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3M Specialty Materials

3M Center
St. Paul, MN 55144-1000
651 733 1100

FYI-0500-1378

May 23, 2000

RECEIVED OPPT NCIC 00 MAY 26 AM 11:40

Dr. Charles Auer, Director
Chemical Control Division
Office of Pollution Prevention and Toxics
United States Environmental Protection Agency
401 M Street, Southwest
Room 403, East Tower (Mail Code 7405)
Washington, D.C. 20460

MR 36139

Contain NO CBI

Re: Information on Perfluorooctane Sulfonates and Related Compounds – Additional Information

Dear Charlie:

I am enclosing additional information that did not get submitted with the recent May 18, 2000 submittal to you on perfluorooctane sulfonates and related compounds. This information includes:

1. An additional summary and report on N-ethylperfluorooctane sulfonamidoethanol (N-EtFOSE Alcohol) and a revised N-EtFOSE Alcohol Bibliography Sheet that includes this report.

2. Information on N-methylperfluorooctanesulfonamidoethyl acrylate (MeFOSEA).

3. Information on Perfluorodecanesulfonate, Ammonium Salt (PFDS).

4. Information on Perfluorooctane sulfonylamido (ethyl) acetate (PFOSAA) and a revised PFOSAA Bibliography Sheet that references FC-127 as another synonym for this compound.

5. Information on N-ethylperfluorooctanesulfanamidoethyl acrylate (EtFOSEA) and a revised EtFOSEA Bibliography Sheet that references a study not included on the bibliography sheet last week.

The Bibliography Sheets for MeFOSEA and PFDS were included as part of the May 18th submittal and remain unchanged.

Very truly yours,

William A. Weppner

William A. Weppner, Ph.D.
Director of Environmental, Health, Safety
And Regulatory Affairs
Specialty Materials Markets
3M Center, Bldg. 236-1B-10
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FYI-00-001378

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This page added by Terry O'Bryan/EPA to expand index of FYI-0500-01378 submitted 5/23/00

1. **Information on N-ethylperfluorooctane sulfonamidoethanol (N-EtFOSE Alcohol):**
   Summary and report of Octanol/Water Partition Coefficient

2. **Information on N-methylperfluorooctanesulfonamidoethyl acrylate (MeFOSEA):**
   Summary and report of Stability in Water (Hydrolysis)
   Summary and report of Octanol/Water Partition Coefficient

3. **Information on Perfluorodecanesulfonate, Ammonium Salt (PFDS):**
   Two summaries and reports on each of the following:
   Biodegradation; Acute Toxicity to Fish; Acute Toxicity to Aquatic Invertebrates; Activated Sludge Respiration Inhibition Test; Microbics Microtox Toxicity Test.

4. **Information on Perfluorooctane sulfonamido (ethyl) acetate (PFOSAA):**
   Several summaries and reports on each of the following:
   Biodegradation; Acute Toxicity to Fish; Acute Toxicity to Aquatic Invertebrates; Activated Sludge Respiration Inhibition Test; Microbics Microtox Toxicity Test.
   Summary and report of Bioaccumulation (analytical only)
   Summary and report of Acute Toxicity to Aquatic Plants (Algae)

5. **Information on N-ethylperfluorooctanesulfonamidoethyl acrylate (EtFOSEA):**
   Summary and report of Physical/Chemical Properties
   Two summaries and reports on Biodegradation
   Summary and report of Acute Toxicity to Fish
   Summary and report of Activated Sludge Respiration Inhibition Test
   Summary and report of Bioaccumulation

N-EtFOSE Alcohol Bibliography

Synonyms: FM 3422, FM 3923, FM 4635, FM 4120, F 9353, FC-10

Physical/Chemical Properties

Title	Laboratory or Author	Date Completed	Type
Water solubility	3M Env. Lab	11/15/77	Robust summary, relevant pages from "Analytical Methodology on FM 3422"
Letter Report. Boiling Point Information	AScl	11/30/98	Robust summary, letter report
Determination of Vapor Pressure Curve by Dynamic Method for U1463 (Et FOSE)	AScl	11/6/98	Robust summary, final report
Internal Memo - Melting Point of FM-3923 N-Ethyl FOSE Alcohol	3M SMMD Analytical Lab	11/22/99	Robust summary, letter report

Environmental Fate and Transport

Title	Laboratory or Author	Completion Date	Type
Tech Report - Biodegradation Studies of Fluorocarbons, Request for Laboratory work - BOD/COD, Memo - Chronological Review of Biodegradation Studies on FM3422, Tech Report - Biodegradation Studies of Fluorocarbons - II. (Robust summary for 4 documents)	3M Env. Lab	8/12/76, July, 1977, 8/12/77, 1/9/78	Robust summary, 2 technical reports, work request, review document
Tech Report - Bioconcentration of FM3422 in Bluegill Sunfish and in Channel Catfish, Tech Report - Aquatic Fate of a Fluorochemical: FM 3422	3M Env. Lab	5/17/1977, 10/14/77, 3/8/93	Robust summary, 2 technical reports, critique by J.W. Gillett (Cornel)
Tech Notebook copies - Analytical from fish bioconcentration studies	G. Vraspir	1/15/76 - 2/1/78	???
Alkaline hydrolysis	3M Env. Lab	11/15/77	Robust summary, relevant pages from "Analytical Methodology on FM 3422"
Pilot studies on soil adsorption	3M Env. Lab	10,13,78, 5/19/93, 12/30/75, 3/7/77, 4/12/77, 5/9/77	Robust summary, page from technical notebook, critique from S. Boyd (MSU) and 2 pages of data summaries.
Evaluation of the Bioconcentration Potential of FM 3422	3M Env. Lab	8/16/78	Robust summary, critique from G.W. Gillett (Cornell), technical report
Tech Report - Adsorption of FM-3422 on Soil	3M Env. Lab	9/1/78	Robust summary, critique from S. Boyd (MSU), technical report

Environmental Fate and Transport

Title	Laboratory or Author	Completion Date	Type
Tech Report - Analysis for Fluorochemicals in Bluegill Fish	3M Env. Lab	5/1/79	Robust summary, critique from J.W. Gillett (Cornell), technical report
Tech Report - Bioaccumulation of Fluorochemicals in Tenn. River, Tech Report - Fluorochemicals in Tenneessee River Fish.	3M Env. Lab	5/22/79, 12/28/79, 3/8/93	Robust summary, 2 technical reports, ciritque from J.W. Gillett (Cornell)
Technical Report - Photolysis of FM 3422 on Soil	3M Env. Lab	12/10/79	Robust summary, technical report, critique from S. Boyd, MSU
Tech Report - FM-3422 Photolysis Study using Simulated Sunlight	3M Ag. Products Lab	8/11/81	Robust summary, technical report
Tech Report - Determination of the Partition Coefficient (N-Octanol/Water) of FM 3422	NOTOX Safety & Environmental Research	6/2/94	Robust summary, technical report

Summary Reports

Title	Laboratory or Author	Completion Date	Type
Tech Report - Analytical Methodology on FM 3422	3M Env. Lab	11/15/77	Technical report., Critique by S. Boyd (MSU) Exerpts from this report appended to solubility and alkaline hydrolysis robust summaries.
Tech Report - Analytical Methodology and Support	3M Env. Lab	1/17/79	Technical report. Exerpts from this report appended to solubility, alkaline hydrolysis,volatility, distribution coefficient, soil adsorption, biodegradation, and fish chronic toxicity robust summaries.
Tech Report - Final comprehensive Report on FM 3422	3M Env. Lab	2/7/1979, 3/8/93	Technical Report, critique by J.W. Gillett (Cornell)

Ecotoxicity Elements

Title	Laboratory or Author	Completion Date	Type
The Effects of Continuous Aqueous Exposure to 78.01 on Hatchability of Eggs and Growth and Survival of Fry of Fathead Minnow (Pimephales promelas), Summary of Histopathological Examinations of Fathead Minnow (Pimephales promelas) Exposed to 78.01 for 30 Days..	EG&G Bionomics	June, 1978, Sept. 1978	Robust summary, 2 technical reports, exerpt from Tech Report - Analytical Methodology and Support (1/17/79)
Tech Report - Multi-Phase Algal Assay Test Method.	3M Env. Lab	12/30/81	Robust summary, technical report.

Perfluorooctane sulfonylamido(ethyl)acetate = PFOSAA

Synonyms: FC-109, FC-109-X, FC-127, FC-128, FC-129, E2113, E2566-2, R1904

Physical/Chemical Properties

Title	Laboratory or Author	Date Completed	Type

Environmental Fate and Transport

Title	Laboratory or Author	Date Completed	Type
Tech. Report - Fate of Fluorochemicals in the Environmental (sic) (Warburg respirometer method for biodegradation of FC-128)	3M Env. Lab	8/12/76	Robust summary, technical report
Ready Biodegradability of FC-128 - BOD/COD	3M Env. Lab	7/8/77	Robust summary, copies of data sheets
Tech report - Analysis for Fluorochemicals in Bluegill Fish	3M Env. Lab	5/1/79	Robust summary, technical report
Ready biodegradability of FC-127 - BOD/COD	3M Env. Lab	8/19/81	Robust summary, copies of data sheets
Ready biodegradability of FC-109-X - BOD/COD	Pace Laboratories, Minneapolis, MN	12/31/87	Robust summary, copy of computer results from archives

Summary Reports

Title	Laboratory or Author	Date Completed	Type

Ecotoxicity Elements

Title	Laboratory or Author	Completion Date	Type
Acute toxicity of FC-128 to fathead minnow	3M Env. Lab	5/18/74	Robust summary, copies of data sheets
Acute toxicity of FC-128 to fathead minnow	3M Env. Lab	6/29/74	Robust summary, copies of data sheets
Acute toxicity of FC-128 to fathead minnow	3M Env. Lab	8/26/77	Robust summary, copies of data sheets
Acute toxicity of FC-127 to fathead minnow	3M Env. Lab	7/24/81	Robust summary, copies of data sheets
Toxicity of FC-127 to activated sludge respiration	3M Env. Lab	8/14/81	Robust summary, copies of data sheets

Ready Biodegradability: "Modified OECD Screening Test" for Fluorad FC-129	RCC, Itingen, Switzerland	9/19/84	Robust summary, memo about study reliability, final report
Microbics Microtox Toxicity Test - FC-109-X	3M Env. Lab	10/15/87	Robust summary, copies of data sheets
Activated Sludge Respiration Inhibition - FC-109-X	3M Env. Lab	10/30/87	Robust summary, copies of data sheets
Acute Toxicity of E2566-2 to Daphnia magna	ABC Laboratory, Columbia, MO	1/9/88	Robust summary, final report
Acute Toxicity of E2566-2 to Fathead Minnow (*Pimephales promelas*)	ABC Laboratory, Columbia, MO	1/12/88	Robust summary, final report
Biodegradation (methylene blue active substance)	Pace Laboratories, Inc., Minneapolis, MN	2/15/89	Robust summary, computer report, copies of data sheets
Acute Toxicity of R1904 to *Daphnia magna*	AScI Corporation, Duluth, MN	6/17/97	Robust summary, final report
Growth Inhibition of R1904 for Green Alga (*Selenastrum capricornutum*)	AScI Corporation, Duluth, MN	6/18/97	Robust summary, final report
Acute Toxicity of R1904 to Fathead Minnow (*Pimephales promelas*)	AScI Corporation, Duluth, MN	6/27/97	Robust summary, final report
Microbics Microtox Toxicity Test - FC-129	AScI Corporation, Duluth, MN	7/9/97	Robust summary, copies of data sheets
Inhibition of R1904 for Activated Sludge Respiration	AScI Corporation, Duluth, MN	7/10/97	Robust summary, final report
R1904 BOD Report for 3M Company	AScI Corporation, Duluth, MN	Jul-97	Robust summary, final report

Ethyl FOSEA

Synonyms: FX-13, B1228, D-1

Physical/Chemical Properties

Title	Laboratory or Author	Date Completed	Type
Determination of Physico-chemical Properties of Sample D-1	Mitsubishi Chemical Safety Institute Ltd., Yokohama, Japan	2/14/96	Robust summary, final report

Environmental Fate and Transport

Title	Laboratory or Author	Completion Date	Type
Ready biodegradability of FX-13 (BOD/COD)	3M Env. Lab	4/26/84	Robust summary, copies of data sheets
Ready Biodegradability Test of D-1	Mitsubishi Chemical Safety Institute Ltd., Yokohama, Japan	10/31/95	Robust summary, final report
Bioaccumulation Study of Sample D-1 with Carp (Cyprinus carpio)	Mitsubishi Chemical Safety Institute Ltd., Yokohama, Japan	11/30/95	Robust summary, final report

Ecotoxicity Elements

Title	Laboratory or Author	Completion Date	Type
Acute toxicity of FX-13 to fathead minnow	3M Env. Lab	4/1/84	Robust summary, copies of data sheets
Activated Sludge Respiration Inhibition Test - FX-13	3M Env. Lab	5/17/84	Robust summary, copies of data sheets

OCTANOL/WATER PARTITION COEFFICIENT

TEST SUBSTANCE

Identity: N-ethylperfluorooctane sulfonamidoethanol; may also be referred to as N-EtFOSE Alcohol or FM-3422. (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: Material is an off-white, waxy solid. The test laboratory identifies the compound as "T-5874" Batch 2334.

METHOD

Method: OECD 117 & EEC-Directive 92/69 EEC, Annex V, Part A.8
GLP (Y/N): Yes
Year completed: 1994
Analytical: HPLC with Spectrophotometric detector

Remarks: Testing used six substances with known log P_{ow} values as references. These substances were used to calibrate the elution time in units of log P_{ow}.

Reference substance	**log P_{ow}**
Benzylalcohol	1.1
Toluene	2.7
1,4-dichlorobenzene	3.4
Fluoranthene (98%)	4.7
Dibenzyl (99%)	4.8
2,4-DDT (99%)	6.2

A stock solution of the test substance was prepared by dissolving 165 mg in 50.0 mL acetonitrile and sonicating for 3 minutes. The stock solution was diluted 10 times with mobile phase, resulting in the test solution.

Mobile phase is a 75/25 (v/v) solution of acetonitrile / Milli-Q water respectively.

RESULTS

Substance	log P_{ow}
Reference chemicals	
Benzylalcohol	1.1
Toluene	2.7
1,4-dichlorobenzene	3.4
Fluoranthene	4.7
Dibenzyl	4.8

2,4-DDT	6.2
Test substance	
Peak 1	5.6*
Peak 2 (major comp.)	4.4*
Several small peaks	2.2/4.4*

* Interpolated from the regression line: y = 0.288x – 0.730 (r=0.972, n=6)

CONCLUSIONS

This study estimates the log P_{ow} for N-EtFOSE alcohol.

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

DATA QUALITY

Reliability: Klimisch ranking 2

-per OECD. HPLC methods enables partition coefficients to be estimated in the log P_{ow} range between 0 and 6.

-Notox states impurities are of minor importance in HPLC method, however OECD states, HPLC method is less sensitive to the presence of impurities in the test substance than the Shake Flask method. Nevertheless, in some cases impurities can make the interpretation of the results difficult due to uncertainty in peak assignments. Mixtures which result in an unresolved band, upper and lower limits of log P_{ow} should be stated. Duplicate analysis should be reported in order to show confidence in the measurement (log P_{ow} values should fall within a range of +/- 0.1 log units) – data provided mean value therefore can't show confidence. pH of eluant is critical of ionizable compounds.
The study did not include any fluorochemicals among the reference substances. Without fluorochemical reference compounds, it is not known whether the same relationship between the capacity factor k" and Kow exists for compounds with perfluoroalkyl portions as exits for nonfluorinated reference materials such as those used in this study. The reference compounds should have been also injected independently to insure appropriate retention time assigned to each reference compound. Reference substances were injected twice, however only the mean retention time data is given for the reference substances thus repeatability of the retention times is not clear. It was not demonstrated that the test material was soluble in the acetonitrile stock solution or in the mobile phase. No demonstration that the major components of the test substance are detectable at UV λ = 210 nm (Generally, UV absorbance detectors poor choice for N-EtFOSE alcohol and related fluorochemicals). UV adsorption spectra is needed of the purified main components of the test substance. Assumptions are made that the major peak from the HPLC of the test substance is the major component. No attempt was made to confirm this (e.g. Mass spec analysis to show the material detected has the appropriate mass). It is not known whether

the major components even come off the column. Some k' values reported are calculated incorrectly.

REFERENCES

Study conducted at the request of 3M Company by NOTOX Safety & Environmental Research B.V., The Netherlands, April 7, 1994.

REPORT

DETERMINATION OF THE PARTITION COEFFICIENT (N-OCTANOL/WATER) OF

T-5874

BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

NOTOX Project 121275
NOTOX substance 38187

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STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice

which are essentially in conformity with:

The United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

The United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

The United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

Study Director: Drs. R. de Vries

.....

Date: June 8, 1994

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QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands.

Study procedures were subject to periodic inspections and general non study specific processes were also inspected at periodic intervals.

This report was audited by the NOTOX Quality Assurance Unit and the methods and results accurately reflect the raw data.

DATES OF QAU INSPECTIONS/ AUDITS	REPORTING DATES
25 March 1994	25 March 1994
28 March 1994	28 March 1994
21 April 1994	21 April 1994

Quality Assurance Manager C.J. Mitchell B.Sc.

Date: 09-06-94

000014

REPORT APPROVAL

STUDY DIRECTOR: Drs. R. de Vries

c/a

.....

Date: June 8, 1994

MANAGEMENT: J.A.M.W. van Helvoirt
Section head Physico Chemistry

PP.

Dr. Ilona C. Enninga
Technical Director

.....

Date: 09/06/1994

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PREFACE

Sponsor	3M Belgium - Chemical EBC Canadastraat 11 B-2070 ZWIJNDRECHT Belgium
Study Monitor	Mr. R.H. Cox
Testing Facility	NOTOX B.V. Hambakenwetering 3 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Drs. R. de Vries
Study plan	Start: 30 March 1994 Completed: 07 April 1994

TEST SUBSTANCE

Identification	T-5874
Description	Cream solid
Batch	2334
Purity	100%
Storage conditions	At room temperature in the dark
Stability under storage conditions	Stable
Expiry date	January 01, 1996

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PURPOSE AND PRINCIPLE

The purpose of the study was to determine the partition coefficient of the test substance between n-octanol and water.

The partition coefficient (n-octanol/water) (P_{OW}) is defined as the ratio of the equilibrium concentrations in a two phase system consisting of n-octanol and water.

The retention time of a substance in a reversed-phase High Performance liquid Chromatography (HPLC) chromatographic system is related to its partition coefficient (n-octanol/water).
The HPLC method is useful for log P_{OW} values between 0 and 6.
Using the HPLC method, impurities are of minor importance. The HPLC method must also be applied if the test substance is a mixture of compounds but is not applicable to strong acids and bases, metal complexes, substances which react with the eluent or surface-active agents.

GUIDELINES

The study procedure described in this report was based on the following guidelines:

Organization for Economic Co-operation and Development (OECD), OECD Guideline for Testing of Chemicals, guideline no. 117: "Partition coefficient (n-octanol/water) High Performance Liquid Chromatography (HPLC) method" (adopted March 30, 1989).

European Economic Community (EEC), EEC-Directive 92/69 EEC, Annex V, Part A, Methods for the determination of physico-chemical properties, A.8: "Partition coefficient". EEC Publication no. L383, December 1992.

ARCHIVING

NOTOX B.V. will archive the following data for at least 10 years: protocol, report, test substance reference sample and raw data.

TEST SYSTEM AND RATIONALE

A High Performance Liquid Chromatograph with a spectrophotometric detector. The stationary phase was bonded silica C_{18}. The mobile phase was 75/25 (v/v) acetonitrile/water. Both HPLC instrumentation and conditions are described in the section "method of chemical analysis".

The test system was recognized by the international guidelines (OECD, EEC).

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REFERENCE SUBSTANCES

Six chemicals for which log P_{OW} has been reported were used to calibrate the elution time in units of log P_{OW}. The mixture of reference substances consisted of:

Reference substance	log P_{OW}[1]
- benzylalcohol (p.a., Merck)	1.1
- toluene (p.a., Merck)	2.7
- 1,4-dichlorobenzene (z.s., Merck)	3.4
- fluoranthene (98%, GC, Aldrich Chemie)	4.7
- dibenzyl (99%, GC, Aldrich Chemie)	4.8
- 2,4-DDT (99%, HPLC, Riedel de Haën, Seelze, FRG)	6.2

[1] log P_{OW} values according to the OECD guideline.

PERFORMANCE OF THE TEST

Preparation of the solutions

Solutions of the reference substances (except for ethylmethylketone) were prepared in methanol (HPLC-grade, Labscan Limited Co., Dublin, Ireland) at a concentration of 1.5-2 g/l. A mixture of the reference substances was prepared by adding 125 µl of each solution to a volumetric flask. Subsequently, this flask was made up with mobile phase to a final volume of 25.0 ml.
For the determination of t_0 (retention time of the unretarded component), a solution of formamide (p.a., Merck, Darmstadt, FRG) in methanol was prepared at a concentration of 1.7 g/l and thereafter diluted 10 times with mobile phase.
A stock solution of T-5874 was prepared by dissolving 165 mg test substance in 50.0 ml acetonitrile (3 minutes sonication). The stock solution was diluted 10 times with mobile phase, resulting in the test solution.

Performance of the test

The solutions were injected in the following sequence: the mixture of reference substances, mobile phase, the formamide solution, the test solution (in duplicate), mobile phase, the mixture of reference substances and the formamide solution.

Temperature of measurement

The temperature of the mobile phase was recorded several times during the measurements.

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METHOD OF CHEMICAL ANALYSIS

The conditions used for the High Performance Liquid Chromatographic method are described below:

Analysis

Column	LiChrospher 100 RP-18; 125 x 4 (I.D.) mm; d_p= 5 µm (Merck, Darmstadt, FRG)
Mobile phase	75/25 (v/v) acetonitrile (HPLC-grade, Labscan Limited Co., Dublin, Ireland)/Milli-Q water (Millipore Corp., Bedford, MA, USA)
Flow	1 ml/min
Detection	UV at λ= 210 nm
Injection volume	10 µl

Instrumentation

HPLC Pump	Series 410 LC (Perkin Elmer, Norwalk, CT, USA)
Autosampler	ISS-200 (Perkin Elmer)
Detector	SpectroMonitor 3100 (LDC Analytical, Riviera Beach, FL, USA)
Integrator	SP 4290 (Spectra Physics, San Jose, CA, USA)

DATA HANDLING

Using High Performance Liquid Chromatography, especially large log P_{ow} values can be accurately determined from the chromatographic retention data. To this end, the capacity factor (k') was used, since it is proportional to the partition coefficient. The capacity factor was calculated from the retention of the substance concerned (t_r) and the unretarded component (t_o):

$$k' = (t_r - t_o)/t_o$$

From the results of the reference substances, a plot of log P_{ow} (x-value) versus log k' (y-value) was constructed, using linear regression analysis. The log k' value of each component of the test substance was compared with the log k' values of the reference substances with known log P_{ow} values.

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RESULTS

Representative HPLC chromatograms of the test solution, the blank (mobile phase), the mixture of reference substances and the formamide solution are shown in Figures 1 to 4.

In the HPLC chromatograms of T-5874, using detection at 210 nm, one large peak at 4.1 minutes and a small peak at 7.7 minutes were observed. Between 1.6 and 4 minutes, several small peaks were observed. It was assumed that the large peak derived from the major component of test substance whereas the small(er) peaks derived from impurities.

The t_0 was determined to be 0.90 minutes as a mean value of both measurements (i.e 0.89 and 0.90 minutes).

The mean values of the retention times, k' values, log k' values, log P_{ow} and P_{ow} values are summarized in Table 1. The plot of log P_{ow} (x-value) versus log k' (y-value) from the reference substances is shown in Figure 5.

Table 1 Results of the test.

Substance	t_r [1]	k'	log k'	log P_{ow}	P_{ow}
benzylalcohol	1.14	0.274	-0.563	1.1	
toluene	2.24	1.503	0.177	2.7	
1,4-dichlorobenzene	2.89	2.229	0.348	3.4	
fluoranthene	5.67	5.330	0.727	4.7	
dibenzyl	4.07	3.547	0.550	4.8	
2,4-DDT	9.12	9.190	0.963	6.2	
test substance					
peak 1	7.70	7.598	0.881	5.6 [2]	39.8×10^4
peak 2 (major comp.)	4.05	3.525	0.547	4.4 [2]	2.51×10^4
several small peaks	1.6 / 4	0.79/ 3.47	-0.10/0.54	2.2/4.4 [2]	1.50×10^2/ 2.51×10^4

[1] Mean value of the retention times of both chromatograms.
[2] Interpolated from the regression line: y = 0.288x - 0.730 (r=0.972, n=6)
Note : The calculations were performed using not rounded values.

The temperature of the mobile phase was 20.2-20.6°C during the measurements.

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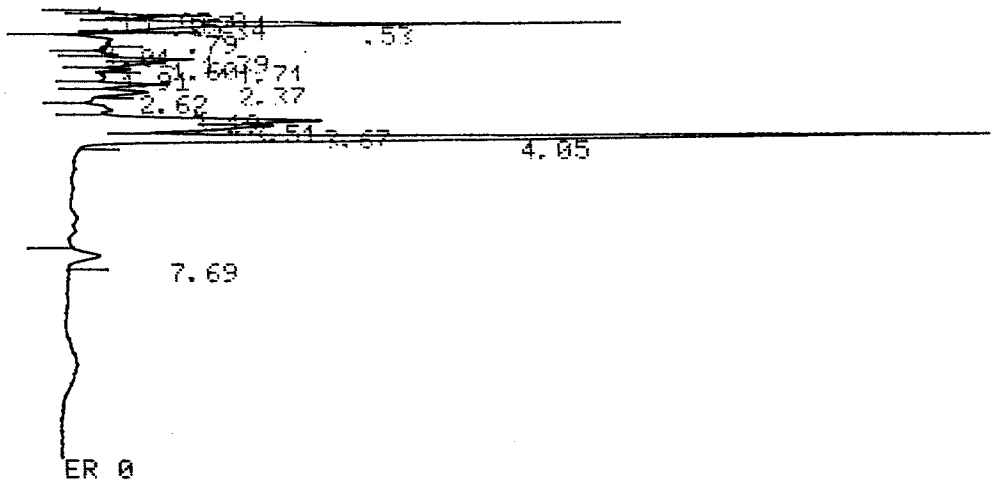
CHANNEL A INJECT 07-04-94 08:44:55 STORED TO BIN # 53



DATA SAVED TO BIN # 53

38196/121286 07-04-94 08:44:55 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 10 INDEX 10 BIN 53

ANALYST: KRH

PEAK#	AREA%	RT	AREA	BC
1	0.495	0.15	1399	02
2	2.459	0.25	6951	02
3	1.083	0.34	3061	02
4	18.792	0.53	53119	08
5	1.288	0.79	3640	05
6	2.538	1.01	7175	05
7	0.468	1.39	1322	02
8	2.476	1.6	7000	02
9	1.977	1.71	5588	02
10	0.636	1.91	1797	03
11	1.864	2.37	5268	02
12	2.067	2.62	5842	03
13	1.718	3.19	4857	02
14	11.232	3.51	31750	02
15	10.435	3.67	29495	02
16	38.458	4.05	108707	03
17	2.014	7.69	5694	01
TOTAL	100.		282665	

Figure 1 HPLC chromatogram of the test solution of 330 mg/l T-5874 in mobile phase.

Column	LiChrospher 100 RP-18; 125x4 (I.D.) mm; d_p=5 µm
Mobile phase	75/25 (v/v) acetonitrile/Milli-Q water
Flow	1 ml/min
Detection	UV, at λ = 210 nm
Injection volume	10 µl

Note: The peaks between 0 and 1.6 minutes were also observed in the chromatograms of the blank (see Figure 2). Therefore it was assumed that these peaks did not derive from the test substance.

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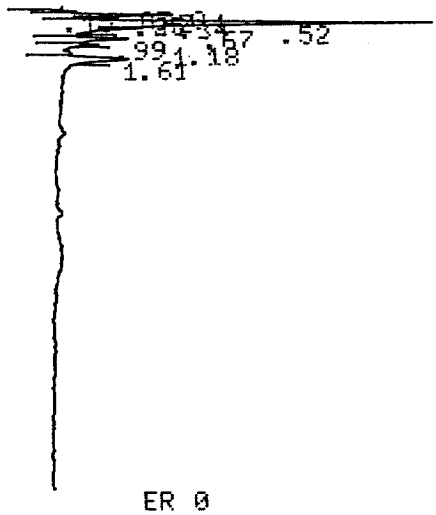
CHANNEL A INJECT 07-04-94 09:34:04 STORED TO BIN # 56



ER 0
DATA SAVED TO BIN # 56

38196/121286 07-04-94 09:34:04 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 13 INDEX 13 BIN 56

ANALYST: KRH

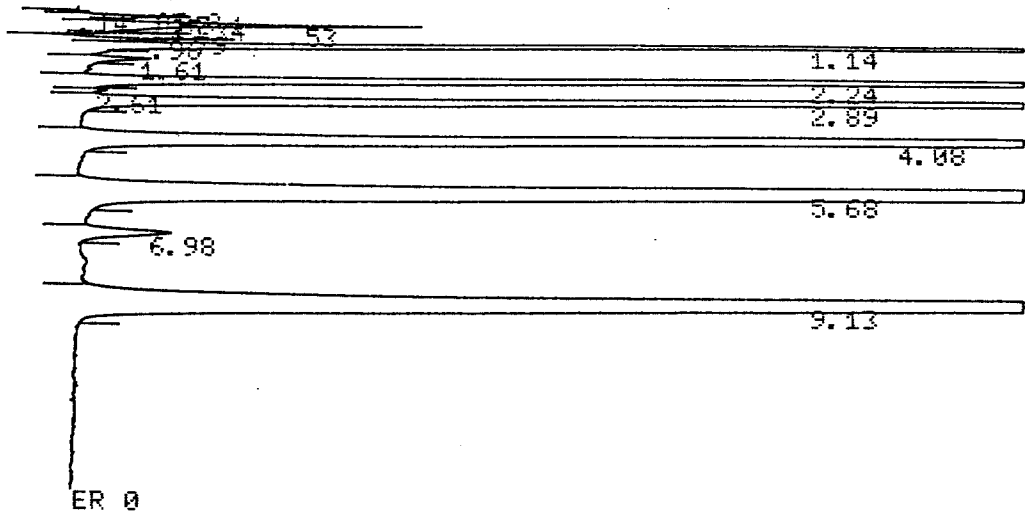
PEAK#	AREA%	RT	AREA	BC
1	1.543	0.15	877	02
2	10.515	0.25	5976	02
3	3.853	0.34	2190	02
4	55.335	0.52	31449	02
5	7.768	0.67	4415	03
6	6.901	0.99	3922	02
7	2.509	1.18	1426	03
8	11.576	1.61	6579	01
TOTAL	100.		56834	

Figure 2 HPLC chromatogram of a blank (mobile phase).

Column	LiChrospher 100 RP-18; 125x4 (I.D.) mm; d_p=5 µm
Mobile phase	75/25 (v/v) acetonitrile/Milli-Q water
Flow	1 ml/min
Detection	UV, at λ = 210 nm
Injection volume	10 µl

000022

CHANNEL A INJECT 07-04-94 09:50:17 STORED TO BIN # 57



ER 0
DATA SAVED TO BIN # 57

38196/121286 07-04-94 09:50:17 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 14 INDEX 14 BIN 57

ANALYST: KRH

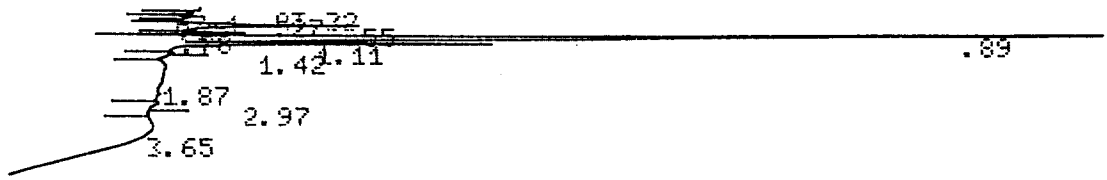
PEAK#	AREA%	RT	AREA	BC
1	0.023	0.14	1131	02
2	0.145	0.25	7039	02
3	0.06	0.34	2917	02
4	0.879	0.53	42751	08
5	0.073	0.79	3542	05
6	0.139	0.96	6771	06
7	8.977	1.14	436774	03
8	0.112	1.61	5460	01
9	11.317	2.24	550640	01
10	0.013	2.61	611	02
11	10.567	2.89	514173	03
12	15.524	4.08	755334	01
13	38.584	5.68	1877339	01
14	0.316	6.98	15361	01
15	13.272	9.13	645770	01
TOTAL	100.		4865613	

Figure 3 HPLC chromatogram of the mixture of reference substances in mobile phase.

Column	LiChrospher 100 RP-18; 125x4 (I.D.) mm; d_p=5 µm
Mobile phase	75/25 (v/v) acetonitrile/Milli-Q water
Flow	1 ml/min
Detection	UV, at λ = 210 nm
Injection volume	10 µl

000023

CHANNEL A INJECT 07-04-94 07:37:44 STORED TO BIN # 49



DATA SAVED TO BIN # 49

38196/121286 07-04-94 07:37:44 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 6 INDEX 6 BIN 49

ANALYST: KRH

PEAK#	AREA%	RT	AREA	BC
1	0.35	0.16	2624	02
2	1.189	0.27	8926	02
3	0.79	[illegible]	5930	02
4	10.44	[illegible]	78363	08
5	1.884	0.78	14143	06
6	54.548	0.89	409437	08
7	6.246	1.11	[illegible]	05
8	0.12	1.42	899	05
9	3.094	1.87	23224	02
10	0.424	2.97	3185	03
11	20.915	3.65	156989	01
TOTAL	100.		750599	

Figure 4 HPLC chromatogram of a formamide solution of 0.2 g/l in mobile phase.

Column	LiChrospher 100 RP-18; 125x4 (I.D.) mm; d_p=5 µm
Mobile phase	75/25 (v/v) acetonitrile/Milli-Q water
Flow	1 ml/min
Detection	UV, at λ = 210 nm
Injection volume	10 µl

000024

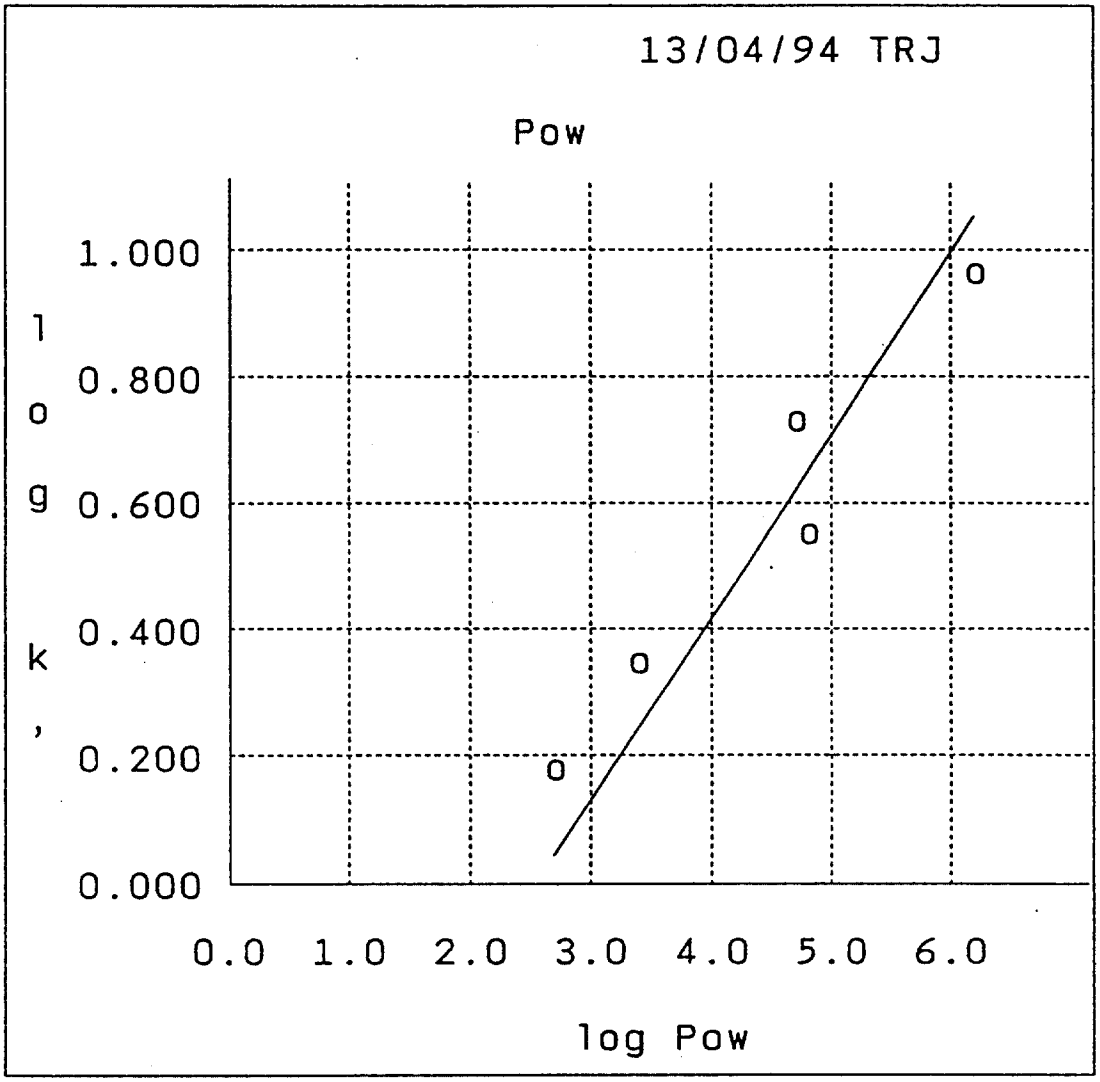


Figure 5 Plot of log P_{ow} (x-value) versus log k' (y-value) from the reference substances.

000025

STABILITY IN WATER (HYDROLYSIS)

TEST SUBSTANCE

Identity: N2-(N-methylperfluorooctanesulfonamido)-ethyl acrylate; may also be referred to as N-MeFOSEA, or MeFOSEA. (2-Propenoic acid, 2-[[(heptadecafluorooctyl)sulfonyl]methylamino]ethyl ester, CAS # 25268-77-3)

Remarks: Material is an amber, waxy solid, coded THAR3F2. Sample purity was not characterized.

METHOD

Method: OPPTS 835.2110
GLP (Y/N): No
Year completed: 1999
Type: Abiotic
Stock solution and test sample preparation: A 10,730 mg/L stock solution of MeFOSEA in acetone was prepared. Test solutions were then prepared by adding 10 uL of the stock solution to 1.0 mL of five solutions individually buffered to pH values of 1.5, 5.0, 7.0, 9.0, and 11.0. This calculates to a 107.3 mg/L concentration of MeFOSEA in each buffered solution.
Remarks: Modification of the test method included using a wider range of pH and longer time periods for a more complete understanding of the behavior of the test substance.

