STABILITY IN WATER (HYDROLYSIS)

TEST SUBSTANCE

Identity: N2-(N-methylperfluorooctanesulfonamido)-ethyl acrylate; may also be referred to as N-MeFOSEA, or MeFOSEA. (2-Propenoic acid, 2-[[(heptadecafluorooctyl)sulfonyl]methylamino]ethyl ester, CAS # 25268-77-3)

Remarks: Material is an amber, waxy solid, coded THAR3F2. Sample purity was not characterized.

METHOD

Method: OPPTS 835.2110
GLP (Y/N): No
Year completed: 1999
Type: Abiotic
Stock solution and test sample preparation: A 10,730 mg/L stock solution of MeFOSEA in acetone was prepared. Test solutions were then prepared by adding 10 uL of the stock solution to 1.0 mL of five solutions individually buffered to pH values of 1.5, 5.0, 7.0, 9.0, and 11.0. This calculates to a 107.3 mg/L concentration of MeFOSEA in each buffered solution.
Remarks: Modification of the test method included using a wider range of pH and longer time periods for a more complete understanding of the behavior of the test substance.

RESULTS

pH impact on Hydrolysis Half-lives at 50°C

pH	Half-life (days)
1.5	9.4
5.0	17.8
7.0	9.9
9.0	9.3
11.0	7.9

pH impact on Hydrolysis Half-lives at 25°C**

pH	Half-life (days)
1.5	94
5.0	178
7.0	99
9.0	93
11.0	79

** Extrapolated from values for 50°C.

Remarks: The analysis showed that concentration of MeFOSEA declined in all aqueous pH buffered solutions with time.
The degradation rates fit second order kinetics better than first order kinetics. Nevertheless, the report authors assumed first order kinetics and calculated the half-life after six time periods. Generally, the calculated half-life increased with time. The half-lives reported above are averages of the half-lives calculated after each of the six time periods.
The report does not explain how the extrapolation was made from the half-lives at 50°C to 25°C. Author states assumed ΔG = 15-18 kcal/mole which would give one order of magnitude increase in $T_{1/2}$ per 25°C.

CONCLUSIONS

This study indicates that MeFOSEA is hydrolytically unstable under environmentally relevant conditions.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking 1

REFERENCES

Study conducted by the 3M Company, Environmental Laboratory, St. Paul, MN.

OTHER

Last changed: 5/17/00

Study of the Stability of MeFOSEA in Aqueous Buffers Using Gas Chromatography with Atomic Emission Detection

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Report Date: June 14, 1999

Report Summary

A study was conducted on 2-(N-methylperfluorooctanesulfonamido)-ethyl acrylate (MeFOSEA) to determine its half-life under environmentally relevant hydrolysis conditions. Its behavior was examined in aqueous solutions buffered at five pHs: 1.5, 5.0, 7.0, 9.0, and 11.0. The analysis was performed according to 3M Environmental Laboratory Method ETS-8-90.0, with modifications that followed the U.S. Environmental Protection Agency's Fate, Transport and Transformation Test Guidelines document OPPTS 835.2110, "Hydrolysis as a Function of pH". Modifications included using a wider range of pH and longer time periods for a more complete understanding of the behavior of MeFOSEA. Data were collected at the start of the study (Time 0) and after 0.94, 1.92, 6.72, 13.90, 20.71, and 27.77 days of sample agitation and incubation at 50°C. After incubation, isopropyl alcohol (IPA) was added and the aqueous samples were extracted with hexane. The hexane extracts were dried with anhydrous sodium sulfate and injected into a gas chromatograph with an atomic emission detector (GC/AED). The analysis showed that concentrations of MeFOSEA declined in all aqueous pH buffers with time. Half-lives increased with time within each pH data set at 50 °C, averaging 9.4 days at pH 1.5, 17.8 days at pH 5.0, 9.9 days at pH 7.0, 9.3 days at pH 9.0, and 7.9 days at pH 11.0. Extrapolation of the 50 °C data to room temperature (25 °C) resulted in a half-lives averaging 94 days at pH 1.5, 178 days at pH 5.0, 99 days at pH 7.0, 93 days at pH 9.0, and 79 days at pH 11.0. These results indicate that MeFOSEA is hydrolytically unstable under environmentally relevant conditions.

1 Introduction

Hydrolysis is one of the main mechanisms by which organic compounds, both natural and synthetic, decompose in the environment. Testing for hydrolysis of compounds is relevant to determining the persistence and environmental fate of many pesticides and polymers such as polyesters, polyethers, and polyamides, and for determining shelf life of products containing such compounds (Appendix A, EPA Guidelines).

Hydrolysis is a reaction between some species (in this case of the type R-CO-X), with water that results in the substitution of X with OH:

Figure 1. Generic Hydrolysis Reaction

R = some hydrocarbon or fluorocarbon material
X = halogen, OR, or NR

In dilute solutions the rate law for chemical loss by a hydrolysis reaction is shown with the following equations, where "ester" signifies the starting material. It is important to note that the equation must be modified in solutions that are not dilute.

$$-\frac{d\,[\text{ester}]}{dt} = k_{hyd}[\text{ester}]$$

or

$$\ln \frac{[A]_t}{[A]_0} = -kt$$

The rate constant k is the negative slope of the line fit to a plot of the natural log of the fraction of compound remaining at time t, versus time. The half-life of the compound at a specific pH is related to the rate of hydrolysis by:

$$t_{1/2} = \frac{0.693}{k}$$

With the above equations and considerations as guides, the study monitored the loss of MeFOSEA over an extended period of time to determine the overall rate of reaction. A predicted product of hydrolysis of MeFOSEA was N-MeFOSE alcohol. Analytical difficulties were encountered in attempting to monitor the appearance of this reaction product in an effort to confirm the degradation route.

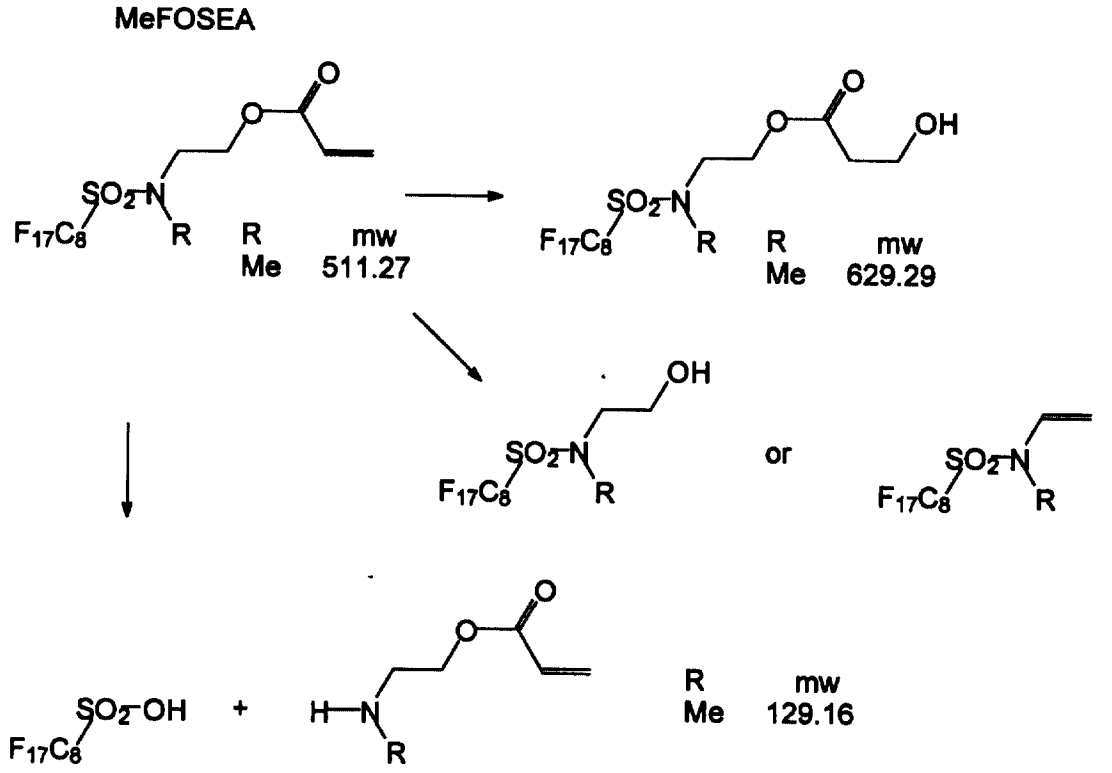
The primary goal of the study was to determine, to a first approximation, the hydrolytic stability of the MeFOSEA. Since levels of MeFOSEA steadily declined over time, data collection stopped after 28 days.

Samples of MeFOSEA were prepared in buffer solutions of pH 1.5, 5.0, 7.0, 9.0, and 11.0 according to published guidelines (Appendix A and references therein). A solution of MeFOSEA in 1.0 mL of each buffer was prepared by adding 10 µL of a 10,730 ppm solution of MeFOSEA in acetone. This corresponds to 107.3 ppm MeFOSEA in each buffer. Significant environmental pHs were chosen (e.g. pH 1.5 is physiologically relevant as stomach pH in case of ingestion). The addition of a pH 11.0 buffer in the present study was made to better understand the behavior of MeFOSEA in basic solutions. The solvent chosen for quenching was hexane, used to extract remaining MeFOSEA from the aqueous matrix. This allowed injection of material onto the gas chromatograph. Previous studies have shown that analysis of diluted aqueous buffers by gas chromatography results in chromatographic anomalies and column degradation.

In this study, biodegradation of MeFOSEA is ruled out for two reasons. First, the 50°C temperature used will impede degradation due to microbial growth in the buffered media. This temperature is hostile to the growth of most mesophillic microorganisms, the type usually inhabiting laboratories. Thermophillic microorganisms, which do thrive at 50°C and above, typically inhabit environments such as hot springs and hydrothermal vents at the bottom of tectonically active ocean basins, but not laboratories. Also, one can hypothesize that the maximum loss rate of MeFOSEA would occur at a different pH if accomplished by hydrolysis versus biodegradation. Since acids or bases typically catalyze hydrolysis, hydrolysis rates are usually maximum under acidic or basic conditions and minimal at or near neutral conditions. This contrasts with the activity of microbes, which typically demonstrate maximum growth rate at or near neutral pH. Maximum rates of hydrolysis were not observed at pH 7.0 in this study.

A proposed mechanism for hydrolytic degradation of MeFOSEA is shown in Figure 2.

Figure 2
MeFOSEA and Possible Degradation Products



2 Sample Receipt

A sample of MeFOSEA (coded THAR3F2) was obtained from C. Elsbernd, 3M Chemicals, on October 13, 1998. The sample was placed into the hydrolysis storage cabinet in the 3M Environmental Laboratory. The sample receiving date, an electronic MSDS, the sample production methodology and predictive chemistries were entered into the 3M proprietary database "Hydrolysis Tracking."

3 Holding Times

The samples were prepared and analyzed within the applicable holding time criteria.

4 Methods: Analytical and Preparatory

3M Environmental Laboratory Method ETS-8-90.0 "Determination of the Stability of MeFOSEA in Aqueous Buffers Using Gas Chromatography with Atomic Emission Detection" was followed except where noted below. This method is presented in Appendix B.

The GC/AED system used was a Hewlett-Packard (HP) 5890 Series II GC equipped with an HP 5921A AED, and an HP 7673 auto-sampler. The column used was a J&W DB5-MS, 30 mm x 0.25 mm x 0.25 μm film thickness. The oven program had an initial temperature of 50°C, initial hold time of 1.0 min., final temperature of 300°C, and a temperature ramp-rate of 15°C per minute.

4.1 Standards:

Test Analyte: 10,730 ppm MeFOSEA in acetone (ID 98086-3-16)
Internal Standard: 9.324 ppm N-EtFOSE alcohol in hexane-1% IPA (ID 98086-3-19, and ID -98060-15-16)
Spiking Standard: 473.4 ppm N-MeFOSE alcohol in methanol (ID W398-1117) (This is an order of magnitude lower than the amount intended due to a calculation error.)

4.2 Sample Preparation and Analysis Dates:

Time 0:	Samples 112398-MeFOSEA-001 to 112398-MeFOSEA-015 Prepared and immediately quenched on 11/24/98. Analysis by GC/AED was begun on 11/25/99.
0.94 Days	Samples 112398-MeFOSEA-016 to 112398-MeFOSEA-030 Incubation started at 3:30 PM on 11/23/98 Quenched at 2:30 PM on 11/24/98 Analysis by GC/AED was begun on 11/25/98 (pH 1.5,5,7) and on 11/30/98 (pH 9,11)
1.92 Days	Samples 112398-MeFOSEA-031 to 112398-MeFOSEA-045 Incubation started at 3:30 PM on 11/23/98 Incubation stopped at 2:10 PM on 11/25/98 Analysis by GC/AED was begun on 11/26/98 (pH 1.5,5,7) and on 12/1/98 (pH 9,11)
6.72 Days	Samples 112398-MeFOSEA-046 to 112398-MeFOSEA-060 Incubation started at 3:30 PM on 11/23/98 Incubation stopped at 8:54 AM on 11/30/98 Analysis by GC/AED was begun on 12/22-23/98 (pH 1.5,5,7), and 12/1/98 (pH 9,11)
13.90 Days	Samples 112398-MeFOSEA-061 to 112398-MeFOSEA-075 Incubation started at 3:30 PM on 11/23/98 Incubation stopped at 1:02 PM on 12/7/98

Analysis by GC/AED was begun on 12/23/98 (pH 1.5,5,7) and on 12/8-9/98 (pH 9,11)

20.71 Days — Samples 112398-MeFOSEA-076 to 112398-MeFOSEA-090
Incubation started at 3:30 PM on 11/23/98
Incubation stopped at 8:38 AM on 12/14/98
Analysis by GC/AED was begun on 12/28/98 (pH 1.5,5,7) and on 12/15-16/98 (pH 9,11)

27.77 Days — Samples 112398-MeFOSEA-091 to 112398-MeFOSEA-105
Incubation started at 3:30 PM on 11/23/98
Incubation stopped at 9:55 AM on 12/21/98
Analysis by GC/AED was begun on 12/29/98 (pH 1.5,5,7, 9) and on 12/30/98 (pH 11)

Sample quenching was completed approximately 1 hour after incubation was stopped. Sample preparation logsheets are presented in Appendix C.

5 Analysis

5.1 Calibration

Standard calibration curves were prepared using a mix of test analyte (MeFOSEA) and degradation product (N-MeFOSE alcohol). Examination of the chromatograms revealed that the intended internal standard (N-EtFOSE alcohol) was in a region of chromatographic overlap of residual components in MeFOSEA. Thus, N-EtFOSE alcohol could not be used as an internal standard with GC/AED testing.

