galactic software. The relative fraction of the reference spectrum, or subtraction factor, was then used to calculate the concentration of the sample in ppmv using the following equation:

$$\text{ppmv} = \frac{(\text{subtraction factor}) * (\text{reference spectrum concentration at cell temp (ppmv*m)})}{\text{pathlength for sample analysis (m)}}$$

3 FTIR Analysis

Spectral features from representative sample spectra were evaluated and carbon monoxide, carbon dioxide, methane, methanol, and water were detected. Ethyl-FOSE was not detected in any chamber exhausts during this study. There were several other unknown spectral features observed at the startup of the testing. These features dropped below detection several minutes after startup. It is suspected these features were related to the white plastic material at the bottom of the exposure chamber. Methanol was also attributed to residual effects within the chamber due most likely to past cleaning activities.

All quality control requirements including daily pathlength determinations and sampling system leak checks met the requirements of EPA Method 320 and ETS-8-31.2.

4 FTIR Test Results – Data Plots

Figure 1 - All Test Compounds Plotted

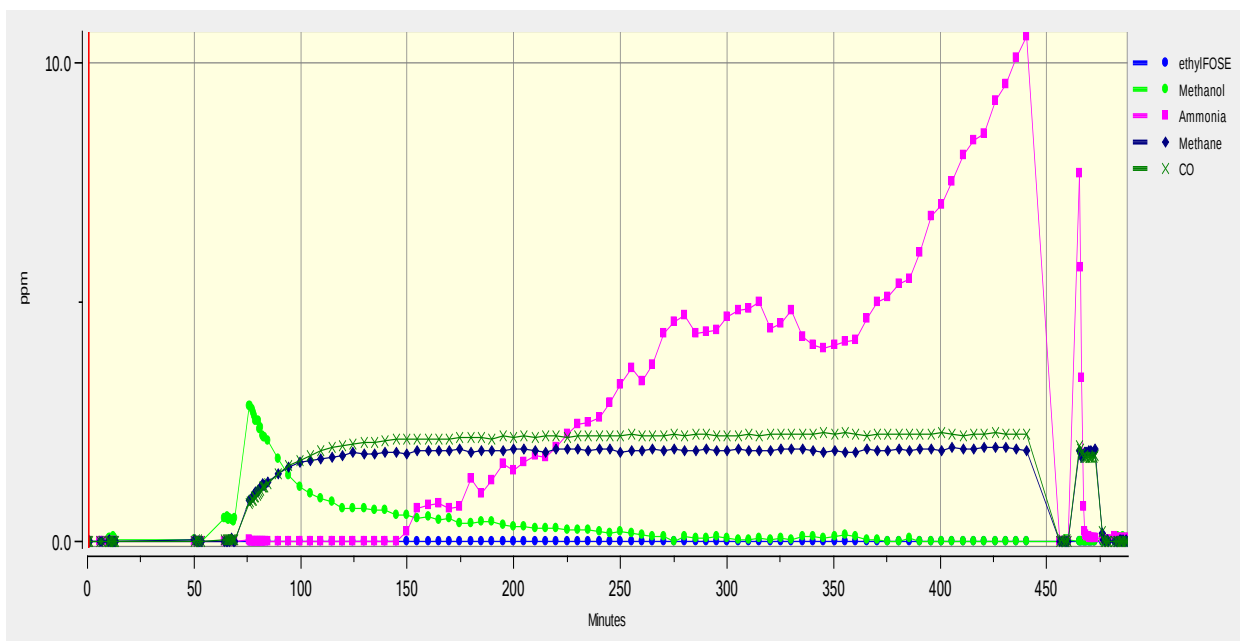


Figure 2 – All Test Compounds Excluding Ammonia

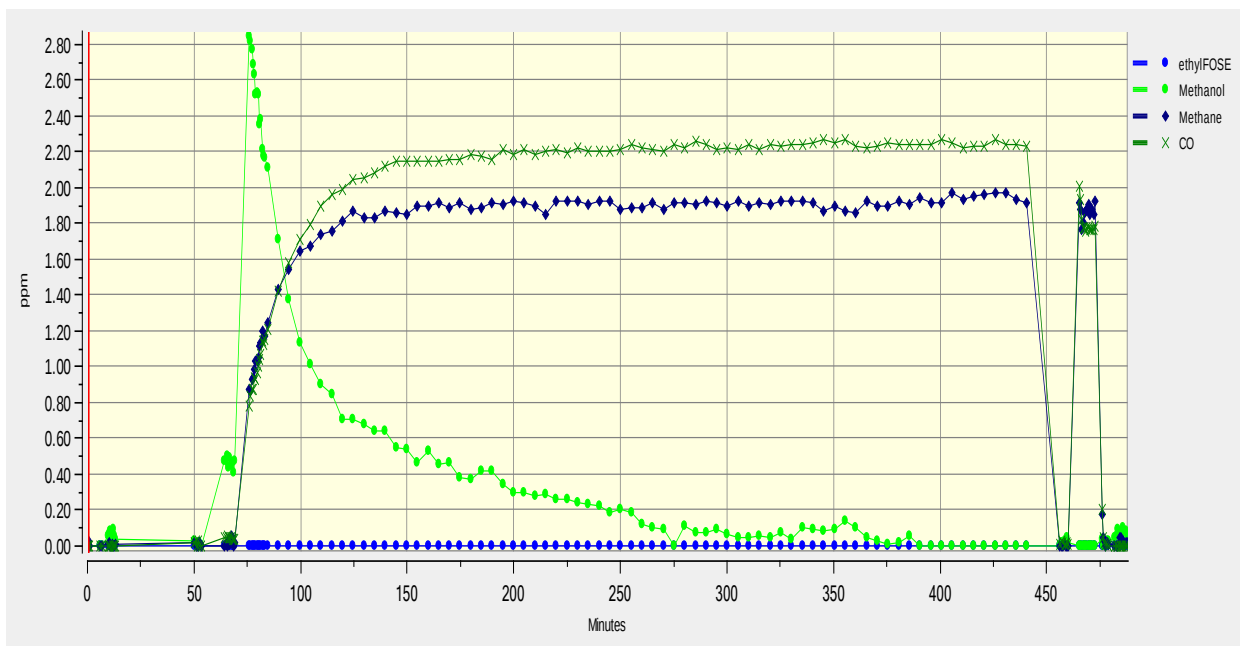
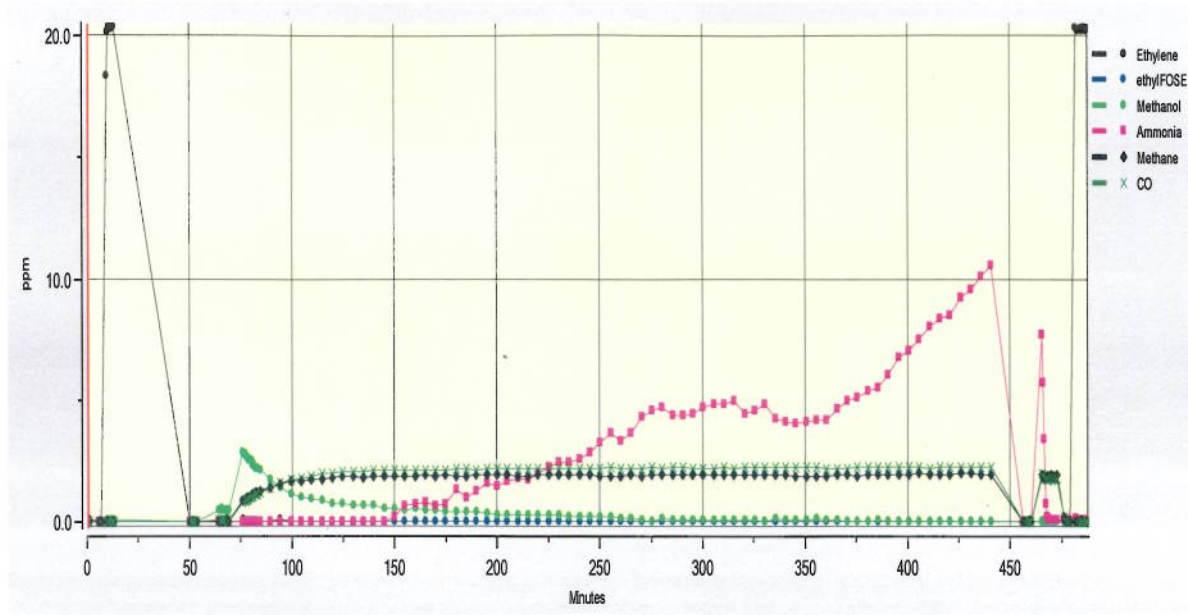


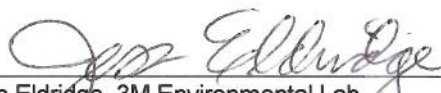
Figure 3 - All Test Compounds plus Ethylene (Pathlength Calc)



5 Data/Sample Retention

Data will be archived in the 3M Environmental Lab archives in accordance with 3M policy for record retention.

