

BIODEGRADATION (MANOMETRIC RESPIROMETRY – ANAEROBIC)

TEST SUBSTANCE

Identity: A mixture containing perfluorooctanesulfonate;, which may also be referred to as PFOS, FC-95, or as a component of FC-3041. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot number was not noted. The test sample is FC-3041. Current information indicates it is a mixture of 6% PFOS, 36% diethylene glycol butyl ether, 36.05% water, 12% Sultone foamer, 9% sodium octyl sulfate, 0.55% sodium lauryl sulfate, and 0.4% tolyltriazole.

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

METHOD

Method: Manometric Respirometry Test

Test type: Anaerobic biodegradability

GLP: No

Year Completed: 1993

Analytical monitoring: Determination of the mixture ratio of methane and carbon dioxide released in the gas phase by gas chromatography, dissolved organic carbon (DOC), and the release of fluoride by fluoride selective electrode and pH values.

Statistical methods: Results were determined by calculation of the percentage biodegradation.

Inoculum source: Anaerobic sludge collected from a community sewage treatment plant (Lennestadt-Maumke, Germany) that was fed almost exclusively with residential wastewater.

Test condition:

Dilution water: Not noted.

Mineral Nutrient Medium: Mineral Basal Medium.

Stock and test solution preparation: Solutions of the test and reference materials were prepared at 100 mg C/L and 50 mg C/L, respectively, with mineral basal medium.

Test vessels: 118 mL serum flasks which contained a liquid phase volume of 40 mL, closed with butyl septa and the gas phase purged with nitrogen.

Incubator Conditions: In the dark.

Temperature: 37 ± 2 °C

Agitation: None

Number of concentrations: Test sample, test sample inhibited, sodium benzoate (reference substance) and blank inoculum, all in triplicate.

Inoculum condition on test initiation: 0.79 g/L dry weight present in test samples.

Element Basis: Biological mineralization by means of the release of methane and carbon dioxide.

Test substance flask conditions:

pH: Not noted in the report.

RESULTS

Nominal concentrations: Blank control, test material at 100 mg C/L and sodium benzoate (reference substance) at 50 mg C/L.

Element values: 60-day % Degradation by Mineralization (methane and carbon dioxide) in test vessels 7, 8*, and 9 were 78.5, 20.7 and 91 respectively.
60-day % Degradation by Determination of DOC in test vessels 7, 8*, and 9 were 81.5, 25.2 and 90.5 respectively.
Fluoride release, by selective fluoride electrode, was not detected in any of the test samples (detection limit 2 µg F/L).
*Could not confirm what caused the low % degradation in this test vessel.

Remarks: Testing was conducted on a 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 under anaerobic conditions was categorized as biologically degradable.

The reference substance obtained 89.5% degradation during the incubation period of 60 days as determined by mineralization (108% by DOC), thus the validation criteria of the test was fulfilled (>60% degradation in sodium benzoate in 60 days).

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DATA QUALITY

Reliability: Klimisch ranking 3. Lacks specific methodology used and documentation. Sample purity was not properly characterized and the study lacks analytical confirmation of the amount of fluorochemical proportion in the solution.

REFERENCES

The studies were conducted by Fraunhofer Institute for Environmental Chemistry and Ecotoxicology, D-57377 Schmallenberg, Germany at the request of the Federal Bureau for Material Technology and Acquisitions, Germany, Grant No.: E/B31E/P0078/N52275, 1993.

OTHER

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FINAL REPORT

**ECO-TOXICOLOGICAL EFFECT OF
FLUORO-SURFACTANTS**

Grant No.:

E/B31E/P0078/N5275

Client:

**Bundesamt für Werktechnik und Beschaffung, Koblenz
(Federal Bureau for Material Technology and Acquisition)**

Investigating Institute:

**Fraunhofer Institute
for Environmental Chemistry and Eco-toxicology
D-57377 Schmallenberg**

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December 1993

**Fraunhofer Institute
for Environmental Chemistry and Eco-toxicology
D-57377 Schmallenberg**

**Investigation for
the Eco-toxicological Evaluation of the Fluoro-surfactant Base FC-3041**

Anaerobic Degradation

Part g

by

Dr. A. Remde

ANAEROBIC DEGRADATION

The determination of the biological degradability of Base FC-3041 under anaerobic conditions was done in the manometric-respirometry test (9).