Solvent/Standard Name	Solvent/Standard Number
Hexane solvent used	TN-A-2009
IPA solvent used	TN-A-2102
MeFOSEA standard	98086-3-16
N-MeFOSE alcohol standard	S398-331
N-EtFOSE alcohol standard	S398-332

The calibration standard set consisted of the following:

Reference #	MeFOSEA (ppm)	N-MeFOSE alcohol (ppm)	N-EtFOSE alcohol (ppm)
98086-46A	64.38	63.12	9.324
98086-46B	48.28	47.34	9.324
98086-46C	32.19	31.56	9.324
98086-46D	16.09	15.78	9.324
98086-46E	6.438	6.312	9.324
98086-46F	4.828	4.734	9.324
98086-46G	3.219	3.156	9.324
98086-46H	1.609	1.578	9.324

The standard curves were linear, with the coefficients of determination (r^2) greater than 0.990. Calibration standards were analyzed in duplicate at the beginning of every sample run. At regular intervals (e.g. after 6 - 8 sample injections) blanks consisting of hexane with 1% IPA and 9.324 ppm N-EtFOSE alcohol were analyzed. A mid-range calibration standard was periodically tested throughout each sequence. When the percent difference of amount of measured analyte was greater than ± 25% from the true value, relative to the initial standard curve, the run was discarded. Only samples analyzed before the last acceptable calibration check-standard were considered as valid results.

5.2 Blanks

The internal standard was a solution of 9.32 ppm N-EtFOSE alcohol in hexane/1% IPA. Approximately 1 mL aliquots were transferred to autovials and used as solvent blanks in the analysis. These blanks served as an instrumental check for analyte carryover. Acceptable values for the blanks were less than half the practical Quantitation Limit of the method (the concentration of the lowest standard used in the curve).

5.3 Surrogates

No surrogate analyte was used in this analysis.

5.4 Matrix Spikes

Matrix spike samples were prepared by introducing a known concentration of the target analyte into a sample. These matrix spike samples were carried through the entire sample preparation process. Comparison of the amount quantified from the matrix spike with that of the unspiked sample provided information on contribution of the sample matrix to the analytical results.

One of the spiking solutions used was a 473.4 ppm N-MeFOSE alcohol standard. This concentration was one-tenth of that which would have been optimal. As such, the amount of N-MeFOSE alcohol added was lower than the lowest calibration standard, precluding quantitation. Spike samples on Day 21 and Day 28 also contained 10 µL of a 10,730 ppm solution of MeFOSEA in acetone. For all the pHs extracted on Days 21 and 28, acceptable MeFOSEA recoveries of 80 to 123% were noted. The average recovery of MeFOSEA was 95%.

5.5 Laboratory Control Samples

Laboratory control samples were not prepared for this analysis.

5.6 Sample Related Comments

The MeFOSEA results for the Day 2, pH 7 samples were rejected, as the mid-range calibration standard that followed these samples did not pass quality control criteria specified in the analytical method (Appendix B).

6 Data Summary

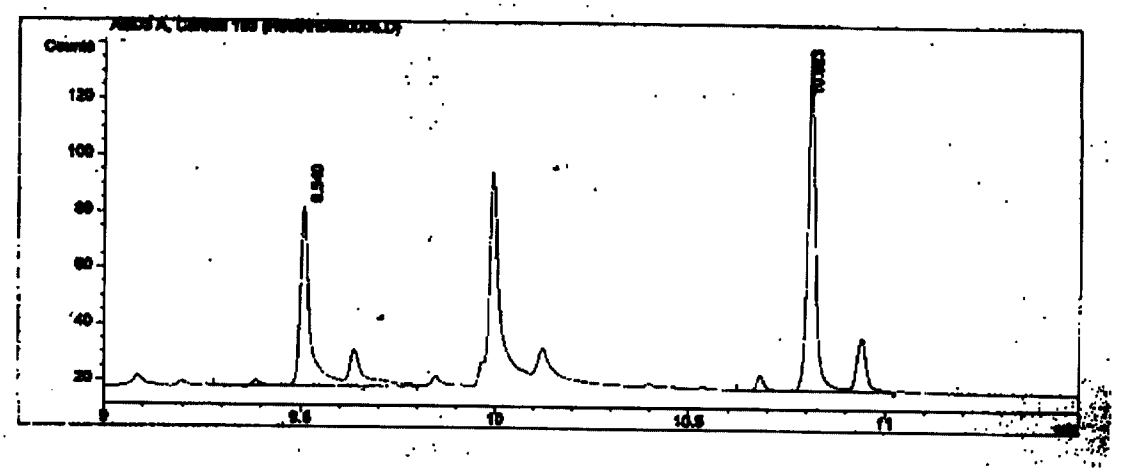
The 3M Environmental Laboratory developed a method for the quantitative determination of MeFOSEA using GC with atomic emission detection. This method was successful using five pH buffer matrices. Sample chromatograms are presented in Appendix D as separate bound

volumes. A representative GC/AED chromatogram of a standard mixture and a sample are presented in Figure 3a and 3b, respectively.

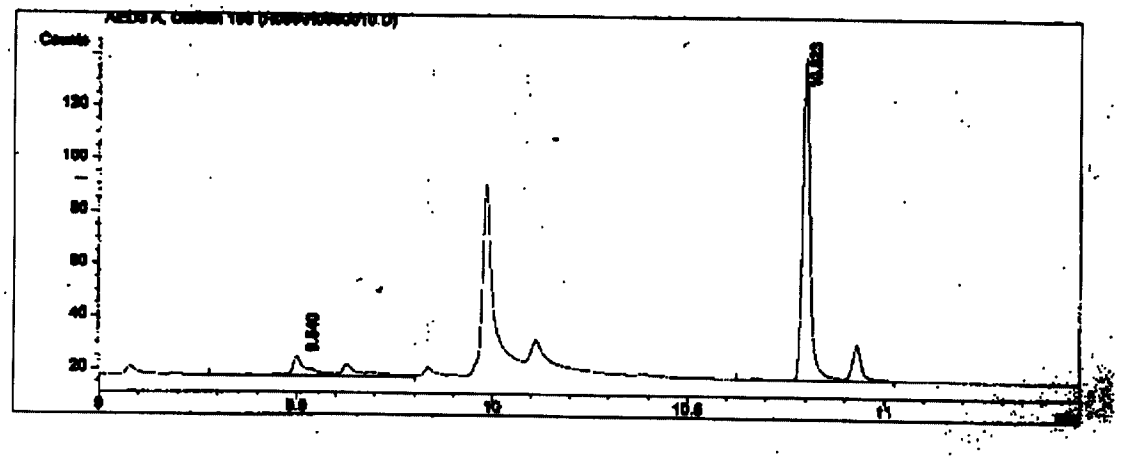
Figure 3
Representative GC/AED Chromatograms

Peak retention times are 9.54 minutes (N-MeFOSE alcohol), 10.0 minutes (internal standard), and 10.82 minutes (MeFOSEA)

a. Standard (6.438 ppm MeFOSEA, 12/22/98, 7:33:14 PM)



b. Sample (MeFOSEA-1.5-1-7, 12/22/98, 9:31:59 PM. Quantified as 5.47 ppm MeFOSEA)



The calculated concentrations of analyte in sample, duplicate, and matrix spike samples are presented in spreadsheet format in Appendix E. These data, plotted as MeFOSEA concentration versus time in Figure 4, show that degradation of MeFOSEA is in general non-linear and a function of pH. Samples from the pH 5.0 and 7.0 series can be fit equally well with either a linear or second-order equation (see Table 1). A second-order equation provides a better fit than a linear equation for the data at pH 1.5, 9.0, and 11.0.

Figure 4
MeFOSEA Concentration Versus Time

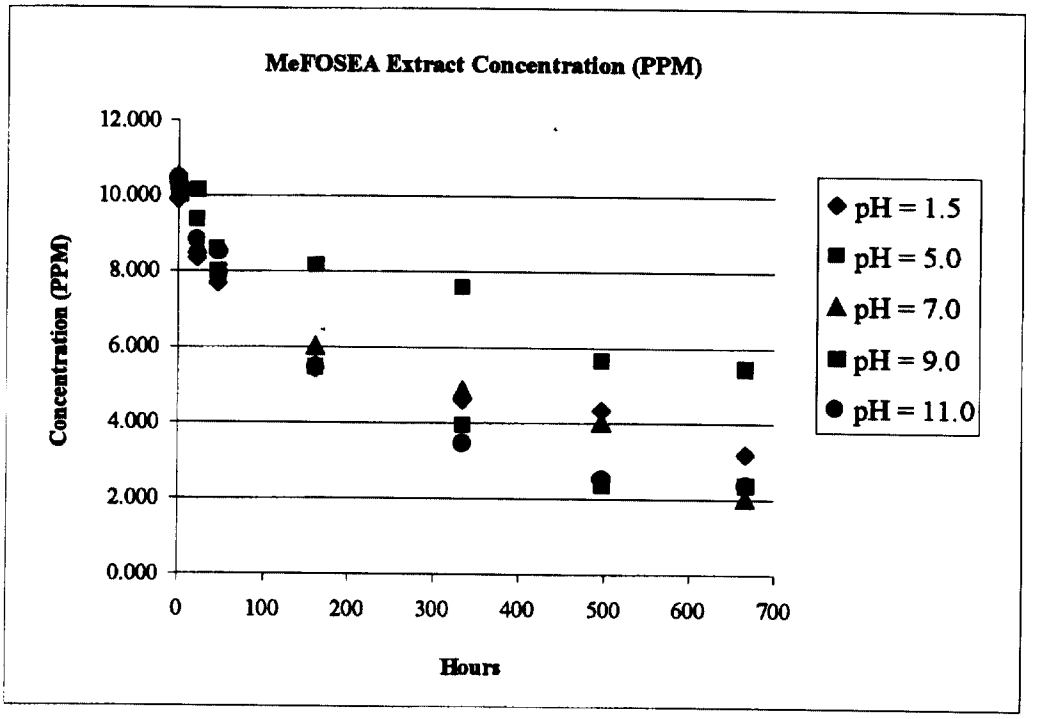


Table 1
Fit of Second-Order and Linear Equations to MeFOSEA Degradation Data

pH	2nd-Order Equation	2nd-Order R^2	Linear Equation	Linear-Fit R^2
1.5	$y = 2e10^{-6}x^2 - 0.0191x + 8.9477$	0.9263	$y = -0.0086x + 8.3400$	0.835
5.0	$y = 5e10^{-6}x^2 - 0.0097x + 9.698$	0.9289	$y = -0.0067x + 9.5239$	0.9153
7.0	$y = 1e10^{-5}x^2 - 0.0186x + 9.6171$	0.9449	$y = -0.0112x + 9.1490$	0.9119
9.0	$y = 2e10^{-5}x^2 - 0.0277x + 9.956$	0.9719	$y = -0.0121x + 9.0474$	0.8645
11.0	$y = 3e10^{-5}x^2 - 0.0283x + 9.832$	0.9859	$y = -0.0118x + 8.8757$	0.8625

At the conclusion of the experiment (27.77 days), approximately 20 - 30% of the original MeFOSEA remained for samples hydrolyzing at pH 1.5, 7.0, 9.0, and 11.0. At pH 5, approximately half of the original MeFOSEA was present at the end of the experiment.

Calculations of kinetic parameters are presented in Appendix F. A general trend is that the half-life of MeFOSEA increased with increasing time, especially at pH 1.5, 5.0, 9.0, and 11.0 (Table 2). This behavior doesn't match the mathematical model for a first order reaction. However, the first order approximation for a hydrolysis reaction assumes a single compound degrades to form only two distinct product species. The results shown in Table 1 indicate this assumption may not be realistic for MeFOSEA. As implied in Figure 2, the degradation of MeFOSEA may involve multiple reaction pathways. MeFOSEA is a 3M control compound, whose behavior is used to predict that of other synthetic chemistries. Overall, the results of this study are sufficient for that purpose.

Table 2
Half-Lives of MeFOSEA Versus Time

Time (Days)	pH = 1.5	5.0	7.0	9.0	11.0
0.94	2.58	4.79	3.09	11.99	3.34
1.92	3.99	5.98	----	4.51	5.72
6.72	6.87	16.94	8.05	6.96	6.97
13.90	11.44	27.93	12.23	9.67	8.51
20.71	15.85	22.64	14.61	9.62	9.97
27.77	15.91	28.45	11.53	12.80	12.81

7 Conclusion

The 3M Environmental Laboratory developed a method for hydrolysis and analysis of MeFOSEA. The hydrolysis study showed pH dependent half-lives which increased with time within each pH data set at 50 °C, averaging 9.4 days at pH 1.5, 17.8 days at pH 5.0, 9.9 days at pH 7.0, 9.3 days at pH 9.0, and 7.9 days at pH 11.0. Extrapolation of the 50 °C data to room temperature (25 °C) resulted in a half-lives averaging 94 days at pH 1.5, 178 days at pH 5.0, 99 days at pH 7.0, 93 days at pH 9.0, and 79 days at pH 11.0. It is the conclusion of this study that MeFOSEA is unstable against hydrolysis under environmentally relevant conditions.

8 Data/Sample Retention

Reserve samples and all original paper data will be retained in the archives of 3M ET&SS for a period of ten years following the signing of the final report.

9 Signatures

Thomas L. Hatfield, Ph.D., Team Leader — Date 6/16/99

Cleston Lange, Ph.D., Analyst — Date 6/16/99

Gregory Maisel, Analyst — Date 6/17/99

Joseph J. S. Tokos, Ph. D., Editor — Date 6/12/99

William K. Reagen, Ph. D., Technical Reviewer — Date 6/21/99

Note: Jeanette Wink, Analyst, did the majority of the laboratory work for this project. She reviewed the original data, but has since left 3M Environmental Laboratory. Her assistance during the course of the study is appreciated.

10 Appendices

Appendix A: EPA Guidelines
Appendix B: MeFOSEA Preparation and Analysis Method
Appendix C: Sample Preparation Logsheets
Appendix D. GC/AED Chromatograms (see separate bound volumes)
Appendix E: Spreadsheets: GC/AED Results
Appendix F: Spreadsheets: Calculation of Kinetic Parameters

Appendix A: Fate, Transport and Transformation Test Guidelines, OPPTS 835.2110, Hydrolysis as a Function of pH, U.S. Environmental Protection Agency document number EPA 712-C-98-057, January 1998

United States
Environmental Protection
Agency

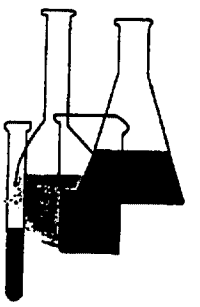
Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–98–057
January 1998

EPA

Fate, Transport and Transformation Test Guidelines

OPPTS 835.2110 Hydrolysis as a Function of pH



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board.* By modem dial 202–512–1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202–512–0132 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading "Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines."

OPPTS 835.2110 Hydrolysis as a function of pH.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline are 40 CFR 796.3500 Hydrolysis as a Function of pH at 25 °C, and OECD guideline 111 Hydrolysis as a Function of pH.

(b) **Guidance information**—(1) **Prerequisites.** Water solubility data; suitable analytical method; vapor pressure curve.

(2) **Qualifying statements.** Pure and commercial grade substances can be tested with the method described here, but the potential effect of impurities on the results should be considered. This test guideline applies only to water soluble compounds. There is uncertainty in extrapolating high temperature results to environmentally relevant temperatures as a change in reaction mechanism could occur.

(3) **Standard documents.** This test guideline is based on methods given in paragraph (f) of this guideline and on the Preliminary Draft Guidance for Premanufacture Notification EPA, August 18, 1978.

(c) **Method**—(1) **Purpose, relevance, application, and limits of test.** (i) The testing of substances for hydrolysis is relevant to their persistence. Hydrolysis is one of the most common reactions controlling abiotic degradation and is therefore one of the main degradation paths of substances in the environment.