6 Signatures


Jess Eldridge, 3M Environmental Lab

2-6-14
Date


Brian Mader, Technical Manager, 3M Environmental Lab

2-6-14
Date

7 Flow Rates, Correction Factors, Test Times

E13-0880 Final Analytical Report, Appendix-A

E13-0722 B270 Tox Chamber Ethyl-FOSE Conditioning

By: JSE 11/26/13

Sweep gas pressure setting = 10 psi

Sweep gas flow rate = 2 lpm; calibrated rotometer

Flow rate checked on inlet connected to pressurized system @ 8:55 11/14/13 = 115 cc/min; not connected @ 8:36 = 104.5 cc/min

Flow rate checked on outlet connected to pressurized system @ 10:15 11/20/13 = 116 cc/min; not connected @ 10:09 = 108 cc/min

Inlet = pump #IH0000150

Outlet = pump #H0000222

Dry-Cal # 3M Instrument # 158675, #E0000003898

Diff, cc/min

Diff, cc/min

Avg, cc/min

EtFOSE-OH P-Chem

Structure C8F17SO2N(CH2CH3)CH2CH2OH

MW 571

10.5 T @ 23

8 P (atm) 1

9.25

Lab Request E13-0722 - Preliminary Chamber Test

Date	Start Time	Stop Time	Elapsed (hr)	Gas	Test	Location	Initial flow, cc/min	Final flow, cc/min	Avg flow, cc/min	Cor. Factor, cc/min	Adj flow, cc/min	Run Time, min	Volume, L	Ethyl FOSE (ng/OVS tube)	Air Conc. Ethyl FOSE (ng/L)	Air Conc. Ethyl FOSE (ppbV)
11/14/2013	10:25	11:25	1.00	Air	1	Inlet	104.5	104	104.25	9.25	113.5	60	6.810	1710	251	11
11/14/2013	10:25	11:25	1.00	Air	1	Outlet	103	107	105	9.25	114.25	60	6.855	6.1	0.89	0.04
11/14/2013	12:20	13:20	1.00	Air	2	Inlet	104	103	103.5	9.25	112.75	60	6.765	3120	461	20
11/14/2013	12:20	13:20	1.00	Air	2	Outlet	107	108	107.5	9.25	116.75	60	7.005	30.6	4.4	0.19
11/14/2013	14:20	15:20	1.00	Air	3	Inlet	103	103	103	9.25	112.25	60	6.735	3510	521	22
11/14/2013	14:20	15:20	1.00	Air	3	Outlet	108	109	108.5	9.25	117.75	60	7.065	72.7	10	0.44
11/14/2013	15:22	16:22	1.00	Air	4	Inlet	103	103	103	9.25	112.25	60	6.735	4040	600	25
11/14/2013	15:22	16:22	1.00	Air	4	Outlet	108	109	108.5	9.25	117.75	60	7.065	124.0	18	0.75
11/15/2013	8:42	9:42	1.00	Air	5	Inlet	104	104	104	9.25	113.25	60	6.795	3990	587	25
11/15/2013	8:42	9:42	1.00	Air	5	Outlet	105	105	105	9.25	114.25	60	6.855	124.0	18	0.8
11/15/2013	10:42	11:42	1.00	Air	6	Inlet	104	104	104	9.25	113.25	60	6.795	4640	683	29
11/15/2013	10:42	11:42	1.00	Air	6	Outlet	105	105	105	9.25	114.25	60	6.855	145.0	21	0.9
11/15/2013	12:42	13:42	1.00	Air	7	Inlet	104	103	103.5	9.25	112.75	60	6.765	5080	751	32
11/15/2013	12:42	13:42	1.00	Air	7	Outlet	105	105	105	9.25	114.25	60	6.855	219.0	32	1.4
11/15/2013	13:44	14:44	1.00	Air	8	Inlet	104	103	103.5	9.25	112.75	60	6.765	5480	810	34
11/15/2013	13:44	14:44	1.00	Air	8	Outlet	105	105	105	9.25	114.25	60	6.855	302.0	44	1.9
11/20/2013	9:06	10:06	1.00	Nitrogen	9	Inlet	104	104	104	9.25	113.25	60	6.795	4590	675	29
11/20/2013	9:06	10:06	1.00	Nitrogen	9	Outlet	108	108	108	9.25	117.25	60	7.035	865.0	123	5.2
11/21/2013	9:05	10:05	1.00	Nitrogen	10	Inlet	105	104	104.5	9.25	113.75	60	6.825	3660	536	23
11/21/2013	9:05	10:05	1.00	Nitrogen	10	Outlet	109	108	108.5	9.25	117.75	60	7.065	1170.0	166	7.0
11/22/2013	8:51	9:51	1.00	Nitrogen	11	Inlet	104	104	104	9.25	113.25	60	6.795	4140	609	26
11/22/2013	8:51	9:51	1.00	Nitrogen	11	Outlet	106	106	106	9.25	115.25	60	6.915	1140.0	165	7.0
11/25/2013	9:54	10:55	1.02	Nitrogen	12	Inlet	104	103	103.5	9.25	112.75	61	6.878	2950	429	18
11/25/2013	9:54	10:55	1.02	Nitrogen	12	Outlet	108	108	108	9.25	117.25	61	7.152	1450.0	203	8.6
12/4/2013	9:18	10:18	1.00	Nitrogen	13	Inlet	104	104	104	9.25	113.25	60	6.795	1770	260	11
12/4/2013	9:18	10:18	1.00	Nitrogen	13	Outlet	105	104	104.5	9.25	113.75	60	6.825	748.0	110	4.7
Lab Request E13-0880 - Exposure Test w Rats																
12/9/2013	9:12	10:12	1.00	Air	1	Inlet	104	103	103.5	9.25	112.75	60	6.765	1397	207	8.8
12/9/2013	9:12	10:12	1.00	Air	1	Outlet	104	106	105	9.25	114.25	60	6.855	524.0	76	3.2
12/9/2013	10:15	11:15	1.00	Air	2	Inlet	104	103	103.5	9.25	112.75	60	6.765	1520	225	9.6
12/9/2013	10:15	11:15	1.00	Air	2	Outlet	104	106	105	9.25	114.25	60	6.855	652.0	95	4.0
12/9/2013	11:18	12:18	1.00	Air	3	Inlet	104	103	103.5	9.25	112.75	60	6.765	1501	222	9.4
12/9/2013	11:18	12:18	1.00	Air	3	Outlet	104	106	105	9.25	114.25	60	6.855	1013.0	148	6.3
12/9/2013	12:21	13:21	1.00	Air	4	Inlet	104	103	103.5	9.25	112.75	60	6.765	1544	228	9.7
12/9/2013	12:21	13:21	1.00	Air	4	Outlet	104	106	105	9.25	114.25	60	6.855	958.0	140	5.9
12/9/2013	13:24	14:24	1.00	Air	5	Inlet	104	103	103.5	9.25	112.75	60	6.765	1505	222	9.5
12/9/2013	13:24	14:24	1.00	Air	5	Outlet	104	106	105	9.25	114.25	60	6.855	919.0	134	5.7
12/9/2013	14:27	15:17	0.83	Air	6	Inlet	104	103	103.5	9.25	112.75	50	5.6375	1271	225	9.6
12/9/2013	14:27	15:17	0.83	Air	6	Outlet	104	106	105	9.25	114.25	50	5.7125	885.0	155	6.6

Inlet
Outlet

Ethyl-FOSE Gas-phase Concentration

Comment	Date/ Time	Generator		Chamber	
		Outlet ppbV	Outlet ppbV	Generator Outlet ng/L	Chamber Outlet ng/L
Pre-test	11/14/13 11:25	11	0.04	251	0.89
Pre-test	11/14/13 13:20	20	0.19	461	4.4
Pre-test	11/14/13 15:20	22	0.44	521	10
Pre-test	11/14/13 16:22	25	0.75	600	18
Pre-test	11/15/13 9:42	25	0.77	587	18
Pre-test	11/15/13 11:42	29	0.90	683	21
Pre-test	11/15/13 13:42	32	1.36	751	32
Pre-test	11/15/13 14:44	34	1.87	810	44
Pre-test	11/20/13 10:06	29	5.23	675	123
Pre-test	11/21/13 10:05	23	7.04	536	166
Pre-test	11/22/13 9:51	26	7.01	609	165
Pre-test	11/25/13 10:55	18	8.62	429	203
Pre-test	12/4/13 10:18	11	4.66	260	110

Ethyl-FOSE Gas-phase Concentration

Comment	Date/ Time	Generator		Chamber	
		Outlet ppbV	Outlet ppbV	Generator Outlet ng/L	Chamber Outlet ng/L
Exposure Test	12/9/2013 10:12	8.8	3.2	207	76
Exposure Test	12/9/2013 11:15	9.6	4.0	225	95
Exposure Test	12/9/2013 12:18	9.4	6.3	222	148
Exposure Test	12/9/2013 13:21	9.7	5.9	228	140
Exposure Test	12/9/2013 14:24	9.5	5.7	222	134
Exposure Test	12/9/2013 15:27	9.6	6.6	225	155

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Start Date Time	Stop Date Time	Elpased (hr)	Elapsed (min)	Flow-rate (LPM)	Volume (L)
11/14/2013 10:25	11/14/2013 16:24	5.98	359	2	718
11/15/2013 8:30	11/15/2013 14:52	12.35	741	2	1482
11/19/2013 15:40	11/26/2013 13:00	177.68	10661	2	21322
11/26/2013 14:44	12/4/2013 10:44	365.68	21941	2	43882
12/9/2013 9:12	12/9/2013 9:19	365.80	21948	2	43896
12/9/2013 9:19	12/9/2013 15:19	371.80	22308	2.1	46847

E13-0722 Prelim Chamber Test
E13-0722 Prelim Chamber Test
E13-0722 Prelim Chamber Test
E13-0722 Prelim Chamber Test
E13-0880 Exposure test w Rats

shut system down @15:19
12/9/13 sampling done @ 15:17

8 FTIR Data, Method Map, Pathlength, Spectra, Leak Checks, QA/QC

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[illegible]

E13-0880 Final Analytical Report, Appendix-A

Method Name: B270 ethyl-FOSE

Method Path: C:\E13-0880 B270 Ethyl-FOSE Rat Exp. Test\Method\B270 ethyl-FOSE\B270 ethyl-FOSE.aq4

Method Type: AutoQuant 4.0

Non-Linear Analysis Mode

MethodParameters

Wavenumber range:	650.00 - 4500.00 cm-1
Default Pathlength =	24.1800 M
Gain =	0
Apodization =	Triangle
Phase Correction =	Mertz
Resolution =	0.5 cm-1
Baseline Correction:	Single Linear
Exclusion Criterion:	2500

Compound: Ethylene

Description:	Ethylene
Molecular Weight:	0
Alarms:	Disabled
Primary Spectrum:	J2KETY.SPC
Reference Concentration:	206.6000 ppm-m
Reference Pathlength:	0.1000 M
Reference Pressure:	0.9699 atm
Reference Temperature:	20.00 C
Region #1:	850.00 - 1100.00 cm-1

Compound: ethylFOSE

Description:	ethylFOSE
Molecular Weight:	0
Alarms:	Disabled
Primary Spectrum:	Ethyl FOSE 17PPB.SPC
Reference Concentration:	0.1700 ppm-m
Reference Pathlength:	10.0000 M
Reference Pressure:	1.0000 atm
Reference Temperature:	25.00 C
Region #1:	1000.00 - 1056.00 cm-1
Region #2:	1057.00 - 1131.00 cm-1
Region #3:	807.00 - 834.00 cm-1