RESULTS

pH impact on Hydrolysis Half-lives at 50°C

pH	Half-life (days)
1.5	9.4
5.0	17.8
7.0	9.9
9.0	9.3
11.0	7.9

pH impact on Hydrolysis Half-lives at 25°C**

pH	Half-life (days)
1.5	94
5.0	178
7.0	99
9.0	93
11.0	79

** Extrapolated from values for 50°C.

Remarks: The analysis showed that concentration of MeFOSEA declined in all aqueous pH buffered solutions with time.
The degradation rates fit second order kinetics better than first order kinetics. Nevertheless, the report authors assumed first order kinetics and calculated the half-life after six time periods. Generally, the calculated half-life increased with time. The half-lives reported above are averages of the half-lives calculated after each of the six time periods.
The report does not explain how the extrapolation was made from the half-lives at 50°C to 25°C. Author states assumed ΔG = 15-18 kcal/mole which would give one order of magnitude increase in $T_{1/2}$ per 25°C.

CONCLUSIONS

This study indicates that MeFOSEA is hydrolytically unstable under environmentally relevant conditions.

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

DATA QUALITY

Reliability: Klimisch ranking 1

REFERENCES

Study conducted by the 3M Company, Environmental Laboratory, St. Paul, MN.

OTHER

Last changed: 5/17/00

Study of the Stability of MeFOSEA in Aqueous Buffers Using Gas Chromatography with Atomic Emission Detection

---

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Requester

3M Company

Report Date: June 14, 1999

Report Summary

A study was conducted on 2-(N-methylperfluorooctanesulfonamido)-ethyl acrylate (MeFOSEA) to determine its half-life under environmentally relevant hydrolysis conditions. Its behavior was examined in aqueous solutions buffered at five pHs: 1.5, 5.0, 7.0, 9.0, and 11.0. The analysis was performed according to 3M Environmental Laboratory Method ETS-8-90.0, with modifications that followed the U.S. Environmental Protection Agency's Fate, Transport and Transformation Test Guidelines document OPPTS 835.2110, "Hydrolysis as a Function of pH". Modifications included using a wider range of pH and longer time periods for a more complete understanding of the behavior of MeFOSEA. Data were collected at the start of the study (Time 0) and after 0.94, 1.92, 6.72, 13.90, 20.71, and 27.77 days of sample agitation and incubation at 50°C. After incubation, isopropyl alcohol (IPA) was added and the aqueous samples were extracted with hexane. The hexane extracts were dried with anhydrous sodium sulfate and injected into a gas chromatograph with an atomic emission detector (GC/AED). The analysis showed that concentrations of MeFOSEA declined in all aqueous pH buffers with time. Half-lives increased with time within each pH data set at 50 °C, averaging 9.4 days at pH 1.5, 17.8 days at pH 5.0, 9.9 days at pH 7.0, 9.3 days at pH 9.0, and 7.9 days at pH 11.0. Extrapolation of the 50 °C data to room temperature (25 °C) resulted in a half-lives averaging 94 days at pH 1.5, 178 days at pH 5.0, 99 days at pH 7.0, 93 days at pH 9.0, and 79 days at pH 11.0. These results indicate that MeFOSEA is hydrolytically unstable under environmentally relevant conditions.

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1 Introduction

Hydrolysis is one of the main mechanisms by which organic compounds, both natural and synthetic, decompose in the environment. Testing for hydrolysis of compounds is relevant to determining the persistence and environmental fate of many pesticides and polymers such as polyesters, polyethers, and polyamides, and for determining shelf life of products containing such compounds (Appendix A, EPA Guidelines).

Hydrolysis is a reaction between some species (in this case of the type R-CO-X), with water that results in the substitution of X with OH:

Figure 1. Generic Hydrolysis Reaction

R = some hydrocarbon or fluorocarbon material
X = halogen, OR, or NR

In dilute solutions the rate law for chemical loss by a hydrolysis reaction is shown with the following equations, where "ester" signifies the starting material. It is important to note that the equation must be modified in solutions that are not dilute.

$$-\frac{d\,[\text{ester}]}{dt} = k_{hyd}[\text{ester}]$$

or

$$\ln \frac{[A]_t}{[A]_0} = -kt$$

The rate constant k is the negative slope of the line fit to a plot of the natural log of the fraction of compound remaining at time t, versus time. The half-life of the compound at a specific pH is related to the rate of hydrolysis by:

$$t_{1/2} = \frac{0.693}{k}$$

With the above equations and considerations as guides, the study monitored the loss of MeFOSEA over an extended period of time to determine the overall rate of reaction. A predicted product of hydrolysis of MeFOSEA was N-MeFOSE alcohol. Analytical difficulties were encountered in attempting to monitor the appearance of this reaction product in an effort to confirm the degradation route.

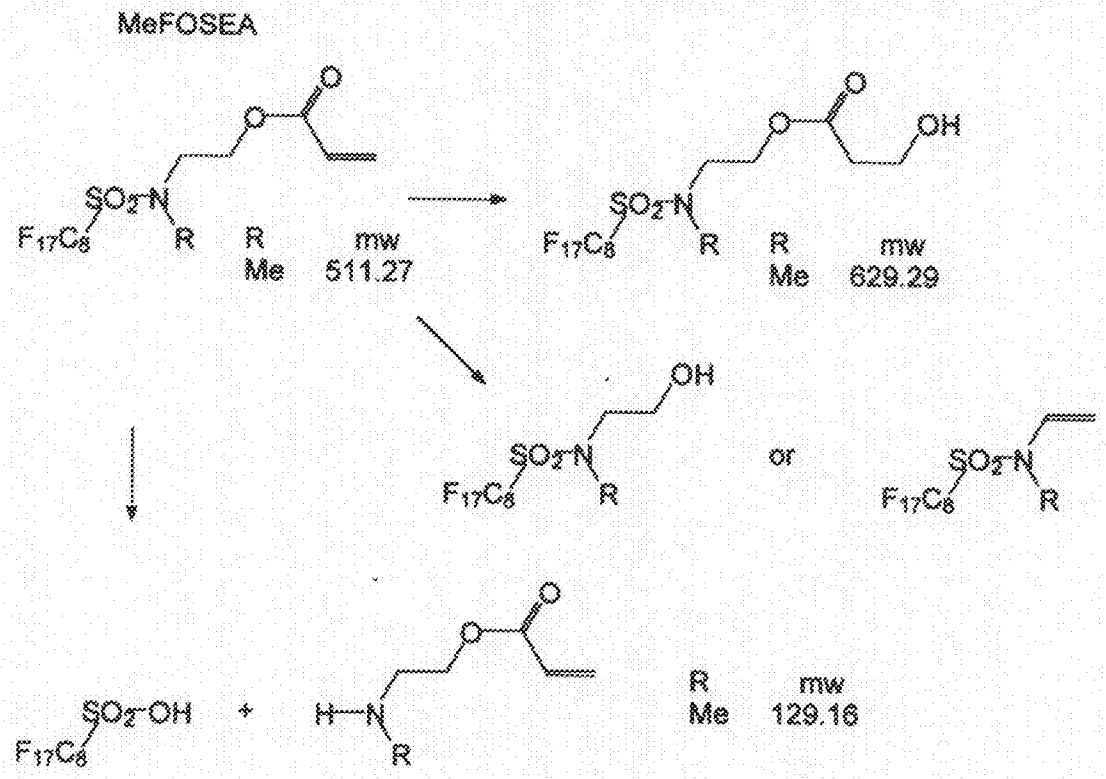
The primary goal of the study was to determine, to a first approximation, the hydrolytic stability of the MeFOSEA. Since levels of MeFOSEA steadily declined over time, data collection stopped after 28 days.

Samples of MeFOSEA were prepared in buffer solutions of pH 1.5, 5.0, 7.0, 9.0, and 11.0 according to published guidelines (Appendix A and references therein). A solution of MeFOSEA in 1.0 mL of each buffer was prepared by adding 10 µL of a 10,730 ppm solution of MeFOSEA in acetone. This corresponds to 107.3 ppm MeFOSEA in each buffer. Significant environmental pHs were chosen (e.g. pH 1.5 is physiologically relevant as stomach pH in case of ingestion). The addition of a pH 11.0 buffer in the present study was made to better understand the behavior of MeFOSEA in basic solutions. The solvent chosen for quenching was hexane, used to extract remaining MeFOSEA from the aqueous matrix. This allowed injection of material onto the gas chromatograph. Previous studies have shown that analysis of diluted aqueous buffers by gas chromatography results in chromatographic anomalies and column degradation.

In this study, biodegradation of MeFOSEA is ruled out for two reasons. First, the 50°C temperature used will impede degradation due to microbial growth in the buffered media. This temperature is hostile to the growth of most mesophillic microorganisms, the type usually inhabiting laboratories. Thermophillic microorganisms, which do thrive at 50°C and above, typically inhabit environments such as hot springs and hydrothermal vents at the bottom of tectonically active ocean basins, but not laboratories. Also, one can hypothesize that the maximum loss rate of MeFOSEA would occur at a different pH if accomplished by hydrolysis versus biodegradation. Since acids or bases typically catalyze hydrolysis, hydrolysis rates are usually maximum under acidic or basic conditions and minimal at or near neutral conditions. This contrasts with the activity of microbes, which typically demonstrate maximum growth rate at or near neutral pH. Maximum rates of hydrolysis were not observed at pH 7.0 in this study.

A proposed mechanism for hydrolytic degradation of MeFOSEA is shown in Figure 2.

Figure 2
MeFOSEA and Possible Degradation Products



2 Sample Receipt

A sample of MeFOSEA (coded THAR3F2) was obtained from C. Elsbernd, 3M Chemicals, on October 13, 1998. The sample was placed into the hydrolysis storage cabinet in the 3M Environmental Laboratory. The sample receiving date, an electronic MSDS, the sample production methodology and predictive chemistries were entered into the 3M proprietary database "Hydrolysis Tracking."

3 Holding Times

The samples were prepared and analyzed within the applicable holding time criteria.

4 Methods-Analytical and Preparatory

3M Environmental Laboratory Method ETS-8-90.0 "Determination of the Stability of MeFOSEA in Aqueous Buffers Using Gas Chromatography with Atomic Emission Detection" was followed except where noted below. This method is presented in Appendix B.

The GC/AED system used was a Hewlett-Packard (HP) 5890 Series II GC equipped with an HP 5921A AED, and an HP 7673 auto-sampler. The column used was a J&W DB5-MS, 30 mm x 0.25 mm x 0.25 µm film thickness. The oven program had an initial temperature of 50°C, initial hold time of 1.0 min., final temperature of 300°C, and a temperature ramp-rate of 15°C per minute.

4.1 Standards:

Test Analyte: 10,730 ppm MeFOSEA in acetone (ID 98086-3-16)
Internal Standard: 9.324 ppm N-EtFOSE alcohol in hexane-1% IPA (ID 98086-3-19, and ID -98060-15-16)
Spiking Standard: 473.4 ppm N-MeFOSE alcohol in methanol (ID W398-1117) (This is an order of magnitude lower than the amount intended due to a calculation error.)

4.2 Sample Preparation and Analysis Dates:

Time 0:	Samples 112398-MeFOSEA-001 to 112398-MeFOSEA-015 Prepared and immediately quenched on 11/24/98. Analysis by GC/AED was begun on 11/25/99.
0.94 Days	Samples 112398-MeFOSEA-016 to 112398-MeFOSEA-030 Incubation started at 3:30 PM on 11/23/98 Quenched at 2:30 PM on 11/24/98 Analysis by GC/AED was begun on 11/25/98 (pH 1.5,5,7) and on 11/30/98 (pH 9,11)
1.92 Days	Samples 112398-MeFOSEA-031 to 112398-MeFOSEA-045 Incubation started at 3:30 PM on 11/23/98 Incubation stopped at 2:10 PM on 11/25/98 Analysis by GC/AED was begun on 11/26/98 (pH 1.5,5,7) and on 12/1/98 (pH 9,11)
6.72 Days	Samples 112398-MeFOSEA-046 to 112398-MeFOSEA-060 Incubation started at 3:30 PM on 11/23/98 Incubation stopped at 8:54 AM on 11/30/98 Analysis by GC/AED was begun on 12/22-23/98 (pH 1.5,5,7), and 12/1/98 (pH 9,11)
13.90 Days	Samples 112398-MeFOSEA-061 to 112398-MeFOSEA-075 Incubation started at 3:30 PM on 11/23/98 Incubation stopped at 1:02 PM on 12/7/98

Analysis by GC/AED was begun on 12/23/98 (pH 1.5,5,7) and on 12/8-9/98 (pH 9,11)

20.71 Days — Samples 112398-MeFOSEA-076 to 112398-MeFOSEA-090
Incubation started at 3:30 PM on 11/23/98
Incubation stopped at 8:38 AM on 12/14/98
Analysis by GC/AED was begun on 12/28/98 (pH 1.5,5,7) and on 12/15-16/98 (pH 9,11)

27.77 Days — Samples 112398-MeFOSEA-091 to 112398-MeFOSEA-105
Incubation started at 3:30 PM on 11/23/98
Incubation stopped at 9:55 AM on 12/21/98
Analysis by GC/AED was begun on 12/29/98 (pH 1.5,5,7, 9) and on 12/30/98 (pH 11)

Sample quenching was completed approximately 1 hour after incubation was stopped. Sample preparation logsheets are presented in Appendix C.

5 Analysis

5.1 Calibration

Standard calibration curves were prepared using a mix of test analyte (MeFOSEA) and degradation product (N-MeFOSE alcohol). Examination of the chromatograms revealed that the intended internal standard (N-EtFOSE alcohol) was in a region of chromatographic overlap of residual components in MeFOSEA. Thus, N-EtFOSE alcohol could not be used as an internal standard with GC/AED testing.

Solvent/Standard Name	Solvent/Standard Number
Hexane solvent used	TN-A-2009
IPA solvent used	TN-A-2102
MeFOSEA standard	98086-3-16
N-MeFOSE alcohol standard	S398-331
N-EtFOSE alcohol standard	S398-332

The calibration standard set consisted of the following:

Reference #	MeFOSEA (ppm)	N-MeFOSE alcohol (ppm)	N-EtFOSE alcohol (ppm)
98086-46A	64.38	63.12	9.324
98086-46B	48.28	47.34	9.324
98086-46C	32.19	31.56	9.324
98086-46D	16.09	15.78	9.324
98086-46E	6.438	6.312	9.324
98086-46F	4.828	4.734	9.324
98086-46G	3.219	3.156	9.324
98086-46H	1.609	1.578	9.324

The standard curves were linear, with the coefficients of determination (r^2) greater than 0.990. Calibration standards were analyzed in duplicate at the beginning of every sample run. At regular intervals (e.g. after 6 - 8 sample injections) blanks consisting of hexane with 1% IPA and 9.324 ppm N-EtFOSE alcohol were analyzed. A mid-range calibration standard was periodically tested throughout each sequence. When the percent difference of amount of measured analyte was greater than ± 25% from the true value, relative to the initial standard curve, the run was discarded. Only samples analyzed before the last acceptable calibration check-standard were considered as valid results.

5.2 Blanks

The internal standard was a solution of 9.32 ppm N-EtFOSE alcohol in hexane/1% IPA. Approximately 1 mL aliquots were transferred to autovials and used as solvent blanks in the analysis. These blanks served as an instrumental check for analyte carryover. Acceptable values for the blanks were less than half the practical Quantitation Limit of the method (the concentration of the lowest standard used in the curve).

5.3 Surrogates

No surrogate analyte was used in this analysis.

5.4 Matrix Spikes

Matrix spike samples were prepared by introducing a known concentration of the target analyte into a sample. These matrix spike samples were carried through the entire sample preparation process. Comparison of the amount quantified from the matrix spike with that of the unspiked sample provided information on contribution of the sample matrix to the analytical results.

One of the spiking solutions used was a 473.4 ppm N-MeFOSE alcohol standard. This concentration was one-tenth of that which would have been optimal. As such, the amount of N-MeFOSE alcohol added was lower than the lowest calibration standard, precluding quantitation. Spike samples on Day 21 and Day 28 also contained 10 µL of a 10,730 ppm solution of MeFOSEA in acetone. For all the pHs extracted on Days 21 and 28, acceptable MeFOSEA recoveries of 80 to 123% were noted. The average recovery of MeFOSEA was 95%.

5.5 Laboratory Control Samples

Laboratory control samples were not prepared for this analysis.

5.6 Sample Related Comments

The MeFOSEA results for the Day 2, pH 7 samples were rejected, as the mid-range calibration standard that followed these samples did not pass quality control criteria specified in the analytical method (Appendix B).

6 Data Summary

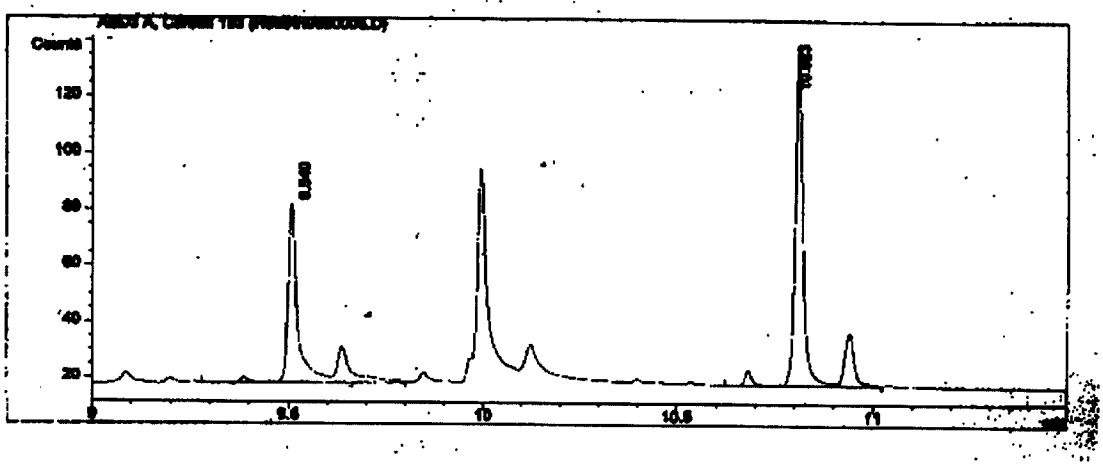
The 3M Environmental Laboratory developed a method for the quantitative determination of MeFOSEA using GC with atomic emission detection. This method was successful using five pH buffer matrices. Sample chromatograms are presented in Appendix D as separate bound

volumes. A representative GC/AED chromatogram of a standard mixture and a sample are presented in Figure 3a and 3b, respectively.

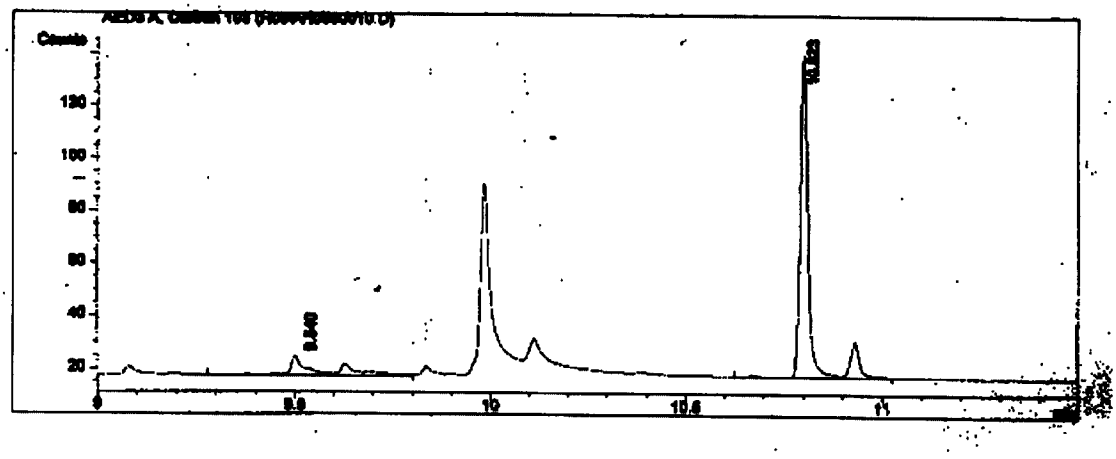
Figure 3
Representative GC/AED Chromatograms

Peak retention times are 9.54 minutes (N-MeFOSE alcohol), 10.0 minutes (internal standard), and 10.82 minutes (MeFOSEA)

a. Standard (6.438 ppm MeFOSEA, 12/22/98, 7:33:14 PM)



b. Sample (MeFOSEA-1.5-1-7, 12/22/98, 9:31:59 PM. Quantified as 5.47 ppm MeFOSEA)



The calculated concentrations of analyte in sample, duplicate, and matrix spike samples are presented in spreadsheet format in Appendix E. These data, plotted as MeFOSEA concentration versus time in Figure 4, show that degradation of MeFOSEA is in general non-linear and a function of pH. Samples from the pH 5.0 and 7.0 series can be fit equally well with either a linear or second-order equation (see Table 1). A second-order equation provides a better fit than a linear equation for the data at pH 1.5, 9.0, and 11.0.

Figure 4
MeFOSEA Concentration Versus Time

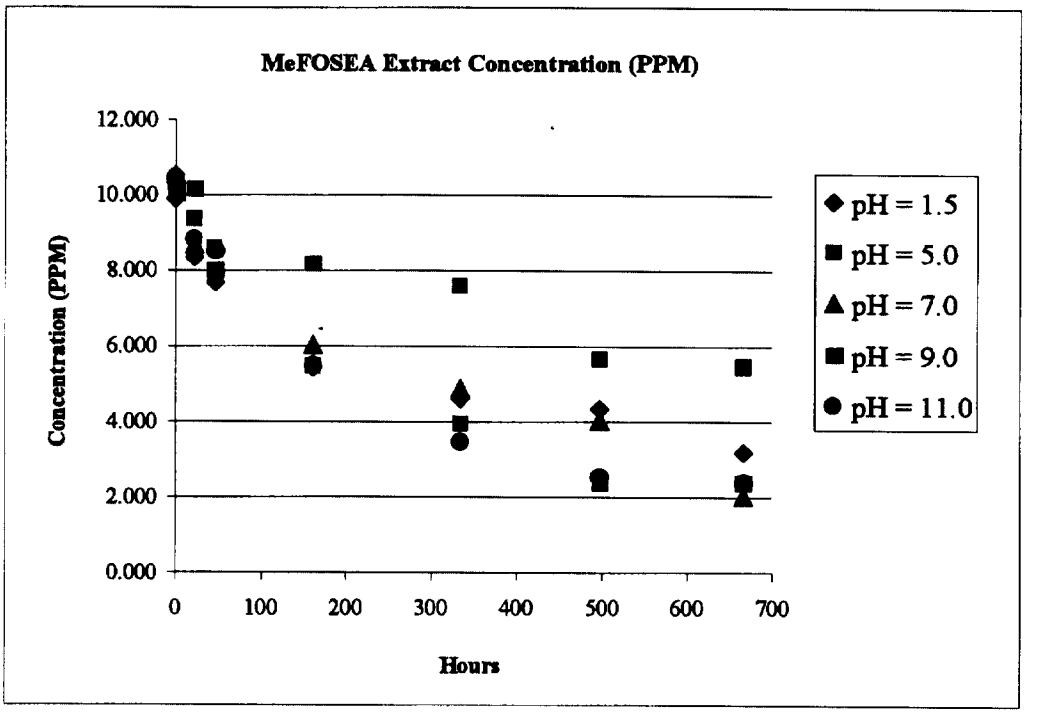


Table 1
Fit of Second-Order and Linear Equations to MeFOSEA Degradation Data

pH	2nd-Order Equation	2nd-Order R^2	Linear Equation	Linear Fit R^2
1.5	$y= 2e10^{-5}x^2 -0.0191x +8.9477$	0.9263	$y= -0.0086x +8.3400$	0.835
5.0	$y= 5e10^{-6}x^2 -0.0097x +9.698$	0.9289	$y= -0.0067x +9.5239$	0.9153
7.0	$y= 1e10^{-5}x^2 -0.0186x +9.6171$	0.9449	$y= -0.0112x +9.1490$	0.9119
9.0	$y= 2e10^{-5}x^2 -0.0277x +9.956$	0.9719	$y= -0.0121x +9.0474$	0.8645
11.0	$y= 3e10^{-5}x^2 -0.0283x +9.832$	0.9859	$y= -0.0118x +8.8757$	0.8625

At the conclusion of the experiment (27.77 days), approximately 20 - 30% of the original MeFOSEA remained for samples hydrolyzing at pH 1.5, 7.0, 9.0, and 11.0. At pH 5, approximately half of the original MeFOSEA was present at the end of the experiment.

Calculations of kinetic parameters are presented in Appendix F. A general trend is that the half-life of MeFOSEA increased with increasing time, especially at pH 1.5, 5.0, 9.0, and 11.0 (Table 2). This behavior doesn't match the mathematical model for a first order reaction. However, the first order approximation for a hydrolysis reaction assumes a single compound degrades to form only two distinct product species. The results shown in Table 1 indicate this assumption may not be realistic for MeFOSEA. As implied in Figure 2, the degradation of MeFOSEA may involve multiple reaction pathways. MeFOSEA is a 3M control compound, whose behavior is used to predict that of other synthetic chemistries. Overall, the results of this study are sufficient for that purpose.

Table 2
Half-Lives of MeFOSEA Versus Time

Time (Days)	pH = 1.5	5.0	7.0	9.0	11.0
0.94	2.58	4.79	3.09	11.99	3.34
1.92	3.99	5.98	----	4.51	5.72
6.72	6.87	16.94	8.05	6.96	6.97
13.90	11.44	27.93	12.23	9.67	8.51
20.71	15.85	22.64	14.61	9.62	9.97
27.77	15.91	28.45	11.53	12.80	12.81

7 Conclusion

The 3M Environmental Laboratory developed a method for hydrolysis and analysis of MeFOSEA. The hydrolysis study showed pH dependent half-lives which increased with time within each pH data set at 50 °C, averaging 9.4 days at pH 1.5, 17.8 days at pH 5.0, 9.9 days at pH 7.0, 9.3 days at pH 9.0, and 7.9 days at pH 11.0. Extrapolation of the 50 °C data to room temperature (25 °C) resulted in a half-lives averaging 94 days at pH 1.5, 178 days at pH 5.0, 99 days at pH 7.0, 93 days at pH 9.0, and 79 days at pH 11.0. It is the conclusion of this study that MeFOSEA is unstable against hydrolysis under environmentally relevant conditions.

8 Data/Sample Retention

Reserve samples and all original paper data will be retained in the archives of 3M ET&SS for a period of ten years following the signing of the final report.

9 Signatures

Thomas L. Hatfield, Ph.D., Team Leader — Date 6/16/99

Cleston Lange, Ph.D., Analyst — Date 6/16/99

Gregory Maisel, Analyst — Date 6/17/99

Joseph J. S. Tokos, Ph. D., Editor — Date 6/18/99

William K. Reagen, Ph. D., Technical Reviewer — Date 6/21/99

Note: Jeanette Wink, Analyst, did the majority of the laboratory work for this project. She reviewed the original data, but has since left 3M Environmental Laboratory. Her assistance during the course of the study is appreciated.

10 Appendices

Appendix A: EPA Guidelines
Appendix B: MeFOSEA Preparation and Analysis Method
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Appendix D. GC/AED Chromatograms (see separate bound volumes)
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Appendix F: Spreadsheets: Calculation of Kinetic Parameters

Appendix A: Fate, Transport and Transformation Test Guidelines, OPPTS 835.2110, Hydrolysis as a Function of pH, U.S. Environmental Protection Agency document number EPA 712-C-98-057, January 1998

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United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

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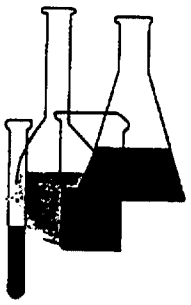
EPA

Fate, Transport and Transformation Test Guidelines

OPPTS 835.2110
Hydrolysis as a Function of pH



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board.* By modem dial 202–512–1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202–512–0132 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading "Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines."

OPPTS 835.2110 Hydrolysis as a function of pH.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline are 40 CFR 796.3500 Hydrolysis as a Function of pH at 25 °C, and OECD guideline 111 Hydrolysis as a Function of pH.

(b) **Guidance information**—(1) **Prerequisites.** Water solubility data; suitable analytical method; vapor pressure curve.

(2) **Qualifying statements.** Pure and commercial grade substances can be tested with the method described here, but the potential effect of impurities on the results should be considered. This test guideline applies only to water soluble compounds. There is uncertainty in extrapolating high temperature results to environmentally relevant temperatures as a change in reaction mechanism could occur.

(3) **Standard documents.** This test guideline is based on methods given in paragraph (f) of this guideline and on the Preliminary Draft Guidance for Premanufacture Notification EPA, August 18, 1978.

(c) **Method**—(1) **Purpose, relevance, application, and limits of test.** (i) The testing of substances for hydrolysis is relevant to their persistence. Hydrolysis is one of the most common reactions controlling abiotic degradation and is therefore one of the main degradation paths of substances in the environment.

(ii) A procedure to determine hydrolysis rates is important also in indicating whether other testing should be carried out on a parent compound or on its hydrolysis products. It is the degradation products that are crucial. Hydrolysis behavior needs to be examined at pH values normally found in the environment (pH 4–9) and under more acidic conditions (pH 1–2) for physiological purposes.

(iii) Surface-controlled reactions can sometimes predominate over bulk solution hydrolysis, especially in the soil environment. This may result in different degradation rates than would be predicted from this guideline based upon rates in homogeneous solutions.

(2) **Definitions and units.** The definitions in section 3 of the Toxic Substances Control Act (TSCA) and the definitions in 40 CFR Part 792—Good Laboratory Practice Standards apply to this test guideline. The following definitions also apply to this test guideline.

Hydrolysis refers to a reaction of a chemical RX with water, with the net exchange of the group X with OH at the reaction center:

$$RX + HOH \longrightarrow ROH + HX$$

Hydrolysis rate, the rate at which the concentration of RX decreases in this simplified process is given by:

second order reaction rate = $k\,[H_2O]\,[RX]$

or

first order reaction rate = $k\,[RX]$

depending on the rate determining step. Because water is present in great excess compared to the chemical, this type of reaction is usually described as a pseudo-first order reaction in which the observed rate constant is given by the relationship:

$$k_{obs} = k\,[H_2O]$$

and can be determined from the expression

$$K_{obs} = 2.303/t \log_{10}(C_0/C_t)$$

where t = time, and C_0 and C_t = concentrations of RX at times 0 and t.

The units of this constant have the dimension of $(\text{time})^{-1}$ and the half-life of the reaction (time for 50 percent of RX to react) is given by

$$t_{1/2} = 0.693/k_{obs}$$

(3) **Reference substances.** (i) Aspirin and Diazinon are used as references. These substances need not be employed in all cases when investigating a new substance. These data are provided primarily so that calibration of the method may be performed from time to time and to offer the chance to compare the results when another method is applied. The results of the OECD/EEC-Laboratory Intercomparison Testing are included in the following Tables 1. and 2.:

Table 1.—Values of Rate Constants and Half-Lives for Aspirin[1,2]

pH	K [10^5 sec^{-1}]	$t_{1/2}$ (h)
3.5	0.065	300
5.0	0.15	130
7.4	0.13	150
9.5	0.37	52
11.3	16	1.2

[1] Data taken at 17 °C
[2] Aspirin (CAS no. 50–78–2) (2-acetylsalicylic acid) data from L.J. Edwards, *Transactions of the Faraday Society* 723–735 (1950).

Table 2.—Values of Rate Constants and Half-Lives for Diazinon[1]

pH	10 °C		20 °C		40 °C		60 °C	
	K [10^5 sec^{-1}]	$t_{1/2}$ (h)	K [10^5 sec^{-1}]	$t_{1/2}$ (h)	K [10^5 sec^{-1}]	$t_{1/2}$ (h)	K [10^5 sec^{-1}]	$t_{1/2}$ (h)
10.43	0.061	310	0.13	150	0.41	48	15	12
9.0	--	--	0.0059	3300	--	--	--	--
7.4	--	--	0.00043	4400	--	--	--	--
5.0	--	--	0.026	740	--	--	--	--
3.1	0.75	260	1.6	120	6.6	29	25	7.8

[1] Diazinon (CAS no. 333–41–5) (*O,O*-diethyl-*O*-(2-isopropyl-4-methyl]-6-pyrimidinyl phosphorothioate) data from H.M. Gomma et al. *Residue Reviews* 29:171 (1969).

(ii) Further results of OECD–EEC Laboratory intercomparison testing follow in Tables 3. through 12. In some cases the results presented have a variability which exceeds what is called for in the test guideline. This may particularly be the result of the use of different buffers in the test systems and the influence of oxygen. These results are presented , however, as they have been obtained using the Test Guidelines in the OECD/EEC Intercomparison Testing Programme, Part II. The guideline has been modified in the light of these results.

(A) Substance: Aspirin

Table 3.—Reaction Rate Constant in $10^5 sec^{-1}$

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
1.2	35–40	1.013	1.278	126.2	0.109–1.916	2
3.0	20	0.080	0.056	70.3	0.040–0.119	2
	40	0.556	0.112	20.1	0.477–0.635	2
7.0	20	0.205	0.033	15.9	0.182–0.228	2
	40	1.339	0.004	0.3	1.336–1.341	2
9.0	20	0.309	0.160	51.7	0.196–0.442	2
	40	0.953	0.006	0.6	0.949–0.957	2

Table 4.—Half-life in hours

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
1.2	35–40	93.71	118.3	126	10.05–177.36	2
3.0	20	319.7	222.5	70	162.3–477.0	2
	40	35.4	7.1	20	30.3–40.4	2 (1 lab)
7.0	20	95.1	15.1	20	84.4–105.8	2
	40	14.4	0.0	–	14.4–14.4	2 (1 lab)
9.0	20	72.1	37.3	50	45.7–98.5	2
	40	20.2	0.1	0.0	20.1–20.3	2 (1 lab)

(B) Substance: Diazinon

Table 5.—Reaction Rate Constant in $10^5 sec^{-1}$

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
1.2	35–40	30.36	8.10	27.0	21.40–38.46	4
3.0	20	2.866	0.825	28.8	1.675–3.841	7
	40	9.038	2.447	27.1	5.708–14.165	11 (10 labs)
	50	5.77	4.27	74.0	0.86–8.58	3 (2 labs)
	60	36.085	11.088	30.7	25.535–51.449	6
7.0	20	0.933	1.796	192.4	0.005–3.626	4
	40	0.231	0.294	127.3	0.042–0.895	8
	50	0.200	0.023	11.3	0.184–0.216	2
	60	1.638	3.154	192.6	0.303–9.413	8 (7 labs)
9.0	20	1.103	2.113	191.6	0.007–4.271	4
	40	2.568	6.900	268.7	0.064–20.955	9
	50	0.292	0.034	11.6	0.268–0.316	2
	60	2.801	4.125	147.3	0.241–12.604	8 (7 labs)

Table 6.—Half-life in hours

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
1.2	35–40	0.672	0.187	27.9	0.501–0.900	4
3.0	20	7.27	2.40	33	5.0–11.5	7
	40	2.25	0.57	25	1.4–3.3	11 (10 labs)
	50	9.00	11.53	128	2.24–22.31	3 (2 labs)
	60	0.57	0.17	29	0.37–0.75	6
7.0	20	1707.75	1875.18	110	5.3–3660.9	4
	40	215.16	166.12	77	21.5–460.7	8
	50	97.07	10.97	11	89.31–104.83	2
	60	38.68	20.98	54	2.0–63.5	8 (7 labs)
9.0	20	1150.75	1335.58	116	4.5–2840.5	4
	40	124.38	102.45	82	0.9–298.7	9
	50	66.40	7.74	12	60.92–71.87	2
	60	25.13	25.67	102	1.5–79.8	8 (7 labs)

(C) Substance: Atrazine

Table 7.—Reaction Rate Constant in $10^5 sec^{-1}$

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
1.2	35–40	0.546	0.416	76.3	0.76–0.948	4
3.0	20	0.014	0.008	56.8	0.005–0.020	3
	40	0.140	0.082	58.4	0.028–0.222	6
	60	0.808	0.397	49.1	0.282–1.283	5
7.0	40	0.009	0.011	124.8	0.001–0.016	2
9.0	20	0.013	0.016	130.1	0.001–0.024	–
	40	0.008	0.010	123.7	0.001–0.015	2

Table 8.—Half-life in hours

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
1.2	35–40	54.76	44.36	81.0	20.3–115.35	4
3.0	20	1867.88	1446.58	77.0	980.7–3537.14	3
	40	240.50	239.61	99.6	111.71–696.79	6
	60	31.42	21.51	69.0	15.0–68.3	5
7.0	40	9000.15	11033.77	123.0	1198.1–16802.2	2
9.0	20	1792105	24188.21	135.0	817.4–35024.7	2
	40	8005.35	9539.51	119.0	1259.9–14750.8	2

(D) Substance: Di(2-ethylhexl) phthalate (DOP)

Table 9.—Reaction Rate Constant in $10^5 sec^{-1}$

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
3.0	20	0.048	0.051	107.8	0.009–0.106	3
9.0	20	0.040	0.047	116.7	0.007–0.073	2
	40	0.166	0.202	121.8	0.023–0.309	2
	60	0.084	0.022	26.3	0.068–0.099	2
7.0	20	0.073	0.064	88.8	0.027–0.118	2
	40	(1.627)	–	–	–	1
	60	(0.092)	–	–	–	1
	40	(0.126)	–	–	–	1

Table 10.—Half-life in hours

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
3.0	20	990.20	996.32	101	182.4–2103.5	3
	40	452.56	551.98	122	62.25–842.86	2
	60	239.33	63.75	27	194.25–284.41	2
7.0	20	440.30	391.35	89	163.57–717.02	2
	40	(11.83)	–	–	–	1
	60	(208.19)	–	–	–	1
	40	(152.53)	–	–	–	1

(E) Substance: Ethyl acetate

Table 11.—Reaction Rate Constant in $10^5 sec^{-1}$

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
3.0	20	(0.0012)	–	–	–	1
	40	(0.012)	–	–	–	1
	60	(0.355)	–	–	–	1
7.0	20	(0.003)	–	–	–	1
	40	(0.008)	–	–	–	1
	60	(0.137)	–	–	–	1
9.0	20	(0.063)	–	–	–	1
	40	(0.153)	–	–	–	1
	60	(1.547)	–	–	–	1

Table 12.—Half-life in hours

pH	temperature, °C	mean value	standard deviation	coefficient of variation, %	range	n° of results
3.0	20	(1656)	–	–	–	1
	40	(1612.85)	–	–	–	1
	60	(54.30)	–	–	–	1
7.0	20	(7553.1)	–	–	–	1
	40	2511.81)	–	–	–	1
	60	(140.38)	–	–	–	1
9.0	20	(305.55)	–	–	–	1
	40	(125.71)	–	–	–	1
	60	(12.44)	–	–	–	1

(4) **Principle of the test method.** (i) In the environment, chemicals usually occur in dilute solution, which means that water is present in large excess, and, therefore, that the concentration of water remains essentially constant during hydrolysis. Hence, the kinetics of hydrolysis are generally pseudo-first order at fixed pH and temperature.