(ii) A procedure to determine hydrolysis rates is important also in indicating whether other testing should be carried out on a parent compound or on its hydrolysis products. It is the degradation products that are crucial. Hydrolysis behavior needs to be examined at pH values normally found in the environment (pH 4–9) and under more acidic conditions (pH 1–2) for physiological purposes.

(iii) Surface-controlled reactions can sometimes predominate over bulk solution hydrolysis, especially in the soil environment. This may result in different degradation rates than would be predicted from this guideline based upon rates in homogeneous solutions.

(2) **Definitions and units.** The definitions in section 3 of the Toxic Substances Control Act (TSCA) and the definitions in 40 CFR Part 792—Good Laboratory Practice Standards apply to this test guideline. The following definitions also apply to this test guideline.

Hydrolysis refers to a reaction of a chemical RX with water, with the net exchange of the group X with OH at the reaction center:

$$RX + HOH \longrightarrow ROH + HX$$

Hydrolysis rate, the rate at which the concentration of RX decreases in this simplified process is given by:

$$\text{second order reaction rate} = k\,[H_2O]\,[RX]$$

or

$$\text{first order reaction rate} = k\,[RX]$$

depending on the rate determining step. Because water is present in great excess compared to the chemical, this type of reaction is usually described as a pseudo-first order reaction in which the observed rate constant is given by the relationship:

$$k_{obs} = k\,[H_2O]$$

and can be determined from the expression

$$K_{obs} = 2.303/t \log_{10}(C_0/C_t)$$

where t = time, and C_0 and C_t = concentrations of RX at times 0 and t.

The units of this constant have the dimension of $(\text{time})^{-1}$ and the half-life of the reaction (time for 50 percent of RX to react) is given by

$$t_{1/2} = 0.693/k_{obs}$$

(3) **Reference substances.** (i) Aspirin and Diazinon are used as references. These substances need not be employed in all cases when investigating a new substance. These data are provided primarily so that calibration of the method may be performed from time to time and to offer the chance to compare the results when another method is applied. The results of the OECD/EEC-Laboratory Intercomparison Testing are included in the following Tables 1. and 2.:

Table 1.—Values of Rate Constants and Half-Lives for Aspirin[1,2]

pH	K [10^5 sec^{-1}]	$t_{1/2}$ (h)
3.5	0.065	300
5.0	0.15	130
7.4	0.13	150
9.5	0.37	52
11.3	16	1.2

[1] Data taken at 17 °C

[2] Aspirin (CAS no. 50–78–2) (2-acetylsalicylic acid) data from L.J. Edwards, *Transactions of the Faraday Society* 723–735 (1950).

Table 2.—Values of Rate Constants and Half-Lives for Diazinon[1]

pH	10 °C		20 °C		40 °C		60 °C	
	K [10^5 sec^{-1}]	$t_{1/2}$ (h)	K [10^5 sec^{-1}]	$t_{1/2}$ (h)	K [10^5 sec^{-1}]	$t_{1/2}$ (h)	K [10^5 sec^{-1}]	$t_{1/2}$ (h)
10.43	0.061	310	0.13	150	0.41	48	15	12
9.0	--	--	0.0059	3300	--	--	--	--
7.4	--	--	0.00043	4400	--	--	--	--
5.0	--	--	0.026	740	--	--	--	--
3.1	0.75	260	1.6	120	6.6	29	25	7.8

[1] Diazinon (CAS no. 333–41–5) (*O,O*-diethyl-*O*-(2-isopropyl-4-methyl]-6-pyrimidinyl phosphorothioate) data from H.M. Gomma et al. *Residue Reviews* 29:171 (1969).

(ii) Further results of OECD–EEC Laboratory intercomparison testing follow in Tables 3. through 12. In some cases the results presented have a variability which exceeds what is called for in the test guideline. This may particularly be the result of the use of different buffers in the test systems and the influence of oxygen. These results are presented , however, as they have been obtained using the Test Guidelines in the OECD/EEC Intercomparison Testing Programme, Part II. The guideline has been modified in the light of these results.

(A) Substance: Aspirin

Table 3.—Reaction Rate Constant in $10^5 sec^{-1}$

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
1.2	35–40	1.013	1.278	126.2	0.109–1.916	2
3.0	20	0.080	0.056	70.3	0.040–0.119	2
	40	0.556	0.112	20.1	0.477–0.635	2
7.0	20	0.205	0.033	15.9	0.182–0.228	2
	40	1.339	0.004	0.3	1.336–1.341	2
9.0	20	0.309	0.160	51.7	0.196–0.442	2
	40	0.953	0.006	0.6	0.949–0.957	2

Table 4.—Half-life in hours

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
1.2	35–40	93.71	118.3	126	10.05–177.36	2
3.0	20	319.7	222.5	70	162.3–477.0	2
	40	35.4	7.1	20	30.3–40.4	2 (1 lab)
7.0	20	95.1	15.1	20	84.4–105.8	2
	40	14.4	0.0	–	14.4–14.4	2 (1 lab)
9.0	20	72.1	37.3	50	45.7–98.5	2
	40	20.2	0.1	0.0	20.1–20.3	2 (1 lab)

(B) Substance: Diazinon

Table 5.—Reaction Rate Constant in $10^5 sec^{-1}$

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
1.2	35–40	30.36	8.10	27.0	21.40–38.46	4
3.0	20	2.866	0.825	28.8	1.675–3.841	7
	40	9.038	2.447	27.1	5.708–14.165	11 (10 labs)
	50	5.77	4.27	74.0	0.86–8.58	3 (2 labs)
	60	36.085	11.088	30.7	25.535–51.449	6
7.0	20	0.933	1.796	192.4	0.005–3.626	4
	40	0.231	0.294	127.3	0.042–0.895	8
	50	0.200	0.023	11.3	0.184–0.216	2
	60	1.638	3.154	192.6	0.303–9.413	8 (7 labs)
9.0	20	1.103	2.113	191.6	0.007–4.271	4
	40	2.568	6.900	268.7	0.064–20.955	9
	50	0.292	0.034	11.6	0.268–0.316	2
	60	2.801	4.125	147.3	0.241–12.604	8 (7 labs)

Table 6.—Half-life in hours

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
1.2	35–40	0.672	0.187	27.9	0.501–0.900	4
3.0	20	7.27	2.40	33	5.0–11.5	7
	40	2.25	0.57	25	1.4–3.3	11 (10 labs)
	50	9.00	11.53	128	2.24–22.31	3 (2 labs)
	60	0.57	0.17	29	0.37–0.75	6
7.0	20	1707.75	1875.18	110	5.3–3660.9	4
	40	215.16	166.12	77	21.5–460.7	8
	50	97.07	10.97	11	89.31–104.83	2
	60	38.68	20.98	54	2.0–63.5	8 (7 labs)
9.0	20	1150.75	1335.58	116	4.5–2840.5	4
	40	124.38	102.45	82	0.9–298.7	9
	50	66.40	7.74	12	60.92–71.87	2
	60	25.13	25.67	102	1.5–79.8	8 (7 labs)

(C) Substance: Atrazine

Table 7.—Reaction Rate Constant in $10^5 sec^{-1}$

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
1.2	35–40	0.546	0.416	76.3	0.76–0.948	4
3.0	20	0.014	0.008	56.8	0.005–0.020	3
	40	0.140	0.082	58.4	0.028–0.222	6
	60	0.808	0.397	49.1	0.282–1.283	5
7.0	40	0.009	0.011	124.8	0.001–0.016	2
9.0	20	0.013	0.016	130.1	0.001–0.024	–
	40	0.008	0.010	123.7	0.001–0.015	2

Table 8.—Half-life in hours

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
1.2	35–40	54.76	44.36	81.0	20.3–115.35	4
3.0	20	1867.88	1446.58	77.0	980.7–3537.14	3
	40	240.50	239.61	99.6	111.71–696.79	6
	60	31.42	21.51	69.0	15.0–68.3	5
7.0	40	9000.15	11033.77	123.0	1198.1–16802.2	2
9.0	20	1792105	24188.21	135.0	817.4–35024.7	2
	40	8005.35	9539.51	119.0	1259.9–14750.8	2

(D) Substance: Di(2-ethylhexl) phthalate (DOP)

Table 9.—Reaction Rate Constant in $10^5 sec^{-1}$

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n° of results
3.0	20	0.048	0.051	107.8	0.009–0.106	3
9.0	20	0.040	0.047	116.7	0.007–0.073	2
	40	0.166	0.202	121.8	0.023–0.309	2
	60	0.084	0.022	26.3	0.068–0.099	2
7.0	20	0.073	0.064	88.8	0.027–0.118	2
	40	(1.627)	–	–	–	1
	60	(0.092)	–	–	–	1
	40	(0.126)	–	–	–	1

Table 10.—Half-life in hours

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n° of results
3.0	20	990.20	996.32	101	182.4–2103.5	3
	40	452.56	551.98	122	62.25–842.86	2
	60	239.33	63.75	27	194.25–284.41	2
7.0	20	440.30	391.35	89	163.57–717.02	2
	40	(11.83)	–	–	–	1
	60	(208.19)	–	–	–	1
	40	(152.53)	–	–	–	1

(E) Substance: Ethyl acetate

Table 11.—Reaction Rate Constant in $10^5 sec^{-1}$

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n° of results
3.0	20	(0.0012)	–	–	–	1
	40	(0.012)	–	–	–	1
	60	(0.355)	–	–	–	1
7.0	20	(0.003)	–	–	–	1
	40	(0.008)	–	–	–	1
	60	(0.137)	–	–	–	1
9.0	20	(0.063)	–	–	–	1
	40	(0.153)	–	–	–	1
	60	(1.547)	–	–	–	1

Table 12.—Half-life in hours

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n° of results
3.0	20	(1656)	–	–	–	1
	40	(1612.85)	–	–	–	1
	60	(54.30)	–	–	–	1
7.0	20	(7553.1)	–	–	–	1
	40	2511.81)	–	–	–	1
	60	(140.38)	–	–	–	1
9.0	20	(305.55)	–	–	–	1
	40	(125.71)	–	–	–	1
	60	(12.44)	–	–	–	1

(4) **Principle of the test method.** (i) In the environment, chemicals usually occur in dilute solution, which means that water is present in large excess, and, therefore, that the concentration of water remains essentially constant during hydrolysis. Hence, the kinetics of hydrolysis are generally pseudo-first order at fixed pH and temperature.

(ii) The hydrolysis reaction may be influenced by acidic or basic species H_3O+, (H+), and OH^-, in which case it is referred to as specific acid or specific base catalysis.

(iii) The concentration of the test substance is determined as a function of time. The logarithms of the concentrations are plotted against time and the slope of the resulting straight line (assuming first-order or pseudo-first order behavior) gives the rate constant from the formula (if $\log_{10}$ is used):

$$k_{obs} = - \text{slope} \times 2.303$$

(iv) When it is not practicable to determine a rate constant for a particular temperature directly, it is usually possible to estimate the constant through the use of the Arrhenius relationship in which the logarithm of rate constants at other temperatures is plotted against the reciprocal of the absolute temperature (K).

(5) **Quality criteria**—(i) **Reproducibility.** Measurements of hydrolysis rate constants on 13 classes of organic structures can be of high precision, often with less than 2 percent standard deviation (see paragraph (f)(2) of this guideline). The rate constants for one pH and one temperature should be determined in duplicate with a deviation of less than 2.5 percent unless unusual circumstances (e.g. analytical difficulties) prevent achieving this and then the details of these circumstances should be reported. The reproducibility can be improved by an improved control of the sensitive parameters, in particular pH and oxygen.

(ii) **Sensitivity.** Most hydrolysis reactions follow apparent first order reaction rates and, therefore, half-lives are independent of concentration

$$t_{1/2} = 0.693/k_{obs}$$

This usually permits the application of laboratory results determined at 10^{-2}–10^{-3} M to environmental conditions ($\leq 10^{-6}$ M) under paragraph (f)(2) of this guideline.

(iii) **Specificity.** Several examples of good agreement between rates of hydrolysis measured in both pure and natural waters for a variety of chemicals providing both pH and temperature have been measured (see paragraph (f)(2) of this guideline).

(d) **Description of the test procedure.** (1) If the water solubility of the substance is less than 2×10^{-2} M, a half-saturated solution in water is prepared. If the solubility is greater than 2×10^{-2}, the test solution is prepared at less than 10^{-2} M. Substances known to be hydrolytically unstable are tested at at least two temperatures between 0–40 °C. Other substances are put through preliminary testing. Substances found to be stable under these conditions are considered hydrolytically stable ($t_{1/2}$ at 25 °C is greater than 1 year). Those not stable at 50 °C for one week are submitted to testing at at least two temperatures between 0–40 °C, or, as appropriate, at at least three elevated temperatures, and the data extrapolated to produce *k* at 25°C.

(2) **Preparations—(i) Materials—(A) Buffer solutions.** (*1*) The hydrolysis test should be performed at four different pH's: at pH 1.2 (if physiologically important); pH 4.0; pH 7.0; and pH 9.0. Either buffers as described below or a pH-stat may be used. Use of a pH-stat avoids potential problems due to buffer catalysis.

(2) For this purpose, 0.05 M sterile buffer solutions should be prepared using reagent grade chemicals and distilled, sterile water. The buffer systems are based upon the analytical requirements for the chemical being tested. It should be noted that the buffer system used may influence the rate of hydrolysis and where this is observed an alternate buffer system should be employed. The use of borate or acetate buffers instead of phosphate has been recommended under paragraph (f)(2) of this guideline. The pH of each buffer solution must be checked with a calibrated pH meter at the required temperature to a precision of at least 0.1 pH units. Some useful buffer systems are presented in the following Tables 13., 14., and 15.:

Table 13.—Buffer Mixtures of Clark and Lubs[1,2]

Composition	pH
0.2 N HCl and 0.2 N KCl	
47.5 ml HC1 + 25 ml KC1 diluted to 100 ml	1.0
32.25 ml HC1 + 25 ml KC1 diluted to 100 ml	1.2

Table 13.—Buffer Mixtures of Clark and Lubs[1,2]—Continued

Composition	pH
20.75 ml HC1 + 25 ml KC1 diluted to 100 ml	1.4
13.15 ml HC1 + 25 ml KC1 diluted to 100 ml	1.6
8.3 ml HC1 + 25 ml KC1 diluted to 100 ml	1.8
5.3 ml HC1 + 25 ml KC1 diluted to 100 ml	2.0
3.35 ml HC1 + 25 ml KC1 diluted to 100 ml	2.2
0.1 M potassium biphthalate + 0.1 N HC1	
46.70 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	2.2
39.60 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	2.4
32.95 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	2.6
26.42 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	2.8
20.32 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	3.0
14.70 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	3.2
9.90 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	3.4
5.97 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	3.6
2.63 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	3.8
0.1 M potassium biphthalate + 0.1 N NaOH	
0.40 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	4.0
3.70 ml 0.1 N NaOH + 50 ml piphthalate to 100 ml	4.2
7.50 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	4.4
12.15 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	4.6
17.70 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	4.8
0.1 M potassium biphthalate + 0.1 NaOH	
23.85 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	5.0
29.95 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	5.2
35.45 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	5.4
39.85 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	5.6
43.00 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	5.8
45.45 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	6.0
0.1 M monopotassium phosphate + 0.1 N NaOH	
5.70 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	6.0
8.60 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	6.2
12.60 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	6.4
17.80 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	6.6
23.45 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	6.8
29.63 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	7.0
35.00 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	7.2
39.50 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	7.4
42.80 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	7.6
45.20 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	7.8
46.80 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	8.0
0.1 M H_2BO_2 in 0.1 M KC1 + 0.1 N NaOH	
2.61 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	7.8
3.97 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	8.0
5.90 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	8.2
8.50 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	8.4
12.00 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	8.6
16.30 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	8.8
21.30 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	9.0
26.70 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	9.2
32.00 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	9.4
36.85 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	9.6
40.80 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	9.8
43.90 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	10.0

[1] Data taken at 20 °C.

