

BIODEGRADATION (OECD 301D)

TEST SUBSTANCE

Identity: A mixture containing perfluorooctanesulfonate, which may also be referred to as PFOS, FC-95, or as a component of FC-780B. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot number was 2. The test sample is FC-780B. Current information indicates it is a mixture of 0.58% PFOS, 14% diethylene glycol monobutyl ether, 76.7% water, 5.5% urea, 1.17% Hydroxy foamer, 1.15% sodium octyl sulfate, 0.8% polyoxyethylene monooctylphenyl ether, 0.05% sodium lauryl sulfate, and 0.05% benzotriazole.

The following summary applies to a mixture with incompletely characterized concentrations of impurities. Data does not accurately reflect degradation potential of the fluorochemical component of the test sample.

METHOD

Method: OECD Closed Bottle Test, OECD Guideline 301D and C 117/79/GER

Test type: Ready Biodegradability

GLP: No

Year Completed: 1980

Analytical monitoring: Dissolved oxygen depletion

Statistical methods: Results were determined by calculation of the % BOD/COD over time

Test organism source: Non-pasturized field soil obtained from 3M Agricultural greenhouse, St. Paul, MN

Test condition:

Dilution water: Deionized water and distilled water.

Reference and test solution preparation: The dilution water was prepared using Hach BOD Slurry Pillows. Nitrification inhibitor, Hach Formula 2533, was added to replicate dilutions of blanks and samples at the recommended concentration of 0.1 g/300 mL.

Test vessels: Not given.

Number of concentrations: 1 plus 2 reference substances, and blank, all in duplicate.

Temperature: 20°C

Total suspended solids and pH on day of testing: Not given.

Element Basis: Decrease in oxygen depletion compared to the blanks.

RESULTS

Nominal concentrations: Blank control, LAS and sodium dodecyl sulfate (reference substances), and test material.

Element values:	5-Day BOD/COD:	21%
	15-Day BOD/COD:	78%
	28-Day BOD/COD:	72%

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 % BOD/COD was 72 after 28 days. The % BOD/COD with nitrification inhibited was 83 after 28 days.

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

DATA QUALITY

Reliability: Klimisch ranking = 3. Study lacks description and records of the method followed. The inoculum is not properly characterized. The sample purity was not properly characterized and the study lacks analytical confirmation of the amount of fluorochemical proportion in the solution.

REFERENCES

The study was conducted by the 3M Company, Environmental Laboratory, St. Paul, MN, Lab Request number 6211S, 1981.

OTHER

Last changed: 6/28/00

Test Guideline C 117/79/GER

Test Guideline for the Closed Bottle Test acc. to Fischer

Date of last revision: August 27, 1979

Level I test for ready biodegradability

1. Prerequisites

*if shall
be soluble*

It has to be known whether the test material is soluble in the concentration range employed in the test (2 - 10 mg/l). If it is not, special measures such as the addition of solubilizing agents, ultrasound dispersion etc may have to be applied.

2. Guidance Information

*is shall
not be a
problem*

Knowledge of the bacterial toxicity or inhibitory properties of the test material is not unequivocally required but constitutes useful information.

3. Qualifying Statements

*is a
refinement but
will not be
needed any way*

The test is applicable for the biodegradability evaluation of organic compounds but not for mixtures thereof. Most experience has been gathered with water soluble compounds, however, the test lends itself, at least in principle, also to the biodegradability evaluation of volatile and insoluble compounds.

August 27, 1979

OECD Chemicals Testing Programme
 Test Guideline for the Closed Bottle
 Test acc. to Fischer

0. The test procedure is based on a paper by W.K. Fischer, P. Gerike, and R. Schmid in Wasser- und Abwasserforschung 7, 99 -118 (1974).

1. Method

- 1.1 Introduction: Purpose, scope, relevance, and application of test and explanation of limits.

The purpose of the method is the measurement of the biodegradability of organic compounds in an aerobic, aqueous medium at a test concentration of 2 (standard concentration) to 10 mg/l of active material. Most data elaborated with this test pertain to water-soluble compounds, however, volatile compounds and those of low water solubility may also, at least in principle, be tested.