MATERIAL AND METHODS

Test substance:

Base FC-3041 (combination of fluoro-surfactants in aqueous solution;
Manufacturer: 3M Company Germany, Neuss).

Test criterion:

Measurement of the biological mineralization by means of the release of methane and carbon dioxide.

Test method:

A defined volume (40 ml) of an inoculated mineral medium with a known concentration of the test substance (100 mg/L) is incubated in the dark in a closed serum flask (volume of 118 ml) at a constant temperature ($37^{\circ}\text{C} \pm 2$). During the incubation period of 60 days the release of methane and carbon dioxide is determined by gas chromatography by extracting regularly 0.5 - 1 ml samples from the gas phase of the test run.

Test procedure:

15 test vessels (serum flasks with a volume of 118 ml, of which 40 ml liquid phase) were prepared for the performance of the test:

Vessel 1, 2 and 3:	mineral basal medium + inoculum
Vessel 4, 5 and 6:	mineral basal medium + reference substance (sodium benzoate 50 mg C/L) + inoculum
Vessel 7, 8 and 9:	mineral basal medium + test substance (100 mg C/L) + inoculum (30 mg solid matter/L)

Vessel 10, 11 and 12: mineral basal medium + test substance (100 mg C/L)
+ inoculum + mercury chloride solution (0.001% final
concentration)

The serum flasks were closed with butylsepta and the gas phase purged with nitrogen. Then the determination of the methane and carbon dioxide content in the gas phase occurred. During the incubation period of 60 days at $37 \pm 2^\circ\text{C}$ gas samples of 0.5 - 1 ml were taken out at regular intervals and the mixture ratio of methane and carbon dioxide in the gas phase was determined. The total amount of the gases released (CCH_4 and CCO_2) was determined through the addition of the concentrations of the gases in the gas phase and the calculated concentration of the gases dissolved in the liquid phase (10).

The total amount of the mineralized carbon ($C_{\min}$) at a certain time of measurement ($t=n$) is calculated from the sum of the release of methane (CCH_4) and carbon dioxide (CCO_2) in the duration of incubation of 60 days, following the equation (a).

$$\text{a)} \quad C_{\min} = [(C_{\text{CH}_4})_{t=n} - (C_{\text{CH}_4})_{t=0}] + [(C_{\text{CO}_2})_{t=n} - (C_{\text{CO}_2})_{t=0}]$$

The bio-degradation was determined according to the following equation (b):

$$\text{b)} \quad \text{Percentage bio-degradation} = \frac{C_{\min}}{C_{\text{TS}}} \times 100$$

$C_{\min}$ = Total amount of the mineralized carbon in mg C

C_{TS} = Total amount of the test substance used in mg C

Additionally, at the start and end of the experiments the pH-value, the dissolved organic carbon and the fluoride concentration in the aqueous phase of the test runs were determined.

Inoculum:

An anaerobic sludge from a community sewage treatment plant (Lennestadt-Maumke) which was fed almost exclusively with residential waste water, was used as inoculum. The sludge was thermostated during the transport. 4 ml sludge were pipetted into the test samples, such that a dry weight of 0.79 g/L was present in the test samples. The sample collection occurred on 9/28/1993.

Analysis:

The determination of the mixture ratio of methane and carbon dioxide in the gas phase was done in a Shimadzu 8A gas chromatograph with a thermal conductivity detector over a Porapak-N analytical separation column. The analysis of the dissolved organic carbon was done in a TOC (Total Organic Carbon Analysis) 500 of the Shimadzu Company. The fluoride was determined by means of a fluoride selective electrode (Orion 69-90).

Reference substance: sodium benzoate (Merck, reagent grade)

Test period: 9/30/1993 - 11/29/1993

RESULTS

During the incubation period of 60 days, 89.5% of the reference substance sodium benzoate was degraded (Tab. g1, Fig. g1). With that, the validity criterion of the test, a degradation of sodium benzoate >60% in 60 days, was fulfilled.