[2] The pH values reported in these tables have been calculated from the potential measurements using Sorensen's standard equations (1909). The corresponding pH values are 0.04 unit higher than the tabulated values.

Table 14.—Citrate Buffers of Kolthoff and Vleeschouwer[1,2]

Composition	pH
0.1 M monopotassium citrate and 0.1 N HCl	
49.7 ml 0.1 N HCl + 50 ml citrate to 100 ml	2.2
43.4 ml 0.1 N HCl + 50 ml citrate to 100 ml	2.4
36.8 ml 0.1 N HCl + 50 ml citrate to 100 ml	2.6
30.2 ml 0.1 N HCl + 50 ml citrate to 100 ml	2.8
23.6 ml 0.1 N HCl + 50 ml citrate to 100 ml	3.0
17.2 ml 0.1 N HCl + 50 ml citrate to 100 ml	3.2
10.7 ml 0.1 N HCl + 50 ml citrate to 100 ml	3.4
4.2 ml 0.1 N HCl + 50 ml citrate to 100 ml	3.6
0.1 M monopotassium citrate and 0.1 N NaOH	
2.0 ml 0.1 N NaOH + 50 ml citrate to 100 ml	3.8
9.0 ml 0.1 N NaOH + 50 ml citrate to 100 ml	4.0
16.3 ml 0.1 N NaOH + 50 ml citrate to 100 ml	4.2
23.7 ml 0.1 N NaOH + 50 ml citrate to 100 ml	4.4
31.5 ml 0.1 N NaOH + 50 ml citrate to 100 ml	4.6
39.2 ml 0.1 N NaOH + 50 ml citrate to 100 ml	4.8
46.7 ml 0.1 N NaOH + 50 ml citrate to 100 ml	5.0
54.2 ml 0.1 N NaOH + 50 ml citrate to 100 ml	5.2
61.0 ml 0.1 N NaOH + 50 ml citrate to 100 ml	5.4
68.0 ml 0.1 N NaOH + 50 ml citrate to 100 ml	5.6
74.4 ml 0.1 N NaOH + 50 ml citrate to 100 ml	5.8
81.2 ml 0.1 N NaOH + 50 ml citrate to 100 ml	6.0

[1] Data taken at 18 °C.
[2] Add tiny crystal of thymol or a few milligrams of mercury or mercuric iodide to prevent growth of molds.

Table 15.—Borate Buffer Mixtures of Sorensen

Composition			Sorensen (18 °C)	Walbum, pH at		
0.05 M borax (ml)	0.01 N HCl (ml)	0.01N NaOH (ml)		10 °C	40 °C	70 °C
5.25	4.75	0.00	7.62	7.64	7.55	7.47
5.50	4.50	0.00	7.94	7.98	7.86	7.76
5.75	4.25	0.00	8.14	8.17	8.06	7.95
6.00	4.00	0.00	8.29	8.32	8.19	8.08
6.50	3.50	0.00	8.51	8.54	8.40	8.28
7.00	3.00	0.00	8.08	8.72	8.56	8.40
7.50	2.50	0.00	8.80	8.84	8.67	8.50
8.00	2.00	0.00	8.91	8.96	8.77	8.59
8.50	1.50	0.00	9.01	9.06	8.86	8.67
9.00	1.00	0.00	9.09	9.14	8.94	8.74
9.50	0.50	0.00	9.17	9.22	9.01	8.80
10.00	0.00	0.00	9.24	9.30	9.08	8.86
10.0	0.00	0.0	9.24	9.30	9.08	8.86
9.0	0.00	1.0	9.36	9.42	9.18	8.94
8.0	0.00	2.0	9.50	9.57	9.30	9.02
7.0	0.00	3.0	9.68	9.76	9.44	9.12
6.0	0.00	4.0	9.97	10.06	9.67	9.28

(B) **Test solutions.** The chemical substance should be dissolved in distilled, sterile water with sterile buffer medium added to it. The concentrations should not exceed the lesser of 0.01 M or half the saturation concentration (see OPPTS 830.7840, Water solubility, shake flask method), and the purest available form of the substance should be employed

in making up the solutions. The use of mixed solvents is recommended only in case substances with low water solubility. The amount of solvent should be less than 1 percent, and the solvent should not interfere with the hydrolysis process.

(C) **Glassware.** All glassware, which must be inert in the pH range studied, should be sterilized. Stoppered volumetric flasks (no grease) should be used for carrying out the hydrolysis reactions. If the chemical or buffer system is volatile, or if the test is being conducted at elevated temperatures, sealed or septum-closed tubes are preferred and head space should be avoided.

(3) **Analytical method.** The analytical method will be determined by the nature of the substance being tested. It must be sufficiently sensitive and specific to allow determination of the different species at the test solution concentrations and may well consist of some combination of pH electrodes, UV-visible spectrophotometry, conductivity, gas chromatography, high pressure liquid chromatography, extraction and formation of derivative(s), and determination by a suitable analytical method.

(4) **Test conditions**—(i) **Temperature.** For extrapolation purposes, it is important to maintain the temperature of the determinations to at least ±0.1 °C. An appropriate constant temperature bath should be employed. If the hydrolytic behavior of the substance is unknown, a preliminary test at 50 °C is required. For tests beyond this preliminary stage data for temperatures in the range 0–40 °C are sought. They may be obtained by measurement at two temperatures in this range or by extrapolation from three higher temperatures. In any event, the determinations should be done at temperatures differing from each other by at least 10 °C.

(ii) **Light and oxygen.** All of the hydrolysis reactions should be carried out using any suitable method to avoid photolytic effects. All suitable measures should be taken to exclude oxygen (e.g. by bubbling nitrogen or argon through the solvent for 5 minutes before preparation of the solution).

(5) **Performance of the test**—(i) **Preliminary test.** A preliminary test should be performed on the substance at 50±0.1 °C at each of pH 4.0, 7.0, and 9.0. If less than 10 percent of the reaction is observed after 5 days ($t_{1/2}$ > 1 year), the chemical is considered hydrolytically stable and no additional testing is required. If the substance is known to be unstable at environmentally relevant temperatures, the preliminary test is not required. The analytical method must be sufficiently precise and sensitive to detect a reduction of 10 percent in the initial concentration.

(ii) **Hydrolysis of unstable substances.** If the substance is unstable as defined by the preliminary test, the test procedure is to be as follows: The buffered test solutions of the substance should be thermostatted at the selected temperatures. To test for first-order behavior each reaction

solution should be analyzed in time intervals which provide a minimum of six spaced data points, normally between 20 percent and 70 percent of hydrolysis of that test chemical. The reaction should be examined at three (4, 7, 9) pH's at each of the selected temperatures with replication at one of them (the middle temperature in the case of elevated temperature determinations).

(iii) **Hydrolysis at pH 1.2.** The above test for a hydrolytically unstable compound should also be carried out at pH 1.2, employing a single, physiologically significant temperature (37 °C).

(e) **Data and reporting**—(1) **Treatment of results**—(i) **Confirmation of first order kinetics.** The data obtained should be plotted at $\log_{10} C_t$ versus t and the reaction rate constant k_{obs} calculated by regression analysis or from the slope:

$$k_{obs} = 2.303 \times \text{slope}$$

(ii) **Interpretation of results.** If the data do not fall on a straight line, the reaction is not first order, and the data must be analyzed by methods beyond the scope of this test principle.

(2) **Test report.** (i) The test report should include information on:

(A) Sample purity.

(B) Any results appropriate to the procedure employing reference substances.

(C) Detailed test procedure including the temperature, pH and, buffer for each set of experiments.

(D) Detailed analytical method used for the tested substance, including detailed method of extraction and recovery data if an extraction method is used to separate the chemical from the aqueous phase.

(E) All concentration-time data points for reactions which were observed to originate a nonlinear log concentration-time plot.

(F) Possibility of acid or base catalysis.

(ii) A suggested format for sample reporting form, which can be duplicated, is presented:

in making up the solutions. The use of mixed solvents is recommended only in case substances with low water solubility. The amount of solvent should be less than 1 percent, and the solvent should not interfere with the hydrolysis process.

(C) **Glassware.** All glassware, which must be inert in the pH range studied, should be sterilized. Stoppered volumetric flasks (no grease) should be used for carrying out the hydrolysis reactions. If the chemical or buffer system is volatile, or if the test is being conducted at elevated temperatures, sealed or septum-closed tubes are preferred and head space should be avoided.

(3) **Analytical method.** The analytical method will be determined by the nature of the substance being tested. It must be sufficiently sensitive and specific to allow determination of the different species at the test solution concentrations and may well consist of some combination of pH electrodes, UV-visible spectrophotometry, conductivity, gas chromatography, high pressure liquid chromatography, extraction and formation of derivative(s), and determination by a suitable analytical method.

(4) **Test conditions**—(i) **Temperature.** For extrapolation purposes, it is important to maintain the temperature of the determinations to at least ±0.1 °C. An appropriate constant temperature bath should be employed. If the hydrolytic behavior of the substance is unknown, a preliminary test at 50 °C is required. For tests beyond this preliminary stage data for temperatures in the range 0–40 °C are sought. They may be obtained by measurement at two temperatures in this range or by extrapolation from three higher temperatures. In any event, the determinations should be done at temperatures differing from each other by at least 10 °C.

(ii) **Light and oxygen.** All of the hydrolysis reactions should be carried out using any suitable method to avoid photolytic effects. All suitable measures should be taken to exclude oxygen (e.g. by bubbling nitrogen or argon through the solvent for 5 minutes before preparation of the solution).

(5) **Performance of the test**—(i) **Preliminary test.** A preliminary test should be performed on the substance at 50±0.1 °C at each of pH 4.0, 7.0, and 9.0. If less than 10 percent of the reaction is observed after 5 days ($t_{1/2}$ > 1 year), the chemical is considered hydrolytically stable and no additional testing is required. If the substance is known to be unstable at environmentally relevant temperatures, the preliminary test is not required. The analytical method must be sufficiently precise and sensitive to detect a reduction of 10 percent in the initial concentration.

(ii) **Hydrolysis of unstable substances.** If the substance is unstable as defined by the preliminary test, the test procedure is to be as follows: The buffered test solutions of the substance should be thermostatted at the selected temperatures. To test for first-order behavior each reaction

solution should be analyzed in time intervals which provide a minimum of six spaced data points, normally between 20 percent and 70 percent of hydrolysis of that test chemical. The reaction should be examined at three (4, 7, 9) pH's at each of the selected temperatures with replication at one of them (the middle temperature in the case of elevated temperature determinations).

(iii) **Hydrolysis at pH 1.2.** The above test for a hydrolytically unstable compound should also be carried out at pH 1.2, employing a single, physiologically significant temperature (37 °C).

(e) **Data and reporting**—(1) **Treatment of results**—(i) **Confirmation of first order kinetics.** The data obtained should be plotted at $\log_{10} C_t$ versus t and the reaction rate constant k_{obs} calculated by regression analysis or from the slope:

$$k_{obs} = 2.303 \times \text{slope}$$

(ii) **Interpretation of results.** If the data do not fall on a straight line, the reaction is not first order, and the data must be analyzed by methods beyond the scope of this test principle.

(2) **Test report.** (i) The test report should include information on:

(A) Sample purity.

(B) Any results appropriate to the procedure employing reference substances.

(C) Detailed test procedure including the temperature, pH and, buffer for each set of experiments.

(D) Detailed analytical method used for the tested substance, including detailed method of extraction and recovery data if an extraction method is used to separate the chemical from the aqueous phase.

(E) All concentration-time data points for reactions which were observed to originate a nonlinear log concentration-time plot.

(F) Possibility of acid or base catalysis.

(ii) A suggested format for sample reporting form, which can be duplicated, is presented:

DATA SHEET FOR HYDROLYSIS STUDIES

Laboratory:_____

Date:_____

Test Substance:_____

Formula:_____

Name (IUPAC):_____

Test protocol

A. Preliminary test

yes_____

no_____

buffer systems used:

pH 4.0_____

pH 7.0_____

pH 9.0_____

Approximate saturation concentration	pH		
mole/L	4.0	7.0	9.0
C_0: Initial concentration	.....	.....	.....
C_t: Final concentration after t days; $t_{max} = 5$	.....	.....	.....
t	.....	.....	.....
$(C_0 - C_t)/c_0 \times 100$ at 50 ±0.1 °C	.....	.....	.....

B. **Determination**

Separate runs at pH 4.0, 7.0, and 9.0 at the chosen temperature(s) *with replication at one of these* (the middle temperature in the case of determination at elevated temperature). The same format whould be used for each pH.

Buffer solution used_____

Temperature_____

Approximate saturation concentration_____

t [ ] 0

C_t [mole/L]	.....	.....	.....	.....	.....	.....
log C_t	.....	.....	.....	.....	.....	.....

Hydrolysis at pH 1–2

Buffer solution used_____

pH_____

Temperature_____

Approximate saturation concentration_____

t [ ] 0

C_t [mole/L]	.....	.....	.....	.....	.....	.....
log C_t	.....	.....	.....	.....	.....	.....

Final data

pH	temperature °C	initial concentration, C_0 [mole/L]	reaction rate constant, k_{obs} [1/s × 10^5]	half-life, $t_{1/2}$ [h]	coefficient of correlation, r^2
.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....

C. **Report on test method**

Provide detailed description of the experimental conditions, e.g. for maintaining sterility; to avoid photolytic effects; to exclude oxygen; to prepare the test solution, etc. (Please use separate sheet of paper.)

Analytical combinations used

h	h
pH electrodes	.....
UV-visible spectrophotometry	.....
Conductivity	.....
Gas chromatography	.....
High pressure liquid chromatography	.....
Extraction and formation of derivative(s)	.....

Details of the analytical performance:

Type of apparatus

Test conditions

Was the accuracy of this result determined in any additional way ?

Particular incidents:

Comments:

(f) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Kolthoff, I.M. and Laitinen, H.A. *pH and Electro-Titrations*, 2nd Ed., Wiley, pp 34–36 (1941).

(2) Mabey, W. and Mill, T., Critical Review of Hydrolysis of Organic Compounds in Water Under Environmental Conditions. *Journal of Physical Chemistry Reference Data* 7:383–415 (1978).

(3) Gomaa, H.M. et al. Kinetics of Hydrolysis of Diazoxon. *Residue Reviews* 29:171 (1969).

(4) OECD Document A80.30, Summary of OECD-EEC Laboratory Intercomparison Testing Programme, Part 2, Umweltbundesamt, Berlin, May 1980.