(ii) The hydrolysis reaction may be influenced by acidic or basic species H_3O+, (H+), and OH^-, in which case it is referred to as specific acid or specific base catalysis.

(iii) The concentration of the test substance is determined as a function of time. The logarithms of the concentrations are plotted against time and the slope of the resulting straight line (assuming first-order or pseudo-first order behavior) gives the rate constant from the formula (if $\log_{10}$ is used):

$$k_{obs} = -\text{slope} \times 2.303$$

(iv) When it is not practicable to determine a rate constant for a particular temperature directly, it is usually possible to estimate the constant through the use of the Arrhenius relationship in which the logarithm of rate constants at other temperatures is plotted against the reciprocal of the absolute temperature (K).

(5) **Quality criteria**—(i) **Reproducibility.** Measurements of hydrolysis rate constants on 13 classes of organic structures can be of high precision, often with less than 2 percent standard deviation (see paragraph (f)(2) of this guideline). The rate constants for one pH and one temperature should be determined in duplicate with a deviation of less than 2.5 percent unless unusual circumstances (e.g. analytical difficulties) prevent achieving this and then the details of these circumstances should be reported. The reproducibility can be improved by an improved control of the sensitive parameters, in particular pH and oxygen.

(ii) **Sensitivity.** Most hydrolysis reactions follow apparent first order reaction rates and, therefore, half-lives are independent of concentration

$$t_{1/2} = 0.693/k_{obs}$$

This usually permits the application of laboratory results determined at 10^{-2}–10^{-3} M to environmental conditions ($\leq 10^{-6}$ M) under paragraph (f)(2) of this guideline.

(iii) **Specificity.** Several examples of good agreement between rates of hydrolysis measured in both pure and natural waters for a variety of chemicals providing both pH and temperature have been measured (see paragraph (f)(2) of this guideline).

(d) **Description of the test procedure.** (1) If the water solubility of the substance is less than 2×10^{-2} M, a half-saturated solution in water is prepared. If the solubility is greater than 2×10^{-2}, the test solution is prepared at less than 10^{-2} M. Substances known to be hydrolytically unstable are tested at at least two temperatures between 0–40 °C. Other substances are put through preliminary testing. Substances found to be stable under these conditions are considered hydrolytically stable ($t_{1/2}$ at 25 °C is greater than 1 year). Those not stable at 50 °C for one week are submitted to testing at at least two temperatures between 0–40 °C, or, as appropriate, at at least three elevated temperatures, and the data extrapolated to produce *k* at 25°C.

(2) **Preparations—(i) Materials—(A) Buffer solutions.** (*1*) The hydrolysis test should be performed at four different pH's: at pH 1.2 (if physiologically important); pH 4.0; pH 7.0; and pH 9.0. Either buffers as described below or a pH-stat may be used. Use of a pH-stat avoids potential problems due to buffer catalysis.

(2) For this purpose, 0.05 M sterile buffer solutions should be prepared using reagent grade chemicals and distilled, sterile water. The buffer systems are based upon the analytical requirements for the chemical being tested. It should be noted that the buffer system used may influence the rate of hydrolysis and where this is observed an alternate buffer system should be employed. The use of borate or acetate buffers instead of phosphate has been recommended under paragraph (f)(2) of this guideline. The pH of each buffer solution must be checked with a calibrated pH meter at the required temperature to a precision of at least 0.1 pH units. Some useful buffer systems are presented in the following Tables 13., 14., and 15.:

Table 13.—Buffer Mixtures of Clark and Lubs[1,2]

Composition	pH
0.2 N HCl and 0.2 N KCl	
47.5 ml HC1 + 25 ml KC1 diluted to 100 ml	1.0
32.25 ml HC1 + 25 ml KC1 diluted to 100 ml	1.2

Table 13.—Buffer Mixtures of Clark and Lubs[1,2]—Continued

Composition	pH
20.75 ml HC1 + 25 ml KC1 diluted to 100 ml	1.4
13.15 ml HC1 + 25 ml KC1 diluted to 100 ml	1.6
8.3 ml HC1 + 25 ml KC1 diluted to 100 ml	1.8
5.3 ml HC1 + 25 ml KC1 diluted to 100 ml	2.0
3.35 ml HC1 + 25 ml KC1 diluted to 100 ml	2.2
0.1 M potassium biphthalate + 0.1 N HC1	
46.70 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	2.2
39.60 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	2.4
32.95 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	2.6
26.42 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	2.8
20.32 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	3.0
14.70 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	3.2
9.90 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	3.4
5.97 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	3.6
2.63 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	3.8
0.1 M potassium biphthalate + 0.1 N NaOH	
0.40 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	4.0
3.70 ml 0.1 N NaOH + 50 ml piphthalate to 100 ml	4.2
7.50 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	4.4
12.15 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	4.6
17.70 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	4.8
0.1 M potassium biphthalate + 0.1 NaOH	
23.85 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	5.0
29.95 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	5.2
35.45 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	5.4
39.85 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	5.6
43.00 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	5.8
45.45 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	6.0
0.1 M monopotassium phosphate + 0.1 N NaOH	
5.70 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	6.0
8.60 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	6.2
12.60 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	6.4
17.80 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	6.6
23.45 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	6.8
29.63 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	7.0
35.00 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	7.2
39.50 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	7.4
42.80 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	7.6
45.20 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	7.8
46.80 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	8.0
0.1 M H_2BO_2 in 0.1 M KC1 + 0.1 N NaOH	
2.61 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	7.8
3.97 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	8.0
5.90 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	8.2
8.50 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	8.4
12.00 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	8.6
16.30 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	8.8
21.30 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	9.0
26.70 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	9.2
32.00 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	9.4
36.85 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	9.6
40.80 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	9.8
43.90 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	10.0

[1] Data taken at 20 °C.

[2] The pH values reported in these tables have been calculated from the potential measurements using Sorensen's standard equations (1909). The corresponding pH values are 0.04 unit higher than the tabulated values.

Table 14.—Citrate Buffers of Kolthoff and Vleeschouwer[1,2]

Composition	pH
0.1 M monopotassium citrate and 0.1 N HCl	
49.7 ml 0.1 N HC1 + 50 ml citrate to 100 ml	2.2
43.4 ml 0.1 N HC1 + 50 ml citrate to 100 ml	2.4
36.8 ml 0.1 N HC1 + 50 ml citrate to 100 ml	2.6
30.2 ml 0.1 N HC1 + 50 ml citrate to 100 ml	2.8
23.6 ml 0.1 N HC1 + 50 ml citrate to 100 ml	3.0
17.2 ml 0.1 N HC1 + 50 ml citrate to 100 ml	3.2
10.7 ml 0.1 N HC1 + 50 ml citrate to 100 ml	3.4
4.2 ml 0.1 N HC1 + 50 ml citrate to 100 ml	3.6
0.1 M monopotassium citrate and 0.1 N NaOH	
2.0 ml 0.1 N NaOH + 50 ml citrate to 100 ml	3.8
9.0 ml 0.1 N NaOH + 50 ml citrate to 100 ml	4.0
16.3 ml 0.1 N NaOH + 50 ml citrate to 100 ml	4.2
23.7 ml 0.1 N NaOH + 50 ml citrate to 100 ml	4.4
31.5 ml 0.1 N NaOH + 50 ml citrate to 100 ml	4.6
39.2 ml 0.1 N NaOH + 50 ml citrate to 100 ml	4.8
46.7 ml 0.1 N NaOH + 50 ml citrate to 100 ml	5.0
54.2 ml 0.1 N NaOH + 50 ml citrate to 100 ml	5.2
61.0 ml 0.1 N NaOH + 50 ml citrate to 100 ml	5.4
68.0 ml 0.1 N NaOH + 50 ml citrate to 100 ml	5.6
74.4 ml 0.1 N NaOH + 50 ml citrate to 100 ml	5.8
81.2 ml 0.1 N NaOH + 50 ml citrate to 100 ml	6.0

[1] Data taken at 18 °C.
[2] Add tiny crystal of thymol or a few milligrams of mercury or mercuric iodide to prevent growth of molds.

Table 15.—Borate Buffer Mixtures of Sorensen

Composition			Sorensen (18 °C)	Walbum, pH at		
0.05 M borax (ml)	0.01 N HCl (ml)	0.01N NaOH (ml)		10 °C	40 °C	70 °C
5.25	4.75	0.00	7.62	7.64	7.55	7.47
5.50	4.50	0.00	7.94	7.98	7.86	7.76
5.75	4.25	0.00	8.14	8.17	8.06	7.95
6.00	4.00	0.00	8.29	8.32	8.19	8.08
6.50	3.50	0.00	8.51	8.54	8.40	8.28
7.00	3.00	0.00	8.08	8.72	8.56	8.40
7.50	2.50	0.00	8.80	8.84	8.67	8.50
8.00	2.00	0.00	8.91	8.96	8.77	8.59
8.50	1.50	0.00	9.01	9.06	8.86	8.67
9.00	1.00	0.00	9.09	9.14	8.94	8.74
9.50	0.50	0.00	9.17	9.22	9.01	8.80
10.00	0.00	0.00	9.24	9.30	9.08	8.86
10.0	0.00	0.0	9.24	9.30	9.08	8.86
9.0	0.00	1.0	9.36	9.42	9.18	8.94
8.0	0.00	2.0	9.50	9.57	9.30	9.02
7.0	0.00	3.0	9.68	9.76	9.44	9.12
6.0	0.00	4.0	9.97	10.06	9.67	9.28

(B) **Test solutions.** The chemical substance should be dissolved in distilled, sterile water with sterile buffer medium added to it. The concentrations should not exceed the lesser of 0.01 M or half the saturation concentration (see OPPTS 830.7840, Water solubility, shake flask method), and the purest available form of the substance should be employed

in making up the solutions. The use of mixed solvents is recommended only in case substances with low water solubility. The amount of solvent should be less than 1 percent, and the solvent should not interfere with the hydrolysis process.

(C) **Glassware.** All glassware, which must be inert in the pH range studied, should be sterilized. Stoppered volumetric flasks (no grease) should be used for carrying out the hydrolysis reactions. If the chemical or buffer system is volatile, or if the test is being conducted at elevated temperatures, sealed or septum-closed tubes are preferred and head space should be avoided.

(3) **Analytical method.** The analytical method will be determined by the nature of the substance being tested. It must be sufficiently sensitive and specific to allow determination of the different species at the test solution concentrations and may well consist of some combination of pH electrodes, UV-visible spectrophotometry, conductivity, gas chromatography, high pressure liquid chromatography, extraction and formation of derivative(s), and determination by a suitable analytical method.

(4) **Test conditions**—(i) **Temperature.** For extrapolation purposes, it is important to maintain the temperature of the determinations to at least ±0.1 °C. An appropriate constant temperature bath should be employed. If the hydrolytic behavior of the substance is unknown, a preliminary test at 50 °C is required. For tests beyond this preliminary stage data for temperatures in the range 0–40 °C are sought. They may be obtained by measurement at two temperatures in this range or by extrapolation from three higher temperatures. In any event, the determinations should be done at temperatures differing from each other by at least 10 °C.

(ii) **Light and oxygen.** All of the hydrolysis reactions should be carried out using any suitable method to avoid photolytic effects. All suitable measures should be taken to exclude oxygen (e.g. by bubbling nitrogen or argon through the solvent for 5 minutes before preparation of the solution).

(5) **Performance of the test**—(i) **Preliminary test.** A preliminary test should be performed on the substance at 50±0.1 °C at each of pH 4.0, 7.0, and 9.0. If less than 10 percent of the reaction is observed after 5 days ($t_{1/2}$ > 1 year), the chemical is considered hydrolytically stable and no additional testing is required. If the substance is known to be unstable at environmentally relevant temperatures, the preliminary test is not required. The analytical method must be sufficiently precise and sensitive to detect a reduction of 10 percent in the initial concentration.

(ii) **Hydrolysis of unstable substances.** If the substance is unstable as defined by the preliminary test, the test procedure is to be as follows: The buffered test solutions of the substance should be thermostatted at the selected temperatures. To test for first-order behavior each reaction

solution should be analyzed in time intervals which provide a minimum of six spaced data points, normally between 20 percent and 70 percent of hydrolysis of that test chemical. The reaction should be examined at three (4, 7, 9) pH's at each of the selected temperatures with replication at one of them (the middle temperature in the case of elevated temperature determinations).

(iii) **Hydrolysis at pH 1.2.** The above test for a hydrolytically unstable compound should also be carried out at pH 1.2, employing a single, physiologically significant temperature (37 °C).

(e) **Data and reporting**—(1) **Treatment of results**—(i) **Confirmation of first order kinetics.** The data obtained should be plotted at $\log_{10} C_t$ versus t and the reaction rate constant k_{obs} calculated by regression analysis or from the slope:

$$k_{obs} = 2.303 \times \text{slope}$$

(ii) **Interpretation of results.** If the data do not fall on a straight line, the reaction is not first order, and the data must be analyzed by methods beyond the scope of this test principle.

(2) **Test report.** (i) The test report should include information on:

(A) Sample purity.

(B) Any results appropriate to the procedure employing reference substances.

(C) Detailed test procedure including the temperature, pH and, buffer for each set of experiments.

(D) Detailed analytical method used for the tested substance, including detailed method of extraction and recovery data if an extraction method is used to separate the chemical from the aqueous phase.

(E) All concentration-time data points for reactions which were observed to originate a nonlinear log concentration-time plot.

(F) Possibility of acid or base catalysis.

(ii) A suggested format for sample reporting form, which can be duplicated, is presented:

in making up the solutions. The use of mixed solvents is recommended only in case substances with low water solubility. The amount of solvent should be less than 1 percent, and the solvent should not interfere with the hydrolysis process.

(C) **Glassware.** All glassware, which must be inert in the pH range studied, should be sterilized. Stoppered volumetric flasks (no grease) should be used for carrying out the hydrolysis reactions. If the chemical or buffer system is volatile, or if the test is being conducted at elevated temperatures, sealed or septum-closed tubes are preferred and head space should be avoided.

(3) **Analytical method.** The analytical method will be determined by the nature of the substance being tested. It must be sufficiently sensitive and specific to allow determination of the different species at the test solution concentrations and may well consist of some combination of pH electrodes, UV-visible spectrophotometry, conductivity, gas chromatography, high pressure liquid chromatography, extraction and formation of derivative(s), and determination by a suitable analytical method.

(4) **Test conditions**—(i) **Temperature.** For extrapolation purposes, it is important to maintain the temperature of the determinations to at least ±0.1 °C. An appropriate constant temperature bath should be employed. If the hydrolytic behavior of the substance is unknown, a preliminary test at 50 °C is required. For tests beyond this preliminary stage data for temperatures in the range 0–40 °C are sought. They may be obtained by measurement at two temperatures in this range or by extrapolation from three higher temperatures. In any event, the determinations should be done at temperatures differing from each other by at least 10 °C.

(ii) **Light and oxygen.** All of the hydrolysis reactions should be carried out using any suitable method to avoid photolytic effects. All suitable measures should be taken to exclude oxygen (e.g. by bubbling nitrogen or argon through the solvent for 5 minutes before preparation of the solution).

(5) **Performance of the test**—(i) **Preliminary test.** A preliminary test should be performed on the substance at 50±0.1 °C at each of pH 4.0, 7.0, and 9.0. If less than 10 percent of the reaction is observed after 5 days ($t_{1/2}$ > 1 year), the chemical is considered hydrolytically stable and no additional testing is required. If the substance is known to be unstable at environmentally relevant temperatures, the preliminary test is not required. The analytical method must be sufficiently precise and sensitive to detect a reduction of 10 percent in the initial concentration.

(ii) **Hydrolysis of unstable substances.** If the substance is unstable as defined by the preliminary test, the test procedure is to be as follows: The buffered test solutions of the substance should be thermostatted at the selected temperatures. To test for first-order behavior each reaction

solution should be analyzed in time intervals which provide a minimum of six spaced data points, normally between 20 percent and 70 percent of hydrolysis of that test chemical. The reaction should be examined at three (4, 7, 9) pH's at each of the selected temperatures with replication at one of them (the middle temperature in the case of elevated temperature determinations).

(iii) **Hydrolysis at pH 1.2.** The above test for a hydrolytically unstable compound should also be carried out at pH 1.2, employing a single, physiologically significant temperature (37 °C).

(e) **Data and reporting**—(1) **Treatment of results**—(i) **Confirmation of first order kinetics.** The data obtained should be plotted at $\log_{10} C_t$ versus t and the reaction rate constant k_{obs} calculated by regression analysis or from the slope:

$$k_{obs} = 2.303 \times \text{slope}$$

(ii) **Interpretation of results.** If the data do not fall on a straight line, the reaction is not first order, and the data must be analyzed by methods beyond the scope of this test principle.

(2) **Test report.** (i) The test report should include information on:

(A) Sample purity.

(B) Any results appropriate to the procedure employing reference substances.

(C) Detailed test procedure including the temperature, pH and, buffer for each set of experiments.

(D) Detailed analytical method used for the tested substance, including detailed method of extraction and recovery data if an extraction method is used to separate the chemical from the aqueous phase.

(E) All concentration-time data points for reactions which were observed to originate a nonlinear log concentration-time plot.

(F) Possibility of acid or base catalysis.

(ii) A suggested format for sample reporting form, which can be duplicated, is presented:

DATA SHEET FOR HYDROLYSIS STUDIES

Laboratory:_____

Date:_____

Test Substance:_____

Formula:_____

Name (IUPAC):_____

Test protocol

A. Preliminary test

yes_____

no_____

buffer systems used:

pH 4.0_____

pH 7.0_____

pH 9.0_____

Approximate saturation concentration	pH		
mole/L	4.0	7.0	9.0
C_0: Initial concentration	.....	.....	.....
C_t: Final concentration after *t* days; t_{max} = 5	.....	.....	.....
t	.....	.....	.....
$(C_0 - C_t)/c_0 \times 100$ at 50 ±0.1 °C	.....	.....	.....

B. **Determination**

Separate runs at pH 4.0, 7.0, and 9.0 at the chosen temperature(s) *with replication at one of these* (the middle temperature in the case of determination at elevated temperature). The same format whould be used for each pH.

Buffer solution used_____

Temperature_____

Approximate saturation concentration_____

t [ ] 0

C_t [mole/L]	.....	.....	.....	.....	.....	.....
log C_t	.....	.....	.....	.....	.....	.....

Hydrolysis at pH 1–2

Buffer solution used_____

pH_____

Temperature_____

Approximate saturation concentration_____

t [ ] 0

C_t [mole/L]	.....	.....	.....	.....	.....	.....
log C_t	.....	.....	.....	.....	.....	.....

Final data

pH	temperature °C	initial concentration, C_0 [mole/L]	reaction rate constant, k_{obs} [1/s × 10^5]	half-life, $t_{1/2}$ [h]	coefficient of correlation, r^2
.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....

C. **Report on test method**

Provide detailed description of the experimental conditions, e.g. for maintaining sterility; to avoid photolytic effects; to exclude oxygen; to prepare the test solution, etc. (Please use separate sheet of paper.)

Analytical combinations used

h	h
pH electrodes	.....
UV-visible spectrophotometry	.....
Conductivity	.....
Gas chromatography	.....
High pressure liquid chromatography	.....
Extraction and formation of derivative(s)	.....

Details of the analytical performance:

Type of apparatus

Test conditions

Was the accuracy of this result determined in any additional way ?

Particular incidents:

Comments:

(f) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Kolthoff, I.M. and Laitinen, H.A. *pH and Electro-Titrations*, 2nd Ed., Wiley, pp 34–36 (1941).

(2) Mabey, W. and Mill, T., Critical Review of Hydrolysis of Organic Compounds in Water Under Environmental Conditions. *Journal of Physical Chemistry Reference Data* 7:383–415 (1978).

(3) Gomaa, H.M. et al. Kinetics of Hydrolysis of Diazoxon. *Residue Reviews* 29:171 (1969).

(4) OECD Document A80.30, Summary of OECD-EEC Laboratory Intercomparison Testing Programme, Part 2, Umweltbundesamt, Berlin, May 1980.

Appendix B: 3M Environmental Laboratory Method ETS-8-90.0 "Determination of the Stability of MeFOSEA in Aqueous Buffers Using Gas Chromatography with Atomic Emission Detection"

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3M ENVIRONMENTAL LABORATORY

METHOD

DETERMINATION OF THE STABILITY OF MEFOSEA IN AQUEOUS BUFFERS USING GAS CHROMATOGRAPHY WITH ATOMIC EMISSION DETECTION

Method Number: ETS-8-90.0 **Adoption date:** 06/07/99

Revision Date: NA

Author: Thomas Hatfield, Ph.D./Cleston Lange, Ph.D./Gregory Maisel/Jeanette Wink

Approved by:

Laboratory Manager — Date 6/7/99

Group Leader — Date 6/4/99

Technical Reviewer — Date 6/3/99

1.0 SCOPE AND APPLICATION

1.1 This procedure defines the steps for preparation and analysis of 2-(*N*-methylperfluorooctanesulfonamido)-ethyl acrylate (MeFOSEA) from aqueous hydrolysis by gas chromatography with atomic emission detection (GC/AED).

1.2 Acceptable Matrices: Aqueous solutions at various buffered pHs.

2.0 SUMMARY OF THE METHOD

2.1 One milliliter of buffered aqueous sample from the hydrolysis of MeFOSEA is quenched with 0.5 mL isopropyl alcohol (IPA) and extracted with 10 mL hexane. A portion (1-2 mL) of the separated hexane phase is dried through an anhydrous sodium sulfate column and then analyzed on a gas chromatograph with an atomic emission detector.

3.0 DEFINITIONS

3.1 **Duplicate analyses**. Analyses or measurements of the analyte of interest performed identically on the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not precision of sampling, preservation or storage internal to the laboratory.

3.2 **Sample duplicates.** Two samples taken from and representative of the same sample source and carried through all steps of the sampling and analytical procedures in an identical manner. Duplicate samples are used to assess variance of the total method, including sampling and analysis.

3.3 **Matrix spike**. Prepared by adding a known mass of target analyte to specified amount of a sample matrix for which an independent estimate of target analyte concentration is available. Matrix spikes are used to determine the effect of the matrix on the method's recovery efficiency.

3.4 **Solvent blank**. A sample of analyte-free medium to which all reagents are added in the same volumes or proportions as used in sample processing but is not carried through the complete sample preparation and analytical procedure.

3.5 **Continuing Calibration Verification (CCV).** A standard analyzed periodically during an analytical run to verify the continued accuracy of the calibration curve. This solution may be prepared from a different source of lot number than the calibration curve standards.

3.6 **Limit of Quantitation (LOQ).** The lowest concentration that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions. It may be nominally chosen within these guidelines to simplify data reporting. For many analytes, the LOQ analyte concentration is selected as the lowest non-zero standard in the calibration curve. Sample LOQs are highly matrix dependent.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

4.1.1 Wear the proper lab attire for all parts of these procedures. Wear gloves at all times.

4.1.2 Handle all solvent in a hood for all parts of the described sample preparation procedure.

4.1.3 For potential hazards of each chemical used, refer to material safety data sheets, packing materials and 3M Environmental Laboratory's Chemical Hazard Review.

4.1.4 Use care when working with GC/AED because high temperatures and ultraviolet light are present.

4.2 Cautions:

4.2.1 Rinse with solvents all glassware used to prepare standards to reduce the possibility of contamination.

5.0 INTERFERENCES

5.1 Contaminants in solvents, reagents, glassware, and other sample processing or analysis hardware may cause interferences. Routinely analyze laboratory solvent blanks to demonstrate that no interferences are present during the analysis.

6.0 EQUIPMENT

6.1 Balance, capable of measuring to 0.1 mg.

6.2 Shaker, incubating, capable of holding at 50°C ± 3°C.

6.3 pH meter, Corning Model 308 pH/Temperature Meter with 3-in-1 gel filled combination electrode (pH/reference/temperature) or equivalent.

6.4 Gas Chromatograph, HP 5890 Series II, with HP 5921A Atomic Emission Detector (AED) or equivalent.

6.5 Column, J & W Scientific Incorporated, DB-5MS, 30 m x .25 mm x .25 µm or equivalent.

6.6 Data Recording System, HP ChemStation Rev. A.05.04 with Windows NT software.

6.7 Autosampler, HP 7673 or equivalent.

6.8 Clock.

7.0 SUPPLIES AND MATERIALS

7.1 Autovials, crimp top, 1.5 mL.

7.2 Vials, glass screw top, 40 mL, I-CHEM or equivalent.

7.3 Labels, Avery 5160 or equivalent.

7.4 Graduated pipettes, glass, disposable, various sizes.

7.5 Pasteur pipettes, glass, disposable.

7.6 Volumetric flasks, various sizes.

7.7 Beakers, glass, various sizes.

8.0 REAGENTS AND STANDARDS

8.1 Buffer solutions

Prepare the buffer solutions in 1000 mL quantities. Adjust pH with NaOH or HCl. Use a portable pH meter to calibrate all buffer solutions. Record final pH measurements of buffers. Store the buffer solutions in sealed 1000 mL flasks. Prepare buffers as follows:

8.1.1 pH 1.5 $\pm$ 0.3

a) 250 mL of 0.1 M HCl,

b) 125 mL of 0.2 M KCl,

c) Bring to a final volume of 1 L with ASTM Type I H_2O.

8.1.2 pH 5.0 ± 0.3

a) Dissolve 3.57 g ammonium acetate in 200 mL ASTM Type I H_2O.

b) Add 230 mL of 0.052 M acetic acid (3 mL glacial acetic acid into 1 L ASTM Type I H_2O)

c) Bring to a final volume of 1 L with ASTM Type I H_2O.

8.1.3 pH 7.0 ± 0.3

a) Dissolve 7.9 g Trizma®-HCl in 200 mL ASTM Type I H_2O.

b) Adjust pH to 7.0 with 0.1N NaOH.

c) Bring to a final volume of 1 L with ASTM Type I H_2O.

8.1.4 pH 9.0 ± 0.3

a) 46 mL of 0.1 N HCl.

b) 125 mL of 0.1 M borax (sodium borate, 10 hydrate).

c) Adjust pH to 9.0.

d) Bring to a final volume of 1 L with ASTM Type I H_2O.

8.1.5 pH 11.0 ± 0.3

a) 250 mL of 0.1 N NaOH.

b) 250 mL of 0.1 M borax.

c) Adjust pH to 11.0.

d) Bring to a final volume of 1 L with ASTM Type I H_2O.

8.2 **Acetone**, spectroscopy grade or equivalent.

8.3 **IPA**, spectroscopy grade or equivalent.

8.4 **Hexane**, 85% n-Hexane, spectroscopy grade or equivalent. Use hexane in post-injection solvent washes for the GC/AED. Prepare a one L hexane solution containing 10 mL of IPA (hexane-1% IPA) to use for extraction and for solvent blanks.

8.5 **MeFOSEA,** prepared in acetone, approximately 10,000 ppm. Weigh 0.1 g of MeFOSEA and dissolve in 10 mL acetone. Use this solution for calibration standards, test analyte samples and spikes.

8.6 **Sodium sulfate**, anhydrous, 60 mesh, reagent grade.

9.0 SAMPLE HANDLING

9.1 Handle all samples and standards in a well-ventilated area. Wear gloves when handling solutions.

9.2 Prepare hydrolysis sample prep worksheets for each sample set. (Attachment A.) Prepare a tracking schedule for sample extraction.

9.3 Analyze all samples as soon as possible after extraction. If samples cannot be analyzed immediately, store in refrigerator at approximately 4°C.

10.0 QUALITY CONTROL

10.1 **Solvent blank.** Prepare solvent blanks (hexane-1% IPA). This serves as an instrumental check for any analyte carryover.

10.2 **Duplicates.** Prepare a sample and a duplicate. Perform two injections of each.

10.3 **Matrix spikes.** Prepare a post-hydrolysis matrix spike for each of the pHs used in the study. Perform two injections of each spike.

10.4 **CCV.** Run a CCV every 15 or fewer injections.

11.0 CALIBRATION AND STANDARDIZATION

11.1 **Standard preparation.** Prepare six calibration standards of MeFOSEA. Standards from approximately 1.6 to 32 ppm are suggested.

11.2 **Calibration standards.** Analyze calibration standards at the beginning of the run.

11.3 **Coefficient of Determination.** The acceptable coefficient of determination (r^2) is 0.990 or greater. Curves should be examined closely for linearity and intercept, particularly for accuracy of quantitation at the high and low ends of the curve.

12.0 PROCEDURE

12.1 Sample preparation.

12.1.1 Establish time points for sample analysis. Typical time points are at days 0, 1, 2, 7, 14, 21 and 28.

12.1.2 For each pH to be tested, set up the 40 mL I-Chem vials to allow for a sample, a duplicate and a spike for the duration of the experiment. Each time point has 3 samples per pH and five pHs for a total of 15 vials per time point.

12.1.3 Create labels and affix to vials. Label information should include date, a sample number; the pH; whether the vial contains sample, duplicate or spike; the time point; and the initials of the analyst. An example using June 15, 1999 is: 61599-MeFOSEA-002

pH 1.5
Duplicate
Day 0
CCL

12.1.4 Remove vial cap. With a pipette, add 1 mL of buffer to the bottom of each vial, taking care to avoid the walls of the vial. Always replace cap immediately after any addition to minimize evaporation.

12.1.5 Using a 25 µL gas-tight syringe, add 10µL of MeFOSEA test analyte solution to each vial with buffer, except for those marked Day 0. Tilt the vial so the buffer runs to one side of the bottom of the vial. Add the test analyte solution on the opposite side of the vial, away from the buffer.

12.1.6 Prepare time zero samples when adequate time is available to immediately quench the samples. Set up each sample individually. Extract each one with hexane-1% IPA before starting another to get a true time zero.

12.1.7 Fill out hydrolysis sample prep worksheets (Attachment A) for each sample set.

12.1.8 Place the samples in the 50°C incubator. Set for 100 rpm shaking. Note time, date, and temperature on worksheets.

12.1.9 At the specified time point, remove samples from the incubator. Let cool to room temperature for 15 minutes.

12.1.10 Add 10 µL of the MeFOSEA spike solution to the bottom of each spike vial so it mixes with buffer.

12.1.11 Add 0.5 mL of IPA to all vials. Swirl to mix.

12.1.12 Add 10 mL of the hexane-1% IPA to vials. Recap vials and shake vigorously for 10-15 seconds. Let settle for 1 minute. The water phase is on the bottom; the hexane phase is on the top.

12.1.13 With a pipette, pull off a portion (1-2 mL) of the top phase. Dispense the hexane onto a column consisting of approximately 1 inch height of Na_2SO_4, in a Pasteur pipette. Allow the hexane extract to flow through the column into a prelabeled autovial. Use two autovials per sample. Cap the vials. Store one set of vials in the freezer at approximately -20°C. Place the other set of vials in the autosampler for analysis. If samples cannot be immediately analyzed, store in refrigerator at approximately 4°C.

12.2 Solvent blanks.

12.2.1 To 40 mL I-Chem vials, add 10 mL of hexane-1% IPA. Analyze in the same manner as the samples.

12.3 Sample analysis.

12.3.1 Install the analytical column and establish instrument conditions. Allow the system to equilibrate for 30 minutes before starting the analysis.

TABLE 1. SUGGESTED GC CONDITIONS FOR SAMPLE ANALYSIS

PARAMETER	SUGGESTED VALUE
Inlet temperature:	180°C
Inlet liner:	4 mm ID, single gooseneck, glass
Detect temperature:	280°
Carrier gas:	Helium
Oven Program:	
Initial temperature:	50°C
Initial time:	1.00 min.
Rate:	15.0°C/min.
Final temperature:	300°C
Final time:	0.00 min.

12.3.2 Set head pressure for a linear velocity through column between 28-32 cm/sec.

TABLE 2. SUGGESTED AED CONDITIONS FOR SAMPLE ANALYSIS

PARAMETER	SUGGESTED VALUE
Transfer line:	280°C
Cavity:	280°C
Min. peak width:	0.054 min.
Data rate:	5.0 hz
Solvent vent program:	
On time:	0.00
Off time:	3.50 minutes
Elements:	Carbon 193, Sulfur 181, Nitrogen 174
Spectra:	Save

12.3.3 **TABLE 3. SUGGESTED AUTOSAMPLER CONDITIONS FOR SAMPLE ANALYSIS**

PARAMETER	SUGGESTED VALUE
Autosampler injector:	7673
Sample washes:	1
Sample pumps:	3
Injection volume:	1.0 µL
Syringe size:	5.0 µL
On column:	Off
Nanoliter adapter:	Off
Post Injection Solv A washes:	4 (hexane)
Post Injection Solv B washes:	4 (hexane)
Viscosity delay:	0 sec
Plunger speed:	Fast

12.3.4 Enter the standard and sample information into the sequence table. Analyze calibration standards first, then a sample set of 15 or fewer injections, followed by either the continuing calibration verification standard or by the full set of calibration standards. Run a solvent blank after the highest calibration standard and after a set of 15 or fewer injections to check for any analyte carryover.

12.3.5 Place the standards, samples and QC (duplicates, matrix spikes, and blanks) into the autosampler tray according to the order they are listed in the sequence table of the HP ChemStation.

12.3.6 Start the sequence.

13.0 DATA ANALYSIS

13.1 Matrix spike recoveries. Calculate spike recoveries using the formula below:

$$\% \text{ recovery} = \frac{(\text{spiked sample result} - \text{sample result})}{\text{actual spiked amount}} \times 100$$

13.2 MeFOSEA concentrations. Calculate the concentration of MeFOSEA in the hexane extract by external method using the calibration curve.

13.3 Data plot. Obtain a plot of MeFOSEA concentration in the hexane extract as a function of time for each pH. Use these data for degradation rate determinations.

14.0 METHOD PERFORMANCE

14.1 Solvent blanks. The measured value for the solvent blank should be less than half the LOQ of the method.

14.2 Sample duplicates. The warning limits are the average ± twice the relative percent difference. The control limits are the average ± three times the relative percent difference. If the lower value of the warning or control limit calculates to be less than zero, use zero as the low limit.

14.3 Matrix spikes. The recovery warning limits are the average spike recovery ± twice the standard deviation. The recovery control limits are the average spike recovery ± three times the standard deviation.

14.4 CCV. Analyze a mid-range calibration standard and a solvent blank after 15 or fewer sample injections and at the end of the run. If the percent difference for the amount of measured analyte exceeds ± 25% of the true value, relative to the initial standard curve, stop the run. Use only those samples analyzed before the last acceptable calibration check standard. Reanalyze the remaining samples with a new calibration curve.

14.5 **Coefficient of Determination.** The coefficient of determination (r^2) should be 0.990 or greater. The curves should be examined closely for linearity and intercept, particularly for accuracy of quantitation at the low and high ends of the curve.

14.6 **LOQ.** The LOQ is equal to the lowest standard in the calibration curve.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

15.1 Dispose of sample waste according to acceptable laboratory practice. Refer to the 3M Waste Stream procedure for further information.

16.0 RECORDS

16.1 Print out hard copies of all graphics and data analysis summaries for archiving.

16.2 Sign and date all graphics and label with instrument ID.

16.3 Record sample weights and extraction information on the hydrolysis sample prep worksheets (Attachment A).

16.4 Print out the sample sequence table, reduce the size with photocopying and tape the photocopy into the instrument log. Keep all the original copies in the raw data files package.

16.5 Print chromatograms and internal standard reports for all analyses.

16.6 Print calibration tables and curve information and store in raw data file.

16.7 Store hydrolysis sample prep worksheets in the raw data file.

16.8 Enter all standard preparation information in the standards preparation logbook.

16.9 Backup all electronic data to appropriate media.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1 Method validation has not been performed for this method.

18.0 REFERENCES

18.1 Fate, Transport and Transformation Test Guidelines, Office of Prevention, Pesticides and Toxic Substances. (OPPTS). 835.2110. Hydrolysis as a Function of pH and Temperature. EPA. 712-C-98-057. January 1998.

18.2 Fate, Transport and Transformation Test Guidelines, Office of Prevention, Pesticides and Toxic Substances. (OPPTS). 835.2130. Hydrolysis as a Function of pH and Temperature. EPA. 712-C-96-059. April 1996.

18.3 *Handbook of Chemical Property Estimation Methods.* Rate of Hydrolysis. pp. 7-1 through 7-48.

18.4 *CRC Handbook of Chemistry and Physics, 1st Student Edition.* "Buffer Solutions Operational Definitions of pH." Robert C. Weast, Ph.D. 1988, p. D-87.

