NITRIFICATION INHIBITION

TEST SUBSTANCE

Identity: Mixture containing perfluorooctanesulfonate, which may also be referred to as PFOS, FC-95, or as a component of FC-203CF-X or FC-203CF. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot number was 501. The test sample is FC-203CF-X. Current information indicates it is a mixture of 1% PFOS, 20% diethylene glycol monobutyl ether, 70.2% water, 2.75% Acrylic foamer, 3% sodium octyl sulfate, 1% triethanolamine, 2% sulfonated alkyl ether salts, and 0.05% tolyltriazole.

The following summary applies to a mixture with incompletely characterized concentrations of impurities. Data may not accurately reflect inhibition by the fluorochemical component of the test sample.

METHOD

Method: ISO/DIS 9509 ISO Nitrification Inhibition Test
Test type: Microbial inhibition
GLP: No
Year Completed: 1995
Analytical monitoring: Oxidation of ammonia, pH and temperature.
Statistical methods: Not given.
Test organism source: Activated sludge mixed liquor collected from the Metro Wastewater Treatment Plant, St. Paul, MN (Prewashed and adjusted prior to use in the test).
Test condition:

Dilution water: Millipore Milli-Q™ water
Mineral medium: Per method.
Reference and test solution preparation: A stock solution of the reference substance, 1-allyl-2-thiourea, was prepared by dissolving 116 mg in 100 mL of Milli-Q™ water. Test substance was added directly to test vessels.
Test vessels: 500 mL glass Erlenmeyer flasks with a total test volume of 250 mL.
Number of concentrations: four plus 1 reference substance and blank control
Temperature: 19.8 – 20.6 °C
Total suspended solids and pH on day of testing: Initial TSS 4.98 g/L which was adjusted to 2.97 g/L. TSS in the final test solution was 1.49 g/L. Initial pH 7.9. Blank control at 0-hr. pH 8.1 and at 4-hr. 8.5..
Element Basis: Reduction in nitrification.

RESULTS

Nominal concentrations: Blank control, 82, 118, 252, and 496 mg/L

Element values: 4-hour EC_{50} = >496 mg/L

Element values based on nominal concentrations.

Remarks: Testing was conducted on the mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical proportion alone.

CONCLUSIONS

The test substance 4-hour EC_{50} for ISO Nitrification Inhibition test was >496 mg/L, the highest concentration tested. This concentration did not have an impact on nitrifying organisms from activated sludge.

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

DATA QUALITY

Reliability: Klimisch ranking 2. Testing meets all criteria for quality testing. However, the sample purity was not properly characterized and the study lacks analytical confirmation of the amount of fluorochemical proportion in the solution.

REFERENCES

This study was conducted by the 3M Environmental Laboratory, St Paul, MN, Lab Request number N2977, 1995.

OTHER

Last changed: 6/28/00

LR N2977
Date 8/24/95
Analyst SAB
Page 1 of 4

ENVIRONMENTAL LABORATORY

ACTIVATED SLUDGE MIXED LIQUOR

Type Test Isc: Activated Sludge Nitrification Inhibition ISO/DIS 9509

ACTIVATED SLUDGE MIXED LIQUOR

Date Obtained 8/21/95 Date Used 8/24/95

Mixed Liquor Suspended Solids (MLSS) 4.98 g/L Adjusted to 2.97 g/L

MLSS in final test solution 1.49 g/L

Initial pH 7.9 (1st test soln. prepared) Final pH 7.91 (last soln. prepared)

Comments _____

TEST SUBSTANCE

Sample Description FC-3030F-X

Initial pH of highest conc. tested (496 mg/L) = 8.2 Adjusted to pH _____

Comments _____

REFERENCE SUBSTANCE

Sample Description 1-Allyl-2-Thiourea Aldrich Red Label 99% Lot 03213LM

Initial pH of stock solution (11.6 mg/L) = 11.4 Adjusted to pH N.A

Comments _____

EXPOSURE CONDITIONS

Test Duration 4-Hours, in the dark

Test Temperature Range: 19.8 - 20.6 °C

Comments _____

ISO Activated Sludge Nitrification Inhibition Test
ISO/DIS 9509

Lab Request No. N 3977

Environmental Lab — Work Sheet

Date: 8/24/95

Analyst: SJB

Sample Description
FC-203CF-X
Lot 501

Test Solutions Preparation

Reference Inhibitor: 1-Allyl-2-thiourea — 1.16 g/L; Each 1.0 mL = 1.16 mg
Dissolved 116 mg in 100 mL Milli-Q water

Microbial Inoculum: Initial pH = 6.1; MLSS = 4980 mg/L; Washed pH = 7.9; Washed MLSS = 4950 mg/L
Want 3.0 g MLSS/L:
$\frac{4950\ mg}{1000\ mL} = \frac{3000\ mg}{x}$ x = 602.41 mL
Want 1000 mL sludge; so wash 602 mL sludge
Diluted washed sludge to 1000 mL with carbon filtered well water

Mineral Medium: 5.04 g $NaHCO_3$ + 2.65 g $(NH_4)_2SO_4$ / L Milli-Q water

Test Solutions: FC-203CF-X added by direct weights

Test Conc, mg/L	mg/250 mL	mL Milli-Q water	mL Medium	Microbial Inoculum, mL	Total Volume, mL
Blank Control	0	100 (SJB 8/24/95)	25	125	250
81.6	20.4	↓	↓	↓	↓
118.4	29.6	↓	↓	↓	↓
251.6	62.9	↓	↓	↓	↓
496.4	124.1	↓	↓	↓	↓