Compound: Methanol

Description:	Methanol
Molecular Weight:	0
Alarms:	Disabled
Spectrum:	MeOh_25T.spc
Reference Concentration:	1.0000 ppm-m
Reference Pathlength:	1.0000 M
Reference Pressure:	1.0000 atm

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Spectrum:	Reference Temperature:	25.00 C
	Region #1:	1028.00 - 1038.00 cm-1
	meoh_z5a.spc	
	Reference Concentration:	224.6000 ppm-m
	Reference Pathlength:	1.0000 M
Primary Spectrum:	Reference Pressure:	1.0000 atm
	Reference Temperature:	25.00 C
	Region #1:	1028.00 - 1038.00 cm-1
	MEOHZ9A.SPC	
	Reference Concentration:	22.5000 ppm-m
	Reference Pathlength:	1.0000 M
	Reference Pressure:	1.0000 atm
	Reference Temperature:	25.00 C
	Region #1:	1028.00 - 1038.00 cm-1

Compound: Temp

Description:	temperature	
Molecular Weight:		0
Alarms:	Disabled	

Compound: Press

Description:	pressure	
Molecular Weight:		0
Alarms:	Disabled	

Compound: Ammonia

Description:	Ammonia	
Molecular Weight:		0
Alarms:	Disabled	
Spectrum:	NH3_25T.spc	

	Reference Concentration:	1.0000 ppm-m
	Reference Pathlength:	1.0000 M
	Reference Pressure:	1.0000 atm
	Reference Temperature:	25.00 C
	Region #1:	800.00 - 1165.00 cm-1

Primary Spectrum:	nh3_12.spc	
	Reference Concentration:	52.6000 ppm-m
	Reference Pathlength:	1.0000 M
	Reference Pressure:	1.0000 atm
	Reference Temperature:	25.00 C
	Region #1:	800.00 - 1165.00 cm-1

Spectrum:	nh3_9.spc	
	Reference Concentration:	99.0000 ppm-m
	Reference Pathlength:	1.0000 M
	Reference Pressure:	1.0000 atm
	Reference Temperature:	25.00 C

	Region #1:	800.00 - 1165.00 cm-1
Compound: Methane		
Description:	Methane	
Molecular Weight:		0
Alarms:	Disabled	
Primary Spectrum:	AIHZME.SPC	
	Reference Concentration:	100.0000 ppm-m
	Reference Pathlength:	1.0000 M
	Reference Pressure:	1.0000 atm
	Reference Temperature:	25.00 C
	Region #1:	2900.00 - 3035.00 cm-1
Spectrum:	CH4_25A.SPC	
	Reference Concentration:	273.0000 ppm-m
	Reference Pathlength:	1.0000 M
	Reference Pressure:	1.0000 atm
	Reference Temperature:	25.00 C
	Region #1:	2900.00 - 3035.00 cm-1
Spectrum:	CH4_25T.spc	
	Reference Concentration:	1.0000 ppm-m
	Reference Pathlength:	1.0000 M
	Reference Pressure:	1.0000 atm
	Reference Temperature:	25.00 C
	Region #1:	2900.00 - 3035.00 cm-1
Compound: CO		
Description:	CO	
Molecular Weight:		0
Alarms:	Disabled	
Spectrum:	7CO.SPC	
	Reference Concentration:	6.6000 ppm-m
	Reference Pathlength:	1.0000 M
	Reference Pressure:	1.0000 atm
	Reference Temperature:	25.00 C
	Region #1:	2100.00 - 2181.00 cm-1
Spectrum:	67CO.SPC	
	Reference Concentration:	66.6900 ppm-m
	Reference Pathlength:	1.0000 M
	Reference Pressure:	1.0000 atm
	Reference Temperature:	25.00 C
	Region #1:	2100.00 - 2181.00 cm-1
Primary Spectrum:	113CO.SPC	
	Reference Concentration:	112.7000 ppm-m
	Reference Pathlength:	1.0000 M
	Reference Pressure:	1.0000 atm
	Reference Temperature:	25.00 C

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Region #1: 2124.00 - 2160.00 cm-1

Compound: Moisture

Description: Moisture

Molecular Weight: 0

Alarms: Disabled

Primary Spectrum: U0000030.spc

Reference Concentration: 1.0000 ppm-m

Reference Pathlength: 24.5700 M

Reference Pressure: 0.9406 atm

Reference Temperature: 22.10 C

Region #1: 2900.00 - 3150.00 cm-1

Region #2: 2200.00 - 2000.00 cm-1

Region #3: 1345.00 - 1200.00 cm-1

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Ethylene Cylinder Number SG9104949BAL
Concentration , ppmV 20.3

Date	Method	Filename	Sample Line	Ethylene (ppm)	SEC (ppm)
12/9/2013 8:08	B270 ethyl-FOSE	U0000006	N/A	21.8714	0.0891
12/9/2013 8:09	B270 ethyl-FOSE	U0000007	N/A	24.0072	0.1004
12/9/2013 8:09	B270 ethyl-FOSE	U0000008	N/A	24.2399	0.1012
12/9/2013 8:10	B270 ethyl-FOSE	U0000009	N/A	24.2151	0.0995
12/9/2013 8:11	B270 ethyl-FOSE	U0000010	N/A	24.1698	0.0996
12/9/2013 8:11	B270 ethyl-FOSE	U0000011	N/A	24.1984	0.0996
12/9/2013 8:12	B270 ethyl-FOSE	U0000012	N/A	24.2151	0.1009
Average				24.21	
12/9/2013 16:00	B270 ethyl-FOSE	U0000139	N/A	24.1959	0.1039
12/9/2013 16:01	B270 ethyl-FOSE	U0000140	N/A	24.0793	0.1048
12/9/2013 16:02	B270 ethyl-FOSE	U0000141	N/A	24.0897	0.1046
12/9/2013 16:03	B270 ethyl-FOSE	U0000142	N/A	24.1542	0.1028
12/9/2013 16:03	B270 ethyl-FOSE	U0000143	N/A	24.1643	0.1024
12/9/2013 16:04	B270 ethyl-FOSE	U0000144	N/A	24.1092	0.1036
12/9/2013 16:04	B270 ethyl-FOSE	U0000145	N/A	24.1952	0.1034
12/9/2013 16:05	B270 ethyl-FOSE	U0000146	N/A	24.1802	0.1036
12/9/2013 16:06	B270 ethyl-FOSE	U0000147	N/A	24.0867	0.1029
12/9/2013 16:06	B270 ethyl-FOSE	U0000148	N/A	24.132	0.1036
Average				24.15	
Overall Average				24.18	

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Manual Validation

2/4/2014

By: JSE

Trial	Compound	Ref Spectrum	Conc, ppm-m	Region	Samp Spectrum	SF	PL, m	AQ value, ppmV	Gramsvalue, ppmV	% recovery
1	Ammonia	NH3_12	52.6	800-980	U80	2.52	24.18	4.71	5.48	116.4%
2	Ammonia	NH3_12	52.6	800-980	U80	2.48	24.18	4.71	5.39	114.5%
1	CO	113CO	112.7	2124-2160	U80	0.44	24.18	2.22	2.05	92.4%
2	CO	113CO	112.7	2124-2160	U80	0.47	24.18	2.22	2.19	98.7%
3	CO	113CO	112.7	2124-2160	U30	0.13	24.18	0.9	0.60	66.7%
1	Methane	AIHZME	100	2900-3035	U80	0.46	24.18	1.91	1.90	99.6%
2	Methane	AIHZME	100	2900-3035	U80	0.46	24.18	1.91	1.90	99.6%
3	Methane	AIHZME	100	2900-3035	U30	0.2	24.18	0.93	0.83	88.9%
1	Methanol	MEOHZ9A	22.5	960-1085	U40	2.05	24.18	1.96	1.91	97.3%
2	Methanol	MEOHZ9A	22.5	960-1085	U40	2.08	24.18	1.96	1.94	98.7%
3	Methanol	MEOHZ9A	22.5	960-1085	U47	0.85	24.18	0.84	0.79	94.2%

Ammonia LOQ = 0.30 ppmV based upon the FMU of 30% and a manual review of spectrum 54.

CO LOQ = 0.90 ppmV based upon FMU of 17 % on spectrum 30 and manual subtraction: matrix interference

Methane LOQ = 0.9 ppmV based upon FMU of 28% on spectrum 30 and manual subtraction

Methanol LOQ = 0.5 ppmV based upon FMU of 30% on spectrum 54 and manual subtraction