19.0 AFFECTED DOCUMENTS

19.1 None

20.0 REVISIONS

Rev number	Revision reason	Rev date

Fluorochemical Degradation (Hydrolysis) Analysis

TEST ANALYTE:

HOURS:

Sample No.		Description	Time of Initial Prep	Buffer pH	Buffer Volume (mL)	Test Analyte Solution (µL)	ISTD Solution (µL)	Spike Solution (µL)	Time of Quenching	Quenching Solvent (mL)
-	-	Sample		1.5	1.0					
-	-	Duplicate		1.5	1.0					
-	-	Spike ☐		1.5	1.0					
-	-	Sample		5	1.0					
-	-	Duplicate		5	1.0					
-	-	Spike ☐		5	1.0					
-	-	Sample		7	1.0					
-	-	Duplicate		7	1.0					
-	-	Spike ☐		7	1.0					
-	-	Sample		9	1.0					
-	-	Duplicate		9	1.0					
-	-	Spike ☐		9	1.0					
-	-	Sample		11	1.0					
-	-	Duplicate		11	1.0					
-	-	Spike ☐		11	1.0					

Date of Initial Prep:

Date of Quenching:

	Test Analyte Solution	ISTD Solution	Spike Solution	Quenching Solution
Standard/Traceability No.				
Component				
Concentration (µg/mL)				NA

Temperature of Incubator (°C):

Incubation Start (Date and Time):

Incubation Stop (Date and Time):

Total Incubation Time:

Buffer Addition by:

ISTD Addition by:

Test Analyte Addition by:

Quenching by:

Spike Addition by:

Autovial Aliquoting by:

Centrifugation Yes / No

Centrifuge:

RPM:

Time: min

By:

Filtration: Yes / No

By:

Pore Size: um, Brand:

Reviewed by:

Page 13 of 13

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Appendix C: MeFOSEA Sample Preparation Logsheets

Attorney Client/Work Product Privileged
Do Not Disclose

Do Not Copy

Fluorochemical Degradation (Hydrolysis) Analysis

TEST ANAYLTE: Methyl FOSEA (THAR3F2) **Date** 11/23/98

HOURS: day 0

Sample No.	Description	Time of Initial Prep	Buffer pH	Buffer Volume (mL)	Test Analyte Solution (µL)	ISTD Solution (µL)	Spike Solution (µL)	Time of Quenching	Quenching Solvent (mL)
101998 -MeFOSEA-001	Sample	2:00pm 11-24-98	1.5	1.0	10	in quench	0	2:00	10
101998 -MeFOSEA-002	Duplicate	2:00pm 11-24	1.5	1.0	10	in quench	0	2:02	10
101998 -MeFOSEA-003	Spike [X]	2:02 11-24	1.5	1.0	10	in quench	10	2:02	10
101998 -MeFOSEA-004	Sample	2:02	5	1.0	10	in quench	0	2:02	10
101998 -MeFOSEA-005	Duplicate	2:04	5	1.0	10	in quench	0	2:04	10
101998 -MeFOSEA-006	Spike [X]	2:04	5	1.0	10	in quench	10	2:04	10
101998 -MeFOSEA-007	Sample	2:06	7	1.0	10	in quench	0	2:06	10
101998 -MeFOSEA-008	Duplicate	2:06	7	1.0	10	in quench	0	2:06	10
101998 -MeFOSEA-009	Spike [X]	2:08	7	1.0	10	in quench	10	2:08	10
101998 -MeFOSEA-010	Sample	2:08	9	1.0	10	in quench	0	2:08	10
101998 -MeFOSEA-011	Duplicate	2:10	9	1.0	10	in quench	0	2:10	10
101998 -MeFOSEA-012	Spike [X]	2:10	9	1.0	10	in quench	10	2:10	10
101998 -MeFOSEA-013	Sample	2:12	11	1.0	10	in quench	0	2:12	10
101998 -MeFOSEA-014	Duplicate	2:12	11	1.0	10	in quench	0	2:12	10
101998 -MeFOSEA-015	Spike [X]	2:12	11	1.0	10	in quench	10	2:12	10

11238-
CCL
5/13/99

Date of Initial Prep: 11-24-98

Date of Quenching: 11-24-98

	Test Analyte Solution	ISTD Solution	Spike Solution	Quenching Solution
Standard/Traceability No.	98086-3-16	98086-3-19	W398-11-17	98086-3-19
Component	MeFOSEA	EFA	MFA	Hexane + ISTD
Concentration (µg/mL)	10,730 ppm	9.324 ppm	[illegible]	9.324 ppm
		473.4 ppm 4,739		

Temperature of Incubator (°C): 50

Incubation Start (Date and Time): 11-24-98 2:00

Incubation Stop (Date and Time): 11-24-98 3:00

Total Incubation Time: Ø

Buffer Addition by: JJD

[redacted] CCL

Test Analyte Addition by: CCL, MLA (pH 9, 11)

[redacted] CCL

[redacted] CCL

[redacted] CCL

[redacted] Yes No

Centrifuge: Roujan

RPM:

Time: min

By:

[redacted] Yes No

By:

Pore Size: um, Brand:

Reviewed by: Emanuel 5/13/99

3M Environmental Laboratory Request No: U2744

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22000

Fluorochemical Degradation (Hydrolysis) Analysis

TEST ANAYLTE: Methyl FOSEA **Date** 11/23/98

HOURS: day 1

12358-

CCL 5/13/99

Sample No.	Description	Time of Initial Prep	Buffer pH	Buffer Volume (mL)	Test Analyte Solution (µL)	ISTD Solution (µL)	Spike Solution (µL)	Time of Quenching	Quenching Solvent (mL)
101998-MeFOSEA-016	Sample	2:50 pm	1.5	1.0	10	in quench	0	2:30	10
101998 -MeFOSEA-017	Duplicate	2:50 pm	1.5	1.0	10	in quench	0		10
101998 -MeFOSEA-018	Spike [X]	2:50 pm	1.5	1.0	10	in quench	10		10
101998 -MeFOSEA-019	Sample	3:05	5	1.0	10	in quench	0		10
101998 -MeFOSEA-020	Duplicate	3:05	5	1.0	10	in quench	0		10
101998 -MeFOSEA-021	Spike [X]	3:05	5	1.0	10	in quench	10		10
101998 -MeFOSEA-022	Sample	3:05	7	1.0	10	in quench	0		10
101998 -MeFOSEA-023	Duplicate	3:05	7	1.0	10	in quench	0		10
101998 -MeFOSEA-024	Spike [X]	3:05	7	1.0	10	in quench	10		10
101998 -MeFOSEA-025	Sample	3:20	9	1.0	10	in quench	0		10
101998 -MeFOSEA-026	Duplicate	3:20	9	1.0	10	in quench	0		10
101998 -MeFOSEA-027	Spike [X]	3:20	9	1.0	10	in quench	10		10
101998 -MeFOSEA-028	Sample	3:40	11	1.0	10	in quench	0		10
101998 -MeFOSEA-029	Duplicate	3:40	11	1.0	10	in quench	0		10
101998 -MeFOSEA-030	Spike [X]	3:40	11	1.0	10	in quench	10	~ [redacted]	10

Date of Initial Prep: 11-23-98

Date of Quenching: 11-24-98 first sample 5/13/99

	Test Analyte Solution	ISTD Solution	SPK Solution	Quenching Solution
Standard/Traceability No.	98086-3-16	98086-3-19	W398-1117	98086-3-19
Component	MeFOSEA	EFA	MFA	Hxan
Concentration (µg/mL)	10,730 ppm	9.324 ppm	9.734 ppm	[redacted]

Temperature of Incubator (°C): 50

[redacted] (Date and Time): 3:30 11/23/98

[redacted] (Date and Time): 2:00 11/24/98

[redacted] Time: 0.94 days

[redacted]: JJD (by CCL)

[redacted]: CCL

[redacted]: CCL

[redacted]: CCL

[redacted]: CCL

[redacted]: CCL

[redacted]: Yes / No

Centrifuge: Roujan

RPM:

Time: min

By:

[redacted]: Yes / No

By:

Pore Size: um, Brand:

Reviewed by: J N Maiul 5/13/99

3M Environmental Laboratory Request No: U2744

000078

Fluorochemical Degradation (Hydrolysis) Analysis

TEST ANAYLTE: Methyl FOSEA **Date** 11/23/98

HOURS: day 2

112398

CCL 11/13/99

Sample No.	Description	Time of Initial Prep	Buffer pH	Buffer Volume (mL)	Test Analyte Solution (µL)	ISTD Solution (µL)	Spike Solution (µL)	Time of Quenching	Quenching Solvent (mL)
101998 -MeFOSEA-031	Sample	2:52	1.5	1.0	10	in quench	0	2:02	10
101998 -MeFOSEA-032	Duplicate	2:52	1.5	1.0	10	in quench	0	~~2:03~~ 2:06	10
101998 -MeFOSEA-033	Spike ☑	2:52	1.5	1.0	10	in quench	10	~~2:03~~ 2:10	10
101998 -MeFOSEA-034	Sample	3:07	5	1.0	10	in quench	0	2:03	10
101998 -MeFOSEA-035	Duplicate	3:07	5	1.0	10	in quench	0	2:07	10
101998 -MeFOSEA-036	Spike ☑	3:07	5	1.0	10	in quench	10	2:10	10
101998 -MeFOSEA-037	Sample	3:07	7	1.0	10	in quench	0	2:03	10
101998 -MeFOSEA-038	Duplicate	3:07	7	1.0	10	in quench	0	2:07	10
101998 -MeFOSEA-039	Spike ☑	3:07	7	1.0	10	in quench	10	2:11	10
101998 -MeFOSEA-040	Sample	3:20	9	1.0	10	in quench	0	2:04	10
101998 -MeFOSEA-041	Duplicate	3:20	9	1.0	10	in quench	0	2:07	10
101998 -MeFOSEA-042	Spike ☑	3:20	9	1.0	10	in quench	10	2:11	10
101998 -MeFOSEA-043	Sample	3:40	11	1.0	10	in quench	0	2:05	10
101998 -MeFOSEA-044	Duplicate	3:40	11	1.0	10	in quench	0	2:08	10
101998 -MeFOSEA-045	Spike ☑	3:40	11	1.0	10	in quench	10	2:12	10

Date of Initial Prep: 11-23-98 **Date of Quenching:** 11-25-98

	Test Analyte Solution	ISTD Solution	SPK Solution	Quenching Solution
Standard/Traceability No.	98086-3-16	EFA	W398-1117	98066-3-19
Component	MeFOSEA	98086-3-19	MFA	Hexane/IPA
Concentration (µg/mL)	10,730 ppm eoc	4324 ppm	4724 ppm	[illegible]

Temperature of Incubator (°C): 50

Incubation Start (Date and Time): [illegible]

Incubation Stop (Date and Time): [illegible]

Total Incubation Time: [illegible]

1.92 days

Buffer Addition by: [illegible]

ISTD Addition by: ~~[illegible]~~ see CCL 5/13/99 JMG

Test Analyte Addition by: CCL

Quenching by: JMG

Spike Addition by: JMG

Autovial Aliquoting by: JMG

Centrifugation Yes / No

Centrifuge: Roujan

RPM:

Time: min

By:

Filtration: Yes / No

By:

Pore Size: um, Brand:

Reviewed by: [illegible] 5/13/99

Added 0.5mL IPA to each vial. JMG prior to addition of quenching solution.

3M Environmental Laboratory Request No: U2744

000079

Fluorochemical Degradation (Hydrolysis) Analysis

TEST ANAYLTE: Methyl FOSEA **Date** 11/23/98

HOURS: day 7

112398-

CCL 5/13/99

Sample No.	Description	Time of Initial Prep	Buffer pH	Buffer Volume (mL)	Test Analyte Solution (µL)	ISTD Solution (µL)	Spike Solution (µL)	Time of Quenching	Quenching Solvent (mL)
101998 -MeFOSEA-046	Sample	2:54	1.5	1.0	10	in quench	0	9:35	10
101998 -MeFOSEA-047	Duplicate	2:54	1.5	1.0	10	in quench	0	9:37	10
101998 -MeFOSEA-048	Spike ☑	2:54	1.5	1.0	10	in quench	10	9:40	10
101998 -MeFOSEA-049	Sample	3:09	5	1.0	10	in quench	0	9:35	10
101998 -MeFOSEA-050	Duplicate	3:09	5	1.0	10	in quench	0	9:38	10
101998 -MeFOSEA-051	Spike ☑	3:09	5	1.0	10	in quench	10	9:41	10
101998 -MeFOSEA-052	Sample	3:09	7	1.0	10	in quench	0	9:36	10
101998 -MeFOSEA-053	Duplicate	3:09	7	1.0	10	in quench	0	9:38	10
101998 -MeFOSEA-054	Spike ☑	3:09	7	1.0	10	in quench	10	9:42	10
101998 -MeFOSEA-055	Sample	3:20	9	1.0	10	in quench	0	9:36	10
101998 -MeFOSEA-056	Duplicate	3:20	9	1.0	10	in quench	0	9:39	10
101998 -MeFOSEA-057	Spike ☑	3:20	9	1.0	10	in quench	10	9:42	10
101998 -MeFOSEA-058	Sample	3:40	11	1.0	10	in quench	0	9:37	10
101998 -MeFOSEA-059	Duplicate	3:40	11	1.0	10	in quench	0	9:40	10
101998 -MeFOSEA-060	Spike ☑	3:40	11	1.0	10	in quench	10	9:43	10

Date of Initial Prep: 11-23-98 **Date of Quenching:** 11-30-98

	Test Analyte Solution	ISTD Solution	SPK Solution	Quenching Solution
Standard/Traceability No.	98086-3-16	98086-3-19	W398-1117	98061-3-19
Component	[illegible]	[illegible]	MeFOSEA	[redacted] IPA
Concentration (µg/mL)	10,730 [redacted]	[redacted]	473.4 ppm	[redacted] ppm

Temperature of Incubator (°C): 50

Incubation Start (Date and Time): 3:30 11/23/98

Incubation Stop (Date and Time): 8:54am 11/30/98

Total Incubation Time: 6.72 days

✓ Buffer Addition by: JJD (put in by CCL)

ISTD Addition by: JMG

✓ Test Analyte Addition by: CCL

Quenching by: JMG

Spike Addition by: JMG

Autovial Aliquoting by: JMG

✓ [redacted] Yes / No

Centrifuge: Roujan

RPM:

Time: min

By:

[redacted] Yes / No

By:

Pore Size: um, Brand:

Reviewed by: [illegible] 5/13/99

Added 0.5 mL IPA to each vial prior to addition of quenching solution. JMG

3M Environmental Laboratory Request No: U2744

000080

Fluorochemical Degradation (Hydrolysis) Analysis

TEST ANAYLTE: Methyl FOSEA **Date** 11/23/98

HOURS: day 14

CCL 5/13/99 112398

Sample No.	Description	Time of Initial Prep	Buffer pH	Buffer Volume (mL)	Test Analyte Solution (µL)	ISTD Solution (µL)	Spike Solution (µL)	Time of Quenching	Quenching Solvent (mL)
101998 -MeFOSEA-061	Sample	2:56	1.5	1.0	10	in quench	0	1:58 pm	10
101998 -MeFOSEA-062	Duplicate	2:56	1.5	1.0	10	in quench	0	1:59	10
101998 -MeFOSEA-063	Spike ☑	2:56	1.5	1.0	10	in quench	10	1:59	10
101998 -MeFOSEA-064	Sample	3:11	5	1.0	10	in quench	0	2:00	10
101998 -MeFOSEA-065	Duplicate	3:11	5	1.0	10	in quench	0	2:00	10
101998 -MeFOSEA-066	Spike ☑	3:11	5	1.0	10	in quench	10	2:01	10
101998 -MeFOSEA-067	Sample	3:11	7	1.0	10	in quench	0	2:01	10
101998 -MeFOSEA-068	Duplicate	3:11	7	1.0	10	in quench	0	2:02	10
101998 -MeFOSEA-069	Spike ☑	3:11	7	1.0	10	in quench	10	2:02	10
101998 -MeFOSEA-070	Sample	3:20	9	1.0	10	in quench	0	2:03	10
101998 -MeFOSEA-071	Duplicate	3:20	9	1.0	10	in quench	0	2:03	10
101998 -MeFOSEA-072	Spike ☑	3:20	9	1.0	10	in quench	10	2:04	10
101998 -MeFOSEA-073	Sample	3:40	11	1.0	10	in quench	0	2:04	10
101998 -MeFOSEA-074	Duplicate	3:40	11	1.0	10	in quench	0	2:05	10
101998 -MeFOSEA-075	Spike ☑	3:40	11	1.0	10	in quench	10	2:05	10

Date of Initial Prep: 11-23-98

Date of Quenching: 12/07/98

	Test Analyte Solution	ISTD Solution	SPK Solution	Quenching Solution
Standard/Traceability No.	98086-3-16	~~98086-3-19~~	98060-15-16	W398-1117
Component	MeFOSEA	~~EtFOSEA~~	EFA	Methyl FOS Alcohol
Concentration (µg/mL)	~~10,730 ppm~~	[illegible]	9.324 µg/mL	~~4,734 µg/mL~~ 473.4 ppm

JMG 12/07/98

Temperature of Incubator (°C): 50

Incubation Start (Date and Time): 11/23/98 CCL 3:30 PM

Incubation Stop (Date and Time): 12/07/98 1:02 pm

Total Incubation Time: 13.90 days

Buffer Addition by: JJD (By CCL 5/13/99)

ISTD Addition by: JMG

Test Analyte Addition by: CCL

Quenching by: JMG

Spike Addition by: JMG

Autovial Aliquoting by: JMG

Added 0.5 mL IPA to each vial prior to addition of quenching solution. JMG

Centrifugation: Yes / No CCL

Centrifuge: Roujan

RPM:

Time: min

By:

Filtration: Yes / No

By:

Pore Size: um, Brand:

Reviewed by: G n maisel 5/13/99

3M Environmental Laboratory Request No: U2744

000031

Fluorochemical Degradation (Hydrolysis) Analysis

TEST ANAYLTE: Methyl FOSEA **Date** 11/23/98

HOURS: day 21

112398-

CCL 5/13/99

Sample No.	Description	Time of Initial Prep	Buffer pH	Buffer Volume (mL)	Test Analyte Solution (µL)	ISTD Solution (µL)	Spike Solution (µL)	Time of Quenching	Quenching Solvent (mL)
101998 -MeFOSEA-076	Sample	2:58	1.5	1.0	10	in quench	0	9:39	10
101998 -MeFOSEA-077	Duplicate	2:58	1.5	1.0	10	in quench	0	9:39	10
101998 -MeFOSEA-078	Spike ☑ (1)	2:58	1.5	1.0	10	in quench	10	9:40	10
101998 -MeFOSEA-079	Sample	3:13	5	1.0	10	in quench	0	9:41	10
101998 -MeFOSEA-080	Duplicate	3:13	5	1.0	10	in quench	0	9:41	10
101998 -MeFOSEA-081	Spike ☑ (1)	3:13	5	1.0	10	in quench	10	9:42	10
101998 -MeFOSEA-082	Sample	3:13	7	1.0	10	in quench	0	9:43	10
101998 -MeFOSEA-083	Duplicate	3:13	7	1.0	10	in quench	0	9:43	10
101998 -MeFOSEA-084	Spike ☑ (1)	3:13	7	1.0	10	in quench	10	9:44	10
101998 -MeFOSEA-085	Sample	3:30	9	1.0	10	in quench	0	9:44	10
101998 -MeFOSEA-086	Duplicate	3:30	9	1.0	10	in quench	0	9:45	10
101998 -MeFOSEA-087	Spike ☑ (1)	3:30	9	1.0	10	in quench	10	9:45	10
101998 -MeFOSEA-088	Sample	3:50	11	1.0	10	in quench	0	9:46	10
101998 -MeFOSEA-089	Duplicate	3:50	11	1.0	10	in quench	0	9:46	10
101998 -MeFOSEA-090	Spike ☑ (1)	3:50	11	1.0	10	in quench	10	9:47	10

Date of Initial Prep: 11-23-98

Date of Quenching: 12/14/98

	Test Analyte Solution	ISTD Solution	SPK (1) Solution	Quenching Solution
Standard/Traceability No.	98086-3-16	98086-3-19	W398-1117	98060-15-16
Component	MeFOSEA	E+FOS-OH	Me-FOS-OH	EFA
Concentration (µg/mL)	10,730 ppm	9,324 ppm	4,739 µg/mL 773.9 CCL	9.324 µg/mL

Temperature of Incubator (°C): 50

Incubation Start (Date and Time): 11/23/98 3:30 pm

Incubation Stop (Date and Time): 12/14/98 8:38 am

Total Incubation Time: 20.71 days

Buffer Addition by: JJD (BxCCL 5/13/99)

ISTD Addition by: JMG

Test Analyte Addition by: CCL

Quenching by: JMG

Spike Addition by: JMG

Autovial Aliquoting by: JMG

[redacted] Yes / No

Centrifuge: Roujan

RPM:

Time: min

By:

Added 0.5 mL IPA to each vial prior to addition of quenching solution. JMG

Filtration: Yes / No

By:

Pore Size: um, Brand:

Reviewed by: [signature] 5/13/99

(1) Also spiked with 10µl of N-methyl fosea: 10,730 ppm in acetone ([illegible] 12/14/JMG [illegible]) 12/14/98 JMG

3M Environmental Laboratory Request No: U2744

(2) A set of these samples was stored in the freezer until future [illegible] 12/14/98 [illegible]

000082

Fluorochemical Degradation (Hydrolysis) Analysis

TEST ANAYLTE: Methyl FOSEA **Date** 11/23/98

HOURS: day 28

11-23-98

CCL 5/13/98

Sample No.	Description	Time of Initial Prep	Buffer pH	Buffer Volume (mL)	Test Analyte Solution (µL)	ISTD Solution (µL)	Spike Solution (µL)	Time of Quenching	Quenching Solvent (mL)
101998 -MeFOSEA-091	Sample	3:00	1.5	1.0	10	in quench	0	10:49	10
101998 -MeFOSEA-092	Duplicate	3:00	1.5	1.0	10	in quench	0	10:49	10
101998 -MeFOSEA-093	Spike ☑ ①	3:00	1.5	1.0	10	in quench	10	10:50	10
101998 -MeFOSEA-094	Sample	3:15	5	1.0	10	in quench	0	10:51	10
101998 -MeFOSEA-095	Duplicate	3:15	5	1.0	10	in quench	0	10:51	10
101998 -MeFOSEA-096	Spike ☑ ①	3:15	5	1.0	10	in quench	10	10:51	10
101998 -MeFOSEA-097	Sample	3:15	7	1.0	10	in quench	0	10:52	10
101998 -MeFOSEA-098	Duplicate	3:15	7	1.0	10	in quench	0	10:53	10
101998 -MeFOSEA-099	Spike ☑ ①	3:15	7	1.0	10	in quench	10	10:53	10
101998 -MeFOSEA-100	Sample	3:30	9	1.0	10	in quench	0	10:54	10
101998 -MeFOSEA-101	Duplicate	3:30	9	1.0	10	in quench	0	10:54	10
101998 -MeFOSEA-102	Spike ☑ ①	3:30	9	1.0	10	in quench	10	10:55	10
101998 -MeFOSEA-103	Sample	3:50	11	1.0	10	in quench	0	10:55	10
101998 -MeFOSEA-104	Duplicate	3:50	11	1.0	10	in quench	0	10:56	10
101998 -MeFOSEA-105	Spike ☑ ①	3:50	11	1.0	10	in quench	10	10:56	10

Date of Initial Prep: 11-23-98 **Date of Quenching:** 12/21/98

	Test Analyte Solution	ISTD Solution	SPK ① Solution	Quenching Solution
Standard/Traceability No.	98086-3-16	98086-3-19	W398-1117	98060-15-16
Component	MeFOSEA	EtFOSEA-011	Me Fos-OH	EtA
Concentration (µg/mL)	[illegible]	[illegible]	4734 ppm	9.324 µg/mL

Temperature of Incubator (°C): 50

Incubation Start (Date and Time): 11/23/98 3:30 AM ccl

Incubation Stop (Date and Time): 9:55 am 12/21/98

Total Incubation Time: 27.77 days

Buffer Addition by: JMG ccl 5/13/98 ccl (JJD) by ccl

ISTD Addition by: JMG

Test Analyte Addition by: CCL

Quenching by: JMG

Spike Addition by: JMG

Autovial Aliquoting by: JMG

[redacted] Yes / No

Centrifuge: Roujan

RPM:

Time: min

By:

Filtration: Yes / No

By:

Pore Size: um, Brand:

Reviewed by: G n Maisel 5/13/99

Added 0.5 mL IPA to each vial prior to addition of quenching solution. JMG

① Also spiked with 10 µl of N-methyl fosea: 10,730 ppm in acetone (98086-3-11) 12/21/98 JMG

② Samples stored in freezer until analysis. Freezer F-2 temp = -18.7°C

3M Environmental Laboratory Request No: U2744

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Appendix D: GC/AED Chromatograms

See separate bound volumes.

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Appendix E: Spreadsheets: GC/AED Results

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MeFOSEA GC/AED results

Note that each extract was injected in duplicate.

pH 1.5 DATA

day	Sample number	MeFOSEA (ppm)	average ppm	% spike recovery for MeFOSEA	%RPD calculations
0	112398-MeFOSEA-001	9.896	9.894		0.3
	112398-MeFOSEA-001	9.891			
0	112398-MeFOSEA-002	9.872	9.865		
	112398-MeFOSEA-002	9.858			
0	112398-MeFOSEA-003	9.722	9.927	n/a	
	112398-MeFOSEA-003	10.132			
0.94	112398-MeFOSEA-016	7.590	7.806		5.3
	112398-MeFOSEA-016	8.021			
0.94	112398-MeFOSEA-017	8.079	8.232		
	112398-MeFOSEA-017	8.385			
0.94	112398-MeFOSEA-018	8.919	8.957	n/a	
	112398-MeFOSEA-018	8.994			
1.92	112398-MeFOSEA-031	7.502	7.554		1.1
	112398-MeFOSEA-031	7.605			
1.92	112398-MeFOSEA-032	7.612	7.639		
	112398-MeFOSEA-032	7.666			
1.92	112398-MeFOSEA-033	7.987	7.861	n/a	
	112398-MeFOSEA-033	7.735			
6.72	112398-MeFOSEA-046	5.537	5.502		3.9
	112398-MeFOSEA-046	5.467			
6.72	112398-MeFOSEA-047	4.752	5.291		
	112398-MeFOSEA-047	5.830			
6.72	112398-MeFOSEA-048	5.561	5.551	n/a	
	112398-MeFOSEA-048	5.540			
13.90	112398-MeFOSEA-061	4.211	4.229		3.8
	112398-MeFOSEA-061	4.246			
13.90	112398-MeFOSEA-062	4.365	4.393		
	112398-MeFOSEA-062	4.420			
13.90	112398-MeFOSEA-063	5.320	5.244	n/a	
	112398-MeFOSEA-063	5.168			
20.71	112398-MeFOSEA-076	3.644	3.647		31.9
	112398-MeFOSEA-076	3.650			
20.71	112398-MeFOSEA-077	5.058	5.031		
	112398-MeFOSEA-077	5.003			
20.71	112398-MeFOSEA-078	12.952	12.874	79.5	
	112398-MeFOSEA-078	12.795			
27.77	112398-MeFOSEA-091	2.764	2.795		25.3
	112398-MeFOSEA-091	2.826			
27.77	112398-MeFOSEA-092	3.543	3.605		
	112398-MeFOSEA-092	3.666			
27.77	112398-MeFOSEA-093	13.411	13.283	94.0	
	112398-MeFOSEA-093	13.154			

MeFOSEA GC/AED results

Note that each extract was injected in duplicate.

pH 5 DATA

day	Sample number	MeFOSEA (ppm)	average ppm	% spike recovery for MeFOSEA	%RPD calculations
0	112398-MeFOSEA-004	10.323	10.266		1.6
	112398-MeFOSEA-004	10.208			
0	112398-MeFOSEA-005	10.368	10.431		
	112398-MeFOSEA-005	10.493			
0	112398-MeFOSEA-006	10.416	10.326	n/a	
	112398-MeFOSEA-006	10.236			
0.94	112398-MeFOSEA-019	9.465	9.437		1.0
	112398-MeFOSEA-019	9.408			
0.94	112398-MeFOSEA-020	9.285	9.344		
	112398-MeFOSEA-020	9.402			
0.94	112398-MeFOSEA-021	9.431	9.315	n/a	
	112398-MeFOSEA-021	9.198			
1.92	112398-MeFOSEA-034	8.491	8.576		0.8
	112398-MeFOSEA-034	8.660			
1.92	112398-MeFOSEA-035	8.467	8.645		
	112398-MeFOSEA-035	8.823			
1.92	112398-MeFOSEA-036	8.496	8.544	n/a	
	112398-MeFOSEA-036	8.592			
6.72	112398-MeFOSEA-049	7.910	8.251		6.8
	112398-MeFOSEA-049	8.592			
6.72	112398-MeFOSEA-050	7.702	7.710		
	112398-MeFOSEA-050	7.718			
6.72	112398-MeFOSEA-051	8.518	8.488	n/a	
	112398-MeFOSEA-051	8.457			
13.90	112398-MeFOSEA-064	7.131	7.281		6.9
	112398-MeFOSEA-064	7.431			
13.90	112398-MeFOSEA-065	7.864	7.804		
	112398-MeFOSEA-065	7.744			
13.90	112398-MeFOSEA-066	7.710	7.710	n/a	
	112398-MeFOSEA-066	bad injection			
20.71	112398-MeFOSEA-079	bad injection	5.789		3.5
	112398-MeFOSEA-079	5.789			
20.71	112398-MeFOSEA-080	5.639	5.593		
	112398-MeFOSEA-080	5.546			
20.71	112398-MeFOSEA-081	18.636	18.845	122.9	
	112398-MeFOSEA-081	19.054			
27.77	112398-MeFOSEA-094	5.448	5.332		4.5
	112398-MeFOSEA-094	5.215			
27.77	112398-MeFOSEA-095	5.636	5.577		
	112398-MeFOSEA-095	5.518			
27.77	112398-MeFOSEA-096	15.199	15.166	90.5	
	112398-MeFOSEA-096	15.132			

MeFOSEA GC/AED results

Note that each extract was injected in duplicate.

pH 7 DATA

day	Sample number	MeFOSEA (ppm)	average ppm	% spike recovery for MeFOSEA	%RPD calculations
0	112398-MeFOSEA-007	10.598	10.586		1.8
	112398-MeFOSEA-007	10.573			
0	112398-MeFOSEA-008	10.440	10.398		
	112398-MeFOSEA-008	10.356			
0	112398-MeFOSEA-009	10.453	10.619	n/a	
	112398-MeFOSEA-009	10.785			
0.94	112398-MeFOSEA-022	8.535	8.519		0.8
	112398-MeFOSEA-022	8.503			
0.94	112398-MeFOSEA-023	8.627	8.587		
	112398-MeFOSEA-023	8.547			
0.94	112398-MeFOSEA-024	9.023	8.953	n/a	
	112398-MeFOSEA-024	8.882			
1.92	112398-MeFOSEA-037	8.660	8.788		
	112398-MeFOSEA-037	8.916			
1.92	112398-MeFOSEA-038	8.762	8.754		
	112398-MeFOSEA-038	8.746			
1.92	112398-MeFOSEA-039	8.524	8.564	n/a	
	112398-MeFOSEA-039	8.604			
6.72	112398-MeFOSEA-052	6.534	6.703		20.1
	112398-MeFOSEA-052	6.871			
6.72	112398-MeFOSEA-053	5.410	5.476		
	112398-MeFOSEA-053	5.542			
6.72	112398-MeFOSEA-054	5.947	5.874	n/a	
	112398-MeFOSEA-054	5.801			
13.90	112398-MeFOSEA-067	4.793	4.867		14.0
	112398-MeFOSEA-067	4.940			
13.90	112398-MeFOSEA-068	4.278	4.229		
	112398-MeFOSEA-068	4.180			
13.90	112398-MeFOSEA-069	5.507	5.546	n/a	
	112398-MeFOSEA-069	5.585			
20.71	112398-MeFOSEA-082	4.506	4.595		28.9
	112398-MeFOSEA-082	4.684			
20.71	112398-MeFOSEA-083	3.512	3.436		
	112398-MeFOSEA-083	3.360			
20.71	112398-MeFOSEA-084	14.152	14.047	93.5	
	112398-MeFOSEA-084	13.941			
27.77	112398-MeFOSEA-097	1.958	2.000		2.0
	112398-MeFOSEA-097	2.041			
27.77	112398-MeFOSEA-098	2.021	2.041		
	112398-MeFOSEA-098	2.060			
27.77	112398-MeFOSEA-099	14.490	14.290	114.3	
	112398-MeFOSEA-099	14.089			

Results for Day 1.92 were not included in the graph, due to failed QC criteria.

MeFOSEA GC/AED results

Note that each extract was injected in duplicate.

pH 9 DATA

day	Sample number	MeFOSEA (ppm)	average ppm	% spike recovery for MeFOSEA	%RPD calculations
0	112398-MeFOSEA-010	9.998	9.734		6.8
	112398-MeFOSEA-010	9.469			
0	112398-MeFOSEA-011	10.312	10.423		
	112398-MeFOSEA-011	10.533			
0	112398-MeFOSEA-012	10.313	10.097	n/a	
	112398-MeFOSEA-012	9.881			
0.94	112398-MeFOSEA-025	10.996	11.145		3.9
	112398-MeFOSEA-025	11.293			
0.94	112398-MeFOSEA-026	11.386	10.723		
	112398-MeFOSEA-026	10.059			
0.94	112398-MeFOSEA-027	8.713	8.615	n/a	
	112398-MeFOSEA-027	8.517			
1.92	112398-MeFOSEA-040	9.897	9.349		24.7
	112398-MeFOSEA-040	8.800			
1.92	112398-MeFOSEA-041	7.263	7.295		
	112398-MeFOSEA-041	7.326			
1.92	112398-MeFOSEA-042	7.457	7.323	n/a	
	112398-MeFOSEA-042	7.189			
6.72	112398-MeFOSEA-055	5.036	5.014		11.0
	112398-MeFOSEA-055	4.992			
6.72	112398-MeFOSEA-056	6.114	5.598		
	112398-MeFOSEA-056	5.082			
6.72	112398-MeFOSEA-057	6.263	5.872	n/a	
	112398-MeFOSEA-057	5.481			
13.90	112398-MeFOSEA-070	4.436	4.415		1.8
	112398-MeFOSEA-070	4.394			
13.90	112398-MeFOSEA-071	4.365	4.495		
	112398-MeFOSEA-071	4.624			
13.90	112398-MeFOSEA-072	2.980	2.970	n/a	
	112398-MeFOSEA-072	2.960			
20.71	112398-MeFOSEA-085	2.601	2.538		10.3
	112398-MeFOSEA-085	2.475			
20.71	112398-MeFOSEA-086	2.312	2.291		
	112398-MeFOSEA-086	2.269			
20.71	112398-MeFOSEA-087	11.365	11.309	82.9	
	112398-MeFOSEA-087	11.252			
27.77	112398-MeFOSEA-100	2.392	2.382		0.3
	112398-MeFOSEA-100	2.371			
27.77	112398-MeFOSEA-101	2.415	2.388		
	112398-MeFOSEA-101	2.361			
27.77	112398-MeFOSEA-102	11.798	11.885	88.5	
	112398-MeFOSEA-102	11.972			

MeFOSEA GC/AED results

Note that each extract was injected in duplicate.

pH 11 DATA

day	Sample number	MeFOSEA (ppm)	average ppm	% spike recovery for MeFOSEA	%RPD calculations
0	112398-MeFOSEA-013	12.496	11.429		13.6
	112398-MeFOSEA-013	10.362			
0	112398-MeFOSEA-014	9.650	9.976		
	112398-MeFOSEA-014	10.302			
0	112398-MeFOSEA-015	10.306	9.990	n/a	
	112398-MeFOSEA-015	9.674			
0.94	112398-MeFOSEA-028	8.971	8.810		3.3
	112398-MeFOSEA-028	8.648			
0.94	112398-MeFOSEA-029	9.218	9.103		
	112398-MeFOSEA-029	8.988			
0.94	112398-MeFOSEA-030	8.596	8.562	n/a	
	112398-MeFOSEA-030	8.527			
1.92	112398-MeFOSEA-043	7.315	8.032		11.2
	112398-MeFOSEA-043	8.749			
1.92	112398-MeFOSEA-044	9.143	8.988		
	112398-MeFOSEA-044	8.833			
1.92	112398-MeFOSEA-045	8.525	8.480	n/a	
	112398-MeFOSEA-045	8.434			
6.72	112398-MeFOSEA-058	5.947	5.789		4.5
	112398-MeFOSEA-058	5.630			
6.72	112398-MeFOSEA-059	5.966	5.535		
	112398-MeFOSEA-059	5.103			
6.72	112398-MeFOSEA-060	5.315	5.175	n/a	
	112398-MeFOSEA-060	5.035			
13.90	112398-MeFOSEA-073	3.360	3.310		2.6
	112398-MeFOSEA-073	3.260			
13.90	112398-MeFOSEA-074	3.471	3.398		
	112398-MeFOSEA-074	3.325			
13.90	112398-MeFOSEA-075	3.545	3.674	n/a	
	112398-MeFOSEA-075	3.802			
20.71	112398-MeFOSEA-088	2.144	2.249		23.2
	112398-MeFOSEA-088	2.354			
20.71	112398-MeFOSEA-089	2.885	2.840		
	112398-MeFOSEA-089	2.795			
20.71	112398-MeFOSEA-090	12.385	12.516	92.9	
	112398-MeFOSEA-090	12.647			
27.77	112398-MeFOSEA-103	2.268	2.284		8.7
	112398-MeFOSEA-103	2.300			
27.77	112398-MeFOSEA-104	2.497	2.493		
	112398-MeFOSEA-104	2.488			
27.77	112398-MeFOSEA-105	12.028	12.235	91.8	
	112398-MeFOSEA-105	12.442			

QA/QC calculations

Matrix spike recovery calculations

pH	Sample	%Recovery
1.5	112398-MeFOSEA-078	79.5
1.5	112398-MeFOSEA-093	94.0
5	112398-MeFOSEA-081	122.9
5	112398-MeFOSEA-096	90.5
7	112398-MeFOSEA-084	93.5
7	112398-MeFOSEA-099	114.3
9	112398-MeFOSEA-087	82.9
9	112398-MeFOSEA-102	88.5
11	112398-MeFOSEA-090	92.9
11	112398-MeFOSEA-105	91.8
	Average recovery	95.1
	Standard deviation	12.7
	%Relative standard deviation	13.4%
	Lower warning limit	69.6
	Upper warning limit	120.5
	Lower control limit	56.9
	Upper control limit	133.3

Sample precision calculations, using the sample and duplicate to calculate the RPD

Average RPD	8.5
Standard deviation	9.0
Lower warning limit	0.0
Upper warning limit	26.6
Lower control limit	0.0
Upper control limit	35.6

Appendix F: Spreadsheets: Calculation of Kinetic Parameters

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Equations used:

The hydrolysis rate constant, $k_H = [\ln(RX)_0 - \ln(RX)_t] / t$
where RX is Molar concentration
$t_{1/2} = 0.693 / k_T$

MeFOSEA was tested by the addition of 10 microliters of 10,730ppm McFOSEA in acetone to 1.0ml of buffer. This is equivalent to 107.3 micrograms/ml of MeFOSEA.

The formular weight of Methyl FOSEA is equal to 611.27
This makes the initial concentration of MeFOSEA equal to 1.7554E-4 Molar.

pH 1.5 Data

Day	Average MeFOSEA (ppm)	[MeFOSEA] (Molar)	$[MeFOSEA]_t/[MeFOSEA]_0$	natural log of $[MeFOSEA]_t/[MeFOSEA]_0$	k	$t_{1/2}$ (days)
0	9.90	1.619E-04	0.922	-0.081	-	-
0.94	8.33	1.363E-04	0.777	-0.253	0.2690	2.6
1.92	7.68	1.257E-04	0.716	-0.334	0.1738	4.0
6.72	5.45	8.912E-05	0.508	-0.678	0.1008	6.9
13.90	4.62	7.561E-05	0.431	-0.842	0.0606	11.4
20.71	4.34	7.098E-05	0.404	-0.905	0.0437	15.9
27.77	3.20	5.235E-05	0.298	-1.210	0.0436	15.9
				Average	0.1152	9.4
				Standard Dev.	0.0900	5.8

pH 5 Data

Day	Average MeFOSEA (ppb)	[MeFOSEA] measured (Molar)	$[MeFOSEA]_t/[MeFOSEA]_0$	natural log of $[MeFOSEA]_t/[MeFOSEA]_0$	k	$t_{1/2}$ (days)
0	10.34	1.692E-04	0.964	-0.037	-	-
0.94	9.36	1.532E-04	0.873	-0.136	0.1445	4.8
1.92	8.59	1.405E-04	0.801	-0.222	0.1159	6.0
6.72	8.15	1.333E-04	0.760	-0.275	0.0409	16.9
13.90	7.60	1.243E-04	0.708	-0.345	0.0248	27.9
20.71	5.69	9.310E-05	0.530	-0.634	0.0306	22.6
27.77	5.45	8.923E-05	0.508	-0.676	0.0244	28.4
				Average	0.0635	17.8
				Standard Dev.	0.0528	10.5

000093

pH 7 Data

Day	Average MeFOSEA (ppb)	[MeFOSEA] measured (Molar)	$[MeFOSEA]_t/[MeFOSEA]_0$	natural log of $[MeFOSEA]_t/[MeFOSEA]_0$	k	$t_{1/2}$ (days)
0	10.53	1.723E-04	0.982	-0.018	-	-
0.94	8.69	1.421E-04	0.810	-0.211	0.2246	3.1
6.72	6.02	9.844E-05	0.561	-0.578	0.0860	8.1
13.90	4.88	7.984E-05	0.455	-0.788	0.0567	12.2
20.71	4.02	6.569E-05	0.374	-0.983	0.0474	14.6
27.77	2.02	3.305E-05	0.188	-1.670	0.0601	11.5
				Average	0.0950	9.9
				Standard Dev.	0.0739	4.5

pH 9 Data

Day	Average MeFOSEA (ppb)	[MeFOSEA] measured (Molar)	$[MeFOSEA]_t/[MeFOSEA]_0$	natural log of $[MeFOSEA]_t/[MeFOSEA]_0$	k	$t_{1/2}$ (days)
0	10.08	1.650E-04	0.940	-0.062	-	-
0.94	10.16	1.662E-04	0.947	-0.054	0.0578	12.0
1.92	7.99	1.307E-04	0.745	-0.295	0.1535	4.5
6.72	5.49	8.989E-05	0.512	-0.669	0.0996	7.0
13.90	3.96	6.478E-05	0.369	-0.997	0.0717	9.7
20.71	2.41	3.950E-05	0.225	-1.491	0.0720	9.6
27.77	2.38	3.901E-05	0.222	-1.504	0.0541	12.8
				Average	0.0848	9.3
				Standard Dev.	0.0373	3.1

pH 11 Data

	Day	Average MeFOSEA (ppb)	[MeFOSEA] measured (Molar)	$[MeFOSEA]_t/[MeFOSEA]_0$	natural log of $[MeFOSEA]_t/[MeFOSEA]_0$	k	$t_{1/2}$ (days)
0	0	10.47	1.712E-04	0.976	-0.025	-	-
-0.94	0.94	8.82	1.444E-04	0.823	-0.195	0.2078	3.3
-1.92	1.92	8.50	1.391E-04	0.792	-0.233	0.1212	5.7
-6.72	6.72	5.50	8.997E-05	0.513	-0.668	0.0994	7.0
-13.9	13.90	3.46	5.661E-05	0.323	-1.131	0.0814	8.5
-20.71	20.71	2.54	4.163E-05	0.237	-1.439	0.0695	10.0
-27.77	27.77	2.39	3.907E-05	0.223	-1.502	0.0541	12.8
					Average	0.1056	7.9
					Standard Dev.	0.0552	3.3

000094

OCTANOL/WATER PARTITION COEFFICIENT

TEST SUBSTANCE

Identity: N2-(N-methylperfluorooctanesulfonamido)-ethyl acrylate; may also be referred to as N-MeFOSEA, or MeFOSEA. (2-Propenoic acid, 2-[[(heptadecafluorooctyl)sulfonyl]methylamino]ethyl ester, CAS # 25268-77-3)

Remarks: Material is an amber, waxy solid. The test laboratory identifies the compound as "T-5869" (Batch 2408). Sample purity is given as "95%". The remaining 5% of the product was not characterized.