Appendix B: 3M Environmental Laboratory Method ETS-8-90.0 "Determination of the Stability of MeFOSEA in Aqueous Buffers Using Gas Chromatography with Atomic Emission Detection"

3M ENVIRONMENTAL LABORATORY

METHOD

DETERMINATION OF THE STABILITY OF MeFOSEA IN AQUEOUS BUFFERS USING GAS CHROMATOGRAPHY WITH ATOMIC EMISSION DETECTION

Method Number: ETS-8-90.0 **Adoption date:** 06/07/99

Revision Date: NA

Author: Thomas Hatfield, Ph.D./Cleston Lange, Ph.D./Gregory Maisel/Jeanette Wink

Approved by:

Laboratory Manager 6/7/99
Date

Group Leader 6/4/99
Date

Technical Reviewer 6/3/99
Date

1.0 SCOPE AND APPLICATION

1.1 This procedure defines the steps for preparation and analysis of 2-(*N*-methylperfluorooctanesulfonamido)-ethyl acrylate (MeFOSEA) from aqueous hydrolysis by gas chromatography with atomic emission detection (GC/AED).

1.2 Acceptable Matrices: Aqueous solutions at various buffered pHs.

2.0 SUMMARY OF THE METHOD

2.1 One milliliter of buffered aqueous sample from the hydrolysis of MeFOSEA is quenched with 0.5 mL isopropyl alcohol (IPA) and extracted with 10 mL hexane. A portion (1-2 mL) of the separated hexane phase is dried through an anhydrous sodium sulfate column and then analyzed on a gas chromatograph with an atomic emission detector.

3.0 DEFINITIONS

3.1 **Duplicate analyses.** Analyses or measurements of the analyte of interest performed identically on the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not precision of sampling, preservation or storage internal to the laboratory.

3.2 **Sample duplicates.** Two samples taken from and representative of the same sample source and carried through all steps of the sampling and analytical procedures in an identical manner. Duplicate samples are used to assess variance of the total method, including sampling and analysis.

3.3 **Matrix spike.** Prepared by adding a known mass of target analyte to specified amount of a sample matrix for which an independent estimate of target analyte concentration is available. Matrix spikes are used to determine the effect of the matrix on the method's recovery efficiency.

3.4 **Solvent blank.** A sample of analyte-free medium to which all reagents are added in the same volumes or proportions as used in sample processing but is not carried through the complete sample preparation and analytical procedure.

3.5 **Continuing Calibration Verification (CCV).** A standard analyzed periodically during an analytical run to verify the continued accuracy of the calibration curve. This solution may be prepared from a different source of lot number than the calibration curve standards.

3.6 **Limit of Quantitation (LOQ).** The lowest concentration that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions. It may be nominally chosen within these guidelines to simplify data reporting. For many analytes, the LOQ analyte concentration is selected as the lowest non-zero standard in the calibration curve. Sample LOQs are highly matrix dependent.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

4.1.1 Wear the proper lab attire for all parts of these procedures. Wear gloves at all times.

4.1.2 Handle all solvent in a hood for all parts of the described sample preparation procedure.

4.1.3 For potential hazards of each chemical used, refer to material safety data sheets, packing materials and 3M Environmental Laboratory's Chemical Hazard Review.

4.1.4 Use care when working with GC/AED because high temperatures and ultraviolet light are present.

4.2 Cautions:

4.2.1 Rinse with solvents all glassware used to prepare standards to reduce the possibility of contamination.

5.0 INTERFERENCES

5.1 Contaminants in solvents, reagents, glassware, and other sample processing or analysis hardware may cause interferences. Routinely analyze laboratory solvent blanks to demonstrate that no interferences are present during the analysis.

6.0 EQUIPMENT

6.1 Balance, capable of measuring to 0.1 mg.

6.2 Shaker, incubating, capable of holding at 50°C ± 3°C.

6.3 pH meter, Corning Model 308 pH/Temperature Meter with 3-in-1 gel filled combination electrode (pH/reference/temperature) or equivalent.

6.4 Gas Chromatograph, HP 5890 Series II, with HP 5921A Atomic Emission Detector (AED) or equivalent.

6.5 Column, J & W Scientific Incorporated, DB-5MS, 30 m x .25 mm x .25 µm or equivalent.

6.6 Data Recording System, HP ChemStation Rev. A.05.04 with Windows NT software.

6.7 Autosampler, HP 7673 or equivalent.

6.8 Clock.

7.0 SUPPLIES AND MATERIALS

7.1 Autovials, crimp top, 1.5 mL.

7.2 Vials, glass screw top, 40 mL, I-CHEM or equivalent.

7.3 Labels, Avery 5160 or equivalent.

7.4 Graduated pipettes, glass, disposable, various sizes.

7.5 Pasteur pipettes, glass, disposable.

7.6 Volumetric flasks, various sizes.

7.7 Beakers, glass, various sizes.

8.0 REAGENTS AND STANDARDS

8.1 Buffer solutions

Prepare the buffer solutions in 1000 mL quantities. Adjust pH with NaOH or HCl. Use a portable pH meter to calibrate all buffer solutions. Record final pH measurements of buffers. Store the buffer solutions in sealed 1000 mL flasks. Prepare buffers as follows:

8.1.1 pH 1.5 ± 0.3

a) 250 mL of 0.1 M HCl,

b) 125 mL of 0.2 M KCl,

c) Bring to a final volume of 1 L with ASTM Type I H_2O.

8.1.2 pH 5.0 ± 0.3

a) Dissolve 3.57 g ammonium acetate in 200 mL ASTM Type I H_2O.

b) Add 230 mL of 0.052 M acetic acid (3 mL glacial acetic acid into 1 L ASTM Type I H_2O)

c) Bring to a final volume of 1 L with ASTM Type I H_2O.

8.1.3 pH 7.0 ± 0.3

a) Dissolve 7.9 g Trizma®-HCl in 200 mL ASTM Type I H_2O.

b) Adjust pH to 7.0 with 0.1N NaOH.

c) Bring to a final volume of 1 L with ASTM Type I H_2O.

8.1.4 pH 9.0 ± 0.3

a) 46 mL of 0.1 N HCl.

b) 125 mL of 0.1 M borax (sodium borate, 10 hydrate).

c) Adjust pH to 9.0.

d) Bring to a final volume of 1 L with ASTM Type I H_2O.

8.1.5 pH 11.0 ± 0.3

a) 250 mL of 0.1 N NaOH.

b) 250 mL of 0.1 M borax.

c) Adjust pH to 11.0.

d) Bring to a final volume of 1 L with ASTM Type I H_2O.

8.2 **Acetone**, spectroscopy grade or equivalent.

8.3 **IPA**, spectroscopy grade or equivalent.

8.4 **Hexane**, 85% n-Hexane, spectroscopy grade or equivalent. Use hexane in post-injection solvent washes for the GC/AED. Prepare a one L hexane solution containing 10 mL of IPA (hexane-1% IPA) to use for extraction and for solvent blanks.

8.5 **MeFOSEA,** prepared in acetone, approximately 10,000 ppm. Weigh 0.1 g of MeFOSEA and dissolve in 10 mL acetone. Use this solution for calibration standards, test analyte samples and spikes.

8.6 **Sodium sulfate**, anhydrous, 60 mesh, reagent grade.

9.0 SAMPLE HANDLING

9.1 Handle all samples and standards in a well-ventilated area. Wear gloves when handling solutions.

9.2 Prepare hydrolysis sample prep worksheets for each sample set. (Attachment A.) Prepare a tracking schedule for sample extraction.

9.3 Analyze all samples as soon as possible after extraction. If samples cannot be analyzed immediately, store in refrigerator at approximately 4°C.

10.0 QUALITY CONTROL

10.1 **Solvent blank.** Prepare solvent blanks (hexane-1% IPA). This serves as an instrumental check for any analyte carryover.

10.2 **Duplicates.** Prepare a sample and a duplicate. Perform two injections of each.

10.3 **Matrix spikes.** Prepare a post-hydrolysis matrix spike for each of the pHs used in the study. Perform two injections of each spike.

10.4 **CCV.** Run a CCV every 15 or fewer injections.

11.0 CALIBRATION AND STANDARDIZATION

11.1 **Standard preparation.** Prepare six calibration standards of MeFOSEA. Standards from approximately 1.6 to 32 ppm are suggested.

11.2 **Calibration standards.** Analyze calibration standards at the beginning of the run.

11.3 **Coefficient of Determination.** The acceptable coefficient of determination (r^2) is 0.990 or greater. Curves should be examined closely for linearity and intercept, particularly for accuracy of quantitation at the high and low ends of the curve.

12.0 PROCEDURE

12.1 Sample preparation.

12.1.1 Establish time points for sample analysis. Typical time points are at days 0, 1, 2, 7, 14, 21 and 28.

12.1.2 For each pH to be tested, set up the 40 mL I-Chem vials to allow for a sample, a duplicate and a spike for the duration of the experiment. Each time point has 3 samples per pH and five pHs for a total of 15 vials per time point.

12.1.3 Create labels and affix to vials. Label information should include date, a sample number; the pH; whether the vial contains sample, duplicate or spike; the time point; and the initials of the analyst. An example using June 15, 1999 is: 61599-MeFOSEA-002

pH 1.5
Duplicate
Day 0
CCL

12.1.4 Remove vial cap. With a pipette, add 1 mL of buffer to the bottom of each vial, taking care to avoid the walls of the vial. Always replace cap immediately after any addition to minimize evaporation.

12.1.5 Using a 25 µL gas-tight syringe, add 10µL of MeFOSEA test analyte solution to each vial with buffer, except for those marked Day 0. Tilt the vial so the buffer runs to one side of the bottom of the vial. Add the test analyte solution on the opposite side of the vial, away from the buffer.

12.1.6 Prepare time zero samples when adequate time is available to immediately quench the samples. Set up each sample individually. Extract each one with hexane-1% IPA before starting another to get a true time zero.

12.1.7 Fill out hydrolysis sample prep worksheets (Attachment A) for each sample set.

12.1.8 Place the samples in the 50°C incubator. Set for 100 rpm shaking. Note time, date, and temperature on worksheets.

12.1.9 At the specified time point, remove samples from the incubator. Let cool to room temperature for 15 minutes.

12.1.10 Add 10 µL of the MeFOSEA spike solution to the bottom of each spike vial so it mixes with buffer.

12.1.11 Add 0.5 mL of IPA to all vials. Swirl to mix.

12.1.12 Add 10 mL of the hexane-1% IPA to vials. Recap vials and shake vigorously for 10-15 seconds. Let settle for 1 minute. The water phase is on the bottom; the hexane phase is on the top.

12.1.13 With a pipette, pull off a portion (1-2 mL) of the top phase. Dispense the hexane onto a column consisting of approximately 1 inch height of Na_2SO_4, in a Pasteur pipette. Allow the hexane extract to flow through the column into a prelabeled autovial. Use two autovials per sample. Cap the vials. Store one set of vials in the freezer at approximately -20°C. Place the other set of vials in the autosampler for analysis. If samples cannot be immediately analyzed, store in refrigerator at approximately 4°C.

12.2 Solvent blanks.

12.2.1 To 40 mL I-Chem vials, add 10 mL of hexane-1% IPA. Analyze in the same manner as the samples.

12.3 Sample analysis.

12.3.1 Install the analytical column and establish instrument conditions. Allow the system to equilibrate for 30 minutes before starting the analysis.

TABLE 1. SUGGESTED GC CONDITIONS FOR SAMPLE ANALYSIS

PARAMETER	SUGGESTED VALUE
Inlet temperature:	180°C
Inlet liner:	4 mm ID, single gooseneck, glass
Detect temperature:	280°
Carrier gas:	Helium
Oven Program:	
Initial temperature:	50°C
Initial time:	1.00 min.
Rate:	15.0°C/min.
Final temperature:	300°C
Final time:	0.00 min.

12.3.2 Set head pressure for a linear velocity through column between 28-32 cm/sec.

TABLE 2. SUGGESTED AED CONDITIONS FOR SAMPLE ANALYSIS

PARAMETER	SUGGESTED VALUE
Transfer line:	280°C
Cavity:	280°C
Min. peak width:	0.054 min.
Data rate:	5.0 hz
Solvent vent program:	
On time:	0.00
Off time:	3.50 minutes
Elements:	Carbon 193, Sulfur 181, Nitrogen 174
Spectra:	Save

12.3.3 TABLE 3. SUGGESTED AUTOSAMPLER CONDITIONS FOR SAMPLE ANALYSIS

PARAMETER	SUGGESTED VALUE
Autosampler injector:	7673
Sample washes:	1
Sample pumps:	3
Injection volume:	1.0 µL
Syringe size:	5.0 µL
On column:	Off
Nanoliter adapter:	Off
Post Injection Solv A washes:	4 (hexane)
Post Injection Solv B washes:	4 (hexane)
Viscosity delay:	0 sec
Plunger speed:	Fast

12.3.4 Enter the standard and sample information into the sequence table. Analyze calibration standards first, then a sample set of 15 or fewer injections, followed by either the continuing calibration verification standard or by the full set of calibration standards. Run a solvent blank after the highest calibration standard and after a set of 15 or fewer injections to check for any analyte carryover.

12.3.5 Place the standards, samples and QC (duplicates, matrix spikes, and blanks) into the autosampler tray according to the order they are listed in the sequence table of the HP ChemStation.

12.3.6 Start the sequence.

13.0 DATA ANALYSIS

13.1 Matrix spike recoveries. Calculate spike recoveries using the formula below:

$$\% \text{ recovery} = \frac{(\text{spiked sample result} - \text{sample result})}{\text{actual spiked amount}} \times 100$$

13.2 MeFOSEA concentrations. Calculate the concentration of MeFOSEA in the hexane extract by external method using the calibration curve.

13.3 Data plot. Obtain a plot of MeFOSEA concentration in the hexane extract as a function of time for each pH. Use these data for degradation rate determinations.

14.0 METHOD PERFORMANCE

14.1 Solvent blanks. The measured value for the solvent blank should be less than half the LOQ of the method.

14.2 Sample duplicates. The warning limits are the average ± twice the relative percent difference. The control limits are the average ± three times the relative percent difference. If the lower value of the warning or control limit calculates to be less than zero, use zero as the low limit.

14.3 Matrix spikes. The recovery warning limits are the average spike recovery ± twice the standard deviation. The recovery control limits are the average spike recovery ± three times the standard deviation.

14.4 CCV. Analyze a mid-range calibration standard and a solvent blank after 15 or fewer sample injections and at the end of the run. If the percent difference for the amount of measured analyte exceeds ± 25% of the true value, relative to the initial standard curve, stop the run. Use only those samples analyzed before the last acceptable calibration check standard. Reanalyze the remaining samples with a new calibration curve.

14.5 Coefficient of Determination. The coefficient of determination (r^2) should be 0.990 or greater. The curves should be examined closely for linearity and intercept, particularly for accuracy of quantitation at the low and high ends of the curve.

14.6 LOQ. The LOQ is equal to the lowest standard in the calibration curve.

15.0 Pollution Prevention and Waste Management

15.1 Dispose of sample waste according to acceptable laboratory practice. Refer to the 3M Waste Stream procedure for further information.