SEC = Sample Error Concentration calculated by AutoQuant software

FMU = Fractional Model Uncertainty

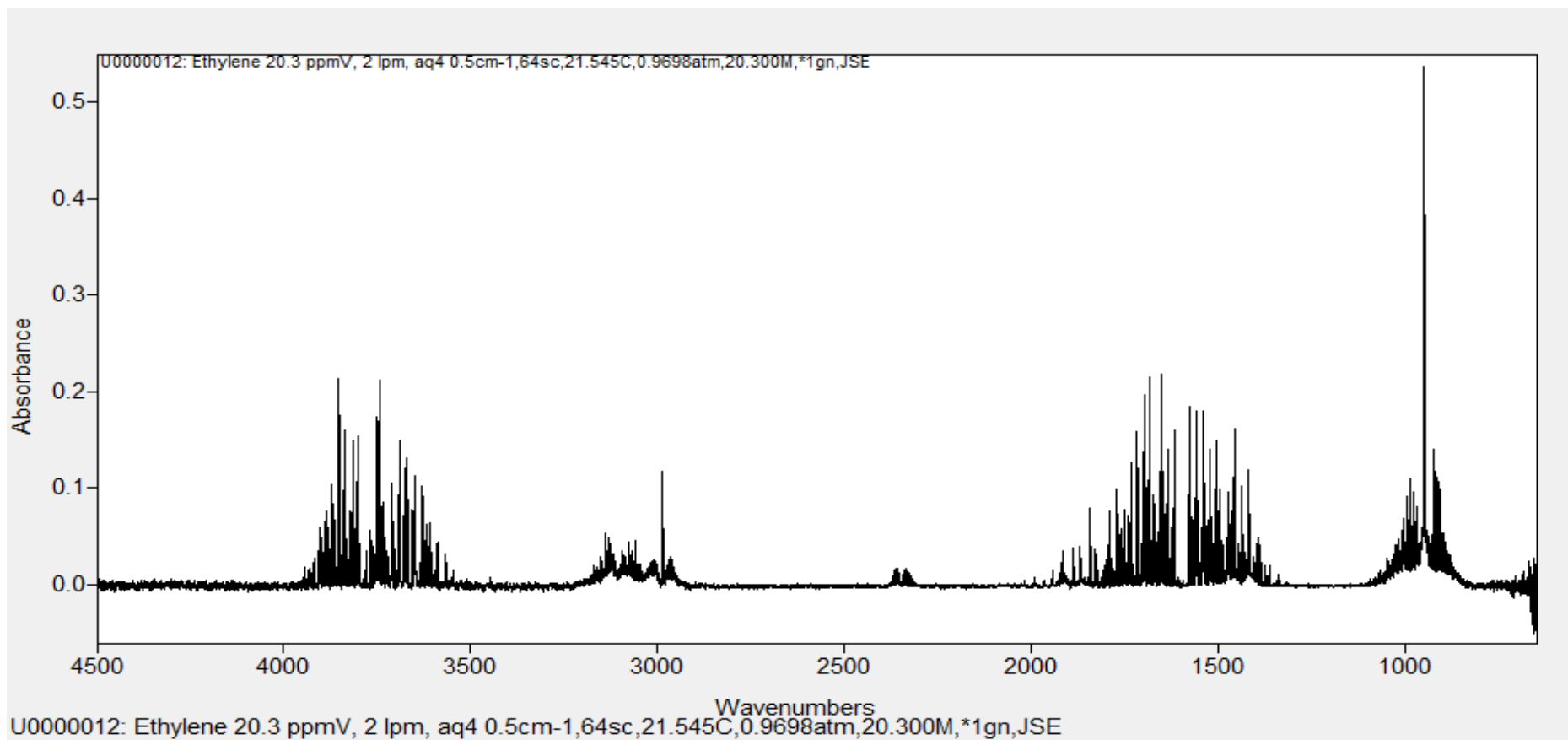
FMU = (2x SEC)/predicted value

The lower the SEC thus FMU the better the predictive model is performing

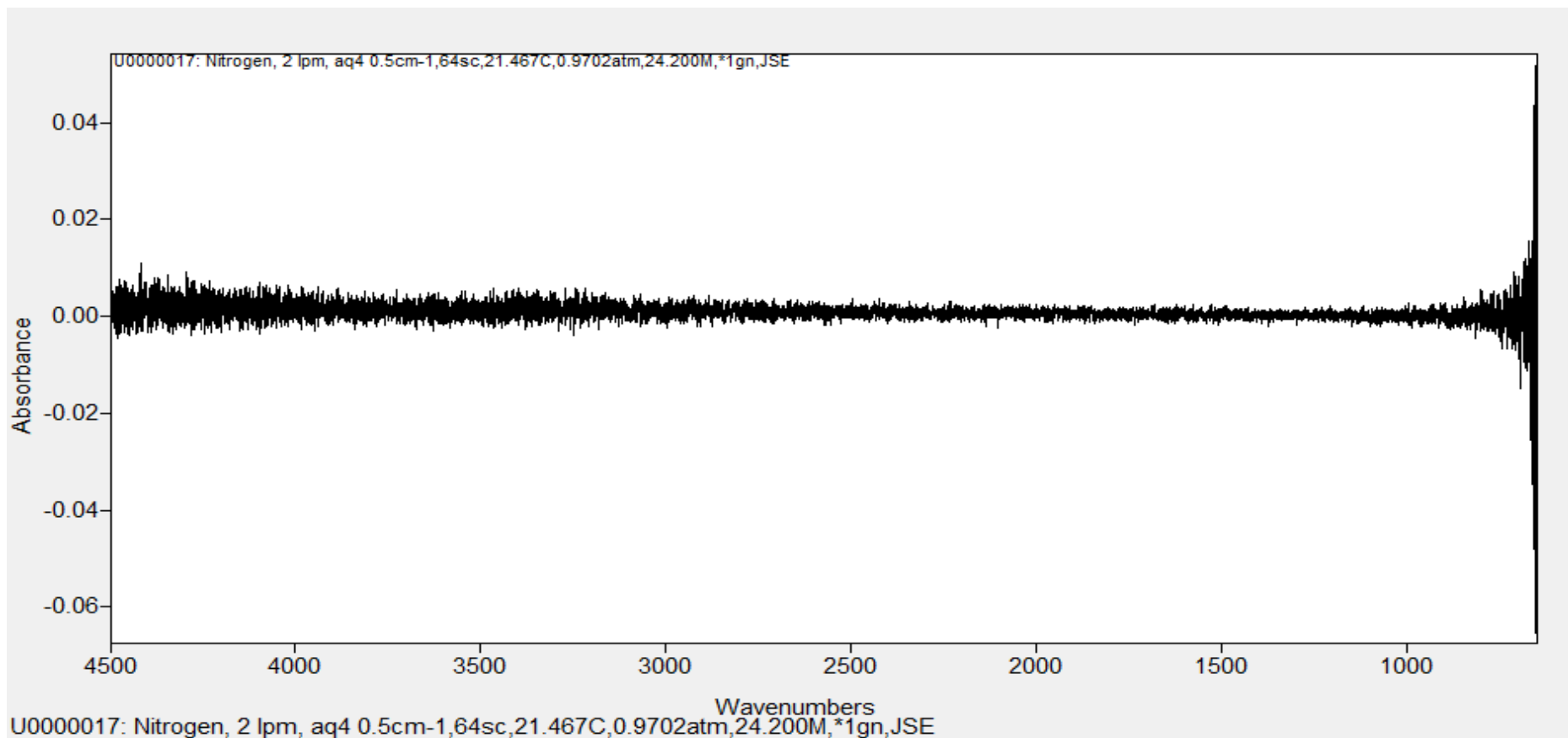
LOQ is based upon manual spectral interpretation and factoring in the FMU values

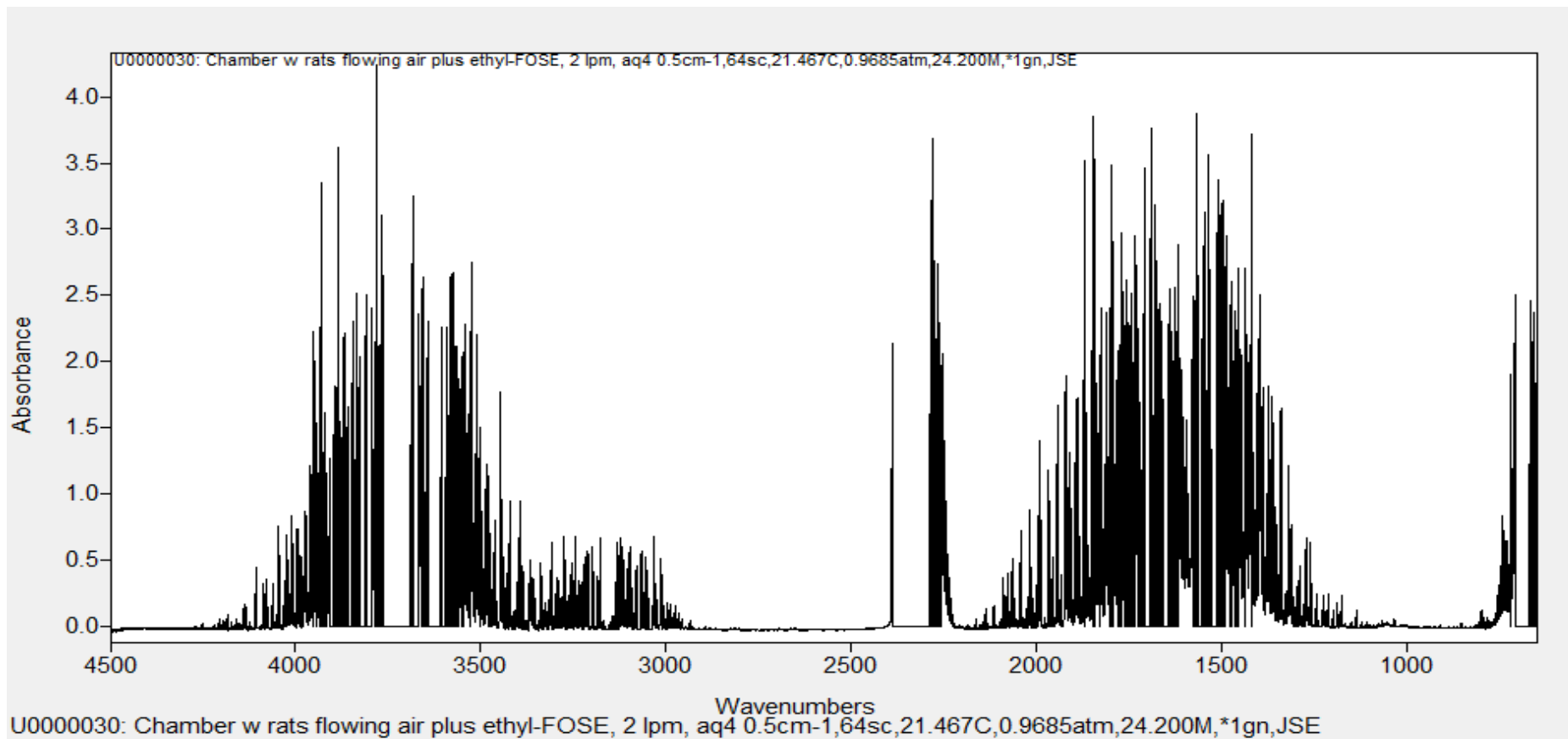
The FMU calculation evaluates the predictive model performance on a real sample matrix per compound