METHOD

Method: OECD 117 & EEC-Directive 92/69 EEC, Annex V, Part A.8
GLP (Y/N): Yes
Year completed: 1994
Analytical: HPLC with Spectrophotometric detector

Remarks: Testing used six substances with known log P_{ow} values as references. These substances were used to calibrate the elution time in units of log P_{ow}.

Reference substance	**log P_{ow}**
Benzylalcohol	1.1
Toluene	2.7
1,4-dichlorobenzene	3.4
Fluoranthene (98%)	4.7
Dibenzyl (99%)	4.8
2,4-DDT (99%)	6.2

A stock solution of the test substance was prepared by dissolving 287 mg in 50.0 mL acetonitrile and sonicating for 2 minutes. The stock solution was diluted 10 times with mobile phase, resulting in the test solution (574 mg/L).

Mobile phase is a 75/25 (v/v) solution of acetonitrile / Millipore-Q water respectively.

RESULTS

Substance	log P_{ow}
Reference chemicals	
Benzylalcohol	1.1**
Toluene	2.7**
1,4-dichlorobenzene	3.4**

Fluoranthene	4.7**
Dibenzyl	4.8**
2,4-DDT	6.2**
Test substance	
Peak 1 (major comp.)	5.6*
Peak 2	5.4*
Peak 3	5.3*
Several small peaks	-0.7/5.2*

- Interpolated from the regression line: y = 0.288x – 0.730 (r=0.972, n=6)

** log P_{ow} values according to the OECD guidelines.

CONCLUSIONS

This study estimates the log P_{ow} for MeFOSEA.

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

DATA QUALITY

Reliability: Klimisch ranking 2

-per OECD. HPLC methods enables partition coefficients to be estimated in the log P_{ow} range between 0 and 6.

-Notox states impurities are of minor importance in HPLC method, however OECD states, HPLC method is less sensitive to the presence of impurities in the test substance than the Shake Flask method. Nevertheless, in some cases impurities can make the interpretation of the results difficult due to uncertainty in peak assignments. Mixtures which result in an unresolved band, upper and lower limits of log P_{ow} should be stated. Duplicate analysis should be reported in order to show confidence in the measurement (log P_{ow} values should fall within a range of +/- 0.1 log units) – data provided mean value therefore can't show confidence. pH of eluant is critical of ionizable compounds.
The study did not include any fluorochemicals among the reference substances. Without fluorochemical reference compounds, it is not known whether the same relationship between the capacity factor k' and Kow exists for compounds with perfluoroalkyl portions as exits for nonfluorinated reference materials such as those used in this study. The reference compounds should have been also injected independently to insure appropriate retention time assigned to each reference compound. Reference substances were injected twice, however only the mean retention time data is given for the reference substances thus repeatability of the retention times is not clear. It was not demonstrated that the test material was soluble in the acetonitrile stock solution or in the mobile phase. No demonstration that the major components of the test substance are detectable at UV λ = 210 nm. UV adsorption spectra is needed of the purified main components of the test substance. Assumptions are made that the major

peak from the HPLC of the test substance is the major component. No attempt was made to confirm this (e.g. Mass spec analysis to show the material detected has the appropriate mass). It is not known whether the major components even come off the column. Some k' values reported are calculated incorrectly.

REFERENCES

Study conducted at the request of 3M Company by NOTOX Safety & Environmental Research B.V., The Netherlands, April 7, 1994.

OTHER

Last changed: 5/17/00

- T-5869.4 Physical Prop - Partition Coefficient -

REPORT

DETERMINATION OF THE PARTITION COEFFICIENT (N-OCTANOL/WATER) OF

T-5869.4

BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

NOTOX Project 121286
NOTOX substance 38196

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NOV - 9 1994
3M
TOXICOLOGY

STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice

which are essentially in conformity with:

The United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

The United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

The United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

Study Director: Drs. R. de Vries

i/a

.....

Date: June 3, 1994

000099

QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands.

Study procedures were subject to periodic inspections and general non study specific processes were also inspected at periodic intervals.

This report was audited by the NOTOX Quality Assurance Unit and the methods and results accurately reflect the raw data.

DATES OF QAU INSPECTIONS/ AUDITS	REPORTING DATES
25 March 1994	25 March 1994
28 March 1994	28 March 1994
21 April 1994	21 April 1994

Quality Assurance Manager C.J. Mitchell B.Sc.

Date: 09-06-94.

000100

REPORT APPROVAL

STUDY DIRECTOR: Drs. R. de Vries

i/a

.....

Date: June 8, 1994

MANAGEMENT: J.A.M.W. van Helvoirt
Section head Physico Chemistry

PP.

.....

Dr. Ilona C. Enninga
Technical Director

Date: 09/06/1994

000101

PREFACE

Sponsor	3M Belgium - Chemical EBC Canadastraat 11 B-2070 ZWIJNDRECHT Belgium
Study Monitor	Mr. R.H. Cox
Testing Facility	NOTOX B.V. Hambakenwetering 3 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Drs. R. de Vries
Study plan	Start: 30 March 1994 Completed: 07 April 1994

TEST SUBSTANCE

Identification	T-5869
Description	Cream solid
Batch	2408
Purity	95%
Storage conditions	At room temperature in the dark
Stability under storage conditions	Stable
Expiry date	January 01, 1996

PURPOSE AND PRINCIPLE

The purpose of the study was to determine the partition coefficient of the test substance between n-octanol and water.

The partition coefficient (n-octanol/water) (P_{ow}) is defined as the ratio of the equilibrium concentrations in a two phase system consisting of n-octanol and water.

The retention time of a substance in a reversed-phase High Performance liquid Chromatography (HPLC) chromatographic system is related to its partition coefficient (n-octanol/water).
The HPLC method is useful for log P_{ow} values between 0 and 6.
Using the HPLC method, impurities are of minor importance. The HPLC method must also be applied if the test substance is a mixture of compounds but is not applicable to strong acids and bases, metal complexes, substances which react with the eluent or surface-active agents.

GUIDELINES

The study procedure described in this report was based on the following guidelines:

Organization for Economic Co-operation and Development (OECD), OECD Guideline for Testing of Chemicals, guideline no. 117: "Partition coefficient (n-octanol/water) High Performance Liquid Chromatography (HPLC) method" (adopted March 30, 1989).

European Economic Community (EEC), EEC-Directive 92/69 EEC, Annex V, Part A, Methods for the determination of physico-chemical properties, A.8: "Partition coefficient". EEC Publication no. L383, December 1992.

ARCHIVING

NOTOX B.V. will archive the following data for at least 10 years: protocol, report, test substance reference sample and raw data.

TEST SYSTEM AND RATIONALE

A High Performance Liquid Chromatograph with a spectrophotometric detector. The stationary phase was bonded silica C_{18}. The mobile phase was 75/25 (v/v) acetonitrile/water. Both HPLC instrumentation and conditions are described in the section "method of chemical analysis".

The test system was recognized by the international guidelines (OECD, EEC).

REFERENCE SUBSTANCES

Six chemicals for which log P_{ow} has been reported were used to calibrate the elution time in units of log P_{ow}. The mixture of reference substances consisted of:

Reference substance	log P_{ow}[1]
- benzylalcohol (p.a., Merck)	1.1
- toluene (p.a., Merck)	2.7
- 1,4-dichlorobenzene (z.s., Merck)	3.4
- fluoranthene (98%, GC, Aldrich Chemie)	4.7
- dibenzyl (99%, GC, Aldrich Chemie)	4.8
- 2,4-DDT (99%, HPLC, Riedel de Haën, Seelze, FRG)	6.2

[1] log P_{ow} values according to the OECD guideline.

PERFORMANCE OF THE TEST

Preparation of the solutions

Solutions of the reference substances were prepared in methanol (HPLC-grade, Labscan Limited Co., Dublin, Ireland) at a concentration of 1.5-2 g/l. A mixture of the reference substances was prepared by adding 125 µl of each solution to a volumetric flask. Subsequently, this flask was made up with mobile phase to a final volume of 25.0 ml.
For the determination of t_0 (retention time of the unretarded component), a solution of formamide (p.a., Merck, Darmstadt, FRG) in methanol was prepared at a concentration of 1.7 g/l and thereafter diluted 10 times with mobile phase.
A stock solution of T-5869 was prepared by dissolving 287 mg test substance in 50.0 ml acetonitrile (2 minutes sonication). The stock solution was diluted 10 times with mobile phase, resulting in the test solution.

Performance of the test

The solutions were injected in the following sequence: the mixture of reference substances, mobile phase, the formamide solution, the test solution (in duplicate), mobile phase, the mixture of reference substances and the formamide solution.

Temperature of measurement

The temperature of the mobile phase was recorded several times during the measurements.

METHOD OF CHEMICAL ANALYSIS

The conditions used for the High Performance Liquid Chromatographic method are described below:

Analysis

Column	LiChrospher 100 RP-18; 125 x 4 (I.D.) mm; d_p= 5 µm (Merck, Darmstadt, FRG)
Mobile phase	75/25 (v/v) acetonitrile (HPLC-grade, Labscan Limited Co., Dublin, Ireland)/Milli-Q water (Millipore Corp., Bedford, MA, USA)
Flow	1 ml/min
Detection	UV at λ= 210 nm
Injection volume	10 µl

Instrumentation

HPLC Pump	Series 410 LC (Perkin Elmer, Norwalk, CT, USA)
Autosampler	ISS-200 (Perkin Elmer)
Detector	SpectroMonitor 3100 (LDC Analytical, Riviera Beach, FL, USA)
Integrator	SP 4290 (Spectra Physics, San Jose, CA, USA)

DATA HANDLING

Using High Performance Liquid Chromatography, especially large log P_{ow} values can be accurately determined from the chromatographic retention data. To this end, the capacity factor (k') was used, since it is proportional to the partition coefficient. The capacity factor was calculated from the retention of the substance concerned (t_r) and the unretarded component (t_o):

$$k' = (t_r - t_o)/t_o$$

From the results of the reference substances, a plot of log P_{ow} (x-value) versus log k' (y-value) was constructed, using linear regression analysis. The log k' value of each component of the test substance was compared with the log k' values of the reference substances with known log P_{ow} values.

RESULTS

Representative HPLC chromatograms of the test solution, the blank (mobile phase), the mixture of reference substances and the formamide solution are shown in Figures 1 to 4.

In the HPLC chromatograms of T-5869, using detection at 210 nm, one large and 2 small test substance peaks were observed. In addition, many small test substance peaks were observed between approximately 1 and 6 minutes. It was assumed that the large peak derived from the major component of test substance whereas the small peaks derived from impurities.

The t_0 was determined to be 0.90 minutes as a mean value of both measurements (i.e 0.89 and 0.90 minutes).

The mean values of the retention times, k' values, log k' values, log P_{ow} and P_{ow} values, are summarized in Table 1. The plot of log P_{ow} (x-value) versus log k' (y-value) from the reference substances is shown in Figure 5.

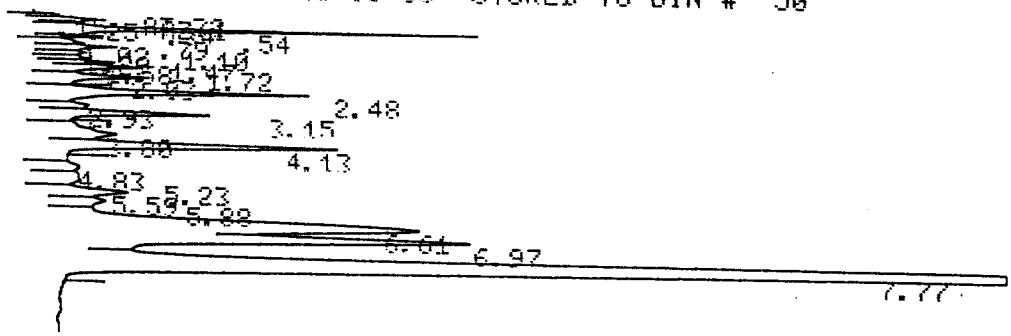
Table 1 Results of the test.

Substance	t_r [1]	k'	log k'	log P_{ow}	P_{ow}
benzylalcohol	1.14	0.274	-0.563	1.1	
toluene	2.24	1.503	0.177	2.7	
1,4-dichlorobenzene	2.89	2.229	0.348	3.4	
fluoranthene	5.67	5.330	0.727	4.7	
dibenzyl	4.07	3.547	0.550	4.8	
2,4-DDT	9.12	9.190	0.963	6.2	
test substance					
peak 1 (major comp.)	7.75	7.659	0.884	5.6 [2]	3.98×10^5
peak 2	6.96	6.777	0.831	5.4 [2]	2.51×10^5
peak 3	6.60	6.374	0.804	5.3 [2]	2.00×10^5
several small peaks	1 - 6	0.12/ 5.67	-0.93/0.75	-0.7/5.2 [2]	$0.2/1.58 \times 10^5$

[1] Mean value of the retention times of both chromatograms.
[2] Interpolated from the regression line: y = 0.288x - 0.730 (r=0.972, n=6)
Note : The calculations were performed using not rounded values.

The temperature of the mobile phase was 20.2-20.6°C during the measurements.

CHANNEL A INJECT 07-04-94 08:03:58 STORED TO BIN # 50



DATA SAVED TO BIN # 50

38196/121286 07-04-94 08:03:58 CH= "A" PS= 1.

.ILE 1. METHOD 0. RUN 7 INDEX 7 BIN 50

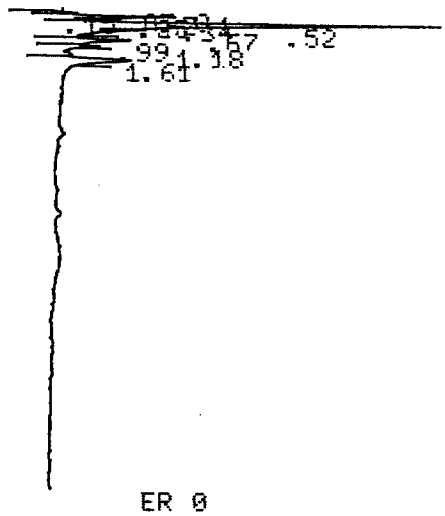
PEAK#	AREA%	RT	AREA	BC
1	0.041	0.15	1321	02
2	0.209	0.25	6658	02
3	0.098	0.34	3122	02
4	3.425	0.54	109239	08
5	0.121	0.79	3874	05
6	0.251	1.02	8011	06
7	0.498	1.1	15893	06
8	0.189	1.29	6033	06
9	0.25	1.47	7978	06
10	0.428	1.58	13648	06
11	0.839	1.72	26774	06
12	0.899	2.03	28669	07
13	3.097	2.48	98772	08
14	0.092	2.93	2936	06
15	1.652	3.15	52708	03
16	1.125	3.8	35886	02
17	4.167	4.13	132925	03
18	0.248	4.83	7895	02
19	0.432	5.23	13795	02
20	1.217	5.59	38811	02
21	0.89	5.88	28393	02
22	13.076	6.61	417083	02
23	11.364	6.97	362494	02
24	55.39	7.77	1766796	03

Figure 1 HPLC chromatogram of the test solution of 574 mg/l T-5869 in mobile phase.

Column	LiChrospher 100 RP-18; 125x4 (I.D.) mm; d_p=5 µm
Mobile phase	75/25 (v/v) acetonitrile/Milli-Q water
Flow	1 ml/min
Detection	UV, at λ = 210 nm
Injection volume	10 µl

Note: All the peaks between 0 and approximately 1 minute and some of the peaks between approximately 1 and 2 minutes were also observed in the chromatograms of the blank (see Figure 2). Therefore it was assumed that these peaks did not derive from the test substance.

CHANNEL A INJECT 07-04-94 09:34:04 STORED TO BIN # 56



ER 0
DATA SAVED TO BIN # 56

38196/121286 07-04-94 09:34:04 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 13 INDEX 13 BIN 56

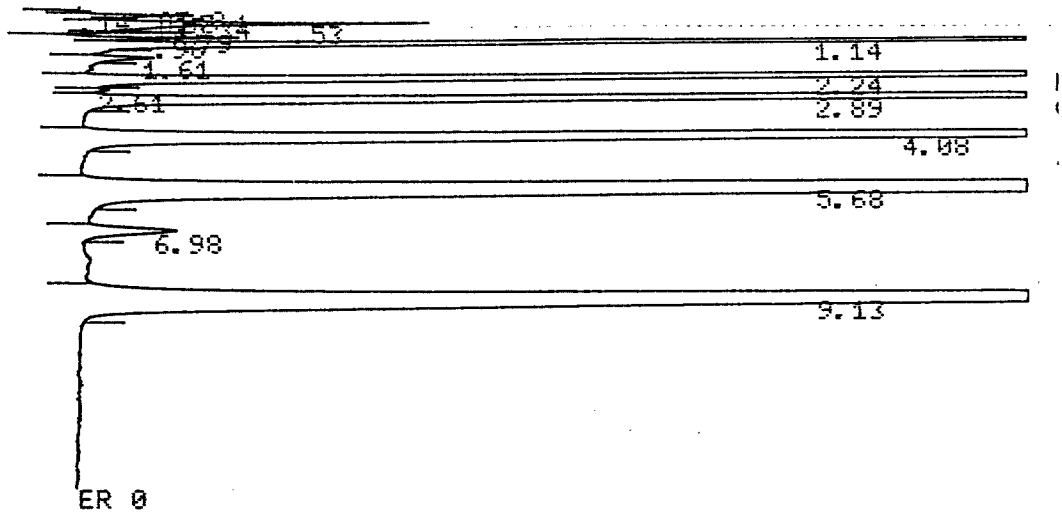
ANALYST: KRH

PEAK#	AREA%	RT	AREA BC
1	1.543	0.15	877 02
2	10.515	0.25	5976 02
3	3.853	0.34	2190 02
4	55.335	0.52	31449 02
5	7.768	0.67	4415 03
6	6.901	0.99	3922 02
7	2.509	1.18	1426 03
8	11.576	1.61	6579 01
TOTAL	100.		56834

Figure 2 HPLC chromatogram of a blank (mobile phase).

Column	LiChrospher 100 RP-18; 125x4 (I.D.) mm; d_p=5 µm
Mobile phase	75/25 (v/v) acetonitrile/Milli-Q water
Flow	1 ml/min
Detection	UV, at λ = 210 nm
Injection volume	10 µl

CHANNEL A INJECT 07-04-94 09:50:17 STORED TO BIN # 57



DATA SAVED TO BIN # 57

38196/121286 07-04-94 09:50:17 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 14 INDEX 14 BIN 57

ANALYST: KRH

PEAK#	AREA%	RT	AREA	BC
1	0.023	0.14	1131	02
2	0.145	0.25	7039	02
3	0.06	0.34	2917	02
4	0.879	0.53	42751	08
5	0.073	0.79	3542	05
6	0.139	0.96	6771	06
7	8.977	1.14	436774	03
8	0.112	1.61	5460	01
9	11.317	2.24	550640	01
10	0.013	2.61	611	02
11	10.567	2.89	514173	03
12	15.524	4.08	755334	01
13	38.584	5.68	1877339	01
14	0.316	6.98	15361	01
15	13.272	9.13	645770	01
TOTAL	100.		4865613	

Figure 3 HPLC chromatogram of the mixture of reference substances in mobile phase.

Column	LiChrospher 100 RP-18; 125x4 (I.D.) mm; d_p=5 µm
Mobile phase	75/25 (v/v) acetonitrile/Milli-Q water
Flow	1 ml/min
Detection	UV, at λ = 210 nm
Injection volume	10 µl

CHANNEL A INJECT 07-04-94 07:37:44 STORED TO BIN # 49

DATA SAVED TO BIN # 49

38196/121286 07-04-94 07:37:44 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 6 INDEX 6 BIN 49

ANALYST: KRH

PEAK#	AREA%	RT	AREA	BC
1	0.35	0.16	2624	02
2	1.189	0.27	8926	02
3	0.79	[illegible]	5930	02
4	10.44	.55	78363	08
5	1.884	0.78	14143	06
6	54.548	0.89	409437	08
7	6.246	1.11	[illegible]79	05
8	0.12	1.42	899	05
9	3.094	1.87	23224	02
10	0.424	2.97	3185	03
11	20.915	3.65	156989	01
TOTAL	100.		750599	

Figure 4 HPLC chromatogram of a formamide solution of 0.2 g/l in mobile phase.

Column	LiChrospher 100 RP-18; 125x4 (I.D.) mm; d_p=5 µm
Mobile phase	75/25 (v/v) acetonitrile/Milli-Q water
Flow	1 ml/min
Detection	UV, at λ = 210 nm
Injection volume	10 µl

000110

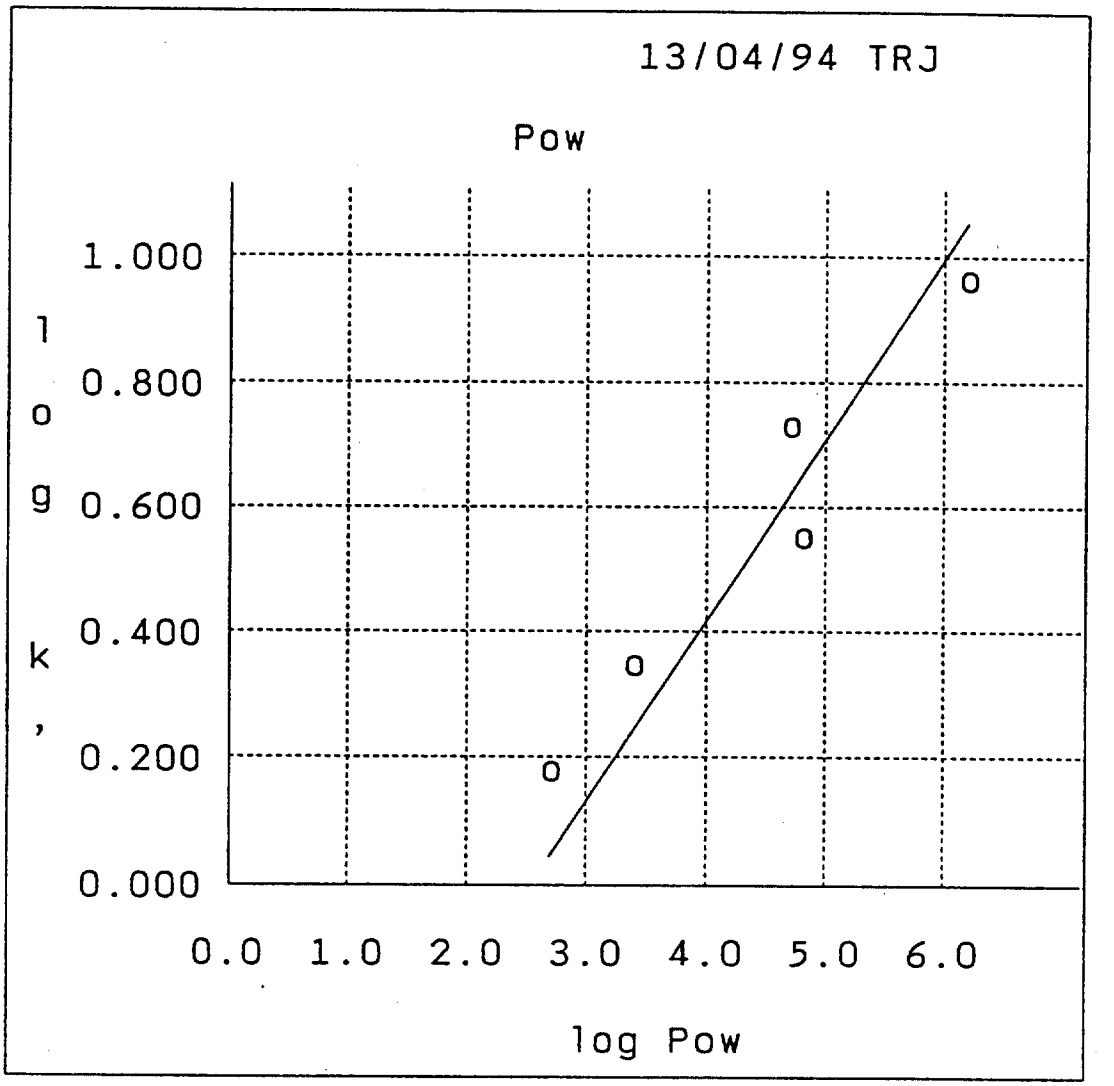


Figure 5 Plot of log P_{OW} (x-value) versus log k' (y-value) from the reference substances.

BIODEGRADATION

TEST SUBSTANCE

Identity: Perfluorodecanesulfonate, ammonium salt; may also be referred to as PFDS, FC-120, or 3M Sample J2905-1. (1-Decanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heneicosafluoro-, ammonium salt, CAS # 67906-42-7)

Remarks: Test sample is a mixture of the test substance in water and butyl cellosolve (approximately 25% test substance, 37.5% butyl cellosolve and 37.5% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample. Test sample was from 3M production lot 164.

METHOD

Method: EPA 405.1
Type: Aerobic
GLP: No
Year completed: 1991
Contact time: 20 days
Inoculum: Source of inoculum not recorded

RESULTS

5-day carbonaceous BOD: 67,000 mg/kg
10-day carbonaceous BOD: 600,000 mg/kg
20-day carbonaceous BOD: 720,000 mg/kg
COD: 1,000,000 mg/kg

Remarks: This material has a perfluorinated moiety and a non-fluorinated moiety. Current knowledge of these chemistries suggests that all biodegradation indicated in this study is likely due to reduction of the non-fluorinated moiety, butyl cellosolve.

Testing was conducted on the "as-sold" mixture of the subject material in water (~25% subject material). The values reported apply to that mixture and not directly to the subject material.

CONCLUSIONS

This testing indicated that the butyl cellosolve moiety is likely to be biodegraded in aerobic environments capable of supporting

biodegradation. This study cannot rule out the possibility that conditions could be found that would allow for biodegradation of the fluorochemical component, nor could it exclude the possibility that a microorganism could eventually be enriched that could metabolically alter this chemical.

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

DATA QUALITY

Reliability: Klimisch ranking 2. This study lacks sufficient documentation, characterization of the test substance purity, and analytical measurement of test concentrations. Because a mixture was tested, the biodegradation of the solvent could not be differentiated from biodegradation of the fluorochemical component.

REFERENCES

Study conducted at the request of 3M Company by Pace Incorporated of Minneapolis, MN, Lab Request number J2905, 12/4/91

OTHER

Last changed: 5/18/00

ARCHIVED FINAL REPORT

LAB REQUEST NO. J2905
REQUESTOR NAME: SAB/KPC
DEPARTMENT: 0535
PROJECT NO: 9970012900
DATE RECEIVED: 10/24/1991
DESC: ECOTOX SCREEN - FC-120

CONTRACT LAB(S): PACE
EXP COMP DATE: 01/24/1992
DATE COMPLETED: 12/05/1991
PROJECT LEAD:
PHONE NO:
3M FAX NO: 651-778-6176

DO NOT REFRIGERATE SAMPLES

TEST BY WEIGHT - NOT VOLUME

SAMPLE	DATE	CODE	DESCRIPTION	RESULT	MDL or (95% C.I.)
1	10/10/91	FC-120	LOT-164		
PACE	C-BOD-05	*BOD-05	(CARBONACEOUS)	67000 MG/KG	6000
PACE	C-BOD-10	*BOD-10	(CARBONACEOUS)	600000 MG/KG	6000
PACE	C-BOD-20	*BOD-20	(CARBONACEOUS)	720000 MG/KG	6000
PACE	COD-RFX	*COD	(DICHROMATE REFLUX)	1000000 MG/KG	50

* = CONTRACT LAB DATA

pace INCORPORATED
THE ASSURANCE OF QUALITY

REPORT OF LABORATORY ANALYSIS

December 04, 1991

RECEIVED
3M ENVIRONMENTAL LABORATORY

Mr. Wade Scheil
3M Environmental Laboratory
935 Bush Avenue
Bldg. 2-3E-09
St. Paul, MN 55133

RE: PACE Project No. 911029.526
Client Reference: J2905

Dear Mr. Scheil:

Enclosed is the report of laboratory analyses for samples received October 29, 1991.

If you have any questions concerning this report, please feel free to contact us.

Sincerely,

Lisa A. Arndt

Lisa A. Arndt
Project Manager

Enclosures

000115

1710 Douglas Drive North
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FAX: 612 525 3377

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REPORT OF LABORATORY ANALYSIS

3M Environmental Laboratory
935 Bush Avenue
Bldg. 2-3E-09
St. Paul, MN 55133

December 04, 1991
PACE Project Number: 911029526

Attn: Mr. Wade Schell

Client Reference: J2905

PACE Sample Number:			10 0386502		
Date Collected:			By Client		
Date Received:			10/29/91		
Client Sample ID:			J2905-1		
Parameter	Units	MDL		METHOD	DATE ANALYZED
INORGANIC ANALYSIS					
INDIVIDUAL PARAMETERS					
3M Chemical Oxygen Demand Method 410	mg/kg	50	1000000	EPA 410.1	11/07/91
Biochemical Oxygen Demand, Carb 05 Day	mg/kg	6000	67000	EPA 405.1	11/13/91
Biochemical Oxygen Demand, Carb 10 Day	mg/kg	6000	600000	EPA 405.1	11/13/91
Biochemical Oxygen Demand, Carb 20 Day	mg/kg	6000	720000	EPA 405.1	11/13/91

MDL Method Detection Limit

These data have been reviewed and are approved for release.

Starla Enger
Inorganic Chemistry Manager

000116

1710 Douglas Drive North
Minneapolis, MN 55422
TEL: 612-544-5543
FAX: 612-525-3377

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Page 1

PACE, INC.

(MW)

5 DAY BOD, BOD-05 _____
SOLUBLE BOD, SOL-BOD _____
CARBONACEOUS BOD 5 DAY, C-BOD-05 X
CARBONACEOUS BOD 10 DAY, C-BOD-10 _____
CARBONACEOUS BOD 20 DAY, C-BOD-20 _____

DATE ANALYZED IN 11-13-91 OUT 11-18-91
ANALYZED BY TMD INCUBATOR # 1
TIME STARTED 15:30
ENTERED BY TMD
BATCH # 14933 QC TMD
REVIEWED BY DOW 4/26/91

SAMPLE	ANALYSIS RESULTS	UNITS	COMMENTS	PH	VOL. (ml)	BOTTLE #	D1	D2	D1-D2 A	A-SC D	(D)(303) / VOLUME
PBW	ND		8138575		PBW	90	8.2	8.2			
			GLUCOSE-GLUTAMIC ACID TV-200-37 8138583		Seed 25	191	8.0	2.0	6.0		
LCS 156-148	208	mgh			LCS 6	53	8.2	3.6	4.6	4.12	208.1
					LCS 6	15	8.2	3.6	4.6	4.12	208.1
38647.2 3M	72,000	mg/kg	1000X		50	70	8.5	<1	—		
					20	32	8.3	3.1	5.2	4.72	71.5
					5	51	8.3	6.5	1.8	<2	—
			J2922-1		0.5	73	8.3	—			
38648.0 3M	530,000	mg/L	1000X SAT'D SOL'N		25	95	8.2	<1	—		
					10	24	8.2	<1	—		
					2	92	8.2	4.2	4.0	3.52	533.3
			J2909-1		0.5	35B	8.2	6.8	1.4	<2	—
38649.9 3M	19,000	mg/kg	1000X		100	60	8.9	2.4	6.5	6.02	18.2
					50	287	8.5	4.7	3.8	3.32	20.1
					25	60A	8.3				
			J2907-1		5	68	8.3				
38650.2 3M	~~<18,000~~ 67,000	TO 11-18 mg/kg	1000X		~~35~~	~~19~~	~~8.3~~	~~8.0~~	~~0.3~~	~~<2~~	~~48~~
					10	188	8.3	5.6	2.7	2.22	67,266
					2	2	8.3	6.1	2.2	<2	
			J2905-1		0.5	31	8.3	—			

D1 = INITIAL DO D2 = FINAL DO F = 2 / 35 SAMPLE SEED VOL. / SEED CONTROL VOL. = 0.08

SEED CONTROL (SC) = SEED CONTROL DO DROP x F 0.48

000117

PACE, INC.

5 DAY BOD, BOD-05 _____
SOLUBLE BOD, SOL-BOD _____
CARBONACEOUS BOD 5 DAY, C-BOD-05 _____
CARBONACEOUS BOD 10 DAY, C-BOD-10 X
CARBONACEOUS BOD 20 DAY, C-BOD-20 _____

DATE ANALYZED IN 11-13-91 OUT 11-23-91 (DAT)
ANALYZED BY TMD INCUBATOR # 1
TIME STARTED 15:30
ENTERED BY TMD
BATCH # 15149 QC —
REVIEWED BY DOW 11/25/91

SAMPLE	ANALYSIS RESULTS	UNITS	COMMENTS	PH	VOL. (ml)	BOTTLE #	D1	D2	D1-D2 A	A-SC D	(D)(303) / VOLUME
38647.2 3M	82,000	mg/kg	1000X SDAY 72,000 J2922-1		50	70	8.5	<1			
					20	32	8.3	2.2	6.1	5.38	81.507
					5	51	8.3	6.0	2.3	<2	
					0.5	73	8.3	7.2	1.1	↓	
38648.0 3M	650,000	mg/L	1000 X SATD SOLN SDAY 530,000 J2909-1		25	95	8.2	<1			
					10	24	8.2	<1		TMD 11-28	648.42
					2	92	8.2	3.2	5.0 4.9	4.28 ~~4.18~~	633.27
					0.5	35B	8.2	6.4	1.8	<2	
38649.9 3M	32,000	mg/kg	1000X SDAY 19,000 J2907-1		100	60	8.9	<1			
					50	287	8.5	2.8	5.7	4.98	30.178
					25	100A	8.3	4.8	3.5	2.78	33.69
					5	68	8.3	7.1	1.2	<2	
38650.2 3M	600,000	mg/kg	1000X SDAY 67,000 TOXIC? J2905-1		35	19	8.3	7.5			
					10	188	8.3	<1			
					2	2	8.3	3.6	4.7	3.98	602.97
					0.5	31	8.3	6.2	2.1	<2	
38651.0 3M	690,000	mg/L	1000 X SATD SOLN SDAY 32,000 J2903-1		25	26	8.2	<1			
					10	106	8.2	<1			
					2	101	8.2	2.9	5.3	4.58	693.87
					0.5	105	8.3	6.9	1.9	<2	
CONTROL					PBW	90	8.2	8.2	0		
					Seed 10	4	8.2	4.6	3.6		

D1 = INITIAL DO D2 = FINAL DO F = 2/10 SAMPLE SEED VOL. = 0.2 / SEED CONTROL VOL.

SEED CONTROL (SC) = SEED CONTROL DO DROP x F 3.6 0.72

PACE, INC.

(Tue)

5 DAY BOD, BOD-05 _____
SOLUBLE BOD, SOL-BOD _____
CARBONACEOUS BOD 5 DAY, C-BOD-05 _____
CARBONACEOUS BOD 10 DAY, C-BOD-10 _____
CARBONACEOUS BOD 20 DAY, C-BOD-20 X

DATE ANALYZED IN 11-13-91 OUT 12-3-91
ANALYZED BY TMD INCUBATOR # 1
TIME STARTED 15:30
ENTERED BY TMD
BATCH # 15150 QC _____
REVIEWED BY _____

SAMPLE	ANALYSIS RESULTS	UNITS	COMMENTS	PH	VOL. (ml)	BOTTLE #	D1	D2	D1-D2 A	A-SC D	(D)(303) / VOLUME
38647.2 3M	110,000	mg/kg	1000X 5 DAY 72,000 10 DAY 82,000 J2922-1		50	70	8.5	<1			
					20	32	8.3	<1			
					5	51	8.3	5.5	2.8	1.88	113.92
					0.5	73	8.3	6.9	1.4	<2	
38648.0 3M	710,000	mg/L	1000X SAT'D SOLN 5 DAY 530,000 10 DAY 654,000 J2909-1		25	95	8.2	<1			
					10	24	8.2	<1			
					2	92	8.2	2.6	5.6	4.68	709.02
					0.5	35B	8.2	6.0	2.2	<2	
38649.9 3M	57,000	mg/kg	1000X 5 DAY 19,000 10 DAY 32,000 J2907-1		100	60	8.9	<1			
					50	287	8.5	<1			
					25	100A	8.3	2.7	5.6	4.68	56.722
					5	68	8.3	6.8	1.5	<2	
38650.7 3M	720,000	mg/kg	1000X 5 DAY 670,00 10 DAY 600,00 TOXIC?! J2905-1	ADJ PH TO 7	35	19	8.3	3.4	4.9	3.98	34.45
					10	188	8.3	<1			
					2	2	8.3	2.6	5.7	4.78	724.17
					0.5	31	8.3	5.9		<2	
38651.0 3M	770,000	mg/L	1000X SAT'D SOLN 5 DAY 32,000 10 DAY 690,000 J2903-1		25	26	8.2	<1			
					10	106	8.2	<1			
					2	101	8.2	2.2	6.0	5.08	769.62
					0.5	105	8.3	6.1	2.2	<2	
CONTROL					PBW	90	8.2	8.1	0.1		
					Seed 10	4	8.2	3.6	4.6		

D1 = INITIAL DO D2 = FINAL DO F = 2/10 SAMPLE SEED VOL. = 0.2 SEED CONTROL VOL.