ATU = 2.9 mg/L, 2.5 mL stock soln + 97.5 mL + 25 mL medium + 125 mL inoculum

BEST COPY AVAILABLE

001289

ENVIRONMENTAL LABORATORY

ISO NITRIFICATION INHIBITION TESTING (ISO/DIS 9509)

RAW DATA

LR N.2977

DATE 8/24/15

ANALYST SMB

TEST SUBSTANCE. EC 3035F-X

READINGS IN TEST SOLUTIONS AT EXPOSURE TIMES - OXIDATION OF AMMONIA

EXPOSURE TIME - HOURS

TEST SUBSTANCE CONCENTRATION mg/L	0-HRS NH3 mg/L	0-HRS pH	0-HRS Temp. °C	2-HRS NH3 mg/L	2-HRS pH	2-HRS Temp. °C	3-HRS NH3 mg/L	3-HRS pH	3-HRS Temp. °C	4-HRS NH3 mg/L	4-HRS pH	4-HRS Temp. °C
BLANK CONTROL	149	8.1	20.2	136	8.5	20.4				104	8.5	20.5
8.2	143	8.1	20.1	141	8.5	20.5				94	8.6	20.3
118	148	8.1	20.6	146	8.5	20.6				74	8.6	20.4
252	147	8.1	20.4	125	8.6	20.2				83	8.6	19.9
496	153	8.2	20.3	122	8.6	19.9				84	8.6	19.8
1111	160	8.1	20.4	141	8.6	20.3				130	8.7	20.1

ISO ACTIVATED SLUDGE

NITRIF CATION INHIBITION TEST ISO/DIS 9509

CALCULATION OF RESULTS

LR N 3977

DATE 8/24/95

ANALYST SAB

Pg 4 of 4

EXPOSURE PERIOD

TEST CONC.	0-HRS NH_3(1) mg/L	2-HRS NH_3 mg/L	2-HRS % Inhibition(2)	4-HRS NH_3 mg/L	4-HRS % Inhibition	NH_3 mg/L	% Inhibition
Blank Control	149	136	-	104	-		
82	142	141	38	94	0		
118	148	146	77	79	0		
232	147	125	0	83	0		
496	153	123	0	84	0		
ATU	160	141	38	130	58		
EC50 (95% CONFIDENCE INTERVAL)(3)							

(1) REFER TO PG. 3

(2) PERCENT INHIBITION OF NITRIFICATION = $\left(\frac{Ct - Cc}{Co - Cc}\right) \times 100$

Where: Ct = NH_3, mg/L in test flask at time t
Cc = NH_3, mg/L in control flask at time t
Co = NH_3, mg/L in control flask at time 0

(3) EC_{50} (95% C.I.) calculated using: > 496 mg/L

SPECIFIC RATE OF NITRIFICATION (R) = $\left(\frac{Cr - Cc}{MLSS \times 4}\right)$ R = 4.36

Where: Cr = NH_3, mg/L in inhibited (ATU) flask, at 4-hrs
Cc = NH_3, mg/L in control flask at 4-hrs
MLSS = Mixed Liquor Suspended Solids, g/L, in test flask

Form 23219 - PWO

Total Suspended Solids [TS] [TSS] [TDS] LRN2977

3M Environmental Laboratory - Raw Data Sheet

No. Tests	
No. Hours	
Analyst and Date Analyze	SAB 8/
QA Check	

Method Reference: Std. Methods (14th ed.) pp. 89-98, sec. 208

Calculation: mg/l Residue = $\frac{\text{mg residue} \times 1000}{\text{ml sample}}$

Sample Description	Reported Result mg/l	ml Sample	Gross Weight after 103 °C	Filter Paper + Pan Crucible Weight (g)	Residue Weight (g)	Residue mg/l	
1 Sludge As-Is		5.0	1.6913	1.6663	0.025	5000	
2 Sludge As-Is		5.0	1.6938	1.6693	0.0245	4900	
3 Sludge As-Is		5.0	1.6825	1.6573	0.0252	5040	
4 Sludge Adj + wash		5.0	1.6687	1.6537	0.0150	3000	
5 Sludge Adj + wash		5.0	1.6841	1.6692	0.0149	2980	
6 Sludge Adj + wash		5.0	1.6688	1.6541	0.0147	2940	
7 N-Seed in test flask		10	1.6632	1.6513	0.0119	1190	
8 ↓		10	1.6680	1.6565	0.0115	1150	
9 ↓		10	1.6634	1.6522	0.0112	1120	

Total Suspended Solids [TS] [TSS] [TDS] LRN2977

3M Environmental Laboratory - Raw Data Sheet

No. Tests	
No. Hours	
Analyst and Date Analyzed	SAB 8/24/95
QA Check	

ds (14th ed.) pp. 89-98, sec. 208

$$\frac{\text{mg residue} \times 1000}{\text{ml sample}}$$

rted Result mg/l	ml Sample	Gross Weight after 103 °C	Filter Paper + Pan Crucible Weight (g)	Residue Weight (g)	Residue mg/l	Comments
	5.0	1.6913	1.6663	0.025	5000	$\bar{x}$ = 4980 mg/L
	5.0	1.6938	1.6693	0.0245	4900	SD = ± 73.11
	5.0	1.6825	1.6573	0.0252	5040	
	5.0	1.6687	1.6537	0.0150	3000	$\bar{x}$ = 2973 mg/L
	5.0	1.6841	1.6692	0.0149	2980	SD = ± 30.6
	5.0	1.6688	1.6541	0.0147	2940	
	10	1.6632	1.6513	0.0119	1190	$\bar{x}$ = 1153 mg/L
	10	1.6580	1.6565	0.0115	1150	SD = ± 33.1
	10	1.6634	1.6522	0.0112	1120	