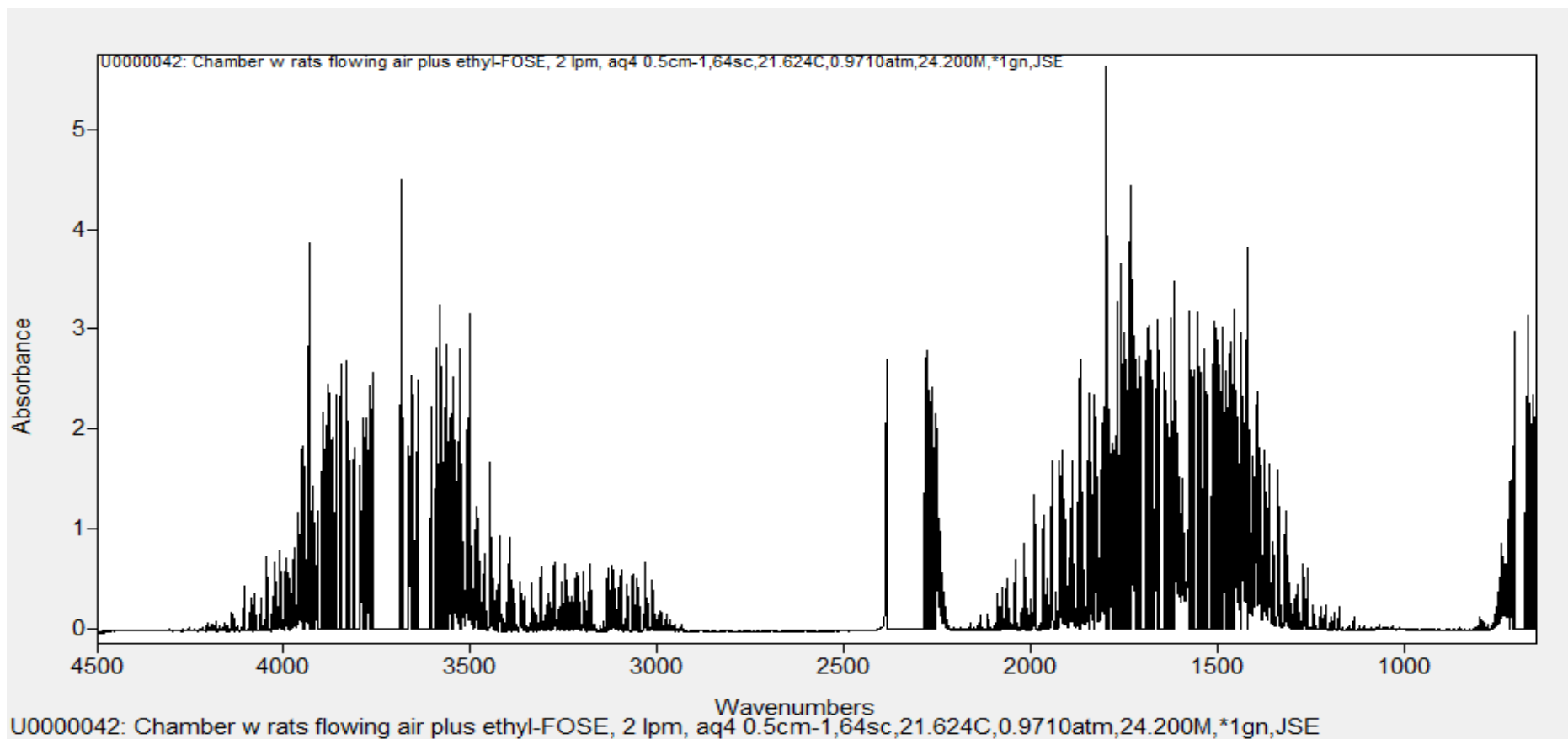
Primary model performance factors are FTIR instrument condition, quality of reference spectra, matrix interference, and spectral regions chosen in method

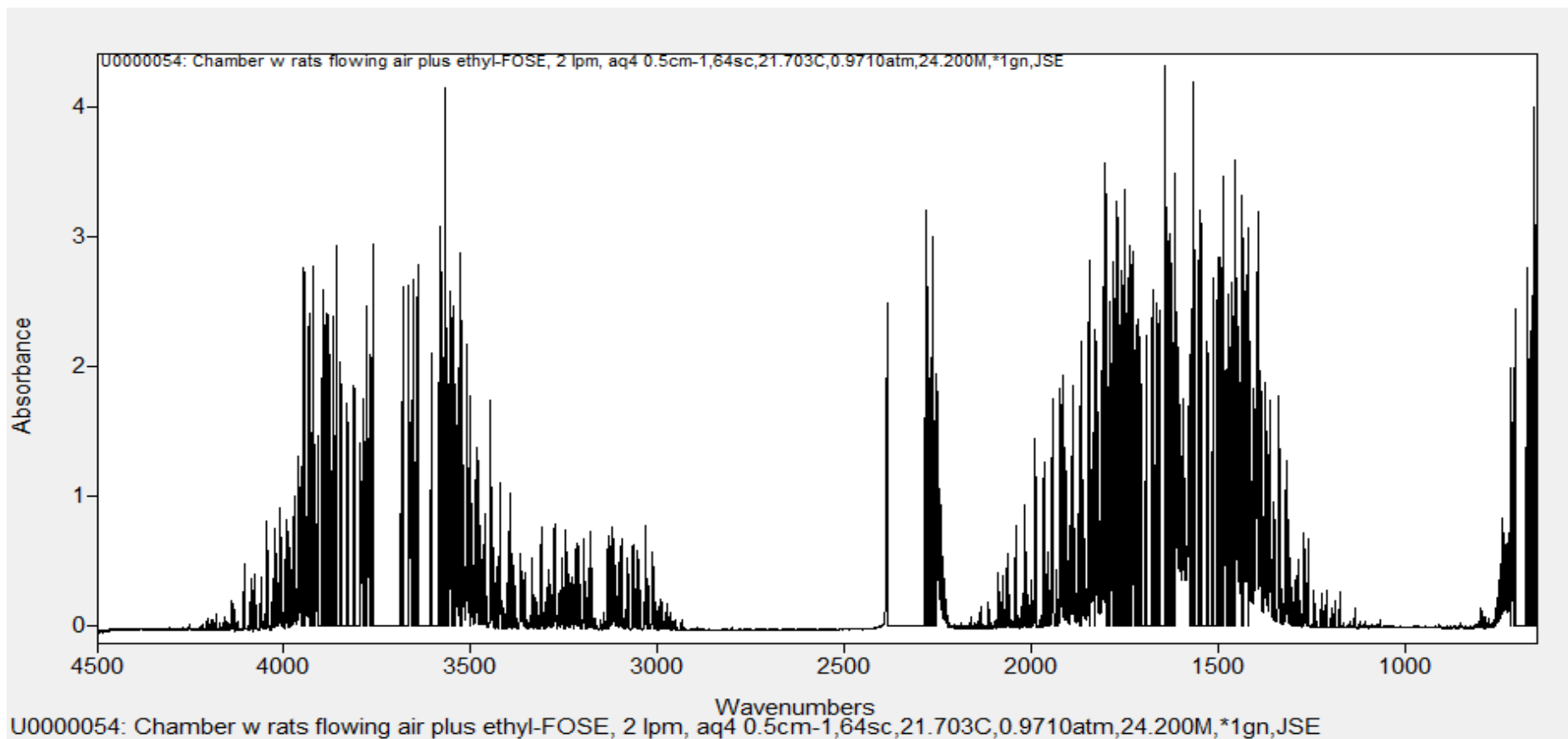


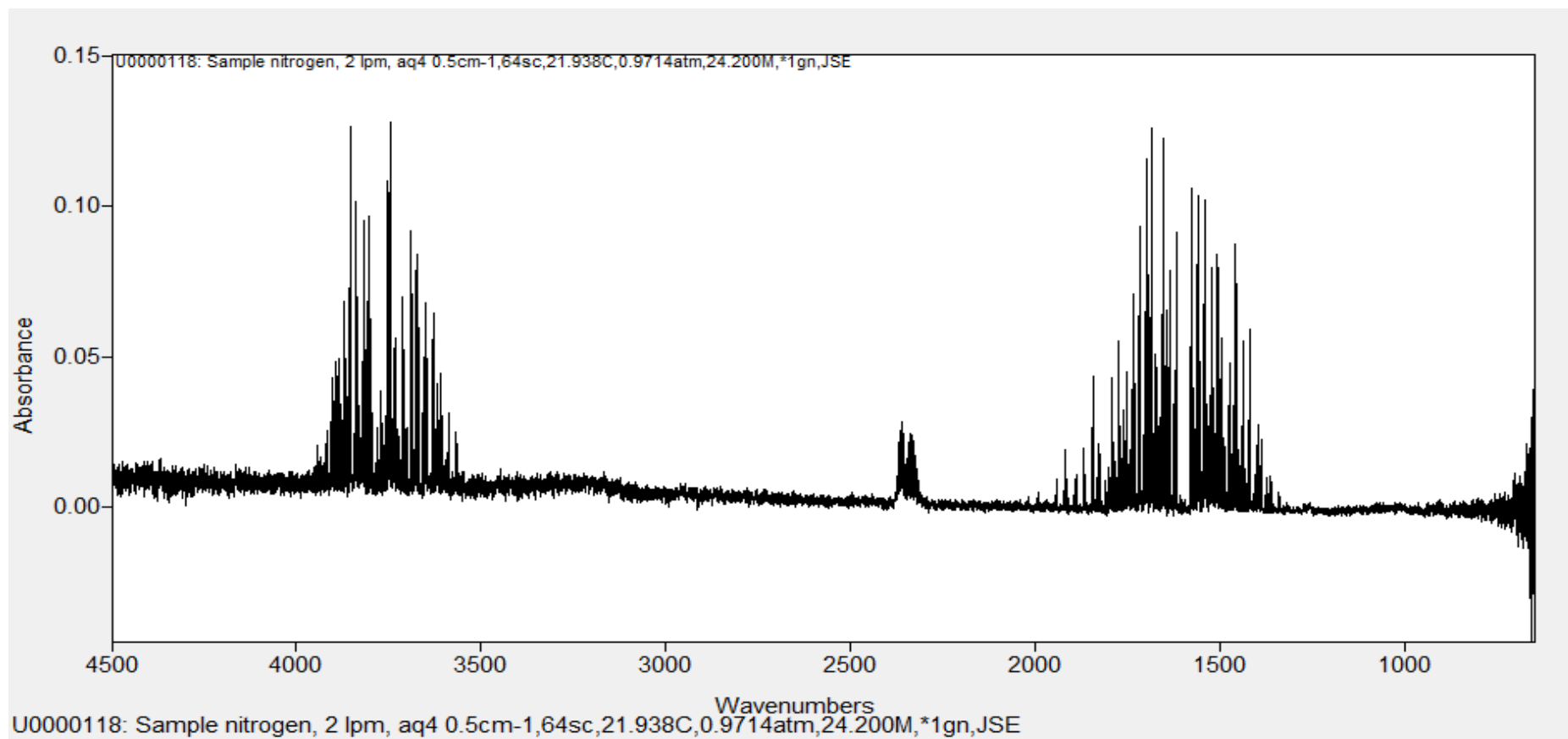
E13-0880 Final Analytical Report, Appendix-A