SEED CONTROL (SC) = SEED CONTROL DO DROP x F 6.92
4.6

ACUTE TOXICITY TO FISH

TEST SUBSTANCE

Identity: Perfluorodecanesulfonate, ammonium salt; may also be referred to as PFDS, FC-120, or 3M Sample J2905-1. (1-Decanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heneicosafluoro-, ammonium salt, CAS # 67906-42-7)

Remarks: Test sample is a mixture of the test substance in water and butyl cellosolve (approximately 25% test substance, 37.5% butyl cellosolve and 37.5% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample. Test sample was from 3M production Lot 164.

METHOD

Method: OECD 203
Type: Static acute
GLP: No
Year completed: 1992
Species: *Pimephales promelas*
Supplier:Aquatic Research Organisms, Hampton, New Hampshire
Analytical monitoring: pH, dissolved oxygen and conductivity and temperature only. Test substance concentration was not measured.
Exposure period: 96-hours
Statistical methods: Probit analysis.
Test fish age: Not noted other than "juvenile".
Length and weight: Average length = 26.5 mm.
Average weight = 0.14 g
Loading: 0.09 g fish / L
Pretreatment: None

Test conditions:

Dilution water: Aerated, filtered natural well water
Dilution water chemistry (from a representative sample):

hardness:	160 mg/L as $CaCO_3$
pH:	7.0
Conductivity:	640 µmhos/cm

Stock and test solution preparation: The test concentrations were prepared by transferring appropriate weights of FC-120

directly to the test chambers. Test concentrations were prepared based on total sample, not on percent concentration of the test substance in the test sample. All test vessels containing FC-120 were initially clear and remained clear throughout the study.
Concentrations dosing rate: Once
Stability of the test chemical solutions: Not noted
Exposure vessels: 20 liter glass aquaria containing 15L of test solution, water depth ~ 18 cm.
Number of replicates: two
Number of fish per replicate: ten
Number of concentrations: five plus a negative control
Water chemistry during the study:
Dissolved oxygen range (0 – 96 hours):
6.8 - 8.5 mg/L (control exposure)
5.7 - 8.6 mg/L (15 mg/L exposure)
pH range (0 – 96 hours)
7.0 - 8.2 (control exposure)
7.0 - 8.0 (15 mg/L exposure)
Test temperature: Mean temperature stated as 23 ± 2°C

RESULTS

Nominal concentrations: Bk control, 2.5, 4.0, 6.0, 10, 15mg/L
Element value: 24-hour LC_{50} = > 15 mg/L
48-hour LC_{50} = > 15 mg/L
72-hour LC_{50} = 11 (9.7 – 12.5) mg/L
96-hour LC_{50} = 4.8 (4.0 – 5.7)mg/L

All element values based on nominal concentrations

Statistical Evaluation of Mortality:
Probit analysis was used to calculate LC_{50} values and the corresponding confidence limits.

Cumulative percent mortality:

Nominal Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Neg. Control	0	0	0	0
2.5	0	0	0	10
4.0	0	0	0	25
6.0	0	0	0	85
10	0	0	40	85
15	0	20	85	100

CONCLUSIONS

The test sample 96-hour LC_{50} for fathead minnow was determined to be 4.8 mg/L with a 95% confidence interval of 4.0 – 5.7 mg/L.

Testing was conducted on the "as-sold" mixture of the subject material in water (~25% subject material). The values reported apply to that mixture and not directly to the subject material.

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

DATA QUALITY

Reliability: Klimisch ranking 2. This study, while well conducted, lacks analytical data for determination of the test substance concentration in the test solutions and determination of the sample purity. Because a mixture was tested, care must be taken when interpreting the results. The solvent in the mixture could have enhanced or restricted biological uptake and/or toxicity of the fluorochemical component.

REFERENCES

This study was conducted by EnviroSystems Division Resource Analysts, Incorporated, Hampton, New Hampshire on behalf of the 3M Company.

OTHER

Last changed: 5/18/00

J2904-1

Study Title

Static Acute Toxicity of FC-120 to the Fathead Minnow, *Pimephales promelas*

Authors

Timothy J. Ward
Robert L. Boeri

Study Completed

March 1992

Performing Laboratory

EnviroSystems Division
Resource Analysts, Incorporated
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Hampton, New Hampshire 03842

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I. SUMMARY

The acute toxicity of FC-120 to the fathead minnow, *Pimephales promelas*, is described in this final report. The test was conducted for 3M Company for 96 hours during February 27 to March 2, 1992, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted by Jeanne Magazu, Peter Kowalski, Ellen Stanford, Robert Boeri, and Timothy Ward.

The test was performed under static conditions with five concentrations of test substance and a dilution water control at a mean temperature of 23 ± 2°C. The dilution water was filtered natural well water collected at Hampton, New Hampshire. Aeration was not employed to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of FC-120 were: 0 mg/L (control), 2.5 mg/L, 4.0 mg/L, 6.0 mg/L, 10.0 mg/L, and 15.0 mg/L. Nominal concentrations were used for all calculations.

Fish used in the test were purchased from a commercial supplier (Aquatic Research Organisms, Hampton, New Hampshire) and acclimated under test conditions for more than 15 days. After 96 hours of exposure the control fish had an average total length of 26.5 mm and an average wet weight (blotted dry) of 0.14 g, resulting in a loading rate of approximately 0.09 g/L. All fish were in good condition at the beginning of the study.

Exposure of fathead minnows to the test substance resulted in a 96 hour LC50 of 4.8 mg/L FC-120, with a 95% confidence interval of 4.0 to 5.7 mg/L. One of the eight surviving fish exposed to 15.0 mg/L at 48 hours and one of the three surviving fish exposed to 15.0 mg/L at 72 hours exhibited erratic swimming. The three surviving fish exposed to 10.0 mg/L at 96 hours exhibited a loss of equilibrium and erratic swimming. No other sublethal effects were observed in any test vessel during the 96 hour exposure.

III. INDEX OF TABLES

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IV. INTRODUCTION

This study was sponsored by 3M Company, St. Paul, Minnesota. The objective of the study was to determine the acute toxicity of FC-120 to the fathead minnow, a freshwater fish. The report contains sections that describe the methods and materials employed in the study, and the results of the investigation. The report also contains an appendix that presents the water quality data collected during the test.

V. METHODS AND MATERIALS

TEST SUBSTANCE:

FC-120 (EnviroSystems Sample Number 5103E) was delivered to EnviroSystems on November 13, 1991. It was contained in a 250 ml plastic bottle that was labelled with the following information: "J2904-I, FC-120, Lot 164, 2-Butoxy Ethanol". FC-120 (a light brown liquid) was supplied by 3M Company, 935 Bush Avenue, St. Paul, Minnesota. Prior to use the test material was stored in the dark at room temperature. A reserve sample (approximately 1.3 grams) will be retained at EnviroSystems for a minimum of 10 years.

DILUTION WATER:

Water used for acclimation of test organisms and for all toxicity testing was collected from wells at EnviroSystems in Hampton, New Hampshire. Water was stored in 500-gallon polyethylene tanks, where it was aerated. Results of chemical analysis of a representative sample of water are presented in Table 1.

TEST ORGANISM:

Juvenile fathead minnows employed as test organisms were from a single source and were identified using an appropriate taxonomic key. They were purchased from a commercial supplier (the Aquatic Research Organisms Division of Resource Analysts, Inc., Hampton, New Hampshire) and acclimated at the EnviroSystems facility for more than 15 days. Control fish were weighed at the conclusion of the toxicity test. Prior to testing, fish were maintained in 100% dilution water under flow through conditions in an all glass aquarium. During acclimation fish were not treated for disease and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. During the acclimation period 14 days prior to the test initiation the temperature ranged from 21.1° to 22.5°C, and the dissolved oxygen concentration was maintained above 8.0 mg/L. Fish were fed a commercial fish food (EnviroSystems lot number TM03) once or twice daily before the test.

Table 1. Chemical characterization of a representative sample of natural well water used as dilution water for toxicity test

Parameter	Unit of Measurement	Reporting Limit	Measured Value
pH	pH units	--	7.0
Conductivity	umhos/cm	--	640
Hardness	mg/L as $CaCO_3$	--	160
Organochlorine pesticides	ug/L	0.5	ND
Organophosphorus pesticides	ug/L	0.5	ND
Polychlorinated biphenyls	ug/L	0.5	ND

Notes: 1. ND = not detected at or above the reporting limit.
2. pH, hardness, and conductivity were measured in dilution water collected from the control test vessel prior to introduction of organisms and pesticide and PCB data is collected during routine biannual water quality testing.

TOXICITY TESTING:

A screening test with the test substance was conducted during January 13 to 17, 1992. The test was conducted at 1, 10, 100, and 1,000 mg/L. After 96 hours there was 0% survival at 100 and 1,000 mg/L and 100% survival at all lower tested concentrations.

A definitive toxicity test was attempted during February 20 to 24, 1992. Nominal concentrations of the test material were: 0 mg/L, 15 mg/L, 25 mg/L, 40 mg/L 60 mg/L, and 100 mg/L. The test was repeated because less than 50% survival occurred at all 5 concentrations after 96 hours.

The definitive toxicity test was performed during February 27 to March 2, 1992 according to procedures of the OECD (1984). The test was conducted at a target temperature of 23 ± 2°C with five concentrations of test substance and a dilution water control. No stock solution was prepared and test material was added directly to dilution water contained in the test vessels without the use of a solvent. Nominal concentrations of the test material were: 0 mg/L (control), 2.5 mg/L, 4.0 mg/L, 6.0 mg/L, 10.0 mg/L, and 15.0 mg/L.

Twenty fish were equally distributed among two replicates of each treatment. The test was performed in 20 liter glass aquaria (approximately 20 cm in width, 40 cm in length, and 25 cm in height) that contained 15 liters of test solution (water depth was approximately 18 cm). Test vessels were randomly arranged in an incubator during the 96 hour test (a random numbers table was used to select the location of each vessel). A 16 hour light and 8 hour dark photoperiod was automatically maintained with cool-white fluorescent lights. Aeration was not employed to maintain dissolved oxygen concentrations above acceptable levels. Fish were not fed during the test.

The number of surviving organisms and the occurrence of sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in behavior) were determined visually and recorded initially and after 24, 48, 72, and 96 hours. Dead test organisms were removed when first observed. Dissolved oxygen (YSI Model 57 meter), pH (Beckman model pHI 12 meter), conductivity (Cole Parmer model number 1481-60), and temperature (ASTM mercury thermometer) were measured and recorded daily in each test chamber that contained live animals.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques (Stephan, 1983). The 24, 48, 72, and 96 hour LC50 values were calculated by the binomial, moving average, or probit method, when warranted. All calculations were performed by the author using nominal concentrations of the test substance.

VI. RESULTS

All test vessels containing FC-120 were initially clear and remained clear throughout the test. Biological and water quality data generated by the acute toxicity test are presented in Table 2 and Appendix A, respectively. One hundred percent survival occurred in the control exposure. Control fish had an average total length of 26.5 mm, and an average wet weight (blotted dry) of 0.14 g at the end of the test. Loading rate during the toxicity test was approximately 0.09 g/L.

The 24, 48, 72, and 96 hour LC50s for fish exposed to FC-120 are presented in Table 3. The 96 hour LC50 value (and associated 95% confidence limits) is 4.8 mg/L FC-120 (4.0 - 5.7 mg/L). One of the eight surviving fish exposed to 15.0 mg/L at 48 hours and one of the three surviving fish exposed to 15.0 mg/L at 72 hours exhibited erratic swimming. The three surviving fish exposed to 10.0 mg/L at 96 hours exhibited a loss of equilibrium and erratic swimming. No other sublethal effects were observed in any test vessel during the 96 hour exposure.

Table 2. Survival data from toxicity test

Nominal Concentration (mg/L)	rep.	Number Alive					Number Affected				
		0hr	24hr	48hr	72hr	96hr	0hr	24hr	48hr	72hr	96hr
0 (control)	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
2.5	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	8	0	0	0	0	0
4.0	1	10	10	10	10	5	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
6.0	1	10	10	10	10	3	0	0	0	0	0
	2	10	10	10	10	0	0	0	0	0	0
10.0	1	10	10	10	6	2	0	0	0	0	2
	2	10	10	10	6	1	0	0	0	0	1
15.0	1	10	10	8	2	0	0	0	0	1	-
	2	10	10	8	1	0	0	0	1	0	-

Note: All affected fish exhibited erratic swimming and affected fish at 96 hours also exhibited a loss of equilibrium.

Table 3. Median lethal concentrations (LC50s) from toxicity test

Exposure period	LC50	95 percent confidence limit	LC50 calculation method
24 hours	>15.0 mg/L	--	--
48 hours	>15.0 mg/L	--	--
72 hours	11.0 mg/L	9.7 - 12.5 mg/L	Probit
96 hours	4.8 mg/L	4.0 - 5.7 mg/L	Probit

VII. REFERENCES

OECD. 1984. Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 203, Fish Acute Toxicity Test. Adopted April 4, 1984.

Stephan, C.E. 1983. Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.

Appendix A. WATER QUALITY DATA FROM TOXICITY TEST

Table A.1. Conductivity, pH, temperature, and dissolved oxygen concentration measured during toxicity test

Nominal concentration (mg/L)	Rep.	Conductivity (umho/cm) 0 hr	24 hr	48 hr	72 hr	96 hr	pH 0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	640	640	650	650	670	7.0	7.8	7.3	8.0	8.1
	2	640	660	670	660	690	7.0	7.8	7.3	8.0	8.2
2.5	1	650	670	670	660	660	7.0	7.9	7.3	8.0	8.2
	2	650	670	670	660	670	7.0	7.9	7.3	8.0	8.2
4.0	1	660	670	670	660	670	7.0	7.9	7.3	7.9	8.2
	2	660	670	670	660	670	7.0	7.9	7.3	7.9	8.1
6.0	1	660	660	670	670	670	7.0	7.9	7.3	7.9	8.1
	2	660	660	670	660	670	7.0	7.9	7.3	7.9	8.1
10.0	1	660	670	670	670	660	7.0	7.9	7.3	7.9	8.0
	2	660	670	670	670	660	7.0	7.9	7.3	7.9	8.1
15.0	1	670	670	670	670	670	7.0	7.9	7.3	7.8	8.0
	2	670	670	670	670	670	7.0	7.9	7.3	7.8	8.0

Table A.1. Continued.

Nominal concentration (mg/L)	Rep.	Temperature (°C) 0 hr	24 hr	48 hr	72 hr	96 hr	Dissolved Oxygen (mg/L) 0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	21.5	23.0	21.5	21.5	22.5	8.5	8.5	7.7	7.4	6.8
	2	21.6	21.0	21.8	21.4	22.9	8.5	8.5	7.7	7.3	7.5
2.5	1	21.5	22.0	21.4	21.4	21.5	8.6	7.9	7.6	7.4	7.0
	2	21.5	22.9	21.5	21.3	22.2	8.7	8.0	7.5	7.3	7.0
4.0	1	21.4	22.5	21.6	21.5	22.5	8.6	7.5	7.6	7.2	6.7
	2	21.5	23.0	21.5	21.3	22.6	8.6	7.6	7.4	7.0	6.0
6.0	1	21.5	22.3	21.7	21.2	21.8	8.6	7.5	7.5	7.1	6.4
	2	21.5	22.3	21.4	21.5	22.3	8.6	7.5	7.3	7.1	5.6
10.0	1	21.5	22.4	21.5	21.2	22.3	8.6	7.6	7.3	6.9	5.8
	2	21.5	22.3	21.7	21.4	22.1	8.6	7.5	7.4	6.8	6.2
15.0	1	21.5	22.7	21.6	21.2	22.2	8.6	7.6	7.2	6.8	5.7
	2	21.5	22.9	21.8	21.3	22.1	8.6	7.6	7.3	7.0	6.0

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (E.G.,DAPHNIA)

TEST SUBSTANCE

Identity: Perfluorodecanesulfonate, ammonium salt; may also be referred to as PFDS, FC-120, or 3M Sample J2905-1. (1-Decanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heneicosafluoro-, ammonium salt, CAS # 67906-42-7)

Remarks: Test sample is a mixture of the test substance in water and butyl cellosolve (approximately 25% test substance, 37.5% butyl cellosolve and 37.5% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample. Test sample was from 3M Lot 164.

METHOD

Method: OECD 202.
Test type: Static acute
GLP: No
Year Completed: 1992
Species: *Daphnia magna*
Analytical monitoring: pH, dissolved oxygen, conductivity, temperature. Test substance concentration was not monitored.
Statistical methods: EC_{50} values calculated using Binomial/nonlinear interpolation
Test daphnid source: In-house cultures, EnvironSystems Division, Resource Analysts, Incorporated, Hampton, NH
Test daphnid age at study initiation: < 24-hours

Test conditions

Dilution water: Aerated, filtered natural well water
Dilution water chemistry (from a representative sample):

hardness:	166 mg/L as $CaCO_3$
pH:	7.2
Conductivity:	540 µmhos/cm

Stock and test solutions preparation: A primary stock solution was prepared in dilution water to yield a test sample concentration of 1000 mg/L. All test solutions were made by diluting the appropriate amount of stock solution with dilution water.
Exposure vessels: 250 mL glass beakers containing 200 mL of test solution.
Number of replicates: 4
Number of daphnids per replicate: 5
Number of concentrations: five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0-48 hours):

8.7 – 9.3 mg/L (control exposure)

8.8 – 9.1 mg/L (100 mg/L exposure)

pH range (0-48 hours):

7.2 – 8.1 (control exposure)

7.2 – 8.2 (100 mg/L exposure)

Test temperature range (0 – 48 hours)

19.4 – 20.8°C

Element basis: mortality and immobilization

RESULTS

Nominal concentrations: Bk control, 15, 25, 40, 60, 100 mg/L

Element value: 24-hour EC_{50} => 100 mg/L

48-hour EC_{50} = 44 (25 – 60) mg/L

Statistical Evaluation: The EC_{50} value and 95% confidence interval was calculated using the binomial/nonlinear interpolation method.

Mortality of controls: None

Remarks: Values reported are for the test sample (mixture). No calculations were made to adjust for the concentration of the test substance in the test sample.

Cumulative percent mortality and immobilization:

Nominal Test Conc., mg/L	24-hours	48-hours
Neg. Control	0	0
15	5	15
25	5	20
40	10	35
60	0	60
100	25	90

CONCLUSIONS

The test sample 48-hour EC_{50} for *Daphnia magna* was determined to be 44 mg/L with a 95% confidence interval of 25 to 60 mg/L.

Testing was conducted on the "as-sold" mixture of the subject material in water (~25% subject material). The values reported apply to that mixture and not directly to the subject material.

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

DATA QUALITY

Reliability: Klimisch ranking 2. This study, while well conducted, lacks analytical data for: determination of the test substance concentration in the test solutions; and determination of the sample purity. Because a mixture was tested, care must be taken when interpreting the results. The solvent in the mixture could have enhanced or restricted biological uptake and/or toxicity of the fluorochemical component.

REFERENCES

This study was conducted by EnviroSystems Division Resource Analysts, Incorporated, Hampton, New Hampshire on behalf of the 3M Company.

OTHER

Last changed: 5/18/00

J2904

Study Title

Static Acute Toxicity of FC-120
to the Daphnid, *Daphnia magna*

Authors

Timothy J. Ward
Robert L. Boeri

Study Completed

February, 1992

Performing Laboratory

EnviroSystems Division
Resource Analysts, Incorporated
One Lafayette Road
Hampton, New Hampshire 03842

I. SUMMARY

The acute toxicity of FC-120 to the daphnid, *Daphnia magna*, is described in this final report. The test was conducted for 3M Company for 48 hours during February 5 to 7, 1992, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted by Jeanne Magazu, Peter Kowalski, Ellen Stanford, Robert Boeri, and Timothy Ward.

The test was performed under static conditions with five concentrations of test substance and a dilution water control at a temperature of 20 ± 1°C. The dilution water was filtered natural well water collected at Hampton, New Hampshire. Aeration was not employed to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of FC-120 were: 0 mg/L (control), 15, 25, 40, 60, and 100 mg/L. Nominal concentrations were used for all calculations.

Daphnids used in the test were produced from an in-house culture and were less than 24 hours old at the start of the test. After 48 hours of exposure the control daphnids had an average wet weight (blotted dry) of 0.0002 g, resulting in a loading rate of 0.005 g/L. All daphnids were in good condition at the beginning of the study.

Exposure of daphnids to the test substance resulted in a 48 hour EC50 of 44 mg/L FC-120, with a 95% confidence interval of 25 to 60 mg/L. Six of the surviving daphnids exposed to 60 mg/L and all of the surviving daphnids exposed to 100 mg/L were immobilized at 48 hours. No other sublethal effects were observed in any test vessel.

II. TABLE OF CONTENTS

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IV. INTRODUCTION

This study was sponsored by 3M Company, St. Paul, Minnesota. The objective of the study was to determine the acute toxicity of FC-120 to the daphnid, a freshwater invertebrate. The report contains sections that describe the methods and materials employed in the study, and the results of the investigation. The report also contains an appendix that presents the water quality data collected during the test.

V. METHODS AND MATERIALS

TEST SUBSTANCE:

FC-120 (EnviroSystems Sample Number 5103E) was delivered to EnviroSystems on November 13, 1991. It was contained in a 250 ml plastic bottle that was labelled with the following information: "J2904-I, FC-120, Lot 164, 2-Butoxy Ethanol." FC-120 was supplied by 3M Company, 935 Bush Avenue, St. Paul, Minnesota. Prior to use the test material was stored in the dark at room temperature. A reserve sample will be retained at EnviroSystems for a minimum of 10 years.

DILUTION WATER:

Water used for acclimation of test organisms and for all toxicity testing was collected from wells at EnviroSystems in Hampton, New Hampshire. Water was stored in 500-gallon polyethylene tanks where it was aerated. Results of chemical analysis of a representative sample of water are presented in Table 1.

TEST ORGANISM:

Juvenile daphnids employed as test organisms were from a single source and were identified using an appropriate taxonomic key. Daphnids used in the test were produced from an in-house culture and were less than 24 hours old at the start of the test. Control daphnids were weighed at the conclusion of the toxicity test. Prior to testing, daphnids were maintained in 100% dilution water under static conditions in 4 liter glass jars. During acclimation daphnids were not treated for disease and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. During the acclimation period 24 hours prior to the test initiation the temperature was 20.3°C. Daphnids were fed yeast, trout chow, and/or the freshwater alga *Selenastrum capricornutum* once or twice daily before the test.

Table 1. Chemical characterization of a representative sample of natural well water used as dilution water for toxicity test

Parameter	Unit of Measurement	Reporting Limit	Measured Value
pH	pH units	--	7.2
Conductivity	umhos/cm	--	540
Hardness	mg/L as $CaCO_3$	--	166
Organochlorine pesticides	ug/L	0.5	ND
Organophosphorus pesticides	ug/L	2	ND
Polychlorinated biphenyls	ug/L	1	ND

Notes:
1. ND= not detected at or above the reporting limit.
2. pH, hardness, and conductivity were measured in dilution water collected from the control test vessel prior to introduction of organisms and pesticide and PCB data is collected during routine biannual water quality testing.

TOXICITY TESTING:

A screening test with the test substance was conducted during January 21 to 23, 1992. Nominal concentrations of test substance were 1, 10, 100, and 1,000 mg/L. After 48 hours of exposure there was 0% survival at 100 and 1,000 mg/L and 80% survival or greater at all lower tested concentrations. A definitive toxicity test was attempted from January 29 to 31, 1992. Nominal concentrations of test substance were 150, 250, 400, 600, and 1,000 mg/L. This test was repeated because less than 50% survival occurred at all tested concentrations.

The definitive toxicity test was performed during February 5 to 7, 1992. The test was conducted at a target temperature of 20 ± 1°C with five concentrations of test substance and a dilution water control. A 1,000 mg/L stock solution was made by combining 0.25 g of the test substance and dilution water in a 250 ml volumetric flask and adjusting the final volume to 250 mls with dilution water. Nominal concentrations of the test material were: 0 mg/L (control), 15, 25, 40, 60, and 100 mg/L.

Twenty daphnids were equally distributed among four replicates of each treatment. The test was performed in 250 ml glass beakers that contained 200 ml of test solution (water depth was approximately 6 cm). Test vessels were randomly arranged in an incubator during the 48 hour test (a random numbers table was used to select the location of each vessel). A 16 hour light and 8 hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of 20 $uEs^{-1}m^{-2}$. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels. Daphnids were not fed during the test.

The number of surviving organisms and the occurrence of immobilization (inability to swim within 15 seconds after gentle agitation) or other sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in behavior) were determined visually and recorded initially and after 24 and 48 hours. Dissolved oxygen (YSI Model 57 meter), pH (Beckman model pHI 12 meter), conductivity (Cole Parmer number 1481-60), and temperature (ASTM mercury thermometer) were measured and recorded daily in each test chamber that contained live animals.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques (Stephan, 1983). The 24 and 48 hour EC50 values were calculated by the binomial, moving average, or probit method. All calculations were performed by the author using nominal concentrations of the test substance.

VI. RESULTS

All test vessels containing FC-120 were clear and remained clear throughout the test. Biological and water quality data generated by the acute toxicity test are presented in Table 2 and Appendix A, respectively. One hundred percent survival occurred in the control exposure. Control daphnids had an average wet weight (blotted dry) of 0.0002 g at the end of the test. Loading rate during the toxicity test was approximately 0.005 g/L.

Exposure of daphnids to the test substance resulted in a 48 hour EC50 of 44 mg/L FC-120, with a 95% confidence interval of 25 to 60 mg/L. Six of the surviving daphnids exposed to 60 mg/L and all of the surviving daphnids exposed to 100 mg/L were immobilized at 48 hours. No other sublethal effects were observed in any test vessel during the test.

Table 2. Survival data from toxicity test

Nominal Concentration (mg/L)	rep.	Number Alive			Number Affected		
		0 hr	24 hr	48 hr	0hr	24hr	48hr
0 (control)	1	5	5	5	0	0	0
	2	5	5	5	0	0	0
	3	5	5	5	0	0	0
	4	5	5	5	0	0	0
15	1	5	5	5	0	0	0
	2	5	5	4	0	0	0
	3	5	4	3	0	0	0
	4	5	5	5	0	0	0
25	1	5	5	3	0	0	0
	2	5	5	5	0	0	0
	3	5	4	3	0	0	0
	4	5	5	5	0	0	0
40	1	5	4	4	0	0	0
	2	5	4	3	0	0	0
	3	5	5	2	0	0	0
	4	5	5	4	0	0	0
60	1	5	5	2	0	0	1
	2	5	5	1	0	0	1
	3	5	5	3	0	0	2
	4	5	5	2	0	0	2
100	1	5	3	0	0	0	0
	2	5	4	0	0	0	0
	3	5	3	1	0	0	1
	4	5	5	1	0	0	1

Note: Affected organism were immobilized (unable to swim within 15 seconds after gentle agitation).

Table 3. Median effective concentrations (EC50s) from toxicity test

Exposure period	EC50	95 percent confidence limits	Calculation method
24 hours	>100 mg/L	--	--
48 hours	44 mg/L	25 - 60 mg/L	Binomial/Nonlinear Interpolation

VII. REFERENCES

OECD. 1984. Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 202, Daphnid sp., Acute Immobilization Test and Reproduction Test. Adopted April 4, 1984.

Stephan, C.E. 1983. Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.

Appendix A. WATER QUALITY DATA FROM TOXICITY TEST

Table A.1. Conductivity, pH, temperature, and dissolved oxygen concentration measured during toxicity test

Nominal concentration (mg/L)	Rep.	Conductivity (umho/cm) 0 hr	24 hr	48 hr	pH 0 hr	24 hr	48 hr
0 (control)	1	540	580	600	7.2	8.0	8.0
	2	550	580	590	7.2	8.1	8.0
	3	570	580	590	7.2	8.1	8.0
	4	570	580	580	7.2	8.1	8.0
15	1	580	580	590	7.2	8.1	8.0
	2	580	570	580	7.2	8.1	8.0
	3	580	570	580	7.2	8.1	8.0
	4	580	570	590	7.2	8.1	8.0
25	1	580	580	590	7.2	8.1	8.0
	2	580	580	580	7.2	8.1	8.0
	3	580	580	580	7.2	8.1	8.0
	4	580	580	580	7.2	8.1	8.0
40	1	580	580	590	7.2	8.2	8.1
	2	580	580	580	7.2	8.2	8.1
	3	580	580	580	7.2	8.2	8.1
	4	580	580	590	7.2	8.2	8.1
60	1	580	580	590	7.2	8.2	8.1
	2	580	580	590	7.2	8.2	8.1
	3	580	570	580	7.2	8.2	8.1
	4	580	580	590	7.2	8.2	8.1
100	1	580	590	590	7.2	8.2	8.1
	2	580	580	580	7.2	8.2	8.1
	3	580	580	580	7.2	8.2	8.1
	4	580	580	590	7.2	8.2	8.1

Table A.1. Continued.

Nominal concentration (mg/L)	Rep.	Temperature (°C) 0 hr	24 hr	48 hr	Dissolved Oxygen (mg/L) 0 hr	24 hr	48 hr
0 (control)	1	19.4	20.5	20.4	8.7	8.9	9.3
	2	19.4	20.3	20.3	8.8	9.0	9.2
	3	19.4	20.3	20.3	8.8	9.1	9.3
	4	19.4	20.2	20.4	8.8	9.0	9.2
15	1	19.4	20.3	20.3	8.9	9.0	9.0
	2	19.4	20.3	20.3	9.0	9.0	9.0
	3	19.4	20.2	20.2	9.0	9.0	9.0
	4	19.4	20.3	20.4	9.0	9.0	9.1
25	1	19.4	20.3	20.4	9.0	9.0	9.1
	2	19.4	20.3	20.3	8.9	9.0	9.1
	3	19.4	20.3	20.3	8.9	9.0	9.1
	4	19.5	20.2	20.3	9.0	8.9	9.1
40	1	19.4	20.6	20.3	8.9	9.0	9.1
	2	19.4	20.6	20.3	8.9	8.9	9.1
	3	19.6	20.4	20.4	8.9	8.8	9.1
	4	19.4	20.3	20.4	8.9	8.8	9.1
60	1	19.4	20.8	20.4	8.9	9.0	9.1
	2	19.4	20.8	20.4	9.0	8.8	9.1
	3	19.4	20.6	20.4	9.0	8.9	9.1
	4	19.4	20.4	20.4	8.8	8.8	9.1
100	1	19.4	20.8	20.4	9.0	9.0	9.1
	2	19.4	20.8	20.4	9.0	9.0	9.1
	3	19.4	20.8	20.4	9.0	8.8	9.1
	4	19.4	20.7	20.4	8.9	8.8	9.1

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST

TEST SUBSTANCE

Identity: Perfluorodecanesulfonate, ammonium salt; may also be referred to as PFDS, FC-120, or 3M Sample J2905-1. (1-Decanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heneicosafluoro-, ammonium salt, CAS # 67906-42-7)

Remarks: Test sample is a mixture of the test substance in water and butyl cellosolve (approximately 25% test substance, 37.5% butyl cellosolve and 37.5% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample. Test sample was from 3M Lot 164.

METHOD

Method: OECD 209
Test type: Static acute
GLP: No
Inoculum source: Metro Waste Treatment Plant, St Paul, MN.
Inoculum holding: None – used the day it was obtained.
Inoculum MLSS: 4.3 g/L.
Year Completed: 1992
Analytical monitoring: Dissolved oxygen concentrations.
Exposure Period: 30-minutes and 3-hours
Measurements: Dissolved oxygen depletion rate after 30-minutes and 3-hours of exposure.

RESULTS

Nominal concentrations: Blank controls and 1000 mg/L test material solution, in duplicate.

Element value: 30-min and 3-hour EC_{50} > 1000 mg/L

All element values based on nominal concentrations.

Inhibition in respiration rate: After 3-hours exposure, the mean of both replicates was 17.3% inhibition at 1000 mg/L

Control response: The largest percent difference between control uptake rates was 1.5%; thus the two controls were within 15% of each other.

Reference substance results: 3,5-dichlorophenol at a concentration of 10 mg/L induced 57% inhibition in respiration rate. This was within the normal range seen in this laboratory with this inoculum.

Remarks: Testing was conducted on the "as-sold" mixture of the subject material (25% subject substance in water). The value reported applies to that mixture and not the test material.

CONCLUSIONS

FC-120 exhibited a 17.3% inhibitory effect at 1000 mg/L after a 3-hour exposure.

Testing was conducted on the "as-sold" mixture of the subject material in water (~25% subject material). The values reported apply to that mixture and not directly to the subject material.

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

DATA QUALITY

Reliability: Klimisch ranking 2. This study, while well conducted, lacks analytical data for: determination of the test substance concentration in the test solutions; and determination of the sample purity. Because a mixture was tested, care must be taken when interpreting the results. The solvent in the mixture could have enhanced or restricted biological uptake and/or toxicity of the fluorochemical component.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, St Paul, MN, Lab Request number J2904, 3/6/92.