1.2 Definitons and units

1.2.1 Definition of biodegradability

$$\% \text{ BODT} = \frac{\text{mg O}_2 / \text{mg AS}}{\text{BODT}} \times 100$$

$$\% \text{ BOD/CCD} = \frac{\text{mg O}_2 / \text{mg AS}}{\text{mg COD/mg AS}} \times 100$$

← We can't calculate %BODT
 ← We will use

ENVIRONMENTAL ENGR. LAB - WORK SHEET

Notes

SAMPLE DESCRIPTION

*Closed Bottle Test*Date: 10-31-80Analyst: Wade A. Schul

Hrs.: _____

1. The dilution water (mineral nutrient solution) was prepared using HACH BOD Slurry Pillows.

2. Nitrification inhibitor - HACH FORMULA 2533, was added to replicate dilutions of blanks and samples at the recommended concentration of 0.1g/300ml.

Reviewed by: _____

002538

Lab request no.: 6211S
Date of start of test: 10-31-80 *EC780 B lot 2*
Analyst: W. A. Scheil
Test / standard material: AFFF-C Theoretical test conc.: 14.7 mg AS/l
BODT of test material: 0.34 mg O₂/mg AS COO of test material: 0.34 mg O₂/mg AS (AS = active substance)
Temp. of the dilution water after aeration: 293 K (20 °C)
O₂-conc. of the distilled (deionized) water after aeration and standing before the start of test: 8.95 mg O₂/l
Dissolved Oxygen analysis: Oxygen electrode method calibrated against the Winkler method
Inoculum: Unfertilized garden soil

A: O₂ DETERMINATIONS:

CULTURE MEDIUM	Flask no.	Analyses	mg O ₂ /l AFTER X DAYS			
			0	5	15	28
Mineral nutrient solution without test material and without inoculum.	1	C ₁	9.10	8.65	8.50	6.90
	2	C ₂	9.10	8.70	8.55	7.20
	3	C ₃	9.10	8.75	8.55	6.85
	4*	C ₄ *	9.10*	8.70*	8.50*	8.25*
	mean control	$m_o = \frac{C_1 + C_2 \dots C_n}{n}$	9.10	8.70	8.53	6.98
Mineral nutrient solution without test material but with inoculum.	1	B ₁	9.10	8.75	8.50	6.80
	2	B ₂	9.10	8.70	8.50	6.90
	3	B ₃	9.15	8.70	8.55	7.10
	4 *	B ₄ *	9.10*	8.75*	8.50*	8.20*
	mean blank	$m_b = \frac{B_1 + B_2 \dots B_n}{n}$	9.12	8.72	8.52	6.93
Mineral nutrient solution with test material and with inoculum.	1	A ₁	9.10	7.70	4.60	4.15
	2	A ₂	9.10	7.60	4.55	2.50
	3 *	A ₃ *	9.10*	8.50*	5.20 *	4.10 *
	4	A ₄				
	mean test material	$m_t = \frac{A_1 + A_2 \dots A_n}{n}$	9.10	7.65	4.58	3.32

*Values not included the mean values; these dilutions contain nitrification inhibitors

*See note above.

B: O₂ DEPLETIONS (mg BOD/l) AFTER X DAYS:

$$BOD_x = (m_o - m_{t_x}) - (m_o - m_{b_x})^*$$

* This difference is important as a check for the validity of the test (see 1.6.3.1.3).

$$MODIFIED FORMULA: BOD_x = (m_{t_o} - m_{t_x}) - (m_{b_o} - m_{b_x})^*$$

The modified formula uses the measured initial O₂ conc. of the test solutions and blanks in place of m_o.

C: EVALUATION:

$$\% BODT = \frac{\text{mg BOD}_x / l}{\text{mg AS} / l \cdot BODT} \cdot 100$$

$$OR \quad \% BOD_x / COO = \frac{\text{mg BOD}_x / l}{\text{mg AS} / l \cdot COO} \cdot 100$$

	after days		
	5	15	28
% BODT _x			
% BOD/COO	21	78	72

mg BOD _x /l after x days		
5	15	28
1.05	3.92	3.59

% BOD/COO nitrification inhibited 82
COMMENTS:

The sample is considered to be 100% active (1mg sample = 1mg AS).