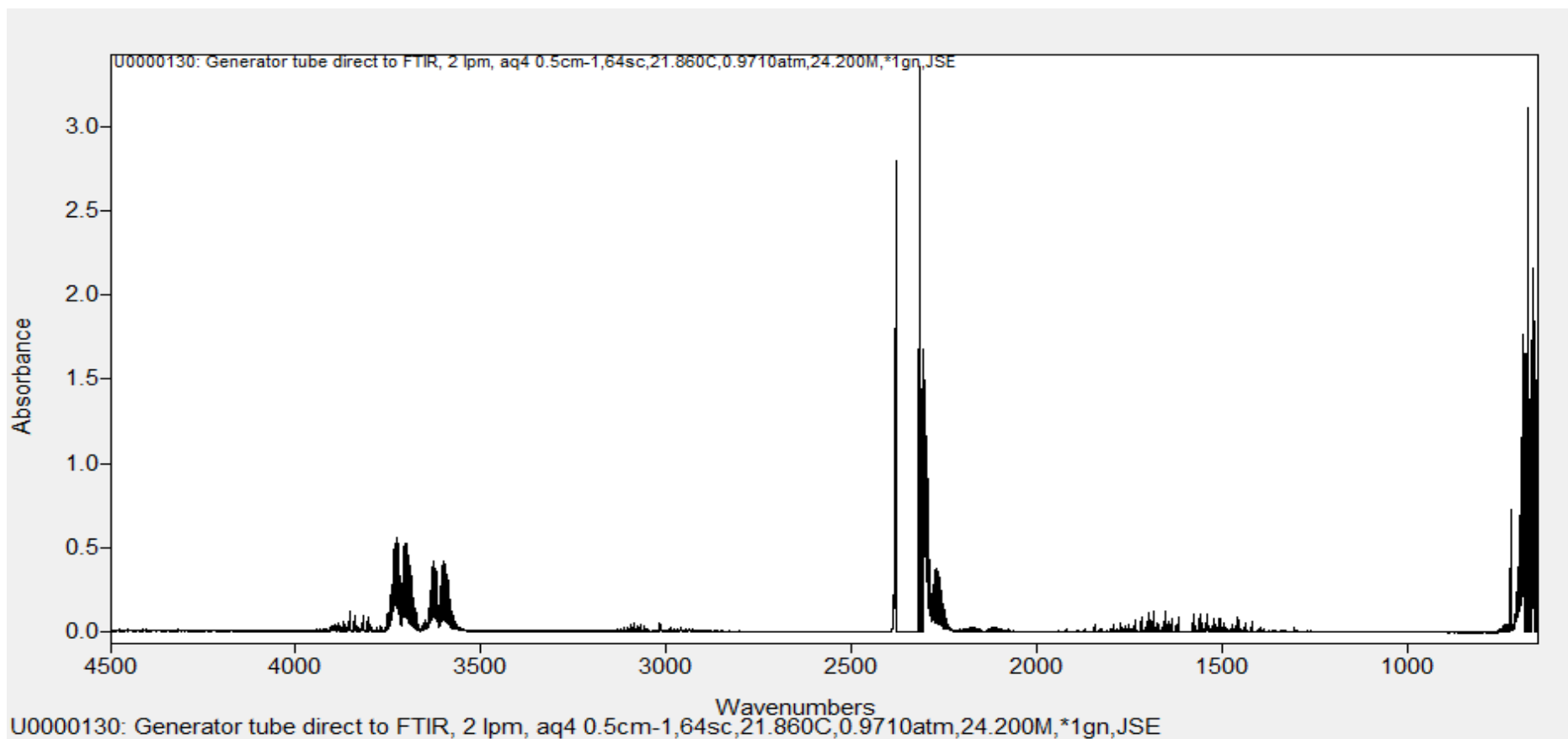




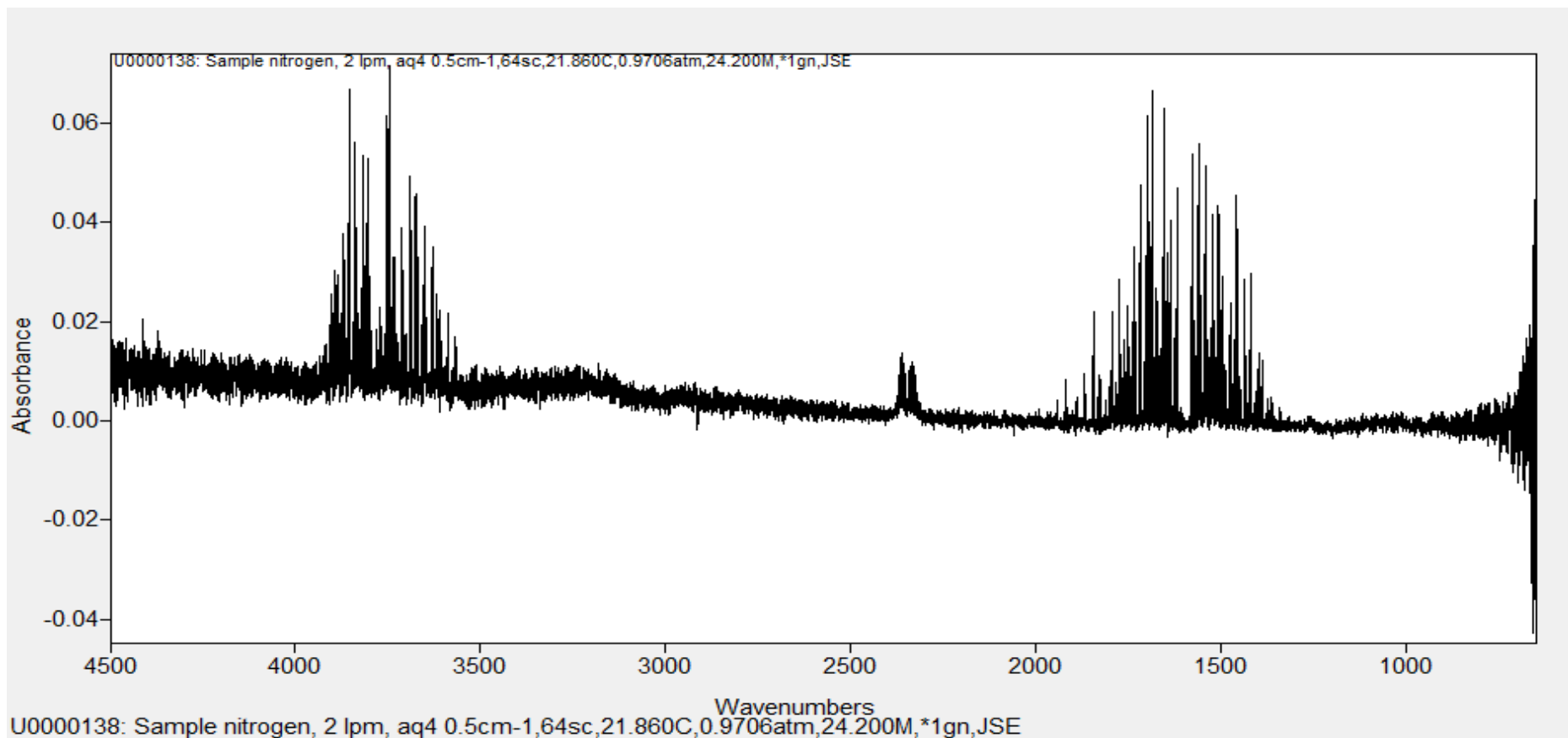








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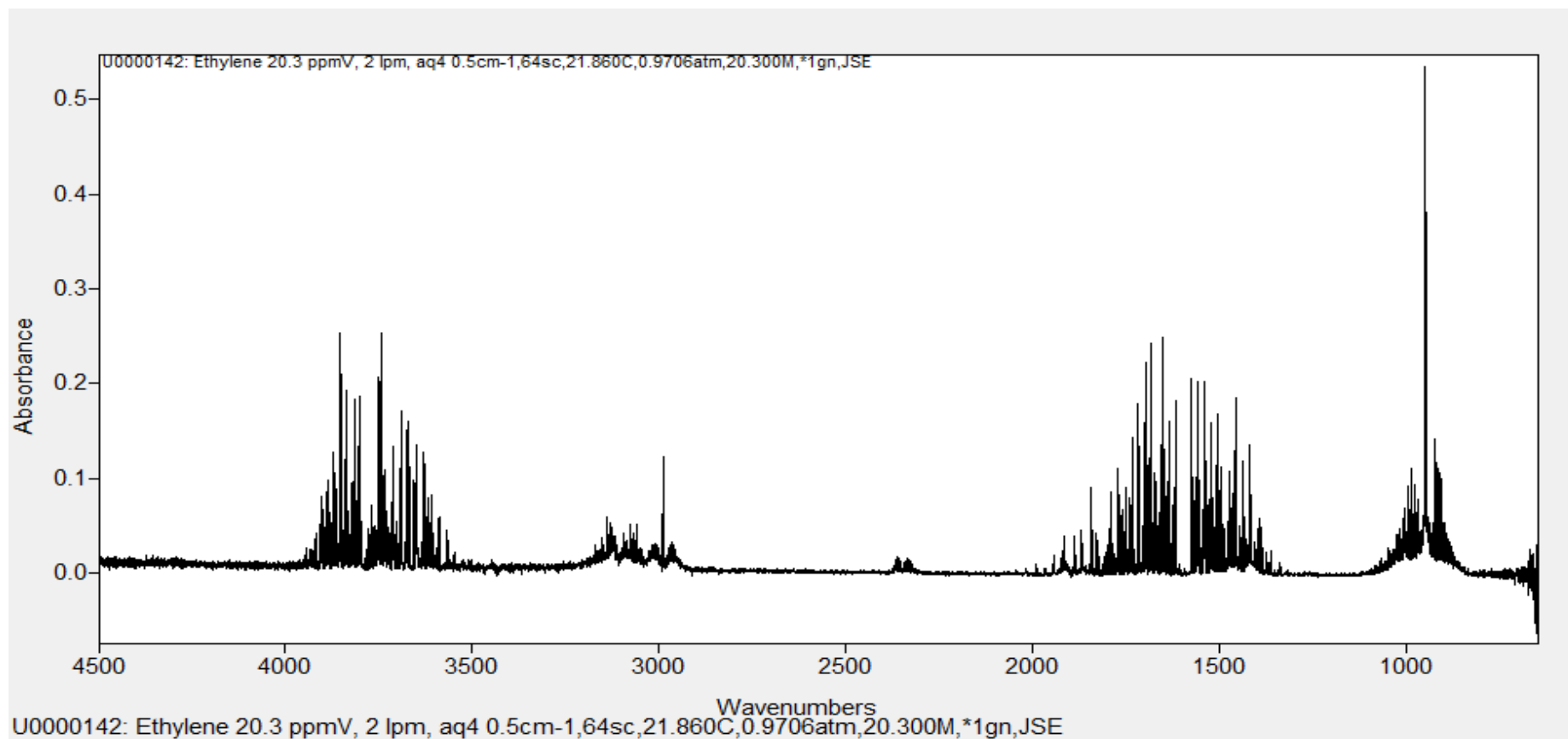
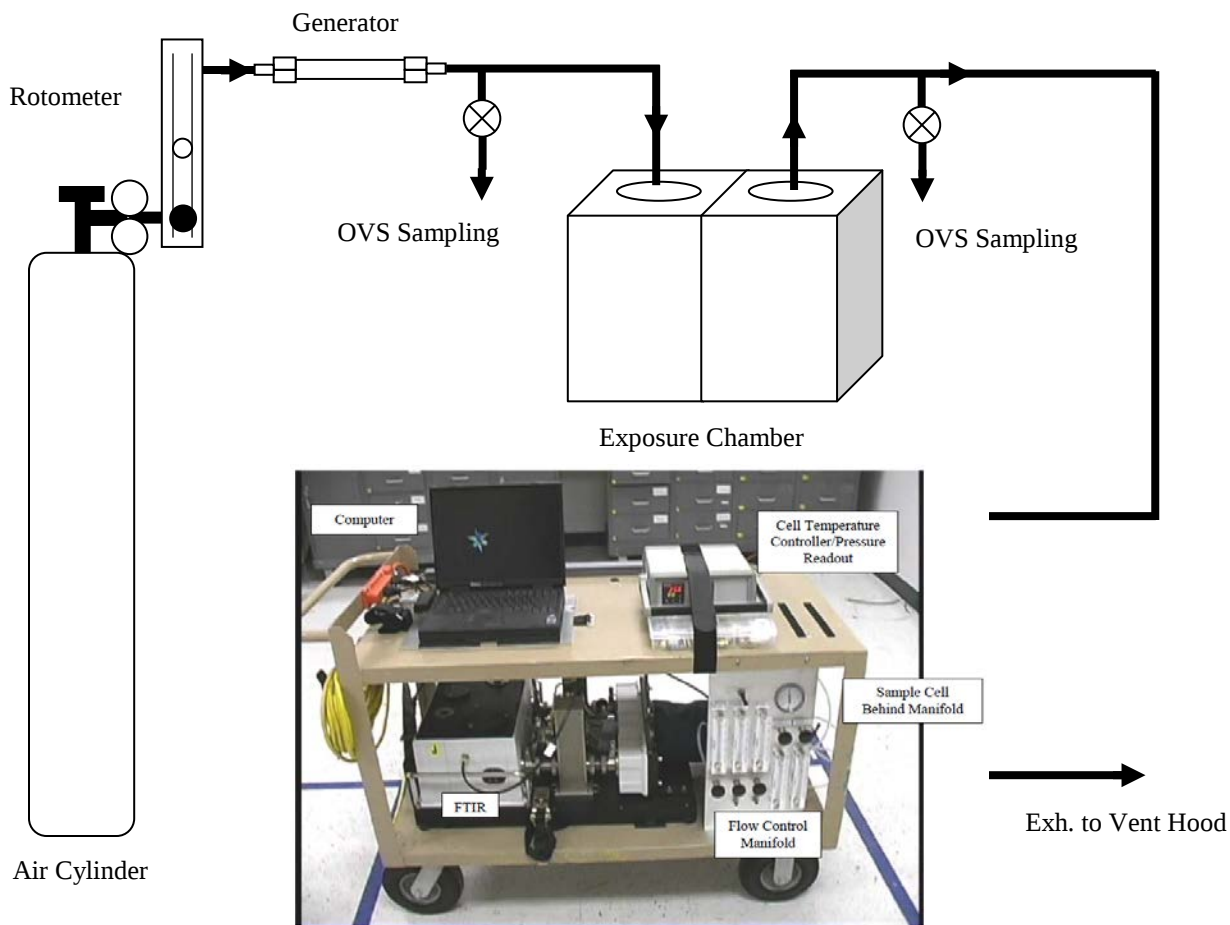


Figure __

Sample System Schematic



Typical FTIR

9 Hand Written Notes

J# 12-9-13 ethy-FOSE rat exposure pg 1 of 3
E13-0880

7:30^{8:15} Arrive - add liq nitrogen to Dewar -
collect background, & ethylene path
length.

Start pre-test air blank @ 8:18:20
flowing @ 1 lpm. Collect for 6 min
Stop @ 8:24:20. Cylinder 89028 Breathing
air quality

Media Blank 8:22 Lot 44440, 20029-U
Exp = May 2015

- Pump #150 @ inlet
Pump #222 @ outlet

8:35 - Inlet flow rate = 104 cc/min avg of 10
Outlet flow rate = 104 cc/min avg of 10
- Leak check $\Rightarrow 15.3$ psi for 5 min.

Inlet Test #1 start @ 9:12 Lot 44440, 20029-U
Outlet Flow rate = 2 lpm into system

9:19 - Bumped inlet flow to 2.1 lpm per
Sue Chang to get 2 lpm thru chamber.
Inlet sample port draws 0.104 lpm for
OVS tube sample
- Test #2 Stop @ 10:12

12-9-13 ethyl-FOSE Rot Evaporator pg 2 of 3
E13-0880

Test #2 Lot #44440, ^{entire run} 12-9-13, 20029-U

Start test #2 @ 10:15 - Inlet + Outlet
Stop test #2 @ 11:15

Start test #3 @ 11:18 - Inlet + Outlet
Stop test #3 @ 12:18

Start test #4 @ 12:21 - Inlet + Outlet
Stop test #4 @ 13:21

All samples from the same lot.

Start test #5 @ 13:24 - Inlet + Outlet
Stop test #5 @ 14:24

Start test #6 @ ¹²⁻⁹⁻¹³ ~~14:27~~ ^{entire run} - Inlet + Outlet
Stop test #6 @ 15:17 50 mins

Shut system down @ 15:19

Start Post-Test air Cylinder Blank @ 15:30
flow rate = 1 Lpm Stop @ ¹²⁻⁹⁻¹³ ~~15:36~~ ^{entire run} 15:37

15:43 - Connected generator tube directly to FTIR

JE 12-9-13 ethyl-FCSE Rot Exp. pg 3 of 3
E13-0880

15:57 - Inlet flow rate = 103 cc/min avg of 10
Outlet flow rate = 106 cc/min avg of 10

- Performed final ethylene check on FTIR
End Test ~ 14:09

JE 12-9-13

10 Chain of Custody Forms

Shipping Address: 3M Center
Bldg. 260-SN-17
St. Paul, MN 55144

Telephone: (651) 736-6559
Sample Receiving: (651) 733-9873
Alternate: (651) 733-5982
FAX: (651) 733-5982

Project ID/Project Name		Template #		Final Report Due Date	