OTHER

Last changed: 5/18/00

LR 52909-1
Date 3/9/92
Analyst KSP
Page 1 of 4

ENVIRONMENTAL LABORATORY

ACTIVATED SLUDGE MIXED LIQUOR

Type Test OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

ACTIVATED SLUDGE MIXED LIQUOR

Date Obtained 3/6/92 Date Used 3/6/92

Mixed Liquor Suspended Solids (MLSS) 4.3 g/L Adjusted to — g/L

MLSS in final test solution 1.7 g/L

Initial pH 7.5 (1st test soln. prepared) Final pH 7.7 (last soln. prepared)

Comments

TEST SUBSTANCE

Sample Description FC-120 LOT 164

Initial pH of highest conc. tested (1000 mg/L) = 7.7 Adjusted to pH —

Comments YELLOW LIQUID

REFERENCE SUBSTANCE

Sample Description 3,5 - DICHLOROPHENOL

Initial pH of stock solution (500 mg/L) = 8.1 Adjusted to pH 7.7

Comments

EXPOSURE CONDITIONS

Test Duration 3 HOURS

Test Temperature Range: 19.6 - 21.0 °C

Comments

Pg 2 of 4

LR J2904-1

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
TEST SOLUTIONS PREPARATION

Test Substance: FC-120 LOT 164

Sample Description: YELLOW LIQUID

Analyst: KSN

Date: 3/9/92

Test Conc. mg/L	mg added per 500 mL	mL MQ(1) water per 500 mL	mL syn. sewage feed per 500 mL	mL microbial inoculum per 500 mL	Total Volume mL
BLANK CONTROL	–	284	16	200	500
1000-A	500	284	16	200	500
1000-B	500	284	16	200	500

INITIAL pH VALUES: A: 7.7

B: 7.7

(1) Millipore Milli-Q^R high purity water

LR 52904

ENVIRONMENTAL LABORATORY

CECO ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: FC-120 LOT 164

Source of Activated Sludge Mixed Liquor: METRO WASTE TREATMENT PLANT ; ST. PAUL, MN

Date Received: 3/6/92 Date Used: 3/6/92

Analyst: KSN

30-MINUTE EXPOSURE DISSOLVED OXYGEN CONCENTRATIONS, mg/L

Test Substance Concentration (mg/L)	TIME-MINUTES 0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	Temp °C
BK CONTROL I	8.4	8.0	7.6	7.2	6.6	6.1	5.6	5.2	4.7	4.2	3.7	20.0
1000-A	4.5	4.0	3.6	3.1	2.6	2.1	1.6	1.0	0.5	—	—	21.0
1000-B	2.8	2.2	1.6	1.0	0.5	—	—	—	—	—	—	20.7
BK CONTROL II	7.6	7.1	6.6	6.2	5.7	5.2	4.9	4.2	3.7	3.1	2.7	20.2

3-HOUR EXPOSURE DISSOLVED OXYGEN CONCENTRATIONS, mg/L

Test Substance Concentration (mg/L)	TIME - MINUTES 0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	Temp °C
BK CONTROL I	8.0	7.5	7.0	6.4	6.0	5.5	5.0	4.5	3.9	3.4	2.9	19.9
1000-A	8.3	7.9	7.5	7.1	6.7	6.3	5.8	5.3	4.9	4.5	4.1	20.5
1000-B	8.5	8.0	7.6	7.2	6.8	6.3	5.9	5.5	5.1	4.6	4.2	19.9
BK CONTROL II	7.1	6.7	6.3	5.8	5.3	4.8	4.2	3.7	3.3	2.8	2.4	19.6

pH of activated sludge mixed liquor: Initial 7.5 Final 7.7

pH of highest concentration tested: Initial 7.6 Final 7.4

Test temperature: 19.6 - 21.0 °C

ENVIRONMENTAL LABORATORY
OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
CALCULATION OF RESULTS

Lr: J2904-1
Date: 3/6/92

Test Substance: FC-120 LOT 164

Analyst: KSN

30-Minute Exposure — Dissolved Oxygen Concentrations, mg/L

mg/L	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	Temp. °C	R (1)	Ra (2)	% Inh. (3)
BK1	8.4	8.0	7.6	7.2	6.6	6.1	5.6	5.2	4.7	4.2	3.7	20.0	0.9661	0.9661	0.0
1000.00	4.5	4.0	3.6	3.1	2.6	2.1	1.6	1.0	-	-	-	21.0	1.0000	0.9328	3.8
1000.00	2.8	2.2	1.6	1.0	-	-	-	-	-	-	-	20.7	1.2000	1.1430	
BK2	7.6	7.1	6.6	6.2	5.7	5.2	4.9	4.2	3.7	3.1	2.7	20.2	0.9867	0.9730	-

% diff in controls (4): 0.7 EC50 (95% CI): >1000 mg/L RaCm (5): 0.9696

3-Hour Exposure — Dissolved Oxygen Concentrations, mg/L

mg/L	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	Temp. °C	R (1)	Ra (2)	% Inh. (3)
BK1	8.0	7.5	7.0	6.4	6.0	5.5	5.0	4.5	3.9	3.4	2.9	19.9	1.0194	1.0265	-
1000.00	8.3	7.9	7.5	7.1	6.7	6.3	5.8	5.3	4.9	4.5	4.1	20.5	0.8594	0.8300	18.5
1000.00	8.5	8.0	7.6	7.2	6.8	6.3	5.9	5.5	5.1	4.6	4.2	19.9	0.8485	0.8544	16.1
BK2	7.1	6.7	6.3	5.8	5.3	4.8	4.2	3.7	3.3	2.8	2.4	19.6	0.9830	1.0108	-

% diff in controls (4): 1.5 EC 50 (95% CI): >1000 mg/L RaCm (5): 1.0186

(1) R= Oxygen uptake rate (mg O2/L-min)
(2) Ra= R adjusted to 20°C= [R/e.Ex]
(3) % Inhibition= [(RaCm)-RaS/(RaCm)]100
(4) % difference in controls: [|RaBk1-RaBk2|/RaCm]100 (Two controls must be within 15% of each other.)
(5) Mean adjusted R, controls= [RaBk1+RaBk2]/2

e= 1.072
RaCm= mean adjusted R, controls
Bk= blank control, S= sample
Tobs= sample temp
x= Tobs-20°C

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LR 52909

ENVIRNOMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST # 209
TEST SOLUTIONS PREPARATION

Test Substance: 3,5 - DICHLOROPHENOL REFERENCE SUBSTANCE

Sample Description: ALDRICH RED LABEL LOT D7,060-0

Analyst: KSN

Date: 3/6/92

Stock Solution Preparation: 500 mg/L ; EACH 1.0 ml = 0.5 mg.
DISSOLVED 500 mg 3,5-DCP IN 10 ml 1.0 N NaOH
ADDED ≈ 20 ml MILLI-Q WATER
ADDED 1.0 N H_2SO_4 DROPWISE, UNDER STIRRING UNTIL INCIPIENT PPT.
ADDED ≈ 500 ml MILLI-Q WATER. STOCK SOLUTION INITIAL pH = 8.1
ADJUSTED TO 7.7 WITH H_2SO_4, DILUTED TO 1 LITER WITH
MILLI-Q WATER. STOCK SOLUTION pH = 7.7

Test Conc. mg/L	mL added per 500 mL	mL MQ(1) water per 500 mL	mL syn. sewage feed per 500 mL	mL microbial inoculum per 500 mL	Total Volume mL
BLANK CONTROL	—	284	16	200	500
10	10	274	16	200	500

(1) Millipore Milli-Q^R high purity water

F. 3-5
Jared
Jones
LR J 3320

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: 3,5- Dichlorophenol Reference Substance

Source of Activated Sludge Mixed Liquor: Metro Waste Treatment Plant; St. Paul, MN

Date Received: 3/6/92 Date Used: 3/6/92

Analyst: KSN

30-MINUTE EXPOSURE DISSOLVED OXYGEN CONCENTRATIONS, mg/L

Test Substance Concentration (mg/L)	TIME-MINUTES 0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	Temp °C
BK CONTROL I	8.4	8.0	7.6	7.2	6.6	6.1	5.6	5.2	4.7	4.2	3.7	20.0
10	8.1	7.9	7.5	7.1	6.9	6.6	6.3	5.9	5.6	5.2	4.9	20.8
BK CONTROL II	7.6	7.1	6.6	6.2	5.7	5.2	4.9	4.2	3.7	3.1	2.7	20.2

3-HOUR EXPOSURE DISSOLVED OXYGEN CONCENTRATIONS, mg/L

Test Substance Concentration (mg/L)	TIME - MINUTES 0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	Temp °C
BK CONTROL I	8.0	7.5	7.0	6.4	6.0	5.5	5.0	4.5	3.9	3.4	2.9	19.9
10	9.4	9.2	9.1	9.0	8.8	8.5	8.2	8.1	7.8	7.6	7.4	19.6
BK CONTROL II	7.1	6.7	6.3	5.8	5.3	4.8	4.2	3.7	3.3	2.8	2.4	20.6

pH of activated sludge mixed liquor: Initial 7.5 Final 7.7
pH of highest concentration tested: Initial 7.6 Final 7.3
Test temperature: 19.6 - 20.8 °C

000160

ENVIRONMENTAL LABORATORY
OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
CALCULATION OF RESULTS

Lr: J2902 & J2904 15330
Date: 3/6/92

Test Substance: 3,5 - DICHLOROPHENOL

Analyst: KSN

30-Minute Exposure — Dissolved Oxygen Concentrations, mg/L

mg/L	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	Temp. °C	R (1)	Ra (2)	% Inh. (3)
BK1	8.4	8.0	7.6	7.2	6.6	6.1	5.6	5.2	4.7	4.2	3.7	20.0	0.9661	0.9661	-
10.00	8.1	7.9	7.5	7.1	6.9	6.6	6.3	5.9	5.6	5.2	4.9	20.8	0.6533	0.6180	36.3
BK2	7.6	7.1	6.6	6.2	5.7	5.2	4.9	4.2	3.7	3.1	2.7	20.2	0.9867	0.9730	-

% diff in controls (4): 0.7 **EC50 (95% CI):** RaCm (5): 0.9696

3-Hour Exposure — Dissolved Oxygen Concentrations, mg/L

mg/L	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	Temp. °C	R (1)	Ra (2)	% Inh. (3)
BK1	8.0	7.5	7.0	6.4	6.0	5.5	5.0	4.5	3.9	3.4	2.9	19.9	1.0194	1.0265	-
10.00	9.4	9.2	9.1	9.0	8.8	8.5	8.2	8.1	7.8	7.6	7.4	19.6	0.4255	0.4375	57.1
BK2	7.1	6.7	6.3	5.8	5.3	4.8	4.2	3.7	3.3	2.8	2.4	19.6	0.9830	1.0108	-

% diff in controls (4): 1.5 **EC 50 (95% CI):** RaCm (5): 1.0186

(1) R= Oxygen uptake rate (mg O2/L-min)
(2) Ra= R adjusted to 20°C= [R/θ.Ex]
(3) % Inhibition= [(RaCm)-RaS/(RaCm)]100
(4) % difference in controls: [(RaBk1-RaBk2)/RaCm]100 (Two controls must be within 15% of each other)
(5) Mean adjusted R, controls= [RaBk1+RaBk2]/2

θ= 1.072
RaCm= mean adjusted R, controls
Bk= blank control, S= sample
Tobs= sample temp
x= Tobs-20°C

MICROBICS MICROTOX® TOXICITY TEST

TEST SUBSTANCE

Identity: Perfluorodecanesulfonate, ammonium salt; may also be referred to as PFDS, FC-120, or 3M Sample J2905-1. (1-Decanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heneicosafluoro-, ammonium salt, CAS # 67906-42-7)

Remarks: Test sample is a mixture of the test substance in water and butyl cellosolve (approximately 25% test substance, 37.5% butyl cellosolve and 37.5% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample. Test sample was from 3M Lot 164.

METHOD

Method: Microbics Microtox® "BASIC" Procedure
Test type: Static acute
GLP: No
Year Completed: 1992
Species: *Photobacterium phosphoreum*
Analytical monitoring: None
Statistical methods: Microbics computer software package
Test organism source: Microbics Microtox® Reagent
Test conditions: Per Microbics method
Replicates: Two

RESULTS

Nominal concentrations: 125, 250, 500, 1000 mg/L
Test Sample Element values: 30-minute EC_{10} = 79 (65 – 96) mg/L
30-minute EC_{50} = 327 (299 – 358) mg/L

CONCLUSIONS

FC-120 exhibited a 30-minute EC_{50} for *Photobacterium phosphoreum* of 327 mg/L.

Testing was conducted on the "as-sold" mixture of the subject material in water (~25% subject material). The values reported apply to that mixture and not directly to the subject material.

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

DATA QUALITY

Reliability: : Klimisch ranking 2. This study, while well conducted, lacks analytical data for: determination of the test substance concentration in the test solutions; and determination of the sample purity. Because a mixture was tested, care must be taken when interpreting the results. The solvent in the mixture could have enhanced or restricted biological uptake and/or toxicity of the fluorochemical component.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, St Paul, MN, Lab Request number J2904, 4/20/92.

OTHER

Last changed: 5/18/00

MICROTOX

Form 10620-6-D-PWO

Lab Request No. J2904-1

Page 1 of 10

Environmental Lab — Work Sheet

Date: 4/20/92

Analyst: KSN

Sample Description

FC-120 LOT 169

YELLOW LIQUID

ADDED 200 mg OF SAMPLE TO FLASK WITH ≈ 50 ml MILLI-Q WATER

INITIAL pH = 7.7 - ADJUSTED TO 6.6 WITH H_2SO_4

ADDED MILLI-Q WATER TO 100 ml

OSMOTIC ADJUSTMENT : ADDED 200 mg NaCl TO 10 ml OF SAMPLE

FINAL APPEARANCE OF TEST SOLUTION : *SLIGHT CLOUDINESS OBSERVED

* NOTE : TEST CONCENTRATIONS ARE CLEAR COLORLESS

BEST COPY AVAILABLE

FINAL RESULTS J2904-1.K5

NUMBER	CONC.	GAMMA	GAMMA EST.	% EFFECT
3	250.0000	0.1277	0.1271	11.324
4	250.0000	0.1315	0.1271	11.619
5	500.0000	0.2287	0.2685	18.611
6	500.0000	0.2920	0.2685	22.603
7	1000.0000	0.5838	0.5670	36.860
8	1000.0000	0.5722	0.5670	36.394

EC 50 =1660.7427 mg/L (95% CONFIDENCE RANGE:1319.8220 TO 2089.7258)

Touch SPACE BAR to continue....

MICROTOX DATA REPORT

FILE NAME: J2904-1.K5

TEST DATE: _____
TEST TIME: _____

Sample Description:

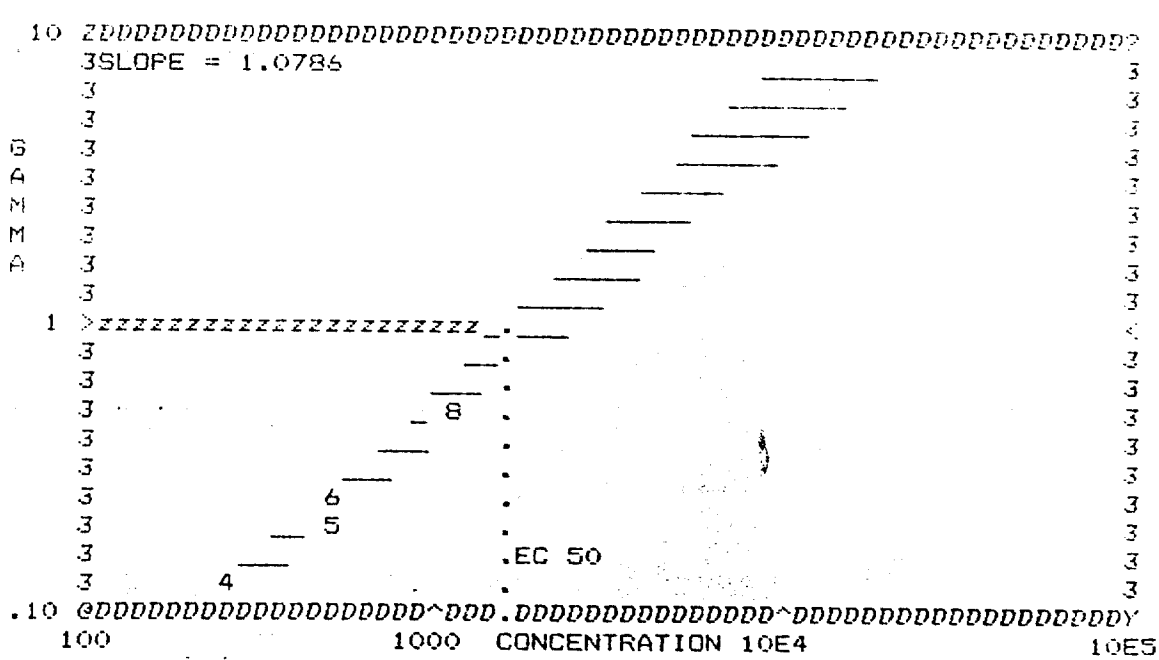
FC-120 LOT 164 YELLOW LIQUID: TEST CONCENTRATIONS ARE CLEAR. COLORLESS

Procedure: BASIC
Initial Concentration : 1000 mg/L
Test Time: 5 minutes

Osmotic Adjustment: NaCl
Dilution Factor : 2
Concentration Units: mg/L

NUMBER	IO/IT	CONC.	GAMMA
1	84/ 48	125.0000	0.06075
2	83/ 49	125.0000	0.02673*
3	80/ 43	250.0000	0.12771#
4	84/ 45	250.0000	0.13146#
5	75/ 37	500.0000	0.22867#
6	81/ 38	500.0000	0.29204#
7	81/ 31	1000.0000	0.58379#
8	83/ 32	1000.0000	0.57218#

CONTROL IT/IO = 55/ 92 BLANK RATIO = 0.6061



EC 50 =1660.7427 (95% CONFIDENCE RANGE:1319.8220 TO 2089.7258)

Used for calculations
* Invalid gammas

STATISTICAL REPORT FOR DATA FILE J2904-1.KS

ESTIMATING EQUATION: LOG C = 0.9129 x LOG b +3.2203

NUMBER OF DATA PAIRS USED: 6

RESIDUAL VARIANCE: 0.00139

95% CONFIDENCE FACTOR: 1.25831 FOR EC50

CORRELATION COEFFICIENT: R = 0.99229

EFFECTIVE CONCENTRATIONS (95% CONFIDENCE RANGE):

EC 50	=1660.7427 mg/L	(1319.8220 TO 2089.7258)***
EC 20	= 468.4890 mg/L	(424.7483 TO 516.7341)
EC 10	= 223.4613 mg/L	(188.4842 TO 264.9290)***
EC 1	= 25.0349 mg/L	(14.7624 TO 42.4556)***

*** EXTRAPOLATED RESULTS

FINAL RESULTS

J2904-1.K15

NUMBER	CONC.	GAMMA	GAMMA EST.	% EFFECT
1	125.0000	0.1686	0.1339	14.429
2	125.0000	0.1175	0.1339	10.511
3	250.0000	0.3356	0.3646	25.126
4	250.0000	0.3484	0.3646	25.839
5	500.0000	0.9564	0.9931	48.886
6	500.0000	0.9886	0.9931	49.714
7	1000.0000	2.7563	2.7046	73.378
8	1000.0000	2.8490	2.7046	74.019

EC 50 = 500.9496 mg/L (95% CONFIDENCE RANGE: 463.6847 TO 541.2094)

Touch SPACE BAR to continue....

MICROTOX DATA REPORT

FILE NAME: J2904-1.K15

TEST DATE: _____
TEST TIME: _____

Sample Description:

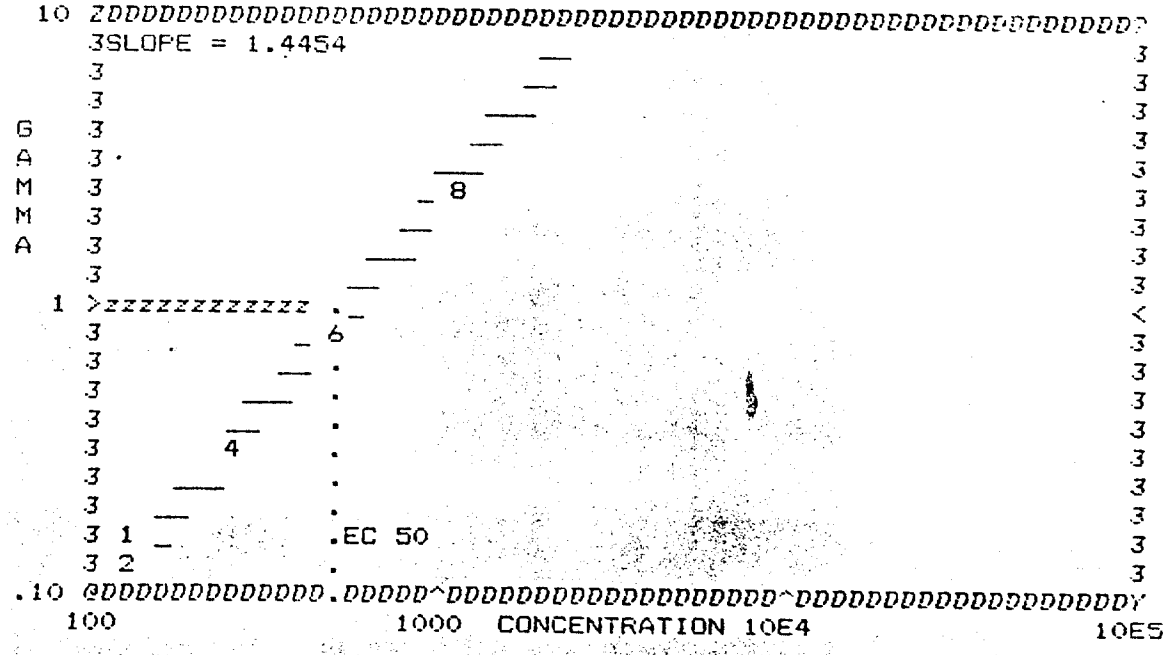
FC-120 LOT 164 YELLOW LIQUID: TEST CONCENTRATIONS ARE CLEAR.
COLORLESS

Procedure: BASIC
Initial Concentration : 1000 mg/L
Test Time: 15 minutes

Osmotic Adjustment: NaCl
Dilution Factor : 2
Concentration Units: mg/L

NUMBER	IO/IT	CONC.	GAMMA
1	84/ 30	125.0000	0.16862#
2	83/ 31	125.0000	0.11746#
3	80/ 25	250.0000	0.33557#
4	84/ 26	250.0000	0.34841#
5	75/ 16	500.0000	0.95640#
6	81/ 17	500.0000	0.98862#
7	81/ 9	1000.0000	2.75629#
8	83/ 9	1000.0000	2.84903#

CONTROL IT/IO = 38/ 92 BLANK RATIO = 0.4174



EC 50 = 500.9496 (95% CONFIDENCE RANGE: 463.6847 TO 541.2094)

Used for calculations

STATISTICAL REPORT FOR DATA FILE J2904-1.K15

ESTIMATING EQUATION: LOG C = 0.6861 x LOG b +2.6998

NUMBER OF DATA PAIRS USED: 8

RESIDUAL VARIANCE: 0.00125

95% CONFIDENCE FACTOR: 1.08037 FOR EC50

CORRELATION COEFFICIENT: R = 0.99585

EFFECTIVE CONCENTRATIONS (95% CONFIDENCE RANGE):

EC 50 =	500.9496 mg/L	(463.6847 TO	541.2094)
EC 20 =	193.5175 mg/L	(176.9699 TO	211.6124)
EC 10 =	110.9398 mg/L	(97.6970 TO	125.9777)***
EC 1 =	21.4085 mg/L	(16.4163 TO	27.9189)***

*** EXTRAPOLATED RESULTS

FINAL RESULTS

J2904-1.K30

NUMBER	CONC.	GAMMA	GAMMA EST.	% EFFECT
1	125.0000	0.1916	0.2306	16.078
2	125.0000	0.2264	0.2306	18.464
3	250.0000	0.7732	0.6639	43.604
4	250.0000	0.6550	0.6639	39.576
5	500.0000	2.3247	1.9118	69.922
6	500.0000	2.1917	1.9118	68.669
7	1000.0000	4.7451	5.5052	82.594
8	1000.0000	4.8870	5.5052	83.013

EC 50 = 327.3192 mg/L (95% CONFIDENCE RANGE: 299.1904 TO 358.0926)

Touch SPACE BAR to continue....

MICROTOX DATA REPORT

FILE NAME: J2904-1.K30

TEST DATE: _____
TEST TIME: _____

Sample Description:

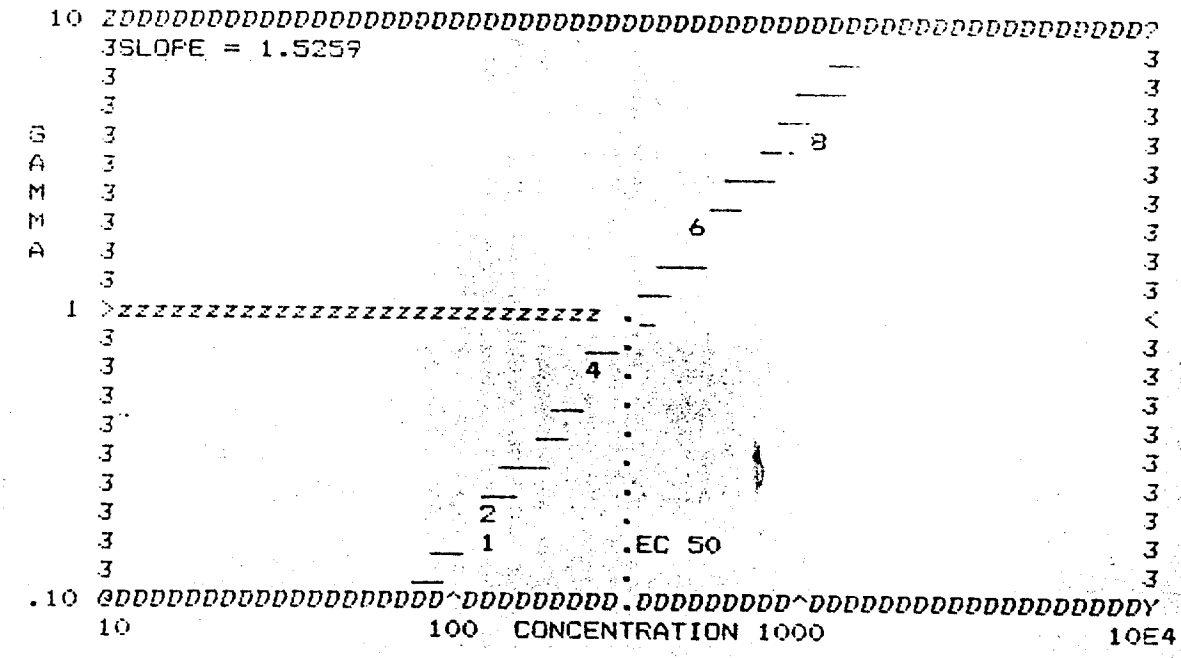
FC-120 LOT 164 YELLOW LIQUID: TEST CONCENTRATIONS ARE CLEAR.
COLORLESS

Procedure: BASIC
Initial Concentration : 1000 mg/L
Test Time: 30 minutes

Osmotic Adjustment: NaCl
Dilution Factor : 2
Concentration Units: mg/L

NUMBER	IO/IT	CONC.	GAMMA
1	84/ 25	125.0000	0.19158#
2	83/ 24	125.0000	0.22645#
3	80/ 16	250.0000	0.77318#
4	84/ 18	250.0000	0.65497#
5	75/ 8	500.0000	2.32471#
6	81/ 9	500.0000	2.19172#
7	81/ 5	1000.0000	4.74510#
8	83/ 5	1000.0000	4.88696#

CONTROL IT/IO = 32/ 92 BLANK RATIO = 0.3546



EC 50 = 327.3192 (95% CONFIDENCE RANGE: 299.1904 TO 358.0926)

Used for calculations

Pg. 10 of 10

STATISTICAL REPORT FOR DATA FILE J2904-1.K30

ESTIMATING EQUATION: LOG C = 0.6466 x LOG b +2.5150

NUMBER OF DATA PAIRS USED: 8

RESIDUAL VARIANCE: 0.00201

95% CONFIDENCE FACTOR: 1.09402 FOR EC50

CORRELATION COEFFICIENT: R = 0.99331

EFFECTIVE CONCENTRATIONS (95% CONFIDENCE RANGE):

EC 50 =	327.3192 mg/L	(299.1904 TO	358.0926)
EC 20 =	133.5555 mg/L	(115.6267 TO	154.2643)
EC 10 =	79.0551 mg/L	(65.0098 TO	96.1349)***
EC 1 =	16.7701 mg/L	(11.6396 TO	24.1620)***

*** EXTRAPOLATED RESULTS

BIODEGRADATION

TEST SUBSTANCE

Identity: Perfluorodecanesulfonate, ammonium salt; may also be referred to as PFDS, FC-121-X, or 3M Sample E2112 and E2566-1. (1-Decanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heneicosafluoro-, ammonium salt, CAS # 67906-42-7)

Remarks: Test sample is a mixture of the test substance in water and 2-propanol, 1-(1,1-dimethylethoxy). This mixture contains approximately 22-26% test substance, 33-37% propanol, 1-(1,1-dimethylethoxy), 0.5-1.5% perfluorononanesulfonate, ammonium salt and 38-42% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample. Test material was from 3M production lot 1.

METHOD

Method: Standard methods #507 for BOD, standard methods #508A for COD
Type: Aerobic
GLP: No
Year completed: 1987
Contact time: 20 days
Inoculum: Source of inoculum not recorded

RESULTS

5-day carbonaceous BOD: 5,800 mg/kg
10-day carbonaceous BOD: 8,000 mg/kg
20-day carbonaceous BOD: 11,000 mg/kg
COD: 870,000 mg/kg

Remarks: This material has a perfluorinated moiety and a non-fluorinated moiety. Current knowledge of these chemistries suggests that all biodegradation indicated in this study is likely due to reduction of the non-fluorinated moiety, propanol, 1-(1,1-dimethylethoxy)

Testing was conducted on the "as-sold" mixture of the subject material in water (~22-26% subject material). The values reported apply to that mixture and not directly to the subject material.

CONCLUSIONS

This testing indicated that the propanol, 1-(1,1-dimethylethoxy) moiety is likely to be biodegraded in aerobic environments capable of supporting biodegradation. This study cannot rule out the possibility that conditions could be found that would allow for biodegradation of the fluorochemical component, nor could it exclude the possibility that a microorganism could eventually be enriched that could metabolically alter this chemical.

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

DATA QUALITY

Reliability: Klimisch ranking 2. This study lacks sufficient documentation, characterization of the test substance purity, and analytical measurement of test concentrations. Because a mixture was tested, the biodegradation of the solvent could not be differentiated from biodegradation of the fluorochemical component.

REFERENCES

Study conducted at the request of 3M Company by Pace Incorporated of Minneapolis, MN, Lab Request number E2112, 11/3/87

OTHER

Last changed: 5/18/00

pace laboratories, inc.

REPORT OF LABORATORY ANALYSIS

Offices:
Minneapolis, Minnesota
Tampa, Florida
Coralville, Iowa

3M Environmental Laboratory
935 Bush Avenue
Bldg. 2-3E
St. Paul, MN 55133

November 03, 1987
PACE Project Number: 870917504

Attn: Mr. Wade Scheil

E2112

Date Sample(s) Collected: By Client
Date Sample(s) Received: 09/16/87

PACE Sample Number: 159240

Parameter	Units	MDL	E2112-1	E2112-2
Biochemical Oxygen Demand, Carb 5 Day(1)	mg/kg	600	5800	-
Biochemical Oxygen Demand, Carb 10 Day(1)	mg/kg	600	8000	-
Biochemical Oxygen Demand, Carb 20 Day(1)	mg/kg	600	11000	-
Chemical Oxygen Demand(2)	mg/kg	15000	850000	890,000

MDL Method Detection Limit
(1) Analysis for biochemical oxygen demand was performed in accordance with standard methods #507
(2) Analysis for chemical oxygen demand was performed in accordance with standard methods #508A

The data contained in this report were obtained using EPA or other approved methodologies. All analyses were performed by me or under my direct supervision.

Thomas L. Halverson
Inorganic Chemistry Manager

000176

ANALYSIS:

FOR CLP USE ONLY? ☐

CHEMICAL OXYGEN DEMAND, COD ✓ COD-LOW ___

DATE ANALYZED: 9/21-22/87
ANALYZED BY: Q/Bo
TIME:
DATA REVIEWED BY: [illegible] 9/23
ENTERED BY: [illegible] 9-23

CLIENT NAME:
PROJECT NUMBER:
PROJECT NAME:
FILE #:
DATE RECEIVED:
DATE COLLECTED:

SAMPLE #	ANALYSIS RESULTS	UNITS	COMMENTS	SAMPLE VOL (mls) A	BLANK B	SAMPLE TITRANT BEG	END	TOTAL C	TITRANT NORMALITY N
Blk	<50	mg/l		50	25.5	0.0	25.5	25.5	0.25
Blk.	<50	↓		50	↓	0.0	25.6	25.6	↓
CPA	148	↓	WP 1284-4 TV-145 mg/l	50	↓	25.5	47.3	21.8	↓
15756	190,000	mg/kg	E 1894-3 Gen. use between .40 → 0.04 g	0.0921 g	↓	25.6	42.5	16.9	↓
128230	2,300	mg/l	Old result 2600 mg/l Rerun	10	↓	0.0	13.8	13.8	↓
15778	950	↓		25	↓	13.8	27.4	13.6	↓
15828	5,400	↓		10	↓	0.0 27.4	.2 50.0	22.8	↓
15924	850,000	mg/kg	E 2112-1 Include Method + Raw Data with report HW.	0.0132 ~~0.0086 g~~ out	↓	0.2	20.1	19.9	↓
↓	890,000	↓	↓	0.0115 ~~0.0167 g~~ out	↓	20.1	40.5	20.4	↓
~~15925~~	~~360,000~~	↓	E 2113-1	0.0112 g	↓	0.0	23.5	23.5	↓
~~15926~~	~~390,000~~	↓	E 2113-1	0.0227 g	↓	23.5	44.6	21.1	↓
15948	2,100	mg/l		10	↓	0.0	15.1	15.1	↓
15949	790	↓		35	↓	0.0	11.7	11.7	↓

A = SAMPLE VOLUME USED (mls OR gms)
B = TITRANT VOL USED FOR BLANK (mls)
C = TITRANT VOL USED FOR SAMPLE (mls)
N = NORMALITY OF TITRANT

$$\frac{B-C \times N \times 8{,}000}{A} = \text{mg COD/l OR mg COD/kg}$$

NOTE: WHEN PERFORMING A COD ON AN EP LEACH SAMPLE, THE COD OF THE ACETIC ACID MUST BE SUBTRACTED TO OBTAIN THE FINAL VALUE. 1 ml OF 0.5 N ACETIC ACID IN LEACHATE EQUALS A COD OF 16 mg/l

0189W

ANALYSIS:

5 DAY BIOCHEMICAL OXYGEN DEMAND, BOD-05 ___
SOLUBLE BOD, SOL-BOD ___
CARBONACEOUS BOD 5 DAY, C-BOD-05 ✓
DISSOLVED OXYGEN, DO ___
CARBONACEOUS BOD 10 DAY, C-BOD-10 ___
CARBONACEOUS BOD 20 DAY, C-BOD-20 ___

DATE ANALYZED: IN 10-2 OUT 10-7-87
ANALYZED BY: ___
TIME: ___
DATA REVIEWED BY: ___
SEED VOL USED ml/l 2
DO DROP 3.0

FOR CLP USE ONLY

CLIENT NAME: ___
PROJECT NAME: ___
FILE #: ___
DATE COLLECTED: ___
DATE RECEIVED: ___
10-23

SAMPLE	ANALYSIS RESULTS	UNITS	COMMENTS	pH	SAMPLE VOL (mls)	BOTTLE #	D1	D2	D1-D2	F FACTOR	SEED CORRECT	DIFF (D1-D2)-SC	(DIFF)(303 SAMP VOL
					BLK	220	7.6	7.4	.2	.08	.024		
					Seed	43	7.7	4.7	3.0				
187	mg/L		T.V = 200 Glucose Glutamic Acid		6	412	7.7	4.0	3.7			3.46	175
					6	33	7.8	3.6	4.2			3.96	200
					Seed	13	7.7	5.5	2.2	.10	.22		
201	mg/L		Poly Pac + Sludge TV = 200 Glucose Glutamic Acid		6	71	7.7	3.6	4.1			3.88	196
					6	30	7.7	3.4	4.3			4.05	206
					50	81	7.6	<1					
16788					35	73	7.7	<1					
	.8400	mg/L	1 → 100	7.4	15	61	7.8	3.4	4.4	.09	.024	4.16	84
					1	302	7.6	6.4	1.2	.08	.24	.96	290

DI = INITIAL DO, D2 = FINAL 5 DAY DO, F = % SEED IN DI/% SEED IN CONTROL
SEED CORRECT (SC) = SEED CONTROL DO DROP x F

ANALYSIS:

5 DAY BIOCHEMICAL OXYGEN DEMAND, BOD-05 ___
SOLUBLE BOD, SOL-BOD ___
CARBONACEOUS BOD 5 DAY, C-BOD-05 ✓
DISSOLVED OXYGEN, DO ___
CARBONACEOUS BOD 10 DAY, C-BOD-10 ___
CARBONACEOUS BOD 20 DAY, C-BOD-20 ___

DATE ANALYZED: IN 10-2 OUT 10-7-87
ANALYZED BY: Tim
TIME:
DATA REVIEWED BY:
SEED VOL USED ml/l 2
DO DROP 3.0

FOR CLP USE ONLY

CLIENT NAME:
PROJECT NAME:
FILE #:
DATE COLLECTED:
DATE RECEIVED:
JD 10-23

SAMPLE	ANALYSIS RESULTS	UNITS	COMMENTS	pH	SAMPLE VOL (mls)	BOTTLE #	D1	D2	D1-D2	F FACTOR	SEED CORRECT	DIFF (D1-D2)-SC	(DIFF)(303) SAMP VOL
5924					50	57	7.6	6.4	1.2	.08	.24	.96	58
≡ 2112-1	5800 ~~56,000~~	mg/L	1 → 1000	7.3	35	53	7.5	7.0	.5				
					15	51	7.6	7.4	~~.2~~				
					1	28 4	7.7	7.3	~~.4~~				
5925					50	12	7.5	<1	—				
≡ 2113-1	31,000	mg/L	1 → 1000	7.3	35	8	7.6	2.3	5.3			5.06	31
					15	428	7.7	5.1	2.6			2.36	24
					1	66	7.6	6.2	1.4				
5926					50	45	7.5	5.7	1.8			1.56	9500
≡ 2114-1	10,000	mg/L	1 → 1000	7.2	35	7	7.6	6.1	1.5			1.26	11,000
					15	15	7.5	6.8					
					1	66	7.5	7.2		✓	✓		

DI = INITIAL DO, D2 = FINAL 5 DAY DO, F = % SEED IN DI/% SEED IN CONTROL
SEED CORRECT (SC) = SEED CONTROL DO DROP x F

0182W

ANALYSIS:

5 DAY BIOCHEMICAL OXYGEN DEMAND, BOD-05 ___
SOLUBLE BOD, SOL-BOD ___
CARBONACEOUS BOD 5 DAY, C-BOD-05 ___
DISSOLVED OXYGEN, DO ___
CARBONACEOUS BOD 10 DAY, C-BOD-10 ✓
CARBONACEOUS BOD 20 DAY, C-BOD-20 ___

DATE ANALYZED: IN 10-2 OUT 10-12-87
ANALYZED BY: Tim
TIME: 6
DATA REVIEWED BY:
SEED VOL USED ml/l 2
DO DROP 3.5

FOR CLP USE ONLY

CLIENT NAME:
PROJECT NAME:
FILE #:
DATE COLLECTED:
DATE RECEIVED: 10-23

SAMPLE	ANALYSIS RESULTS	UNITS	COMMENTS	pH	SAMPLE VOL (mls)	BOTTLE #	D1	D2	D1-D2	F FACTOR	SEED CORRECT	DIFF (D1-D2)-SC	(DIFF)(303) SAMP VOL
15924					50	286	7.4	5.8	1.6	.08	.28	1.32	8.80
					35	223	7.5	6.7					
E2112-1	8,000	mg/L	1 → 1000	7.3	15	65	7.6	6.8					
					1	72	7.7	7.0					
15925					50	227	7.5	<1					
					35	432	7.6	<1					
E 2113-1	61,000	mg/L	1 → 1000	7.3	15	50	7.7	4.4	3.3			3.02	61
					1	56	7.7	5.6					
15926					50	61	7.5	5.2	2.3			2.02	12
					35	2	7.5	5.6	1.9			1.62	14
E 2114-1	13,000	mg/L	1 → 1000	7.2	15	59	7.6	6.2					
					1	52	7.7	6.4					

DI = INITIAL DO, D2 = FINAL 5 DAY DO, F = % SEED IN DI/% SEED IN CONTROL
SEED CORRECT (SC) = SEED CONTROL DO DROP x F

ANALYSIS:

FOR CLP USE ONLY

5 DAY BIOCHEMICAL OXYGEN DEMAND, BOD-05 ___
SOLUBLE BOD, SOL-BOD ___
CARBONACEOUS BOD 5 DAY, C-BOD-05 ___
DISSOLVED OXYGEN, DO ___
CARBONACEOUS BOD 10 DAY, C-BOD-10 ___
CARBONACEOUS BOD 20 DAY, C-BOD-20 ✓

DATE ANALYZED: IN 10-2 OUT 10-22-87
ANALYZED BY: T.
TIME:
DATA REVIEWED BY:
SEED VOL USED ml/l 2
DO DROP 3.7

CLIENT NAME:
PROJECT NAME:
FILE #:
DATE COLLECTED:
DATE RECEIVED: 10-23

SAMPLE	ANALYSIS RESULTS	UNITS	COMMENTS	pH	SAMPLE VOL (mls)	BOTTLE #	D1	D2	D1-D2	F FACTOR	SEED CORRECT	DIFF (D1-D2)-SC	(DIFF)(303) SAMP VOL
15924	11,000	mg/L	1 → 1000	7.3	50	72	7.5	5.4	2.1	.08	.296	1.804	11
E 2112-1					35	210	7.4	6.0	1.4			1.104	10
					15	266	7.3	6.1					
					1	58	7.4	6.2					
15925	69,000	mg/L	1 → 1000	7.3	50	62	7.5	<1					
E 2113-1					35	29	7.6	<1					
					15	280	7.5	3.8	3.7			3.404	69
					1	217	7.5	4.4					
15926	17000	mg/L	1 → 1000	7.2	50	70	7.4	4.6	2.8			2.504	15
E 2114-1					35	61	7.5	4.9	2.6			2.304	20
					15	17	7.5	6.3					
					1	157	7.6	6.2		✓	✓		

DI = INITIAL DO, D2 = FINAL 5 DAY DO, F = % SEED IN DI/% SEED IN CONTROL
SEED CORRECT (SC) = SEED CONTROL DO DROP x F

ACUTE TOXICITY TO FISH

TEST SUBSTANCE

Identity: Perfluorodecanesulfonate, ammonium salt; may also be referred to as PFDS, FC-121-X, or 3M Sample E2112 and E2566-1. (1-Decanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heneicosafluoro-, ammonium salt, CAS # 67906-42-7)

Remarks: Test sample is a mixture of the test substance in water and 2-propanol, 1-(1,1-dimethylethoxy). This mixture contains approximately 22-26% test substance, 33-37% propanol, 1-(1,1-dimethylethoxy), 0.5-1.5% perfluorononanesulfonate, ammonium salt and 38-42% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample. Test material was from 3M production lot 1.

Note: Only the summary pages of this report were available.

METHOD

Method: OECD 203
Type: Static acute
GLP: Not known
Year completed: 1988
Species: *Pimephales promelas*
Supplier: ABC Laboratories In-house cultures, Columbia, MO
Analytical monitoring: Dissolved oxygen, temperature and pH. Test substance concentration was not measured.
Exposure period: 96-hours
Statistical methods:Binomial method.
Length and weight: Average length = 18 mm.
Average weight = 0.087 g

Test conditions:

Dilution water chemistry:

hardness:	44 mg/L
alkalinity:	30 mg/L
pH:	7.5

Stock and test solution preparation: Details not available. All test vessels containing FC-121 contained a thin layer of foam at 0-

hours. The amount of foam increased with the concentration. No foam or film was present after 24-hours.
Number of fish per concentration: ten
Number of concentrations: five plus a negative control
Water chemistry during the study: within acceptable limits throughout the study.
Temperature during the study: 22 ± 1°C

RESULTS

Nominal concentrations: Bk control, 3.2, 5.6, 10, 18, and 32 mg/L
Element value: 24-hour LC_{50} = 26 (18-32) mg/L
48-hour LC_{50} = 24 (18-32) mg/L
96-hour LC_{50} = 13 (10-18) mg/L
96-hour NOEL = 3.2 mg/L
All element values based on nominal concentrations

Statistical Evaluation of Mortality:
Binomial method used to calculate LC_{50} values and corresponding confidence intervals.