Lab request no.: 6211S

Date of start of test: 10-31-80

Analyst: W. A. Scheil

Test / standard material: DODECYL SULFATE, SODIUM SALT Theoretical test conc.: 2.0 mg AS/l

(AS = active substance)

BODT of test material: mg O₂/mg AS COD of test material: 2.0 mg O₂/mg AS

Temp. of the dilution water after aeration: 293 K (20 °C)

O₂-conc. of the distilled (deionized) water after aeration and standing before the start of test: 8.95 mg O₂/l

Dissolved Oxygen analysis: Oxygen electrode method calibrated against the Winkler method

Inoculum: Unfertilized garden soil

A: O₂ DETERMINATIONS:

CULTURE MEDIUM	Flask no.	Analyses	mg O ₂ /l AFTER X DAYS			
			0	5	15	28
Mineral nutrient solution without test material and without inoculum.	1	C ₁	9.10	8.65	8.50	6.90
	2	C ₂	9.10	8.70	8.55	7.20
	3	C ₃	9.10	8.75	8.55	6.85
	4*	C ₄ *	9.10*	8.70*	8.50*	8.25*
	mean control	$m_o = \frac{C_1 + C_2 \dots C_n}{n}$	9.10	8.70	8.53	6.98
Mineral nutrient solution without test material but with inoculum.	1	B ₁	9.10	8.75	8.50	6.80
	2	B ₂	9.10	8.70	8.50	6.90
	3	B ₃	9.15	8.70	8.55	7.10
	4 *	B ₄ *	9.10*	8.75*	8.50*	8.20*
	mean blank	$m_b = \frac{B_1 + B_2 \dots B_n}{n}$	9.12	8.72	8.52	6.93
Mineral nutrient solution with test material and with inoculum.	1	A ₁	9.10	6.40	5.40	4.10
	2	A ₂	9.10	6.30	5.70	3.60
	3 *	A ₃ *	9.05*	6.30*	5.45 *	4.80 *
	4	A ₄				
	mean test material	$m_t = \frac{A_1 + A_2 \dots A_n}{n}$	9.10	6.35	5.55	3.85

*Values not included the mean values; these dilutions contain nitrification inhibitor

*See note above.

B: O₂ DEPLETIONS (mg BOD/l) AFTER X DAYS:

$$BOD_x = (m_o - m_{t_x}) - (m_o - m_{b_x})^*$$

$$MODIFIED FORMULA: BOD_x = (m_{t_o} - m_{t_x}) - (m_{b_o} - m_{b_x})^*$$

* This difference is important as a check for the validity of the test (see 1.6.3.1.3).

The modified formula uses the measured initial O₂ conc. of the test solutions and blanks in place of m_o.

C: EVALUATION:

$$\% BODT = \frac{\text{mg BOD}_x/\text{l}}{\text{mg AS/l} \cdot BODT} \cdot 100$$

$$OR \quad \% BOD_x/COD = \frac{\text{mg BOD}_x/\text{l}}{\text{mg AS/l} \cdot COD} \cdot 100$$

	after days		
	5	15	28
% BODT _x			
% BOD/COD	59	74	76

mg BOD _x /l after X days		
5	15	28
2.35	2.95	3.06

nitritification inhibited
COMMENTS:

83

Lab request no.: 6211S

Date of start of test: 10-31-80 Analyst: W. A. Scheil

Test / standard material: LAS REFERENCE SAMPLE Theoretical test conc.: 2.52 mg AS/l
(AS = active substance)
BODT of test material: 2.24 mg O₂/mg AS COD of test material: 2.45 mg O₂/mg AS

Temp. of the dilution water after aeration: 293 K (20 °C)

O₂-conc. of the distilled (deionized) water after aeration and standing before the start of test: 8.95 mg O₂/l

Dissolved Oxygen analysis: Oxygen electrode method calibrated against the Winkler method

Inoculum: Unfertilized garden soil

A: O₂ DETERMINATIONS:

CULTURE MEDIUM	Flask no.	Analyses	mg O ₂ /l AFTER X DAYS			
			0	5	15	28
Mineral nutrient solution without test material and without inoculum.	1	C ₁	9.10	8.65	8.50	6.90
	2	C ₂	9.10	8.70	8.55	7.20
	3	C ₃	9.10	8.75	8.55	6.85
	4*	C ₄ *	9.10*	8.70*	8.50*	8.25*
	mean control	$m_o = \frac{C_1 + C_2 \dots C_n}{n}$	9.10	8.70	8.53	6.98
Mineral nutrient solution without test material but with inoculum.	1	B ₁	9.10	8.75	8.50	6.80
	2	B ₂	9.10	8.70	8.50	6.90
	3	B ₃	9.15	8.70	8.55	7.10
	4 *	B ₄ *	9.10*	8.75*	8.50*	8.20*
	mean blank	$m_b = \frac{B_1 + B_2 \dots B_n}{n}$	9.12	8.72	8.52	6.93
Mineral nutrient solution with test material and with inoculum.	1	A ₁	9.10	8.65	6.05	3.70
	2	A ₂	9.10	8.65	6.10	4.55
	3 *	A ₃ *	9.05*	8.60*	6.50 *	4.85 *
	4	A ₄				
	mean test material	$m_t = \frac{A_1 + A_2 \dots A_n}{n}$	9.10	8.65	6.08	4.12

*Values not included the mean values; these dilutions contain nitrification inhibitors

*See note above.

B: O₂ DEPLETIONS (mg BOD/l) AFTER X DAYS:

$$BOD_x = (m_o - m_{t_x}) - (m_o - m_{b_x})^*$$

* This difference is important as a check for the validity of the test (see 1.6.3.1.3).

$$\text{MODIFIED FORMULA: } BOD_x = (m_{t_o} - m_{t_x}) - (m_{b_o} - m_{b_x})^*$$

The modified formula uses the measured initial O₂ conc. of the test solutions and blanks in place of m_o.

C: EVALUATION:

$$\% \text{ BODT} = \frac{\text{mg BOD}_x/\text{l}}{\text{mg AS/l} \cdot \text{BODT}} \cdot 100$$

$$\text{OR } \% \text{ BOD}_x/\text{COD} = \frac{\text{mg BOD}_x/\text{l}}{\text{mg AS/l} \cdot \text{COD}} \cdot 100$$

	after days		
	5	15	28
% BODT _x	0.9	43	49
% BOD/COD	0.8	39	45

mg BOD _x /l after X days		
5	15	28
0.05	2.42	2.79

% BOD/COD nitrification inhibited, 53
COMMENTS:

- The BODT of the LAS Reference sample was calculated by using the average M.W. of 318.
- LAS Reference Sample, Lot # (7181, Date 715, avg. M.W = 318, 5.55% active LAS; Prepared by MDQARL, NERC - Cincinnati, US-EPA in cooperation with the Soap and Detergent Association. 002541

3M Environmental Laboratory

FILE LR # 62115

Data Sheet

ANALYST:

ENG.

W. A. Schiefel
M. Roberts

DATE

SHEET

'Closed Bottle Test'

Date of start of Test: 10/31

(dissolved oxygen readings - mg/l O₂)