16.0 Records

16.1 Print out hard copies of all graphics and data analysis summaries for archiving.

16.2 Sign and date all graphics and label with instrument ID.

16.3 Record sample weights and extraction information on the hydrolysis sample prep worksheets (Attachment A).

16.4 Print out the sample sequence table, reduce the size with photocopying and tape the photocopy into the instrument log. Keep all the original copies in the raw data files package.

16.5 Print chromatograms and internal standard reports for all analyses.

16.6 Print calibration tables and curve information and store in raw data file.

16.7 Store hydrolysis sample prep worksheets in the raw data file.

16.8 Enter all standard preparation information in the standards preparation logbook.

16.9 Backup all electronic data to appropriate media.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Method validation has not been performed for this method.

18.0 REFERENCES

18.1 Fate, Transport and Transformation Test Guidelines, Office of Prevention, Pesticides and Toxic Substances. (OPPTS). 835.2110. Hydrolysis as a Function of pH and Temperature. EPA. 712-C-98-057. January 1998.

18.2 Fate, Transport and Transformation Test Guidelines, Office of Prevention, Pesticides and Toxic Substances. (OPPTS). 835.2130. Hydrolysis as a Function of pH and Temperature. EPA. 712-C-96-059. April 1996.

18.3 *Handbook of Chemical Property Estimation Methods.* Rate of Hydrolysis. pp. 7-1 through 7-48.

18.4 *CRC Handbook of Chemistry and Physics, 1st Student Edition.* "Buffer Solutions Operational Definitions of pH." Robert C. Weast, Ph.D. 1988, p. D-87.

19.0 AFFECTED DOCUMENTS

19.1 None

20.0 REVISIONS

Rev number	Revision reason	Rev date

Fluorochemical Degradation (Hydrolysis) Analysis

TEST ANALYTE:

HOURS:

Sample No.		Description	Time of Initial Prep	Buffer pH	Buffer Volume (mL)	Test Analyte Solution (µL)	ISTD Solution (µL)	Spike Solution (µL)	Time of Quenching	Quenching Solvent (mL)
-	-	Sample		1.5	1.0					
-	-	Duplicate		1.5	1.0					
-	-	Spike ☐		1.5	1.0					
-	-	Sample		5	1.0					
-	-	Duplicate		5	1.0					
-	-	Spike ☐		5	1.0					
-	-	Sample		7	1.0					
-	-	Duplicate		7	1.0					
-	-	Spike ☐		7	1.0					
-	-	Sample		9	1.0					
-	-	Duplicate		9	1.0					
-	-	Spike ☐		9	1.0					
-	-	Sample		11	1.0					
-	-	Duplicate		11	1.0					
-	-	Spike ☐		11	1.0					

Date of Initial Prep:

Date of Quenching:

	Test Analyte Solution	ISTD Solution	Spike Solution	Quenching Solution
Standard/Traceability No.				
Component				
Concentration (µg/mL)				NA

Temperature of Incubator (°C):

Incubation Start (Date and Time):

Incubation Stop (Date and Time):

Total Incubation Time:

Buffer Addition by:

ISTD Addition by:

Test Analyte Addition by:

Quenching by:

Spike Addition by:

Autovial Aliquoting by:

Centrifugation Yes / No

Centrifuge:

RPM:

Time: min

By:

Filtration: Yes / No

By:

Pore Size: um, Brand:

Reviewed by:

Page 13 of 13

Appendix C: MeFOSEA Sample Preparation Logsheets

Fluorochemical Degradation (Hydrolysis) Analysis

TEST ANAYLTE: Methyl FOSEA (THAR3F2) **Date** 11/23/98

HOURS: day 0

Sample No.	Description	Time of Initial Prep	Buffer pH	Buffer Volume (mL)	Test Analyte Solution (µL)	ISTD Solution (µL)	Spike Solution (µL)	Time of Quenching	Quenching Solvent (mL)
101998 -MeFOSEA-001	Sample	2:00pm 11-24-98	1.5	1.0	10	in quench	0	2:00	10
101998 -MeFOSEA-002	Duplicate	2:00pm 11.24	1.5	1.0	10	in quench	0	2:02	10
101998 -MeFOSEA-003	Spike [X]	2:02 11-24	1.5	1.0	10	in quench	10	2:02	10
101998 -MeFOSEA-004	Sample	2:02	5	1.0	10	in quench	0	2:02	10
101998 -MeFOSEA-005	Duplicate	2:04	5	1.0	10	in quench	0	2:04	10
101998 -MeFOSEA-006	Spike [X]	2:04	5	1.0	10	in quench	10	2:04	10
101998 -MeFOSEA-007	Sample	2:06	7	1.0	10	in quench	0	2:06	10
101998 -MeFOSEA-008	Duplicate	2:06	7	1.0	10	in quench	0	2:06	10
101998 -MeFOSEA-009	Spike [X]	2:08	7	1.0	10	in quench	10	2:08	10
101998 -MeFOSEA-010	Sample	2:08	9	1.0	10	in quench	0	2:08	10
101998 -MeFOSEA-011	Duplicate	2:10	9	1.0	10	in quench	0	2:10	10
101998 -MeFOSEA-012	Spike [X]	2:10	9	1.0	10	in quench	10	2:10	10
101998 -MeFOSEA-013	Sample	2:12	11	1.0	10	in quench	0	2:12	10
101998 -MeFOSEA-014	Duplicate	2:12	11	1.0	10	in quench	0	2:12	10
101998 -MeFOSEA-015	Spike [X]	2:12	11	1.0	10	in quench	10	2:12	10

Date of Initial Prep: 11-24-98 **Date of Quenching:** 11-24-98

11238- CCL 5/13/99

22.0000

	Test Analyte Solution	ISTD Solution	Spike Solution	Quenching Solution
Standard/Traceability No.	98086-3-16	98086-3-19	W398-111	98086-3-19
Component	MeFOSEA	EFA	MFA	Hexane + ISTD
Concentration (µg/mL)	10,730 ppm	9.324 ppm	473.4 ppm	9.324 ppm

Temperature of Incubator (°C): 50

Incubation Start (Date and Time): 11-24-98 2:00

Incubation Stop (Date and Time): 11-24-98 2:00

Total Incubation Time: 0

Buffer Addition by: JJD

[redacted] CCL

Test Analyte Addition by: CCL, MLA (pH 9, 11)

Quenching by: CCL

Spike Addition by: CCL

Retrieval Aliquoting by: CCL

[redacted] Yes (No)

Centrifuge: Roujan

RPM:

Time: min

By:

[redacted] Yes (No)

By:

Pore Size: um, Brand:

Reviewed by: [signature] 5/13/99

3M Environmental Laboratory Request No: U2744

Fluorochemical Degradation (Hydrolysis) Analysis

TEST ANAYLTE: Methyl FOSEA **Date** 11/23/98

HOURS: day 1

Sample No.	Description	Time of Initial Prep	Buffer pH	Buffer Volume (mL)	Test Analyte Solution (µL)	ISTD Solution (µL)	Spike Solution (µL)	Time of Quenching	Quenching Solvent (mL)
101998-MeFOSEA-016	Sample	2:50 pm	1.5	1.0	10	in quench	0	2:30	10
101998 -MeFOSEA-017	Duplicate	2:50 pm	1.5	1.0	10	in quench	0		10
101998 -MeFOSEA-018	Spike [X]	2:50 pm	1.5	1.0	10	in quench	10		10
101998 -MeFOSEA-019	Sample	3:05	5	1.0	10	in quench	0		10
101998 -MeFOSEA-020	Duplicate	3:05	5	1.0	10	in quench	0		10
101998 -MeFOSEA-021	Spike [X]	3:05	5	1.0	10	in quench	10		10
101998 -MeFOSEA-022	Sample	3:05	7	1.0	10	in quench	0		10
101998 -MeFOSEA-023	Duplicate	3:05	7	1.0	10	in quench	0		10
101998 -MeFOSEA-024	Spike [X]	3:05	7	1.0	10	in quench	10		10
101998 -MeFOSEA-025	Sample	3:20	9	1.0	10	in quench	0		10
101998 -MeFOSEA-026	Duplicate	3:20	9	1.0	10	in quench	0		10
101998 -MeFOSEA-027	Spike [X]	3:20	9	1.0	10	in quench	10		10
101998 -MeFOSEA-028	Sample	3:40	11	1.0	10	in quench	0		10
101998 -MeFOSEA-029	Duplicate	3:40	11	1.0	10	in quench	0	[illegible]	10
101998 -MeFOSEA-030	Spike [X]	3:40	11	1.0	10	in quench	10	~[illegible] for [illegible] of first sample	10

Date of Initial Prep: 11-23-98 **Date of Quenching:** 11-24-98

5/13/99

	Test Analyte Solution	ISTD Solution	SPK Solution	Quenching Solution
Standard/Traceability No.	98086-3-16	98086-3-19	W398-1117	98086-3-19
Component	MeFOSEA	EFA	MFA	Hexane
Concentration (µg/mL)	10,730 ppm	9.324 ppm	9734 ppm	[illegible]

Temperature of Incubator (°C): 50

[illegible] Start (Date and Time): 3:30 11/23/98

[illegible] Stop (Date and Time): 2:00 11/24/98

[illegible] Time: 0.94 days

[illegible] Addition by: JJD (by CCL)

[illegible] Addition by: CCL

[illegible] Addition by: CCL

[illegible]ing by: CCL

[illegible] Addition by: CCL

[illegible] Aliquoting by: CCL

[illegible] Yes (No)

Centrifuge: Roujan

RPM:

Time: min

By:

[illegible]: Yes / No

By:

Pore Size: um, Brand:

Reviewed by: JMmaine 5/13/99

3M Environmental Laboratory Request No: U2744

Fluorochemical Degradation (Hydrolysis) Analysis

TEST ANAYLTE: Methyl FOSEA **Date** 11/23/98

HOURS: day 2

112398-

Sample No.	Description	Time of Initial Prep	Buffer pH	Buffer Volume (mL)	Test Analyte Solution (µL)	ISTD Solution (µL)	Spike Solution (µL)	Time of Quenching	Quenching Solvent (mL)
101998-MeFOSEA-031	Sample	2:52	1.5	1.0	10	in quench	0	2:02	10
101998 -MeFOSEA-032	Duplicate	2:52	1.5	1.0	10	in quench	0	2:06	10
101998 -MeFOSEA-033	Spike ☑	2:52	1.5	1.0	10	in quench	10	2:10	10
101998 -MeFOSEA-034	Sample	3:07	5	1.0	10	in quench	0	2:03	10
101998 -MeFOSEA-035	Duplicate	3:08	5	1.0	10	in quench	0	2:07	10
101998 -MeFOSEA-036	Spike ☑	3:07	5	1.0	10	in quench	10	2:10	10
101998 -MeFOSEA-037	Sample	3:08	7	1.0	10	in quench	0	2:03	10
101998 -MeFOSEA-038	Duplicate	3:07	7	1.0	10	in quench	0	2:07	10
101998 -MeFOSEA-039	Spike ☑	3:07	7	1.0	10	in quench	10	2:11	10
101998 -MeFOSEA-040	Sample	3:20	9	1.0	10	in quench	0	2:04	10
101998 -MeFOSEA-041	Duplicate	3:20	9	1.0	10	in quench	0	2:07	10
101998 -MeFOSEA-042	Spike ☑	3:20	9	1.0	10	in quench	10	2:11	10
101998 -MeFOSEA-043	Sample	3:40	11	1.0	10	in quench	0	2:05	10
101998 -MeFOSEA-044	Duplicate	3:40	11	1.0	10	in quench	0	2:08	10
101998 -MeFOSEA-045	Spike ☑	3:40	11	1.0	10	in quench	10	2:12	10

Date of Initial Prep: 11-23-98

Date of Quenching: 11-25-98

	Test Analyte Solution	ISTD Solution	SPK Solution	Quenching Solution
Standard/Traceability No.	[illegible]	[illegible]	[illegible]	[illegible]
Component	MeFOSEA	[illegible]	MeFA	Hexane/IPA
Concentration (µg/mL)	10,730 ppm eoc	[illegible]	[illegible]	[illegible]

Temperature of Incubator (°C): 50

Incubation Start (Date and Time): [illegible]

Incubation Stop (Date and Time): [illegible]

Total Incubation Time: 1.92 days

Buffer Addition by:	TDT
ISTD Addition by:	see calculations JMG
Test Analyte Addition by:	[illegible]
Quenching by:	JMG
Spike Addition by:	JMG
Autovial Aliquoting by:	JMG

Centrifugation Yes / No

Centrifuge: Roujan

RPM:

Time: min

By:

Filtration: Yes / No

By:

Pore Size: um, Brand:

Reviewed by: [illegible] 5/13/99

Added 0.5mL IPA to each vial. JMG
prior to addition of quenching solution.

3M Environmental Laboratory Request No: U2744

620000

Fluorochemical Degradation (Hydrolysis) Analysis

TEST ANAYLTE: Methyl FOSEA **Date** 11/23/98

HOURS: day 7

112398-

CCL 5/13/99

Sample No.	Description	Time of Initial Prep	Buffer pH	Buffer Volume (mL)	Test Analyte Solution (µL)	ISTD Solution (µL)	Spike Solution (µL)	Time of Quenching	Quenching Solvent (mL)
101998 -MeFOSEA-046	Sample	2:54	1.5	1.0	10	in quench	0	9:35	10
101998 -MeFOSEA-047	Duplicate	2:54	1.5	1.0	10	in quench	0	9:37	10
101998 -MeFOSEA-048	Spike ☑	2:54	1.5	1.0	10	in quench	10	9:40	10
101998 -MeFOSEA-049	Sample	3:09	5	1.0	10	in quench	0	9:35	10
101998 -MeFOSEA-050	Duplicate	3:09	5	1.0	10	in quench	0	9:38	10
101998 -MeFOSEA-051	Spike ☑	3:09	5	1.0	10	in quench	10	9:41	10
101998 -MeFOSEA-052	Sample	3:09	7	1.0	10	in quench	0	9:36	10
101998 -MeFOSEA-053	Duplicate	3:09	7	1.0	10	in quench	0	9:38	10
101998 -MeFOSEA-054	Spike ☑	3:09	7	1.0	10	in quench	10	9:42	10
101998 -MeFOSEA-055	Sample	3:20	9	1.0	10	in quench	0	9:36	10
101998 -MeFOSEA-056	Duplicate	3:20	9	1.0	10	in quench	0	9:39	10
101998 -MeFOSEA-057	Spike ☑	3:20	9	1.0	10	in quench	10	9:42	10
101998 -MeFOSEA-058	Sample	3:40	11	1.0	10	in quench	0	9:37	10
101998 -MeFOSEA-059	Duplicate	3:40	11	1.0	10	in quench	0	9:40	10
101998 -MeFOSEA-060	Spike ☑	3:40	11	1.0	10	in quench	10	9:43	10

Date of Initial Prep: 11-23-98 **Date of Quenching:** 11-30-98

080000

	Test Analyte Solution	ISTD Solution	SPK Solution	Quenching Solution
Standard/Traceability No.	98086-3-16	98086-3-14	W398-1117	98061-3-19
Component	[illegible]	[illegible]	MeFOSEA	[illegible]
Concentration (µg/mL)	10,730 ppm	[illegible]	473.4 ppm	[illegible]

Temperature of Incubator (°C): 50

Incubation Start (Date and Time): 3:30 11/23/98

Incubation Stop (Date and Time): 8:45am 11/30/98

Total Incubation Time: (6.72 days)