The degradation of BASE FC-3041 was very different in the three test samples started in parallel to each other (Tab g1, Fig. g2). According to the methane and carbon dioxide release, 4 days after starting the test a degradation of 10 - 13% could be observed in all three vessels. In vessel 9, the test substance BASE FC-3041 was degraded at a constant rate of degradation. After 33 days, 91% of the test substance was mineralized.

In the test vessel 7 the test substance BASE FC-3041 did at first not further degrade from day 4 to day 25. Only after 33 days could a significant degradation be observed again. After an incubation period of 60 days, a degradation of 78.5% was achieved.

Parallel to vessel 7, the test substance BASE FC-3041 in vessel 8 did at first not further degrade from day 4 to day 25. Here too, a restart in degradation could be observed after 33 days, however, with a substantially lower rate of degradation in comparison to vessel 8⁴. After 60 days, only 20.7% of the test substance had degraded in this test sample.

⁴sic. Must be "7".

The cause for this degradative behavior in the test vessels 7 and 8 is not known. The possibility exists that during the sample collection or during incubation oxygen diffused in various amounts into the test samples, through which the anaerobic mineralization was inhibited to different degrees. A confirmatory test for oxygen in the vessels 7 and 8 with a suitable color indicator (resazurin) was not possible due to the dark black coloration of the test samples. However, the different degradation of BASE FC-3041 observed in the test samples may also be traced to the inoculum. Sludge represents a very complex inoculum with a large diversity. Not all organisms are capable of degrading the test substance to the same extent. Therefore, the possibility exists that a different distribution of various organisms in the test vessels occurred during the addition of the inoculum, which led to the difference in degradation of the test substance observed.

Tab. g2 shows the results of the determination of the anaerobic degradation of BASE FC-3041 by means of the mineralization to methane and carbon dioxide in comparison to the determination of the decrease of dissolved organic carbon. A very good agreement between the two methods was found in all test samples for the test substance as well as for the reference substance.

A release of fluoride by means of a selective fluoride electrode (detection limit 2 µg fluoride) was not detectable in any of the test samples. During the mineralization of fluorinated compounds, fluorine can in principle be released only as fluoride ion. A volatilization of fluoride, which is observed for low pH-values, can be excluded under the given slightly alkaline conditions, measured at the start and the end of the experiment. The possibility exists that released fluoride was bonded to the glass vessels or to the organisms.

EVALUATION

Based on the present test results, the test substance BASE FC-3041 may be categorized as biologically degradable under anaerobic conditions.

Tab. g1: Mineralization of BASE FC-3041 under anaerobic conditions

Day	Inoculum Cmin 1) mg C	Reference substance Cmin 2) mg C	% degradation	Base FC-3041 + HgCl ₂ Cmin 1) mg C
0	0	0	0	0
4	0,279	0	0	nb
11	0,703	0	0	nb
25	1,206	0,4	20	nb
33	1,343	1,337	66,8	0,04
60	1,392	1,789	89,5	0,05

Day	Test Substance Vessel 7 Cmin 3) mg C	Base FC-3041 % degradation	Vessel 8 Cmin 3) mg C	% degradation	Vessel 9 Cmin 3) mg C	% degradation
0	0	0	0	0	0	0
4	0,428	10,7	0,534	13,3	0,481	12,1
11	0,394	9,8	0,533	13,3	1,657	41,1
25	0,442	11,1	0,407	10,2	3,412	85,1
33	1,913	47,9	0,612	15,3	3,641	91
60	3,141	78,5	0,831	20,7		

1) Average value from 3 experimental runs

2) Average value from 3 experimental runs minus Cmin of the inoculum control

3) Cmin of the experimental runs minus Cmin of the inoculum control

nb = not determined

Tab. g2: Determination of the anaerobic degradation of BASE FC-3041 and sodium benzoate by means of mineralization and measurement of the dissolved organic carbon (DOC)

	Mineralization % degradation	Determination of the DOC % degradation
Na - benzoate 1)	89,5	106
BASE FC-3041		
Vessel 7	78,5	81,5
Vessel 8	20,7	25,2
Vessel 9	91	90,5

