

BIODEGRADATION

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate; may also be referred to as PFOS or $\text{C}_8\text{F}_{17}\text{SO}_3^-\text{K}^+$. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

Remarks: PFOS was a white powder. The original 3M production lot number was not noted. The PFOS was HPLC purified and assigned 3M standard identification # TCR-00017-046. Upon receipt at the testing laboratory, the test article was given a test, control and reference (TCR) number CA-TCR02-014. An Interim Certificate of Analysis, reports the purity to be 97.9%. All results in this study were calculated assuming 100% purity.

METHOD:

Method: Based on EPA Guidelines OPPTS 835.3200

Test Type: Aerobic

GLP: No

Year Initiated: 2000

Year Completed: 2001

Contact time: 18 days

Note: a previous study ⁽¹⁾, conducted to evaluate the biodegradability of N-EtFOSE alcohol and to determine its metabolites, was conducted for 35 days. This robust summary describes the test conditions for an 18-day study of the N-EtFOSE alcohol microbial metabolite, PFOS.

Inoculum: Activated sludge collected 7/31/00 from the aeration basin at the Metro Wastewater Treatment Plant, St Paul, MN. The MLSS was determined to be 2,280 mg/L when first collected. The MLSS was stored at 4° C for approximately 5 weeks prior to being used for this study. The sludge was allowed to settle and the solids used for inoculum. The settled sludge constituted approximately 20% of the volume (~200 mL) of the MLSS used.

Test medium: Test flasks were prepared using a mineral salts medium defined in EPA Guideline OPPTS 835.3200. Methanol (1 mL per liter) was added per liter of mineral medium. Fifty mL of settled sludge was added per liter of mineral salts medium. Mineral medium plus sludge was prepared 9/7/00, while fresh mineral medium without sludge (abiotic controls) was prepared 8/10/00.

Study design:

<u>Blank Sludge Controls</u>	(mineral medium, inoculum)
<u>Abiotic Controls</u>	(mineral medium, PFOS)
<u>Test Substance</u>	(mineral medium, inoculum, PFOS)

Test vessels were set in duplicate. Additional quality control samples (blanks) were prepared and analyzed as appropriate.

Test concentration: 2.455 mg/L.

Incubation conditions:

Temperature: 25°C +/- 3°C

Agitation: ~200 rpm

Test vessels: Sterile 125 mL Nalgene polycarbonate culture flasks containing

25 mL of media

Dosing procedure: Test vessels were spiked with 6 µL of an 10,230 mg/L solution of PFOS in methanol yielding 2.455 mg/L.

Sampling Frequency: Days 0 and 18.

Analytical method: The day zero test vessels were prepared and immediately placed in a freezer that was maintained at –20°C until analyzed. After 18 days, the test vessels were removed from the incubator and frozen until final sample preparation by solid phase extraction (SPE). Following thawing, test vessel contents were adjusted to 1% acetic acid and then passed through a conditioned SEP-VAC C18 6cc SPE cartridge. Methanol was then added to the emptied culture flask, shaken vigorously and then passed through the SPE cartridge to extract adsorbed analytes. A second methanol wash was collected separately for analysis to ensure quantitative extraction.

Quantitative analysis was conducted on an HP1100 high performance liquid chromatograph with mass spectrometer detector (HPLC/MSD) system. The MSD was operated in electrospray ionization in negative-ion mode using selected-ion monitoring (SIM) for quantitation.

In addition to PFOS, the additional compounds quantified are specified below. In the case of the compounds that are potassium or ammonia salts, only the concentration of the fluorochemical anion was quantified and reported.

Compound Name	Acronym	Chemical Formula
2-(N-Ethyl Perfluorooctane sulfonamido) ethyl alcohol	N-EtFOSE Alcohol	$C_8F_{17}SO_2N(C_2H_5)CH_2CH_2OH$
2-(N-Ethyl Perfluorooctane sulfonamido) acetic acid	N-EtFOSAA	$C_8F_{17}SO_2N(C_2H_5)(CH_2COOH)$
2-(Perfluorooctane sulfonamido) acetic acid	M556	$C_8F_{17}SO_2NH(CH_2COOH)$
N-Ethyl Perfluorooctane sulfonamide	N-EtFOSA	$C_8F_{17}SO_2NH(C_2H_5)$
Perfluorooctane sulfinate, potassium salt	PFOSulfinate	$C_8F_{17}SO_2^- K^+$
Perfluorooctanoate, ammonium salt	PFOA	$C_7F_{15}COO^- NH_4^+$
Perfluorooctane sulfonamide	FOSA	$C_8F_{17}SO_2NH_2$

Reference substance: None. When results from an EtFOSE Alcohol study conducted at the same time are compared to the previous EtFOSE Alcohol 35-day study, the viability of the microbial inoculum is confirmed.

RESULTS

After 18 days, the analytical results² demonstrate that when exposed to municipal wastewater treatment sludge, 2.455 mg/L PFOS was not measurably degraded biotically or abiotically. Mass balance for PFOS test vessels was excellent and ranged from 104 - 108%.

CONCLUSIONS

No loss of PFOS was demonstrated. Mass balance was 104 - 108%. The results from this study confirm the results from other aerobic biodegradation studies of PFOS.

DATA QUALITY

Klimisch ranking = 2. The study was conducted as a non-GLP study but with the understanding that good data quality objectives be met.

Determination of analyte recovery from spiked sample matrices was not deemed necessary as they have been determined twice previously at several different concentrations, and in both instances recoveries were near 100% with some exceptions for M556. Methanol and sample (mineral medium) blanks contained no detected target analytes.

Analyses of the blank sludge controls (mineral media plus inoculum) at days 0 and 18 demonstrated that the inoculum source did not contain endogenous concentrations of test substance or its metabolites.

A calculation error was discovered. The PFOS molar conversion calculation should have used 538 ng/nmole rather than 522 ng/nmole. The corrected value for PFOS on page 13 of the report should be 92.9 nM.

REFERENCES

¹Biodegradation Study Report, "*The Aerobic Biodegradation of N-EtFOSE Alcohol by the Microbial Activity Present in Municipal Wastewater Treatment Sludge*", Contract Analytical Project ID: CA058, October 30, 2000. LIMS E00-2252

²Biodegradation Study Report, "*The 18-Day Aerobic Biodegradation Study of Perfluorooctanesulfonyl-Based Chemistries*", Contract Analytical Project ID: CA097, February 23, 2001.

Both of the above studies were conducted at the request of the 3M Company by Pace Analytical Services, Inc., Minneapolis, MN.

OTHER

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