BIODEGRADATION

TEST SUBSTANCE

Identity: N-ethylperfluorooctane sulfonamidoethanol; may also be referred to as N-EtFOSE alcohol. (1-Octanesulfonamide, N-ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-N-(2-hydroxyethyl)-, CAS # 1691-99-2).

Remarks: N-EtFOSE alcohol was a white powder originally from 3M production lot number 30107. It was HPLC purified. An Interim Certificate of Analysis, reports the purity to be 99.9%. All results in this study were calculated assuming 100% purity.

METHOD:

Method: Based on EPA Guidelines OPPTS 835.3200, OPPTS 835.3210, OPPTS 835.5045.
Test Type: Aerobic
GLP: No, but many GLP procedures followed
Year Completed: 2000
Contact time: 35 days

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. The suspended solids were allowed to settle overnight and the settled sludge, approximately 20% of the volume, was used to prepare cultures for the biodegradation study.
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 to the mineral salts medium. Fifty mL of settled sludge was added per liter of mineral salts medium.
Study design:

Blank Sludge Controls	(mineral medium, inoculum)
Sterile/inhibited Controls	(mineral medium, inoculum, N-EtFOSE alcohol, autoclaved, chloramphenicol added)
Abiotic Controls	(mineral medium, N-EtFOSE alcohol)
Positive Controls	(mineral medium, inoculum, sodium dodecyl sulfate)
Test Substance	(mineral medium, inoculum, N-EtFOSE alcohol)

Test vessels were set in triplicate. Additional quality control samples including analyte spikes and blanks were prepared and analyzed as appropriate.

Test concentrations: 0.047 mg/L and 2.380 mg/L N-EtFOSE alcohol.
Incubation conditions:
Temperature: 28^{o}C +/- 2^{o}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 either 5 µL of 11,900 mg/L solution of N-EtFOSE alcohol in methanol or 5 µL of 238 mg/L solution of N-EtFOSE alcohol in methanol yielding 2.380 mg/L and 0.047 mg/L solutions, respectively.

Sampling Frequency: Days 0, 1, 3, 7, 14, 21, 28 and 35.

Analytical method: The day zero test vessels were prepared and immediately placed in a freezer that was maintained at –19°C +/- 7°C. Upon removal from the incubator, test vessels on other days were either immediately frozen, or prepared for analysis by solid phase extraction (SPE). Following thawing, if needed, 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 the parent, N-EtFOSE alcohol, the additional compounds quantified are specified below. In the case of the compounds that are potassium or ammonia salts, only the concentration of the fluorochemcial anion was quantified and reported.

Compound Name
Acronym
Chemical Formula

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 sulfonamide
FOSA
$C_8F_{17}SO_2NH_2$

Perfluorooctane sulfinate, potassium salt
PFOSulfinate
$C_8F_{17}SO_2^- K^+$

Perfluorooctanoate, ammonium salt
PFOA
$C_7F_{15}COO^- NH_4^+$

Perfluorooctane sulfonate potassium salt
PFOS
$C_8F_{17}SO_3^- K^+$

RESULTS

The analytical results[1] demonstrate that when exposed to municipal wastewater treatment sludge the 0.047 mg/L N-EtFOSE alcohol samples completely degrade in 14 days and that 90% of the 2.380 mg/L samples degrade in 35 days. The formation of breakdown products can be followed through a series of oxidation and dealkylation reactions yielding quantifiable products. The measured metabolites observed in this study were: N-EtFOSAA, M556, N-EtFOSA, FOSA, PFOSulfinate, PFOA and PFOS. N-EtFOSAA and M556 together accounted for 85% of the total metabolic products observed in the day 35 test vessels.

CONCLUSIONS

The 35-day biodegradation of 0.047 mg/L and 2.380 mg/L N-EtFOSE alcohol by aerobic microorganisms present in municipal sludge, versus the abiotic and sterile/inhibited controls, demonstrated significant biodegradation. The major metabolites identified were N-EtFOSAA and M556, accounting for 84% of the total products observed in the Day 35 cultures. Minor metabolites include N-EtFOSA, FOSA, PFOSulfinate, PFOS and PFOA. Mass balance between parent material and measured products was achieved, implying that all significant degradation products of N-EtFOSE alcohol under conditions studied were identified.

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.

Analytical results for sterile/inhibited controls and no-sludge controls confirm that the observed biodegradation is due to the aerobic microorganisms present in the municipal sludge used for this study. Analyses of the blank sludge controls (mineral media plus inoculum) throughout the study demonstrated that the inoculum source did not contain endogenous concentrations of test substance or its metabolites. One blank control sample replicate on Day 21 showed very low concentrations of PFOS, M556, N-EtFOSAA and N-EtFOSE alcohol. It appears that that the wrong bottle was sampled in this event. These analytes were not noted in this replicate on days 28 or 35.

A calculation error was discovered subsequent to the issuance of the study report. The PFOS molar conversion calculation should have used 538 ng/nmole rather than 522 ng/nmole. The corrected values on page 15 of the 35-day study report should be 0.929 µM and 92.9 nM. This does not affect any of the reported results or conclusions.

REFERENCES

[1]Biodegradation 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

[2]Biodegradation Study Report, "*The 18-Day Aerobic Biodegradation Study of Perfluoroctanesulfonyl-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

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

Last changed: 6/21/01