Solution Addition by: JJD (put in by CCL)

ISTD Addition by: JMG

Test Analyte Addition by: CCL

Quenching by: JMG

Spike Addition by: JMG

Autovial Aliquoting by: JMG

[illegible] Yes / (No)

Centrifuge: Roujan

RPM:

Time: min

By:

[illegible] Yes / (No)

By:

Pore Size: um, Brand:

Reviewed by: Jr mainel 5/13/99

Added 0.5 mL IPA to each vial prior to addition of quenching solution. JMG

3M Environmental Laboratory Request No: U2744

Fluorochemical Degradation (Hydrolysis) Analysis

TEST ANAYLTE: Methyl FOSEA **Date** 11/23/98

HOURS: day 14

11/23/98 CCL 5/13/99

Sample No.	Description	Time of Initial Prep	Buffer pH	Buffer Volume (mL)	Test Analyte Solution (µL)	ISTD Solution (µL)	Spike Solution (µL)	Time of Quenching	Quenching Solvent (mL)
101998-MeFOSEA-061	Sample	2:56	1.5	1.0	10	in quench	0	1:58 pm	10
101998 -MeFOSEA-062	Duplicate	2:56	1.5	1.0	10	in quench	0	1:59	10
101998 -MeFOSEA-063	Spike ☑	2:56	1.5	1.0	10	in quench	10	1:59	10
101998 -MeFOSEA-064	Sample	3:11	5	1.0	10	in quench	0	2:00	10
101998 -MeFOSEA-065	Duplicate	3:11	5	1.0	10	in quench	0	2:01	10
101998 -MeFOSEA-066	Spike ☑	3:11	5	1.0	10	in quench	10	2:01	10
101998 -MeFOSEA-067	Sample	3:11	7	1.0	10	in quench	0	2:01	10
101998 -MeFOSEA-068	Duplicate	3:11	7	1.0	10	in quench	0	2:02	10
101998 -MeFOSEA-069	Spike ☑	3:11	7	1.0	10	in quench	10	2:02	10
101998 -MeFOSEA-070	Sample	3:20	9	1.0	10	in quench	0	2:03	10
101998 -MeFOSEA-071	Duplicate	3:20	9	1.0	10	in quench	0	2:03	10
101998 -MeFOSEA-072	Spike ☑	3:20	9	1.0	10	in quench	10	2:04	10
101998 -MeFOSEA-073	Sample	3:40	11	1.0	10	in quench	0	2:04	10
101998 -MeFOSEA-074	Duplicate	3:40	11	1.0	10	in quench	0	2:05	10
101998 -MeFOSEA-075	Spike ☑	3:40	11	1.0	10	in quench	10	2:05	10

Date of Initial Prep: 11-23-98

✓ **Date of Quenching:** [redacted] 12/7/98

	Test Analyte Solution	ISTD Solution	SPK Solution (JMG 12/7/98)	Quenching Solution
Standard/Traceability No.	98af6-316	~~[redacted]~~	98060-15-16	W398-1117
Component	MeFOSEA	~~[redacted]~~	EFA	Methyl FOS Alcohol
Concentration (µg/mL)	~~[redacted]~~ 730 ppm	~~[redacted]~~	9.324 µg/mL	4.734 µg/mL 473.4 ppm CCL

Temperature of Incubator (°C): 50

Incubation Start (Date and Time): 11/23/98 CCL 3:30pm

Incubation Stop (Date and Time): 12/07/98 1:02pm

Total Incubation Time: 13.90 days

~~Buffer~~ Addition by: JJD (By CCL 5/13/99)

~~ISTD~~ Addition by: JMG

~~Test Analyte~~ Addition by: CCL

Quenching by: JMG

Spike Addition by: JMG

Autovial Aliquoting by: JMG

~~[redacted]~~ Yes (No)

Centrifuge: Roujan

RPM:

Time: min

By:

~~Filtration~~: Yes (No)

By:

Pore Size: um, Brand:

Added 0.5 mL IPA to each vial prior to addition of quenching solution. JMG

Reviewed by: Jnmaisel 5/13/99

3M Environmental Laboratory Request No: U2744

Fluorochemical Degradation (Hydrolysis) Analysis

TEST ANAYLTE: Methyl FOSEA **Date** 11/23/98

HOURS: day 21

Sample No.	Description	Time of Initial Prep	Buffer pH	Buffer Volume (mL)	Test Analyte Solution (µL)	ISTD Solution (µL)	Spike Solution (µL)	Time of Quenching	Quenching Solvent (mL)
101998 -MeFOSEA-076	Sample	2:58	1.5	1.0	10	in quench	0	9:39	10
101998 -MeFOSEA-077	Duplicate	2:58	1.5	1.0	10	in quench	0	9:39	10
101998 -MeFOSEA-078	Spike ☑ (1)	2:58	1.5	1.0	10	in quench	10	9:40	10
101998 -MeFOSEA-079	Sample	3:13	5	1.0	10	in quench	0	9:41	10
101998 -MeFOSEA-080	Duplicate	3:13	5	1.0	10	in quench	0	9:41	10
101998 -MeFOSEA-081	Spike ☑ (1)	3:13	5	1.0	10	in quench	10	9:42	10
101998 -MeFOSEA-082	Sample	3:13	7	1.0	10	in quench	0	9:43	10
101998 -MeFOSEA-083	Duplicate	3:13	7	1.0	10	in quench	0	9:43	10
101998 -MeFOSEA-084	Spike ☑ (1)	3:13	7	1.0	10	in quench	10	9:44	10
101998 -MeFOSEA-085	Sample	3:30	9	1.0	10	in quench	0	9:44	10
101998 -MeFOSEA-086	Duplicate	3:30	9	1.0	10	in quench	0	9:45	10
101998 -MeFOSEA-087	Spike ☑ (1)	3:30	9	1.0	10	in quench	10	9:45	10
101998 -MeFOSEA-088	Sample	3:50	11	1.0	10	in quench	0	9:46	10
101998 -MeFOSEA-089	Duplicate	3:50	11	1.0	10	in quench	0	9:46	10
101998 -MeFOSEA-090	Spike ☑ (1)	3:50	11	1.0	10	in quench	10	9:47	10

Date of Initial Prep: 11-23-98 **Date of Quenching:** 12/14/98

	Test Analyte Solution	ISTD Solution	SPK (1) Solution	Quenching Solution
Standard/Traceability No.	98AE-3-16	98OE-3-19	W398-1117	98060-15-16
Component	MeFOSEA	Et-FOSE-OH	Me-NOS-OH	EFA
Concentration (µg/mL)	10,738ppm	9,324ppm	4239 µg/mL 773.9 CCL	9.324 µg/mL

Temperature of Incubator (°C): 50

Incubation Start (Date and Time): 11/23/98 3:30pm

Incubation Stop (Date and Time): 12/14/98 8:33am

Total Incubation Time: 20.71 days

Buffer Addition by:	JJB (By CCL 5/13/99)
ISTD Addition by:	JMG
Test Analyte Addition by:	CCL
Quenching by:	JMG
Spike Addition by:	JMG
Autovial Aliquoting by:	JMG

Yes (No)

Centrifuge: Roujan

RPM:

Time: min

By:

Added 0.5 mL IPA to each vial prior to addition of quenching solution. JMG

Filtration: Yes (No)

By:

Pore Size: um, Brand:

Reviewed by: Gmaisel 5/13/99

① Also spiked with 10µl of N-methyl fosea: 10,738ppm in acetone (CCL 12/14/98) 12/14/98 JMG

② A set of these samples was stored in the freezer until future (CCL 12/14/98) 12/14/98 JMG

280000

Fluorochemical Degradation (Hydrolysis) Analysis

TEST ANAYLTE: Methyl FOSEA **Date** 11/23/98

HOURS: day 28

11-23-98

CCL 5/13/98

Sample No.	Description	Time of Initial Prep	Buffer pH	Buffer Volume (mL)	Test Analyte Solution (µL)	ISTD Solution (µL)	Spike Solution (µL)	Time of Quenching	Quenching Solvent (mL)
101998 -MeFOSEA-091	Sample	3:00	1.5	1.0	10	in quench	0	10:49	10
101998 -MeFOSEA-092	Duplicate	3:00	1.5	1.0	10	in quench	0	10:49	10
101998 -MeFOSEA-093	Spike ☑ (1)	3:00	1.5	1.0	10	in quench	10	10:50	10
101998 -MeFOSEA-094	Sample	3:15	5	1.0	10	in quench	0	10:51	10
101998 -MeFOSEA-095	Duplicate	3:15	5	1.0	10	in quench	0	10:51	10
101998 -MeFOSEA-096	Spike ☑ (1)	3:15	5	1.0	10	in quench	10	10:51	10
101998 -MeFOSEA-097	Sample	3:15	7	1.0	10	in quench	0	10:52	10
101998 -MeFOSEA-098	Duplicate	3:15	7	1.0	10	in quench	0	10:53	10
101998 -MeFOSEA-099	Spike ☑ (1)	3:15	7	1.0	10	in quench	10	10:53	10
101998 -MeFOSEA-100	Sample	3:30	9	1.0	10	in quench	0	10:54	10
101998 -MeFOSEA-101	Duplicate	3:30	9	1.0	10	in quench	0	10:54	10
101998 -MeFOSEA-102	Spike ☑ (1)	3:30	9	1.0	10	in quench	10	10:55	10
101998 -MeFOSEA-103	Sample	3:50	11	1.0	10	in quench	0	10:55	10
101998 -MeFOSEA-104	Duplicate	3:50	11	1.0	10	in quench	0	10:56	10
101998 -MeFOSEA-105	Spike ☑ (1)	3:50	11	1.0	10	in quench	10	10:56	10

Date of Initial Prep: 11-23-98 **Date of Quenching:** 12/21/98

	Test Analyte Solution	ISTD Solution	SPK(1) Solution	Quenching Solution
Standard/Traceability No.	980616	980618-319	U398-1117	980610-15-16
Component	MeFOSEA	EtFOSE-OH	MeFOS-OH	IPA
Concentration (µg/mL)	[redacted]	[redacted]	473 ppm	9.324 µg/mL

Temperature of Incubator (°C): 50

Incubation Start (Date and Time): 11/23/98 3:30 AM CCL

Incubation Stop (Date and Time): 9:55 AM, 12/21/98

Total Incubation Time: 27.77 days

Buffer Addition by:	JMG CCL 5/13/99	[redacted] Yes	(No)
ISTD Addition by:	JMG	Centrifuge: Roujan	
Test Analyte Addition by:	CCL	RPM:	
Quenching by:	JMG	Time:	min
Spike Addition by:	JMG	By:	
Autovial Aliquoting by:	JMG		

Filtration: Yes (No)

By:

Pore Size: um, Brand:

Reviewed by: [redacted] 5/13/99

Added 0.5 mL IPA to each vial prior to addition of quenching solution. JMG

3M Environmental Laboratory Request No: U2744

(1) Also spiked with 10 µL of N-methyl fosea: 10,730 ppm in acetone [redacted] 12/21/98 JMG

(2) Samples stored in freezer until analysis. Freezer F-2 temp = -18.7°C

Appendix D: GC/AED Chromatograms

See separate bound volumes.

Appendix E: Spreadsheets: GC/AED Results

MeFOSEA GC/AED results

Note that each extract was injected in duplicate.

pH 1.5 DATA

day	Sample number	MeFOSEA (ppm)	average ppm	% spike recovery for MeFOSEA	%RPD calculations
0	112398-MeFOSEA-001	9.896	9.894		0.3
	112398-MeFOSEA-001	9.891			
0	112398-MeFOSEA-002	9.872	9.865		
	112398-MeFOSEA-002	9.858			
0	112398-MeFOSEA-003	9.722	9.927	n/a	
	112398-MeFOSEA-003	10.132			
0.94	112398-MeFOSEA-016	7.590	7.806		5.3
	112398-MeFOSEA-016	8.021			
0.94	112398-MeFOSEA-017	8.079	8.232		
	112398-MeFOSEA-017	8.385			
0.94	112398-MeFOSEA-018	8.919	8.957	n/a	
	112398-MeFOSEA-018	8.994			
1.92	112398-MeFOSEA-031	7.502	7.554		1.1
	112398-MeFOSEA-031	7.605			
1.92	112398-MeFOSEA-032	7.612	7.639		
	112398-MeFOSEA-032	7.666			
1.92	112398-MeFOSEA-033	7.987	7.861	n/a	
	112398-MeFOSEA-033	7.735			
6.72	112398-MeFOSEA-046	5.537	5.502		3.9
	112398-MeFOSEA-046	5.467			
6.72	112398-MeFOSEA-047	4.752	5.291		
	112398-MeFOSEA-047	5.830			
6.72	112398-MeFOSEA-048	5.561	5.551	n/a	
	112398-MeFOSEA-048	5.540			
13.90	112398-MeFOSEA-061	4.211	4.229		3.8
	112398-MeFOSEA-061	4.246			
13.90	112398-MeFOSEA-062	4.365	4.393		
	112398-MeFOSEA-062	4.420			
13.90	112398-MeFOSEA-063	5.320	5.244	n/a	
	112398-MeFOSEA-063	5.168			
20.71	112398-MeFOSEA-076	3.644	3.647		31.9
	112398-MeFOSEA-076	3.650			
20.71	112398-MeFOSEA-077	5.058	5.031		
	112398-MeFOSEA-077	5.003			
20.71	112398-MeFOSEA-078	12.952	12.874	79.5	
	112398-MeFOSEA-078	12.795			
27.77	112398-MeFOSEA-091	2.764	2.795		25.3
	112398-MeFOSEA-091	2.826			
27.77	112398-MeFOSEA-092	3.543	3.605		
	112398-MeFOSEA-092	3.666			
27.77	112398-MeFOSEA-093	13.411	13.283	94.0	
	112398-MeFOSEA-093	13.154			

MeFOSEA GC/AED results

Note that each extract was injected in duplicate.

pH 5 DATA

day	Sample number	MeFOSEA (ppm)	average ppm	% spike recovery for MeFOSEA	%RPD calculations
0	112398-MeFOSEA-004	10.323	10.266		1.6
	112398-MeFOSEA-004	10.208			
0	112398-MeFOSEA-005	10.368	10.431		
	112398-MeFOSEA-005	10.493			
0	112398-MeFOSEA-006	10.416	10.326	n/a	
	112398-MeFOSEA-006	10.236			
0.94	112398-MeFOSEA-019	9.465	9.437		1.0
	112398-MeFOSEA-019	9.408			
0.94	112398-MeFOSEA-020	9.285	9.344		
	112398-MeFOSEA-020	9.402			
0.94	112398-MeFOSEA-021	9.431	9.315	n/a	
	112398-MeFOSEA-021	9.198			
1.92	112398-MeFOSEA-034	8.491	8.576		0.8
	112398-MeFOSEA-034	8.660			
1.92	112398-MeFOSEA-035	8.467	8.645		
	112398-MeFOSEA-035	8.823			
1.92	112398-MeFOSEA-036	8.496	8.544	n/a	
	112398-MeFOSEA-036	8.592			
6.72	112398-MeFOSEA-049	7.910	8.251		6.8
	112398-MeFOSEA-049	8.592			
6.72	112398-MeFOSEA-050	7.702	7.710		
	112398-MeFOSEA-050	7.718			
6.72	112398-MeFOSEA-051	8.518	8.488	n/a	
	112398-MeFOSEA-051	8.457			
13.90	112398-MeFOSEA-064	7.131	7.281		6.9
	112398-MeFOSEA-064	7.431			
13.90	112398-MeFOSEA-065	7.864	7.804		
	112398-MeFOSEA-065	7.744			
13.90	112398-MeFOSEA-066	7.710	7.710	n/a	
	112398-MeFOSEA-066	bad injection			
20.71	112398-MeFOSEA-079	bad injection	5.789		3.5
	112398-MeFOSEA-079	5.789			
20.71	112398-MeFOSEA-080	5.639	5.593		
	112398-MeFOSEA-080	5.546			
20.71	112398-MeFOSEA-081	18.636	18.845	122.9	
	112398-MeFOSEA-081	19.054			
27.77	112398-MeFOSEA-094	5.448	5.332		4.5
	112398-MeFOSEA-094	5.215			
27.77	112398-MeFOSEA-095	5.636	5.577		
	112398-MeFOSEA-095	5.518			
27.77	112398-MeFOSEA-096	15.199	15.166	90.5	
	112398-MeFOSEA-096	15.132			