1) Average value from 3 parallel [runs]

Fig. g1: Anaerobic degradation of sodium benzoate (average value from 3 parallel [runs])

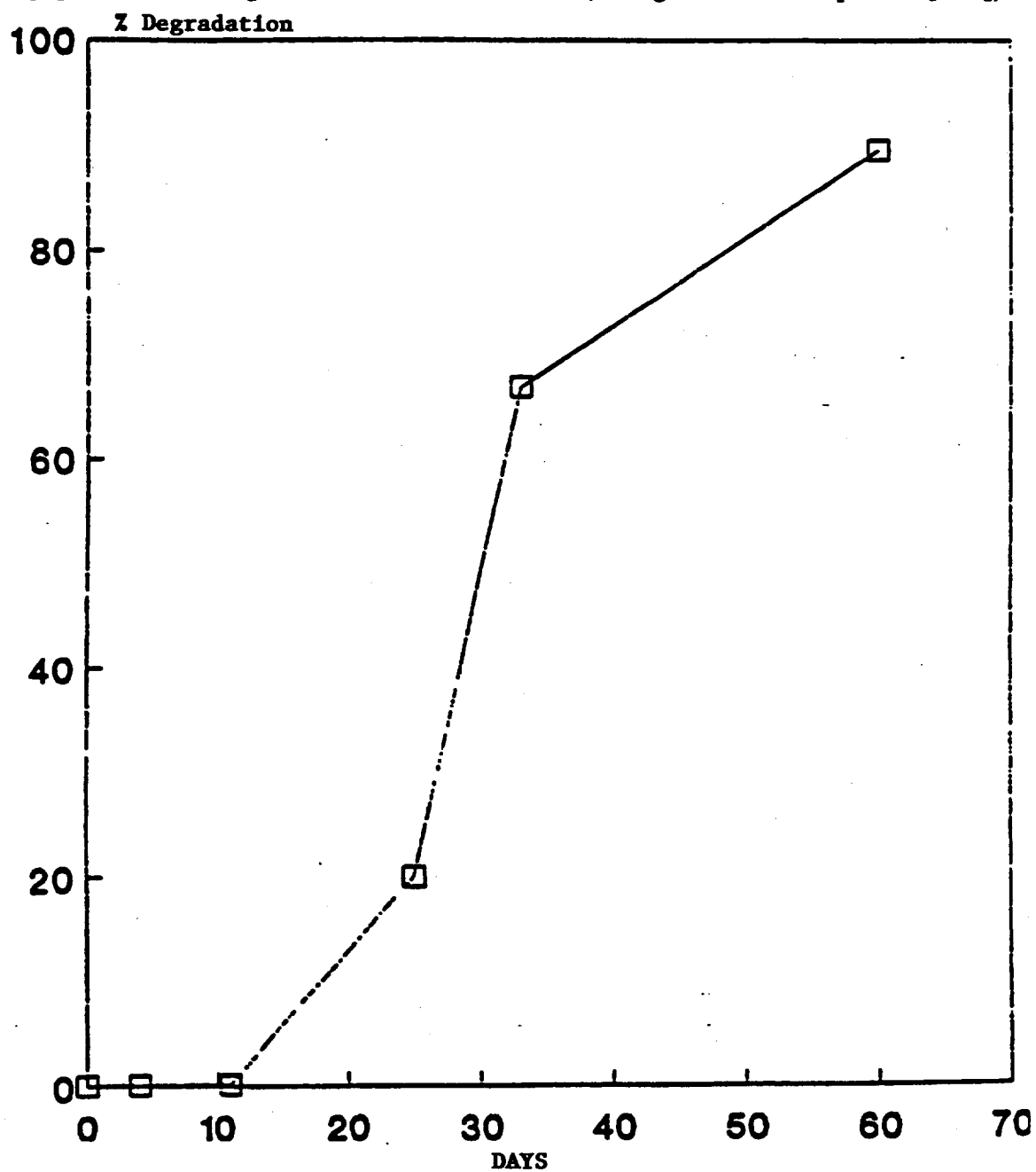


Fig. g2: Anaerobic degradation of BASE FC-3041 in 3 parallel samples