ANALYSIS FORM

TEST SOLUTIONS		BOTTLE NO.			DAY 0 (10-11-80)	DAY 5 (11-05-80)	DAY 15	DAY 28 (11/28/80)				
1	AFFF-A	54			9.05	7.35	4.50	2.40				
2	1.0 ml stock solution diluted	14			9.05	7.75	4.65	2.30				
3	to 1.0 l with inoculated	3*			9.05	8.15	4.00	3.35				
4	dilution H ₂ O.											
5												
6												
7												
8	AFFF-B	75			9.00	7.00	5.55	3.65				
9	1.0 ml stock solution diluted	14			9.05	7.20	5.65	3.90				
10	to 1.0 l with inoculated	74*			9.05	7.70	5.65	5.05				
11	dilution H ₂ O.											
12												
13												
14	AFFF-C	51			9.10	7.70	4.60	4.15				
15	1.0 ml stock solution	76			9.10	7.60	4.55	2.50				
16	diluted to 1.0 l with	7*			9.10	8.50	5.20	4.10				
17	inoculated dilution H ₂ O.											
18												
19	Dodecyl sulfate, sodium salt	140			9.10	6.40	5.40	4.10				
20	1.0 ml of stock solution	15			9.10	6.30	5.70	3.60				
21	diluted to 1.0 l with	96*			9.05	6.30	5.45	4.80				
22	inoculated dilution H ₂ O.											
23												
24												
25	LAS Reference sample	94(48)			9.10	8.65	6.05	3.70				
26	1.0 ml stock solution diluted	61			9.10	8.65	6.10	4.55				
27	to 1.0 ml with inoculated	19*			9.05	8.60	6.50	4.85				
28	dilution H ₂ O.											
29												
30												
31	Dilution H ₂ O - with	28	0		9.10	8.75	8.58	6.80				
32	inoculum, but without	2			9.10	8.70	8.50	6.90				
33	test material.	85			9.15	8.70	8.55	7.10				
34		82*			9.10	8.75	8.50	8.20				
35												
36	Dilution H ₂ O without	68			9.10	8.65	8.50	6.90				
37	inoculum and without	17			9.10	8.70	8.55	7.20				
38	test material.	41			9.10	8.75	8.55	6.85				
39		92*			9.10	8.70	8.50	8.25				
40												
41	DO concentration of DI. H ₂ O											
42	after aeration and standing	8.95										
43	before start of test											
44												
45												

BEST COPY AVAILABLE

* BOTTLES CONTAINING NITRIFICATION
INHIBITOR (NACH FORMULA 2533)

002542

BEST COPY AVAILABLE

File: Page No. 62115

ENVIRONMENTAL ENGR. LAB - WORK SHEET

SAMPLE DESCRIPTION

Closed Bottle Test

Start Date: 10-31-80Analyst: W. A. Schief

Hrs.: _____

Extra Blanks

Environmental Lab DI H₂O (Not Distilled) with nutrients and with inoculum.

Bottle #	Day 0	Day 5	Day 15	Day 28
94	9.10	8.65	8.40	8.00
98	9.15	8.80	8.50	6.85
74	9.20	8.80	8.45	6.85
9*	9.20	8.80	8.50	8.10

Env. Lab DI H₂O (distilled) with nutrients and with inoculum

Bottle #	Day 0	Day 28
119	9.00	7.15
78	9.10	7.70
17	9.00	8.30
86*	9.10	8.30
4*	9.10	8.35
6*	9.10	8.30

This series only opened on day 28.

* Bottles containing nitrification inhibitor -
HACH - formula 2533, 0.1g/300 ml.

Reviewed by: _____

002543

BEST COPY AVAILABLE

LR#
File: Page No. 62115

ENVIRONMENTAL ENGR. LAB - WORK SHEET

SAMPLE DESCRIPTION

Date: 10-31-80

Analyst: W. G. Schell

Hrs.: _____

Preparation of Stock Solutions used in
the 'Closed Bottle Test' - OECD, Tokyo, 12-01-79, C 117/79 / GAGER

The following amounts of each sample were
separately diluted to 100 ml in a volumetric
flask with deionized water:

Sample Description	grams sample per 100ml	mg sample per ml stock	mg COD per 1.0 ml stock solution
AFFF - A	1.54	15.4	6.0
AFFF - B	2.14	21.4	6.0
AFFF - C	1.47	14.7	5.0
Dodecyl sulfate, ⁽¹⁾ sodium salt	0.200	2.0	4.0
LAS Reference sample ⁽²⁾	4.54	45.4	6.2 OR (2.5 mg A

prepared 10-31-80 by W. G. Schell

Date of Start of Test - 10-31-80

(1) Dodecyl sulfate, sodium salt (sodium lauryl sulfate), Aldrich Chem. Co.,
Lot # BE, MW 288.28, $\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}$.

(2) LAS REFERENCE sample, lot # (7181, Date 715, avg MW 318, Active LAS 5.5
Prepared by MDQARL, NERC - Cincinnati, US-EPA in
cooperation with the Soap & Detergent Assoc.

Reviewed by: _____

002544