Project Lead		Internal Due Date		Class/Job/Project #	
Dept. # (main)					

Report Results to:		Contact Name		Date Available	
Mailing Address		Company		Date Due	
City, State, Zip		Telephone #		Contract Lab	
FAX #					

Special Instructions and/or Specific Regulatory Requirements:
(method, limit of detection, reporting units, etc.)

Preservatives:					Total Number of Containers	Analysis Requested: Complete below. Attach any associated information.
HNO ₃	H ₂ SO ₄	VOCs	None	Other		
Enter the number of containers of each						(Enter an 'X' in the box below to indicate request)

Item #	Client Sample Identification	3M LIMS#	Date Sampled	Time Sampled	Matrix/Media					
1.	001	001	12/13	9:30 AM	W					
2.	Blank media	002		9:30 AM	W					
3.	Test #1 - Blank	003		12:15 PM	W					
4.	Test #1 - Blank	004		12:15 PM	W					
5.	Test #2 - Blank	005		12:15 PM	W					
6.	Test #2 - Blank	006		12:15 PM	W					
7.	Test #3 - Blank	007		12:15 PM	W					
8.	Test #3 - Blank	008		12:15 PM	W					
9.	Test #4 - Blank	009		12:15 PM	W					
10.	Test #4 - Blank	010		12:15 PM	W					

Chain of Custody	Collected by (print):	Relinquished by/Affiliation	Time	Date	Shipped Via:	Received by/Affiliation	Time	Date
	Jess E. Hodge							

Sample Condition Upon Receipt:	O Acceptable	O Other:	Comments:
Temperature: 'C	O Received on Ice		
Other Associated CoCs:	Copies to:		

Form 38778 - A - PWO

Shipping Address:
3M Center
Bldg 260-SN-17
St. Paul, MN 55144

Telephone:
Sample Receiving: (651) 736-6559
Alternate: (651) 733-9873
Fax: (651) 733-5982

Project ID/Project Name
Template #
Project Lead
Dept. # (main)

Final Report Due Date
Internal Due Date
Class/Job/Project #

E13-0280

Report Results to:
Contact Name
Company
Mailing Address
City, State, Zip
Telephone #

FAX #

Date Available
Date Due
Contract Lab

Special Instructions and/or Specific Regulatory Requirements:
(method, limit of detection, reporting units, etc.)

Item #	Client Sample Identification	3M LIMS#	Date Sampled	Time Sampled	Matrix/Media	Preservatives:					Total Number of Containers	Analysis Requested:									
						HNO ₃	H ₂ SO ₄	VOCs	None	Other		Complete below. Attach any associated information.									