MeFOSEA GC/AED results

Note that each extract was injected in duplicate.

pH 7 DATA

day	Sample number	MeFOSEA (ppm)	average ppm	% spike recovery for MeFOSEA	%RPD calculations
0	112398-MeFOSEA-007	10.598	10.586		1.8
	112398-MeFOSEA-007	10.573			
0	112398-MeFOSEA-008	10.440	10.398		
	112398-MeFOSEA-008	10.356			
0	112398-MeFOSEA-009	10.453	10.619	n/a	
	112398-MeFOSEA-009	10.785			
0.94	112398-MeFOSEA-022	8.535	8.519		0.8
	112398-MeFOSEA-022	8.503			
0.94	112398-MeFOSEA-023	8.627	8.587		
	112398-MeFOSEA-023	8.547			
0.94	112398-MeFOSEA-024	9.023	8.953	n/a	
	112398-MeFOSEA-024	8.882			
1.92	112398-MeFOSEA-037	8.660	8.788		
	112398-MeFOSEA-037	8.916			
1.92	112398-MeFOSEA-038	8.762	8.754		
	112398-MeFOSEA-038	8.746			
1.92	112398-MeFOSEA-039	8.524	8.564	n/a	
	112398-MeFOSEA-039	8.604			
6.72	112398-MeFOSEA-052	6.534	6.703		20.1
	112398-MeFOSEA-052	6.871			
6.72	112398-MeFOSEA-053	5.410	5.476		
	112398-MeFOSEA-053	5.542			
6.72	112398-MeFOSEA-054	5.947	5.874	n/a	
	112398-MeFOSEA-054	5.801			
13.90	112398-MeFOSEA-067	4.793	4.867		14.0
	112398-MeFOSEA-067	4.940			
13.90	112398-MeFOSEA-068	4.278	4.229		
	112398-MeFOSEA-068	4.180			
13.90	112398-MeFOSEA-069	5.507	5.546	n/a	
	112398-MeFOSEA-069	5.585			
20.71	112398-MeFOSEA-082	4.506	4.595		28.9
	112398-MeFOSEA-082	4.684			
20.71	112398-MeFOSEA-083	3.512	3.436		
	112398-MeFOSEA-083	3.360			
20.71	112398-MeFOSEA-084	14.152	14.047	93.5	
	112398-MeFOSEA-084	13.941			
27.77	112398-MeFOSEA-097	1.958	2.000		2.0
	112398-MeFOSEA-097	2.041			
27.77	112398-MeFOSEA-098	2.021	2.041		
	112398-MeFOSEA-098	2.060			
27.77	112398-MeFOSEA-099	14.490	14.290	114.3	
	112398-MeFOSEA-099	14.089			

Results for Day 1.92 were not included in the graph, due to failed QC criteria.

MeFOSEA GC/AED results

Note that each extract was injected in duplicate.

pH 9 DATA

day	Sample number	MeFOSEA (ppm)	average ppm	% spike recovery for MeFOSEA	%RPD calculations
0	112398-MeFOSEA-010	9.998	9.734		6.8
	112398-MeFOSEA-010	9.469			
0	112398-MeFOSEA-011	10.312	10.423		
	112398-MeFOSEA-011	10.533			
0	112398-MeFOSEA-012	10.313	10.097	n/a	
	112398-MeFOSEA-012	9.881			
0.94	112398-MeFOSEA-025	10.996	11.145		3.9
	112398-MeFOSEA-025	11.293			
0.94	112398-MeFOSEA-026	11.386	10.723		
	112398-MeFOSEA-026	10.059			
0.94	112398-MeFOSEA-027	8.713	8.615	n/a	
	112398-MeFOSEA-027	8.517			
1.92	112398-MeFOSEA-040	9.897	9.349		24.7
	112398-MeFOSEA-040	8.800			
1.92	112398-MeFOSEA-041	7.263	7.295		
	112398-MeFOSEA-041	7.326			
1.92	112398-MeFOSEA-042	7.457	7.323	n/a	
	112398-MeFOSEA-042	7.189			
6.72	112398-MeFOSEA-055	5.036	5.014		11.0
	112398-MeFOSEA-055	4.992			
6.72	112398-MeFOSEA-056	6.114	5.598		
	112398-MeFOSEA-056	5.082			
6.72	112398-MeFOSEA-057	6.263	5.872	n/a	
	112398-MeFOSEA-057	5.481			
13.90	112398-MeFOSEA-070	4.436	4.415		1.8
	112398-MeFOSEA-070	4.394			
13.90	112398-MeFOSEA-071	4.365	4.495		
	112398-MeFOSEA-071	4.624			
13.90	112398-MeFOSEA-072	2.980	2.970	n/a	
	112398-MeFOSEA-072	2.960			
20.71	112398-MeFOSEA-085	2.601	2.538		10.3
	112398-MeFOSEA-085	2.475			
20.71	112398-MeFOSEA-086	2.312	2.291		
	112398-MeFOSEA-086	2.269			
20.71	112398-MeFOSEA-087	11.365	11.309	82.9	
	112398-MeFOSEA-087	11.252			
27.77	112398-MeFOSEA-100	2.392	2.382		0.3
	112398-MeFOSEA-100	2.371			
27.77	112398-MeFOSEA-101	2.415	2.388		
	112398-MeFOSEA-101	2.361			
27.77	112398-MeFOSEA-102	11.798	11.885	88.5	
	112398-MeFOSEA-102	11.972			

MeFOSEA GC/AED results

Note that each extract was injected in duplicate.

pH 11 DATA

day	Sample number	MeFOSEA (ppm)	average ppm	% spike recovery for MeFOSEA	%RPD calculations
0	112398-MeFOSEA-013	12.496	11.429		13.6
	112398-MeFOSEA-013	10.362			
0	112398-MeFOSEA-014	9.650	9.976		
	112398-MeFOSEA-014	10.302			
0	112398-MeFOSEA-015	10.306	9.990	n/a	
	112398-MeFOSEA-015	9.674			
0.94	112398-MeFOSEA-028	8.971	8.810		3.3
	112398-MeFOSEA-028	8.648			
0.94	112398-MeFOSEA-029	9.218	9.103		
	112398-MeFOSEA-029	8.988			
0.94	112398-MeFOSEA-030	8.596	8.562	n/a	
	112398-MeFOSEA-030	8.527			
1.92	112398-MeFOSEA-043	7.315	8.032		11.2
	112398-MeFOSEA-043	8.749			
1.92	112398-MeFOSEA-044	9.143	8.988		
	112398-MeFOSEA-044	8.833			
1.92	112398-MeFOSEA-045	8.525	8.480	n/a	
	112398-MeFOSEA-045	8.434			
6.72	112398-MeFOSEA-058	5.947	5.789		4.5
	112398-MeFOSEA-058	5.630			
6.72	112398-MeFOSEA-059	5.966	5.535		
	112398-MeFOSEA-059	5.103			
6.72	112398-MeFOSEA-060	5.315	5.175	n/a	
	112398-MeFOSEA-060	5.035			
13.90	112398-MeFOSEA-073	3.360	3.310		2.6
	112398-MeFOSEA-073	3.260			
13.90	112398-MeFOSEA-074	3.471	3.398		
	112398-MeFOSEA-074	3.325			
13.90	112398-MeFOSEA-075	3.545	3.674	n/a	
	112398-MeFOSEA-075	3.802			
20.71	112398-MeFOSEA-088	2.144	2.249		23.2
	112398-MeFOSEA-088	2.354			
20.71	112398-MeFOSEA-089	2.885	2.840		
	112398-MeFOSEA-089	2.795			
20.71	112398-MeFOSEA-090	12.385	12.516	92.9	
	112398-MeFOSEA-090	12.647			
27.77	112398-MeFOSEA-103	2.268	2.284		8.7
	112398-MeFOSEA-103	2.300			
27.77	112398-MeFOSEA-104	2.497	2.493		
	112398-MeFOSEA-104	2.488			
27.77	112398-MeFOSEA-105	12.028	12.235	91.8	
	112398-MeFOSEA-105	12.442			

QA/QC calculations

Matrix spike recovery calculations

pH	Sample	%Recovery
1.5	112398-MeFOSEA-078	79.5
1.5	112398-MeFOSEA-093	94.0
5	112398-MeFOSEA-081	122.9
5	112398-MeFOSEA-096	90.5
7	112398-MeFOSEA-084	93.5
7	112398-MeFOSEA-099	114.3
9	112398-MeFOSEA-087	82.9
9	112398-MeFOSEA-102	88.5
11	112398-MeFOSEA-090	92.9
11	112398-MeFOSEA-105	91.8
	Average recovery	95.1
	Standard deviation	12.7
	%Relative standard deviation	13.4%
	Lower warning limit	69.6
	Upper warning limit	120.5
	Lower control limit	56.9
	Upper control limit	133.3

Sample precision calculations, using the sample and duplicate to calculate the RPD

Average RPD	8.5
Standard deviation	9.0
Lower warning limit	0.0
Upper warning limit	26.6
Lower control limit	0.0
Upper control limit	35.6

Appendix F: Spreadsheets: Calculation of Kinetic Parameters

Equations used:

The hydrolysis rate constant, $k_H = [\ln(RX)_0 - \ln(RX)_t] / t$
where RX is Molar concentration
$t_{1/2} = 0.693 / k_H$

MeFOSEA was tested by the addition of 10 microliters of 10,730ppm MeFOSEA in acetone to 1.0ml of buffer. This is equivalent to 107.3 micrograms/ml of MeFOSEA.

The formular weight of Methyl FOSEA is equal to 611.27
This makes the initial concentration of MeFOSEA equal to 1.7554E-4 Molar.

pH 1.5 Data

Day	Average MeFOSEA (ppm)	[MeFOSEA] (Molar)	$[MeFOSEA]_t/[MeFOSEA]_0$	natural log of $[MeFOSEA]_t/[MeFOSEA]_0$	k	$t_{1/2}$ (days)
0	9.90	1.619E-04	0.922	-0.081	-	-
0.94	8.33	1.363E-04	0.777	-0.253	0.2690	2.6
1.92	7.68	1.257E-04	0.716	-0.334	0.1738	4.0
6.72	5.45	8.912E-05	0.508	-0.678	0.1008	6.9
13.90	4.62	7.561E-05	0.431	-0.842	0.0606	11.4
20.71	4.34	7.098E-05	0.404	-0.905	0.0437	15.9
27.77	3.20	5.235E-05	0.298	-1.210	0.0436	15.9
				Average	0.1152	9.4
				Standard Dev.	0.0900	5.8

pH 5 Data

Day	Average MeFOSEA (ppb)	[MeFOSEA] measured (Molar)	$[MeFOSEA]_t/[MeFOSEA]_0$	natural log of $[MeFOSEA]_t/[MeFOSEA]_0$	k	$t_{1/2}$ (days)
0	10.34	1.692E-04	0.964	-0.037	-	-
0.94	9.36	1.532E-04	0.873	-0.136	0.1445	4.8
1.92	8.59	1.405E-04	0.801	-0.222	0.1159	6.0
6.72	8.15	1.333E-04	0.760	-0.275	0.0409	16.9
13.90	7.60	1.243E-04	0.708	-0.345	0.0248	27.9
20.71	5.69	9.310E-05	0.530	-0.634	0.0306	22.6
27.77	5.45	8.923E-05	0.508	-0.676	0.0244	28.4
				Average	0.0635	17.8
				Standard Dev.	0.0528	10.5

pH 7 Data

Day	Average MeFOSEA (ppb)	[MeFOSEA] measured (Molar)	$[MeFOSEA]_t/[MeFOSEA]_0$	natural log of $[MeFOSEA]_t/[MeFOSEA]_0$	k	$t_{1/2}$ (days)
0	10.53	1.723E-04	0.982	-0.018	-	-
0.94	8.69	1.421E-04	0.810	-0.211	0.2246	3.1
6.72	6.02	9.844E-05	0.561	-0.578	0.0860	8.1
13.90	4.88	7.984E-05	0.455	-0.788	0.0567	12.2
20.71	4.02	6.569E-05	0.374	-0.983	0.0474	14.6
27.77	2.02	3.305E-05	0.188	-1.670	0.0601	11.5
				Average	0.0950	9.9
				Standard Dev.	0.0739	4.5

pH 9 Data

Day	Average MeFOSEA (ppb)	[MeFOSEA] measured (Molar)	$[MeFOSEA]_t/[MeFOSEA]_0$	natural log of $[MeFOSEA]_t/[MeFOSEA]_0$	k	$t_{1/2}$ (days)
0	10.08	1.650E-04	0.940	-0.062	-	-
0.94	10.16	1.662E-04	0.947	-0.054	0.0578	12.0
1.92	7.99	1.307E-04	0.745	-0.295	0.1535	4.5
6.72	5.49	8.989E-05	0.512	-0.669	0.0996	7.0
13.90	3.96	6.478E-05	0.369	-0.997	0.0717	9.7
20.71	2.41	3.950E-05	0.225	-1.491	0.0720	9.6
27.77	2.38	3.901E-05	0.222	-1.504	0.0541	12.8
				Average	0.0848	9.3
				Standard Dev.	0.0373	3.1

pH 11 Data

	Day	Average MeFOSEA (ppb)	[MeFOSEA] measured (Molar)	$[MeFOSEA]_t/[MeFOSEA]_0$	natural log of $[MeFOSEA]_t/[MeFOSEA]_0$	k	$t_{1/2}$ (days)
0	0	10.47	1.712E-04	0.976	-0.025	-	-
-0.94	0.94	8.82	1.444E-04	0.823	-0.195	0.2078	3.3
-1.92	1.92	8.50	1.391E-04	0.792	-0.233	0.1212	5.7
-6.72	6.72	5.50	8.997E-05	0.513	-0.668	0.0994	7.0
-13.9	13.90	3.46	5.661E-05	0.323	-1.131	0.0814	8.5
-20.71	20.71	2.54	4.163E-05	0.237	-1.439	0.0695	10.0
-27.77	27.77	2.39	3.907E-05	0.223	-1.502	0.0541	12.8
					Average	0.1056	7.9
					Standard Dev.	0.0552	3.3