(Enter an 'X' in the box below to indicate request)

1.	Test #15 - 10/10/04	011	10/10/04	15:00	Water						1										
2.	Test #15 - 10/10/04	012	10/10/04	15:00	Water						1										
3.	Test #16 - 10/10/04	013	10/10/04	15:00	Water						1										
4.	Test #16 - 10/10/04	014	10/10/04	15:00	Water						1										
5.	Test #16 - 10/10/04	015	10/10/04	15:00	Water						1										
6.	Test #16 - 10/10/04	016	10/10/04	15:00	Water						1										
7.																					
8.																					
9.																					
10.																					

Chain of Custody
Collected by (print): *John E. Hodge*
Relinquished by/Affiliation: *John E. Hodge / EPA*
Time: *10/10/04*
Date: *10/10/04*
Shipped Via: *Express*
Received by/Affiliation: *John M. Hodge*
Time: *10/10/04*
Date: *10/10/04*

Sample Condition Upon Receipt: ☐ Acceptable ☐ Other: *Sample is OK, analyzed only 50 mva. See chain - then wanted to get going on blood work in lab.*

Temperature: *10°C* ☐ Received on Ice ☐ Other: *See chain - then wanted to get going on blood work in lab.*

Other Associated CoCs: *Copies to:*

11 General Project Outline (GPO)

3M Field Analytical Services & Technologies / General Project Outline

To: Sue Chang – 3M Toxicology

cc: Paul Lieder – 3M Toxicology
Jill Hart – 3M Toxicology
Kris Murphy – Pace Analytical Services
Cleston Lange – 3M EHS Operations, Environmental Laboratory 260-5N-17
Jess Eldridge – 3M EHS Operations, Environmental Laboratory 260-5N-17

From: Brian Mader – 3M EHS Operations, Environmental Laboratory 260-5N-17

Date: Dec 6, 2013

Subject: 3M B270 ethyl-FOSE Rat Exposure Chamber Test

- **Department No.** 108022
- **Lab Request No.** E13-0880
- **Test Date(s)** Dec 9, 2013
- **Monitoring Requested by:**
 - Sue Chang
 - 3M Toxicology
 - 3M Center, Building 220-6W-08
 - St. Paul, MN 55144
 - Email: s.chang@mmm.com
 - Phone No: 651-737-9073
- **Monitoring Coordinated by:**
 - Brian Mader, Ph.D.
 - 3M EHS Opns Environmental Laboratory
 - 3M Center, Building 260-5N-17
 - St. Paul, MN 55144
 - E-mail:bmader@mmm.com
 - Phone No.: (651) 733-9866
 - Fax: (651) 778-6176

All verbal and written correspondence will be directed to the Monitoring Coordinator.

➤ **Plant Location / Contact(s)**

3M Center
Building 270 Room SB322/324
St. Paul, MN 55144
3M Company B270-2A-8
Plant Contact: Jill Hart
Phone Number: 651-733-8586

➤ **Project Objective**

The purpose of this testing is to support 3M Strategic Toxicology exposure study and perform FTIR and OVS-based measurements of the concentration of the target compound ethyl-FOSE generated from a generator cartridge and in the exhaust of an exposure chamber during a rat exposure chamber test. The goal of the test is to measure the gas-phase concentration of ethyl-FOSE. The test will be done at the 3M Strategic toxicology lab at SB322/324 located on the third floor of B270. The initial target concentration for the ethyl-FOSE is approximately 5-10 ppbV for a 6 hour rat exposure. The material will be introduced into the chamber via a generator tube containing ~ 65 gm of glass beads coated with ~ 700 mg of ethyl-FOSE and flowing 2 lpm air through the tube. In addition to FTIR, samples will be collected on OVS adsorbant sample tubes for analysis in the 3M Env Lab via HPLC/MS. If other compounds are present as detected by the FTIR, they will be analyzed and reported.

➤ **Project Schedule**

<i>December 6th, 2013</i>	<i>Equipment Preparation, Set-up</i>
<i>December 9th, 2013</i>	<i>Begin Monitoring</i>
<i>December 10th, 2013</i>	<i>Replicate tests(if needed), demobilization</i>
<i>December 11-13th, 2013</i>	<i>Analysis of Samples</i>
<i>December 20th, 2013</i>	<i>Preliminary Data needed.</i>
<i>January 6th, 2014</i>	<i>Final Report Due</i>

➤ **Process(es) Requiring Testing**

Exposure chamber air sampled inline on the new “flow through” system.

➤ **Test Conditions and Locations**

Condition 1: Quality control – no test chemicals nor animals

- (1) Breathing Air Background - cylinder
 - a. Prior to and after the toxicity test take a 5 minute OVS tube sample of the cylinder breathing air; flow set at 1 lpm for each sample. The pressure of the cylinder will drive the sampling – no pump will be used.

Condition 2: Steady-State Chamber Testing

- (1) Chamber Test
 - a. Collect samples continuously with the FTIR during the testing with target chemical present.

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- b. Two locations will be sampled with OVS tubes ; immediately downstream of the generator tube and immediately downstream of the test chamber(s).
- c. During the 6 hour run time, six 60 min samples will be collected contiguously at both locations. There will be several minutes between removing one set of samples and re-setting new tubes in place for the next one hour period.
- d. Sample rates through the OVS tubes will be set to approximately 0.1 lpm; measured and set on each pump. The actual flow rates and samples volume will be documented.

➤ **Gas Stream Characteristics**

Experiments will be conducted with ethyl-FOSE as the target compound. Other compounds may be present as well and will be analyzed and identified on-site. Based on the preliminary generator cartridge monitoring performed under lab request E13-0722 the expected concentration will be in the range of 5-10 ppbV.

Table 1. ethyl-FOSE Standard Information

1. INGREDIENT	C.A.S. NO.	PERCENT
PERFLUOROOCTANESULFONAMIDO ALCOHOL.....	1691-99-2	80.0 - 90.0
RESIDUAL ORGANIC FLUOROchemicals.....	TradeSecret	< 12
PERFLUOROHEXANESULFONAMIDO ALCOHOL.....	34455-03-3	3.0 - 7.0
PERFLUOROHEPTANESULFONAMIDO ALCOHOL.....	68555-73-7	2.0 - 6.0
PERFLUOROBUTANESULFONAMIDO ALCOHOL.....	34449-89-3	2.0 - 6.0
PERFLUOROPENTANESULFONAMIDO ALCOHOL.....	68555-72-6	1.0 - 3.0

➤ **Test Methods**

+ OVS Analysis by LC/MS ETS-8-105.0

Analysis of the Ethyl FOSE concentration will be conducted at the 3M Environmental Lab using the appropriate 3M Env. Lab SOP. The analysis will include analysis of blanks as well as the use of multipoint calibrations curves and QA/QC procedures that may include LCS and LMS spikes. The target compound is ethyl-FOSE but the presence of other compounds will be reported as will the relative distribution of branched versus liner materials present in the OVS samples. The MS/MS ion masses monitored are for EtFOSE-OH acetate adduct, and is 630 > 59 (acetate adduct)

+EPA Method 320 modified for speciated FTIR analysis

PQL 2 Quantitative Monitoring

Project Quality Level 2 (PQL 2) is appropriate for emission factor estimates and non-compliance test measurements. PQL 2 is appropriate when the project objectives specify the data will not be incorporated in compliance tests of manufacturing emissions, but can be used for use in certain environmental permitting and regulatory activities such as emission factor estimation.

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As per SOP ETS-8-31 the project quality level (PQL) for this project has been identified as **PQL 2** with the following project specific goals to be met:

- + Selection and preparation of the relevant equipment, supplies and spectral libraries.
 - Midac Fourier Transform Infrared Spectrometer (FTIR), heated sample cell, non-Intrinsically Safe (non-IS) heated sample lines, and rotary vane or diaphragm pumps for sample ports.
 - EPA, MIDAC and 3M library standards. etc
- + Use of quantitative FTIR spectral libraries and methods provided by the 3M Environmental Lab with a documented concentration. For each compound the series of reference spectra should span the range of ppmV-m values expected to be observed during the testing.
- + Pre-test calculations and documentation:
 - Estimated absorption path-length.
 - Determination of the detector linearity as per SOP ETS-8-031.
 - FTIR maintenance as per SOP ETS-9-58
- + Testing procedures and documentation:
- + As per SOP ETS-8-031 including but not limited to:
 - + Direct and system calibration with calibration transfer standard (CTS).
 - + Desired Analytical Uncertainty (AU) value = $\pm 50\%$
 - + Direct and system calibration with calibration transfer standard (CTS).
 - + Potential Interferents:
 - Water
 - Carbon Dioxide
- + Data Analysis:
 - The concentration determinations using AutoQuant 4.0 CLS analytical program and/or MD Grams.
- + Reporting:
 - Comprehensive report (See Reporting Requirements section)

➤ **Estimated Project Cost**

All managed outsourced testing expense for this project will be charged to the department number identified on page one of the GPO.

Estimated Total Project Cost ~ \$3,000 - \$5,000

Actual project costs are determined on a time and materials basis in accordance with the existing 3M Environmental Laboratory contract.

➤ **Reporting Requirements**

Type of report(s) required:

- + On-site results will be reported to the Project Requestor as requested during the project
- + Final Reports
 - The “Comprehensive Final” report due to the 3M Project Requestor 4 weeks from the test date.

➤ **Supplemental Information**

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- + 3M facility personnel must provide:
 - One dedicated 20-amp, 110-120 VAC electrical power circuit (reachable with extension cords) at the test location.
 - Designated representatives
 - Daily work permits as necessary.
- + 3M facility must identify proper PPE and if necessary the type of full face respirator that is to be worn.
- + 3M facility must verify non-intrinsically safe equipment is acceptable at the test locations.
- + 3M facility must ensure that the test conditions are maintained during the test and properly documented.
- + 3M facility must provide OSHA approved safe access to test locations.
- + 3M facility must install all test ports that will be needed prior to the test.
- + Perform all process operations and attempt to maintain consistent operating parameters throughout the testing time frame including pressures, temperature, etc.

Contract Lab Requirements:

- + Contract technicians must follow all applicable safety protocols while on-site at the 3M facility (see plant safety officer for appropriate safety procedures).
 - + Invoicing must be sent to monitoring coordinator including;
 - Invoice Date
 - Project Description (Must be similar to the description found on the 3M Lab Request)
 - Test Date(s)
 - 3M 6 Digit Department No.
 - 3M Site Source No. (If a site source has been assigned it will be found on the 3M Lab Request)
 - 3M Project No. (If a project number has been assigned it will be found on the 3M Lab Request)
 - 3M Lab Request No.
 - Cost Itemization