CONCLUSIONS

The test sample 96-hour LC_{50} for fathead minnow was determined to be 13 mg/L with a 95% confidence interval of 10 – 18 mg/L.

Testing was conducted on the "as-sold" mixture of the subject material in water (~22-26% subject material. The values reported apply to that mixture and not directly to the subject material.

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

DATA QUALITY

Reliability: Klimisch ranking 4. A portion of the final report is missing. Because a mixture was tested, care must be taken when interpreting the results. The solvent in the mixture could have enhanced or restricted biological uptake and/or toxicity of the fluorochemical component. Characterization of the test substance purity and analytical measurement of tested concentrations was lacking.

REFERENCES

This study was conducted by ABC Laboratories, Columbia, MO on behalf of the 3M Company.

OTHER

Last changed: 5/18/00

SPONSOR

3M Company
Attn: Mr. Mohamed Elnabarawy
EE & PC Environmental Labs
Building 21-2W-05, 900 Bush Avenue
St. Paul, Minnesota 55106

STUDY TITLE

Acute Toxicity of E2566-1
to Fathead Minnow (*Pimephales promelas*)

DATA REQUIREMENT

OECD Guideline #203

AUTHOR

Jane H. Bowman
Biologist II

STUDY COMPLETED ON

January 15, 1988

PERFORMING LABORATORY

Analytical Bio-Chemistry Laboratories, Inc.
Aquatic Toxicology Division
7200 East ABC Lane
P.O. Box 1097
Columbia, Missouri 65205

ABC LABORATORY PROJECT ID

#36615

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Submitted By: Analytical Bio-Chemistry Laboratories, Inc.
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Carl M. Thompson — 1-29-88 Date
Aquatic Toxicology Manager

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FISH ACUTE TOXICITY COMPENDIUM

Subject: "Acute Toxicity of E2366-1 to Fathead Minnow (*Pimephales promelas*)," ABC Study Number 36615.

Sponsor: 3M Company

Testing Facility: Analytical Bio-Chemistry Laboratories, Inc.
Aquatic Toxicology Division
P.O. Box 1097
Columbia, Missouri 65205 (314-474-6579)

Location of Original Raw Data and Final Report:

3M Company
St. Paul, Minnesota 55106

Test Material: E2366-1

Test Concentrations: Control, 3.2, 5.6, 10, 18 and 32 mg/l

Dilution Water: 44 mg/l (hardness), 30 mg/l (alkalinity), 7.5 (pH)

Test Dates: Initiation - January 11, 1988

Termination - January 15, 1988

Length of Study: 96-hours

Results: 96-hour LC_{50} = 13 mg/l (95% C.I. = 10-18 mg/l)

96-hour NOEL = 3.2 mg/l

96-hour Slope of the Dose-Response Line was 32

Test Species: Fathead Minnow (*Pimephales promelas*)

Source of Organisms: ABC Laboratories in-house culture

Mean Weight: 0.087 g (±0.027 g)

Mean Length: 18 mm (±1.7 mm)

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SUMMARY

The acute toxicity of E2566-1 to fathead minnow (*Pimephales promelas*) was assessed using the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms. Water quality parameters of temperature, dissolved oxygen and pH were measured throughout the test and were within acceptable limits. Culture and acclimation records indicated the fish were in good condition for testing.

The study was conducted at the following nominal concentrations of E2566-1: 3.2, 5.6, 10, 18 and 32 mg/l. A thin layer of foam was present in all chambers at 0-hour. The amount of foam increased with the concentration. No foam or film was present after 24 hours. Ten fish, with a mean weight of 0.087 (±0.027) g and a mean standard length of 18 (±1.7) mm, were exposed to each test concentration and control.

The results of the four day static fish toxicity study using E2566-1 are summarized below. The 24-, 48- and 72-hour LC_{50} values were also determined. The 96-hour slope of the dose response line was 32.

Compound	96-Hour LC_{50} (95% C.I.)
E2566-1	13 mg/l
	(10-18 mg/l)

Also, the results indicated a 96-hour, no-observed effect concentration could be estimated at 3.2 mg/l, which was based on the lack of mortality and abnormal effects. Abnormal effects of mortality, loss of equilibrium, light discoloration and/or fish on the bottom of test chamber were observed during the 96-hour exposure period in the 5.6, 18 and 32 mg/l test concentrations.

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TABLE 2

Acute Toxicity of E2566-1 to Fathead Minnow
(*Pimephales promelas*)

Compound	LC_{50} in milligrams/liter (ppm)		
	24 hours	48 hours	96 hours
E2566-1[a]	26[(1)]	24[(1)]	13[(1)]
	(18-32)[b]	(18-32)[b]	(10-18)[b]

N = 10 fish per concentration.

[a]Bioassay as conducted at 22°C (±1.0), mean fish weight and length, 0.087 (±0.027) g and 18 (±1.7) mm.

[b]95% confidence limits.

The 96-hour no-observed effect concentration could be estimated at 3.2 mg/l, based on the lack of mortality and abnormal effects at the lowest concentration tested.

The slope of the 96-hour dose response curve as calculated by least squares regression analysis was 32.

All values were rounded to two significant figures following ABC S.O.P #8.7.

LC_{50} calculated using:

(1) Binomial Method

(2) Moving Average Method

(3) Probit Method

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ACUTE TOXICITY TO AQUATIC INVERTEBRATES (E.G.,DAPHNIA)

TEST SUBSTANCE

Identity: Perfluorodecanesulfonate, ammonium salt; may also be referred to as PFDS, FC-121-X, or 3M Sample E2112 and E2566-1. (1-Decanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heneicosafluoro-, ammonium salt, CAS # 67906-42-7)

Remarks: Test sample is a mixture of the test substance in water and 2-propanol, 1-(1,1-dimethylethoxy). This mixture contains approximately 22-26% test substance, 33-37% propanol, 1-(1,1-dimethylethoxy), 0.5-1.5% perfluorononanesulfonate, ammonium salt and 38-42% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample. Test sample was from 3M production lot 1.

METHOD

Method: EPA 660/3-75-009 and APHA (1980) Standard Methods for the Examination of Water and Wastewater
Test type: Static acute
GLP: Yes
Year Completed: 1988
Species: *Daphnia magna*
Analytical monitoring: pH, dissolved oxygen, temperature. Test substance concentration was not monitored.
Statistical methods: EC_{50} values calculated using Binomial method
Test daphnid source: ABC Laboratories In-house cultures, Columbia, MO
Test daphnid age at study initiation: < 24-hours

Test conditions

Dilution water: Well water
Dilution water chemistry (from a representative sample):

hardness:	246 mg/L
alkalinity	178 mg/L
pH:	8.0

Stock and test solutions preparation: Not given. A cloudy appearance and surface foam present at preparation in the 560 and 1000 mg/L concentrations was not noted at the 24- and 48-hour observation periods.
Exposure vessels: 250 mL glass beakers containing 200 mL of test solution.

Number of replicates: 2
Number of daphnids per replicate: 10
Number of concentrations: five plus a negative control
Water chemistry during the study:
Dissolved oxygen range (0-48 hours):
8.7 – 9.2 mg/L (control exposure)
8.6 – 9.2 mg/L (1000 mg/L exposure)
pH range (0-48 hours):
8.2 – 8.2 (control exposure)
8.2 – 8.4 (1000 mg/L exposure)
Test temperature range (0 – 48 hours)
19 – 20 °C
Element basis: mortality and sublethal effects

RESULTS

Nominal concentrations: Bk control, 100, 180, 320, 560, 1000 mg/L
Element value: 24-hour LC_{50} = 200 (100-320) mg/L
48-hour LC_{50} = 130 (100–180) mg/L
48-hour NOEC (sublethal effects) = < 100 mg/L
Sublethal effects included quiescence and/or daphnids tending to the bottom of test vessels.

Statistical Evaluation: The LC_{50} value and 95% confidence interval was calculated using the binomial method.

All element values based on nominal concentrations.

Mortality of controls: None
Remarks: Values reported are for the test sample (mixture). No calculations were made to adjust for the concentration of the test substance in the test sample.

Cumulative percent mortality:

Nominal Test Conc., mg/L	24-hours	48-hours
Neg. Control	0	0
100	0	0
180	35	100
320	100	100
560	100	100
1000	100	100

CONCLUSIONS

The test sample 48-hour EC_{50} for *Daphnia magna* was determined to be 130 mg/L with a 95% confidence interval of 100 – 180 mg/L.

Testing was conducted on the "as-sold" mixture of the subject material in water (~22-26% subject matherial) The values reported apply to that mixture and not directly to the subject material.

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

DATA QUALITY

Reliability: Klimisch ranking 2. This study, while well conducted, lacks analytical data for: determination of the test substance concentration in the test solutions; and determination of the sample purity. Because a mixture was tested, care must be taken when interpreting the results. The solvent in the mixture could have enhanced or restricted biological uptake and/or toxicity of the fluorochemical component.

REFERENCES

This study was conducted by ABC Laboratories, Columbia, MO on behalf of the 3M Company.

OTHER

Last changed: 5/18/00

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SPONSOR

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EE & PC Environmental Labs
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St. Paul, Minnesota 55106

STUDY TITLE

Acute Toxicity of E2566-1 to
Daphnia magna

DATA REQUIREMENT

OECD Guideline 202

AUTHOR

Scott Frazier
Biologist II

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STUDY COMPLETED ON

January 9, 1988

PERFORMING LABORATORY

Analytical Bio-Chemistry Laboratories, Inc.
Aquatic Toxicology Division
7200 East ABC Lane
P.O. Box 1097
Columbia, Missouri 65205

ABC LABORATORY PROJECT ID

#36614

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1/15/88

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1/18/88

Kent Ediger — Date
Quality Assurance Officer

1-18-88

Carl M. Thompson — Date
Aquatic Toxicology Manager

:dab28

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PROJECT PERSONNEL

The Study Director of the project for Analytical Bio-Chemistry Laboratories, Inc. was Scott Frazier, Biologist II. Supervising the conduct of the study for 3M Company was Mr. Mohamed Elnabarawy. The following ABC personnel assisted with various phases of the study.

Name	Title	Initials
Jeffrey Latham	Biological Technician I	JL
Michael Ruddy	Biological Technician II	MR
Scott Frazier	Biologist II	SF

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INVERTEBRATE ACUTE TOXICITY COMPENDIUM

Subject: "Acute Toxicity of E2566-1 to *Daphnia magna*."
ABC Study Number 36614.

Sponsor: 3M Company

Testing Facility: Analytical Bio-Chemistry Laboratories, Inc.
Aquatic Toxicology Division
P.O. Box 1097
Columbia, Missouri 65205 (314-474-8579)

Location of Original Raw Data and Final Report:

3M Company
EE & PC Environmental Labs
Building 21-2W-05, 900 Bush Avenue
St. Paul, Minnesota

Test Material: E2566-1

Test Concentrations: Control, 100, 180, 320, 560 and 1000 mg/l.

Dilution Water: 246 mg/l (hardness), 178 mg/l (alkalinity), 8.0 (pH).

Test Dates: Initiation - January 7, 1988

Termination - January 9, 1988

Length of Study: 48 hours

Results: 24-hour LC_{50} = 200 mg/l (95% C.I. = 100-320 mg/l)

24-hour NOEL = 100 mg/l

48-hour LC_{50} = 130 mg/l (95% C.I. = 100-180 mg/l)

48-hour NOEL = <100 mg/l (estimated)

Test Species: *Daphnia magna*

Source of Organisms: ABC Laboratories in-house culture.

Age of Organisms at Study Initiation: First-instar (≤24 hours old)

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SUMMARY

The acute toxicity of E2566-1 to *Daphnia magna* was assessed using the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms. Water quality parameters of temperature, dissolved oxygen and pH were measured at the termination of the test and were within acceptable limits.

The results of the 48-hour static *Daphnia magna* toxicity study are summarized below. All reported values were based upon nominal concentrations.

Compound	24-Hour LC_{50} (95% C.I.)	48-Hour LC_{50} (95% C.I.)
E2566-1	200 mg/l (100-320 mg/l)	130 mg/l (100-180 mg/l)

The no-effect level observed for E2566-1 at 24 hours was 100 mg/l based on the absence of all abnormal effects. The 48-hour no-effect level was estimated to be <100 mg/l since abnormal effects were observed in all treated test concentrations.

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INTRODUCTION

This definitive static bioassay was performed by the Aquatic Toxicology Division of Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri, for 3M Company, from January 7, 1988 to January 9, 1988. The purpose of this test was to determine the 24- and 48-hour LC_{50} levels for E2566-1 to *Daphnia magna*. The study was performed using ABC Protocol #7806 as approved by Mr. Mohamed Elnabarawy, 3M Company, on January 7, 1988.

METHODS AND MATERIALS

The procedures for static bioassay, as described in *Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians* (1) and *Standard Methods for Examination of Water and Wastewater* (2), were used in this experiment.

I. Test Organisms

The *Daphnia magna* used in the test were cultured at the ABC facilities in well water (Table 1). The adult daphnids were fed algae (*Selenastrum capricornutum*) at least every three days prior to testing and supplemented with a suspension of Tetramin®, cereal leaves, vitamin solution and yeast. The daphnids were identified to species using the taxonomic key presented by Pennak (3).

II. Test Procedure

The static *Daphnia* bioassay was conducted in 250-ml glass beakers containing 200 ml of daphnid culture/test water. Test vessels were covered with loose-fitting petri dish covers to minimize evaporation and protect against contamination during the study. These vessels were kept at 20 (±2.0) °C in a temperature controlled area. The lighting was maintained at 50-70 footcandles on a 16-hour daylight photoperiod with 30-minute simulated dawn and dusk periods.

An initial range-finding experiment was conducted using ten *Daphnia* each in exposure concentrations of 1.0, 10, and 100 mg/l. From these results, five treatment concentrations in duplicate with ten *Daphnia* (first instar less than 24 hours old) per beaker were selected for the definitive bioassay. These concentrations were a logarithmic series ranging from 100 to 1000 mg/l and included a control. All concentrations were observed once every 24 hours for mortality and abnormal effects such as surfacing, clumping of the daphnids together and daphnids tending to the bottom of test chambers. Water chemistry parameters of temperature, dissolved oxygen and pH were measured in the control, low, middle and high concentrations at 0 and 48 hours.

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III. Test Compound

The E2366-1 sample was received on December 28, 1987 as a light brown liquid and was stored at room temperature in darkness. Sample purity was unspecified. Test concentrations were prepared based on total product. All standard weights and dilutions can be found in the Appendix. Daphnid culture/test water was used in the preparation of all working stock solutions.

IV. Statistics

Statistical analysis of the concentration vs. effect data (generally mortality) was obtained by employing a computerized LC_{50} program developed by Stephan et al. (4). This program calculated the LC_{50} statistic and its 95 percent confidence limits using the Binomial, the moving average and the probit tests. However, if no mortality occurred or if a dose response could not be demonstrated over a reasonable range (<3% to >63%) an LC_{50} and/or its 95 percent confidence limits could not be calculated. Three different methods of analyzing the data were used since no one method of analysis is appropriate for all possible sets of data that may be obtained. The method of calculation selected for presentation in this report was that which gave the narrowest confidence limits for the LC_{50} (4, 5) although all three models are valid.

RESULTS

Table 2 presents the calculated LC_{50} values and 95-percent confidence intervals for E2366-1. The 24- and 48-hour LC_{50} values were 200 and 130 mg/l, respectively. All results were based on the nominal concentrations of 100, 180, 320, 560 and 1000 mg/l. The no-effect concentration based on the absence of mortality and abnormal effects was 100 mg/l at 24 hours. At 48 hours the no-effect level was estimated to be <100 mg/l since all treated concentrations exhibited abnormal effects. The abnormal effects observed during the study were mortality, quiescence and/or daphnids tending to the bottom of test vessels. A cloudy appearance and surface foam present at preparation in the 560 and 1000 mg/l concentrations was not noted at the 24- and 48-hour observation periods.

Table 3 presents the percent mortality and water quality parameters measured during the test. The dissolved oxygen concentrations ranged between 8.6 and 9.2 mg/l. These values represented 99 and 103-percent saturation at 20 and 19 °C, respectively (corrected for local altitudinal pressure), and were considered adequate for testing (1). The pH values of the test solutions ranged from 8.2 to 8.4 and were consistent with the control.

The study was conducted following the intent of the Good Laboratory Practice Regulations (6) and the final report was reviewed by Analytical Bio-Chemistry Laboratories' Quality Assurance Unit. All original raw data were provided to 3M Company, with a copy retained at Analytical Bio-Chemistry Laboratories.

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TABLE 1

Chemical Characteristics of Well Water Used by ABC's Aquatic Toxicology Division.

Parameter	Concentration
Temperature	17 - 22°C[b]
Dissolved Oxygen[a]	7.4 - 9.1 ppm[b]
pH	7.6 - 8.4[b]
Hardness ($CaCO_3$)	206 - 275 ppm[b]
Alkalinity ($CaCO_3$)	224 - 336 ppm[b]
Conductivity	500 - 650 umhos/cm[b]
Total Organic Carbon	1.4 ppm[c] (0.9 - 2.8 ppm)[d]
Suspended Solids	0 - 1.3 ppm[b]
Aluminum	<10 ppb
Arsenic	<1 ppb
Boron	2.25 ppm
Cadmium	<3 ppb
Chromium	<7 ppb
Cobalt	<3 ppb
Copper	<3 ppb
Iron	14 ppb
Lead	<30 ppb
Mercury	0.43 ppb
Nickel	<8 ppb
Silver	<4 ppb
Zinc	2 ppb
Measured organophosphorus pesticides	<0.14 ppb
Measured organochlorine pesticides plus PCB's	<1.0 ppb

[a] After aeration

[b] Represents seasonal variation, difference from one month to the next, will not exceed 10%

[c] Average of all well water values from January-September, 1987

[d] Total monthly range of organic carbon

Sample dates: Trace elements - June 3, 1987, organophosphate and organochlorine analyses - May 28, 1987.

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TABLE 2

Acute Toxicity of E2566-1
to *Daphnia magna*[a]

Compound	LC_{50} (mg/l) 24 hours	LC_{50} (mg/l) 48 hours
E2566-1	200 (1)	130 (1)
	(100-320)[b]	(100-180)[b]

[a]Bioassay conducted at 20°C (±2.0).

[b]95% confidence limits.

The 24-hour no-effect concentration was 100 mg/l, based on the absence of all abnormal effects. The 48-hour no-effect concentration was estimated to be <100 mg/l since abnormal effects were observed in all test concentrations.

All values were rounded to two significant figures following the technique provided in ABC S.O.P. #8.7.

LC_{50} calculated using:

(1) Binomial Method

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TABLE 3

Percent Mortality and Water Quality Measurements During the Acute Toxicity Test of E2566-1 to *Daphnia magna*.

Nominal Concentration (mg/l)	Percent Mortality		Water Quality					
			0 hours			48 hours		
	24 hr	48 hr	Temp.[a] °C	D.O.[b] mg/l	pH[c]	Temp. °C	D.O. mg/l	pH
Control	0	0	19	9.2	8.2	20	8.7	8.2
100	0	0	19	9.2	8.2	20	8.7	8.2
180	35	100						
320	100	100	19	9.2	8.2	20	8.8	8.4
560	100	100						
1000	100	100	19	9.2	8.2	20	8.6	8.4

[a]Temperature - mercury thermometer.

[b]Dissolved oxygen concentrations - YSI 54 ARC dissolved oxygen meter.

[c]pH - Corning 140 pH/mV meter with Beckman 39831 electrode.

NOTE: Dissolved oxygen saturations at the test temperatures of 19 and 20°C are 8.9 and 8.7 mg/l, respectively (corrected for altitudinal pressure at ABC Labs).

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LITERATURE CITED

(1) Committee on Methods for Toxicity Tests with Aquatic Organisms. Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. 1975. U.S. EPA, Ecol. Res. Ser. 660/3-75-009.

(2) American Public Health Association. 1980. Standard Methods for the Examination of Water and Wastewater. 15th ed. Washington, DC. 1134 p.

(3) Pennak, R. W. 1978. Freshwater Invertebrates of the United States. John Wiley & Sons, New York, 2nd ed. 803 p.

(4) Stephan, C. E., K. A. Busch, B. Smith, J. Burke and R. W. Andrews. 1978. A Computer Program for Calculating an LC_{50}. U. S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript, August, 1978.

(5) Stephan, C. 1977. Methods for Calculating an LC_{50}. p. 65-84. In F. L. Mayer and J. L. Hamelink (eds.). Aquatic Toxicology and Hazard Evaluation. ASTM Special Technical Publication 634. ASTM. Philadelphia.

(6) Organization for Economic Cooperation and Development. 1981. OECD Guidelines for Testing of Chemicals, Principles of Good Laboratory Practice Annex 2, C(81) 30(Final):7-28.

QUALITY ASSURANCE STATEMENT

Quality Assurance Statement for report #36614 entitled, "Acute Toxicity of E2566-1 to *Daphnia magna*," for Mr. Mohamed Elnabarawy, 3M Company, St. Paul, Minnesota.

In accordance with ABC Laboratories' intent that all studies conducted at our facilities are designed and function in conformance with good laboratory practice regulations and the protocols for individual laboratory studies, an inspection of the final report for E2566-1 was conducted and found to be in an acceptable form by a member of our Quality Assurance Unit. A procedure audit was conducted on December 14, 1987. Results were reported to the study director on the same day and to management on December 30, 1987. A final inspection of all data and records on January 13, 1988, indicated that the report submitted to you is an accurate reflection of the study as it was conducted by ABC Laboratories.

Should you have any questions relating to the information provided in this statement or the function of our Quality Assurance Unit, please contact the Quality Assurance Unit at your convenience.

Kent Ediger 1/13/88

Kent Ediger / Date
Quality Assurance Officer

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STUDY COMPLIANCE STATEMENT

Study Compliance Statement for ABC report #36614 entitled, "Acute Toxicity of EZ566-1 to *Daphnia magna*," for Mr. Mohamed Elmabarawy, 3M Company, St. Paul, Minnesota.

In accordance with ABC Laboratories' intent that all aquatic toxicity tests conducted by our facility follow good laboratory practices, ABC's study director for the above test herein confirms that the study was conducted in compliance with the OECD Guidelines for Testing of Chemicals, Principles of Good Laboratory Practice Annex 2, C(81) 30(Final):7-28.

All original raw data were provided to 3M Company, and a copy retained at Analytical Bio-Chemistry Laboratories.

Scott Frazier 1/12/88

Scott Frazier Date
ABC Study Director

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ACTIVATED SLUDGE RESPIRATION INHIBITION TEST

TEST SUBSTANCE

Identity: Perfluorodecanesulfonate, ammonium salt; may also be referred to as PFDS, FC-121-X, or 3M Sample E2112 and E2566-1. (1-Decanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heneicosafluoro-, ammonium salt, CAS # 67906-42-7)

Remarks: Test sample is a mixture of the test substance in water and 2-propanol, 1-(1,1-dimethylethoxy). This mixture contains approximately 22-26% test substance, 33-37% propanol, 1-(1,1-dimethylethoxy), 0.5-1.5% perfluorononanesulfonate, ammonium salt and 38-42% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample. Test material was from 3M production lot 1.

Note: Only the summary page was available for this study

METHOD

Method: OECD 209
Test type: Static acute
GLP: No
Year Completed: 1987
Analytical monitoring: Dissolved oxygen concentrations.
Exposure Period: 30-minutes and 3-hours
Measurements: Dissolved oxygen depletion rate after 30-minutes and 3-hours of exposure.

RESULTS

Nominal concentrations: Blank control, 100, 180, 320, 560, 1000 mg/L, in duplicate.
Element value: 30-min EC_{50} > 1000 mg/L
3-hour EC_{50} = 780 (630-1050) mg/L

All element values based on nominal concentrations.

Statistical method: Probit analysis

Control response: The largest percent difference between control uptake rates was 7.5%; thus the two controls were within 15% of each other.

Reference substance results: Not available
Remarks: Testing was conducted on the "as-sold" mixture of the subject material (~22-26% subject substance in water). The value reported applies to that mixture and not the test material. No attempt was made to determine the impact of the presence of the organic solvents or what portion of the toxicity can be contributed to the perfluorodecanesulfonate.

CONCLUSIONS

The 3-hour EC_{50} for FC-121 was determined to be 780 (630 – 1050) mg/L

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

DATA QUALITY

Reliability: Klimisch ranking 4. Most of the supporting material for this study is missing. Because a mixture was tested, care must be taken when interpreting the results. The solvent in the mixture could have enhanced or restricted biological uptake and/or toxicity of the fluorochemical component. The study lacked analytical confirmation of concentrations and characterization of the sample purity.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, St Paul, MN, Lab Request numberE2112, 10/30/87

OTHER

Last changed: 5/18/00

ENVIRONMENTAL LABORATORY

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

CALCULATION OF RESULTS

LR E2112
Date 10-30-97
Analyst SHR

Test Substance FC-121-X (41-2601-0429-8 Lot 1) FC Surfactant

Pg 3 of 3

30 - MINUTE EXPOSURE

Test Substance Concentration (mg/L)	Oxygen Uptake Rate R	R Corrected(1) to 20°C	% Inhibition in Respiration Rate(2)
CONTROL I	0.4776	0.4776	—
100	0.4248	0.4248	13
180	0.4297	0.4297	12
320	0.4273	0.4273	13
560	0.3721	0.3721	24
1000	0.3642	0.3642	25
CONTROL II	0.5000	0.5000	—

Percent Difference in Controls(3) 4.6%

EC_{50} (95% Confidence Limits) mg/L >1000 mg/L

3 - HOUR EXPOSURE

Test Substance Concentration (mg/L)	Oxygen Uptake Rate R	R Corrected(1) to 20°C	% Inhibition in Respiration Rate(2)
CONTROL I	0.5067	0.5067	—
100	0.5127	0.4897	7.0
180	0.4218	0.4218	20
320	0.4127	0.4127	22
560	0.3285	0.3285	38
1000	0.2091	0.2091	60
CONTROL II	~~0.5495~~ 0.5232	0.5461	—

Percent Difference in Controls(3) 7.5%

EC_{50} (95% Confidence Limits) mg/L 780 (630-1050) mg/L Probit Analysis

(1) Temp. Correction $= \frac{R_{T,obs}}{\theta^x}$ (mg O_2/L-min.)

(2) % Inhibition $= \frac{\left(\frac{RC_1 + RC_2}{2}\right) - R_S}{\left(\frac{RC_1 + RC_2}{2}\right)} \times 100$

(3) % Difference $= \frac{|RC_1 - RC_2|}{\left(\frac{RC_1 + RC_2}{2}\right)} \times 100$

The two control respiration rates should be within 15% of each other

Where:
R = Oxygen uptake rate (mg O_2/L-min.)
T,obs = Sample temp., C°
θ = 1.047, x = T,obs - 20°C
C = Control

See Pg 1 for test soln. prep

000220

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MICROBICS MICROTOX® TOXICITY TEST

TEST SUBSTANCE

Identity: Perfluorodecanesulfonate, ammonium salt; may also be referred to as PFDS, FC-121-X, or 3M Sample E2112 and E2566-1. (1-Decanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heneicosafluoro-, ammonium salt, CAS # 67906-42-7)

Remarks: Test sample is a mixture of the test substance in water and 2-propanol, 1-(1,1-dimethylethoxy). This mixture contains approximately 22-26% test substance, 33-37% propanol, 1-(1,1-dimethylethoxy), 0.5-1.5% perfluorononanesulfonate, ammonium salt and 38-42% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample. Test material was from 3M production lot 1.

Note: Only the summary page was available for this study

METHOD

Method: Microbics Microtox® Procedure
Test type: Static acute
GLP: No
Year Completed: 1987
Species: *Photobacterium phosphoreum*
Analytical monitoring: None
Statistical methods: Microbics computer software package
Test organism source: Microbics Microtox® Reagent
Test conditions: Per Microbics method
Replicates: Two

RESULTS

Nominal concentrations: 125, 250, 500, 1000 mg/L
Test Sample Element values: 5-minute EC_{50} =640 (605-670)) mg/L
15-minute EC_{50} =290 (260-310) mg/L

All element values based on nominal concentrations.

CONCLUSIONS

FC-121 exhibited a 15-minute EC_{50} for *Photobacterium phosphoreum* of 290 mg/L with a 95% confidence interval of 260- - 310 mg/L.

Testing was conducted on the "as-sold" mixture of the subject material in water (~22 - 26% subject material). The values reported apply to that mixture and not directly to the subject material.

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

DATA QUALITY

Reliability: : Klimisch ranking 4. Most of the supporting material for this study is missing. Because a mixture was tested, care must be taken when interpreting the results. The solvent in the mixture could have enhanced or restricted biological uptake and/or toxicity of the fluorochemical component.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, St Paul, MN, Lab Request number E2112.

OTHER

Last changed: 5/18/00

LR E 2112
S&B

Toxicity of FC-121X (41-2601-0429-8 Lot 1)
to Microbial Systems

Microtox®

Test Conc. mg/L	Percent Light Loss 5-MIN EXP	15-MIN EXP
125	24	29
250	34	44
500	45	65
1000	59	83
EC_{20}	96	92
EC_{50} (95% CI)	640 (605-670)	290 (260-310)
EC_{80}	>1000	890

OECD Activated Sludge Respiration Inhibition Test (#209)

Test Conc. mg/L	Percent Inhibition 30-MIN EXP	3-HR EXP
100	13	7.0
180	12	20
320	13	22
560	24	38
1000	25	60
EC_{50} (95% CI)	>1000	780 (630-1050)

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BIODEGRADATION

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, or FC-109-X. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-127. Current information indicates it is a mixture of 40-44% test substance, 32% water, 14% isopropanol, 4% ethanol, and 6-10% C_4-C_7 fluoroalkyl carboxylate compounds.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data will not accurately relate biodegradation of the test sample with that of the test substance.

METHOD:

Method: Not noted.
Type: Aerobic BOD with Dichromate reflux determination of COD
GLP: No
Year completed: 1982
Contact time: 20 days
Innoculum: Not noted

RESULTS

Chemical Oxygen Demand: 415,000 mg O_2 / kg chemical (mean value)
Biochemical Oxygen Demand:

5-Day:	240,000 mg/L
10-Day:	310,000 mg/L
20-Day:	320,000 mg/L

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The values reported apply to that mixture and not the test substance. The BOD and COD values are expected to be the result of biodegradation / oxidation of the organic solvent components, but it's not understood what portion of the degradation can be attributed to the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

This testing indicated that some portion of the test sample biodegraded in aerobic environments capable of supporting biodegradation.

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

DATA QUALITY

Reliability: Klimisch ranking 3. This study lacks information on the methodology. Testing cannot conclude what portions of the test sample were responsible for the apparent biodegradation values. The analytical method is not chemical specific.

REFERENCES

This study was conducted by the 3M Environmental Laboratory, Lab Request Numbers 7009 (August 19, 1981) and 7633 (February 3, 1982)

OTHER

Last changed: 5/18/00

8-25-81 ENVIRONMENTAL LABORATORY FINAL REPORT

LAB REQUEST NO. 7009

EQUESTER NAME: EAR
EPT: 0535
ROJECT NO: 9970012600
ATE RECEIVED: 7-09-81
ESC: FC-127

CONTRACT LAB:
CONTRACT LAB COST: 0
3M E-L HOURS: 18
DATE COMPLETED: 8-19-81

SAMPLE	DATE	CODE	DESCRIPTION	TEST	RESULT
1		FC-127	F-6390 LOT 1	96 HR LC50	~~833~~ 860 MG/L
			.190 g/g	BOD 05	240000 MG/L
			.25 g/g	BOD 10	310000 MG/L
			.25 g/g	BOD 20	320000 MG/L
				COD	1100000 MG/L
				OX UP	SEE ATTACHED
				96 HR LC50	816 MG/L
				COMB LC50	824 MG/L
2		DUP		COD	~~1100000~~ MG/L

check C Wade

* = CONTRACT LAB

APPROVED AND SUBMITTED BY [signature] DATED 8-25-81

12-30-81

Kieth

This COD is ~ 100% greater than the calculated theoretical COD for this product. Please Recheck calculation. If you don't see an error redo using the standard Methods Dichromate reflux method.

additional Data indicate this must be in error

Dansets

COD updated on attached

EAR.

In ~~fact~~ future please make sure I get copies of COD Data sheets.

2-04-82 ENVIRONMENTAL LABORATORY FINAL REPORT

LAB REQUEST NO. 7009

EQUESTER NAME: EAR
EPT: 0535
ROJECT NO: 9970012600
ATE RECEIVED: 7-09-81
ESC: FC-127

CONTRACT LAB:
CONTRACT LAB COST: 0
3M E-L HOURS: 19
EST CMPLT DATE: 2-03-82
DATE COMPLETED: 2-03-82

RIGINAL COD'S RUN ON 7-22-81 ARE VOIDED -- ANALYST APPEARS TO HAVE MADE A ILUTION ERROR. SAMPLES #1 AN #2 WERE ANALYZED 1-6-82 AND SAMPLE #3 AND #4 WERE NALYZED 1-11-82. SEE LR 7633 FOR MORE RECHECKS.

SAMPLE	DATE	CODE	DESCRIPTION	TEST	RESULT
1		FC-127	F-6390 LOT 1	96 HR LC50	833 MG/L
				BOD 05	240000 MG/L
				COD-RFX	403000MG/KG
				OX UP	SEE ATTACHED
				96 HR LC50	816 MG/L
				COMB LC50	824 MG/L
2		DUP		COD-RFX	416000MG/KG
3		DUP	NO DILUTION	COD-RFX	380000MG/KG
4		DUP	NO DILUTION	COD-RFX	380000MG/KG

* = CONTRACT LAB

APPROVED AND SUBMITTED BY _____ DATED 2-8-82

1-25-82
DCS

CoD's on FC 127

7-22-81	1,100,000 mg/L	Ampule	7009-1	Dilution
	1,120,000 mg/L	"	" Dup	
~~1-30-84~~				
1-6-82	402,700 mg/kg	Reflux	7009-1	Dilution
	416,000 mg/kg	"	7009-2	
1-11-82	380,000 mg/kg	"	7009-1	Direct
	380,000 mg/kg	"	7009-2	
1-21-82	430,000 mg/kg	Ampule	7633-1	Dilut
	430,000 mg/kg	"	7633-2	
1-21-82	560,000 mg/L (Sp. Grav. 1.26 → 440,000 mg/kg)	Ampule	7009-1	Dilut

Mean 415,000 mg/kg
(s = 27,000 mg/kg)

409
380
430
440

000218

Isopropanol

2.39 g/g × 0.14 = .322

Ethanol 2.08 g/g × 0.04 = .083

$$5.5O_2 + C_8F_{17}SO_2N\begin{matrix}C_2H_5\\CH_2COOH\end{matrix} \rightarrow C_8F_{17}SO_3^- + NH_4^+ + 4CO_2 + 2H_2O$$

MW 585

0.3 g/g × .5 = .15

Σ = 0.555 = theoretical COD

if .19 then COD is 0.50 g/g

2-04-82 ENVIRONMENTAL LABORATORY FINAL REPORT

LAB REQUEST NO. 7633

EQUESTER NAME: EAR
EPT: 0535
ROJECT NO: 9970012600
ATE RECEIVED: 1-15-82
ESC: RECHECK OF COD'S ON FC-127

CONTRACT LAB:
CONTRACT LAB COST: 0
3M E-L HOURS: 3
EST CMPLT DATE: 2-03-82
DATE COMPLETED: 2-03-82

REVIOUS ANALYSES: REFLUX 1-6-82 AND 1-11-82; AMPULE 7-22-81 (SEE LR 7009).
C-127 SPECIFIC GRAVITY (FROM EAR) 1.26 G/ML -- #4 EQUAL TO 440,000 MG/KG COD.

SAMPLE	DATE	CODE	DESCRIPTION	TEST	RESULT
1		FC-127	7009-1	COD	430000MG/KG
2			DUP OF 1	COD	430000MG/KG
4		7009-1		COD	560000 MG/L

* = CONTRACT LAB

APPROVED AND SUBMITTED BY [signature] DATED 2-8-82

ACTIVATED SLUDGE RESPIRATION INHIBITION

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, or FC-109-X. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-127. Current information indicates it is a mixture of 40-44% test substance, 32% water, 14% isopropanol, 4% ethanol, and 6-10% C_4-C_7 fluoroalkyl carboxylate compounds.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD:

Methods: Protocol Reference EAR, 11/8/79.
Test type: Not noted.
GLP: No
Year Completed: 1981
Test organism source: Activated Sludge mixed liquor from St. Paul, Minnesota Metro Wastewater Treatment Plant

RESULTS

Nominal concentrations: Bk control, 1000 mg/L
Element values: No acute inhibitory effect on activated sludge respiration rate at 1000 mg/L with a contact time of 3.25 minutes.

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The value reported applies to that mixture and not the test substance. No attempt was made to determine the impact of the presence of the organic solvents or what portion of the toxicity can be contributed to the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

Limited contact time estimates that FC-127 is not expected to inhibit the activity of activated sludge.

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

DATA QUALITY

Reliability: Klimisch ranking 3. Testing lacks record of methodology used. The method was not an agency approved method. There is a lack of characterization of the test sample. Test solutions not analyzed for test substance concentrations. Nominal values used.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, St. Paul, MN, Lab Request number 7009.

OTHER

Last changed: 5/18/00

3M Environmental Laboratory

Acute Toxicity of a Compound (Substance) to Activated Sludge Mixed Liquor

Protocol Reference: EAR 11/8/79

Lab request no.: 7009 Date: 8-14-81 Analyst: W.A. Schil

The activated sludge mixed liquor was collected from the St. Paul/Minneapolis Metro Wastewater Plant on 8-14-81.

Sample description: FC-127, F-6390, LOT-1

Preparation of stock solution: 0.60 grams of sample were diluted to 100 ml with deionized reagent water. Date of preparation: 8-14-81.

TIME min.	TIME sec.	CONCENTRATION OF TEST MATERIAL IN ACTIVATED SLUDGE MIXTURE DI H2O CONTROL	1000 mg/l	100 mg/l	10 mg/l	DI H2O Control	0.10 mg/l	0.010 mg/l
	15	7.85	7.30					
	30	7.55	6.90					
1	-	6.90	5.85					
2	-	5.60	3.60					
3	-	4.35	1.25					
4	-	3.05	0.50	3 minutes 20 seconds				
5	-	1.80						
6	-	0.65						

COMMENTS:

after the dissolved oxygen was monitored, the dilutions were poured into 500-ml graduated cylinders to observe the sludge settling. The cylinders were shaken and photos were taken after the 5, 10, 20, 30, 40 and 60 min. intervals

ENVIRONMENTAL LABORATORY SUMMARY SHEET

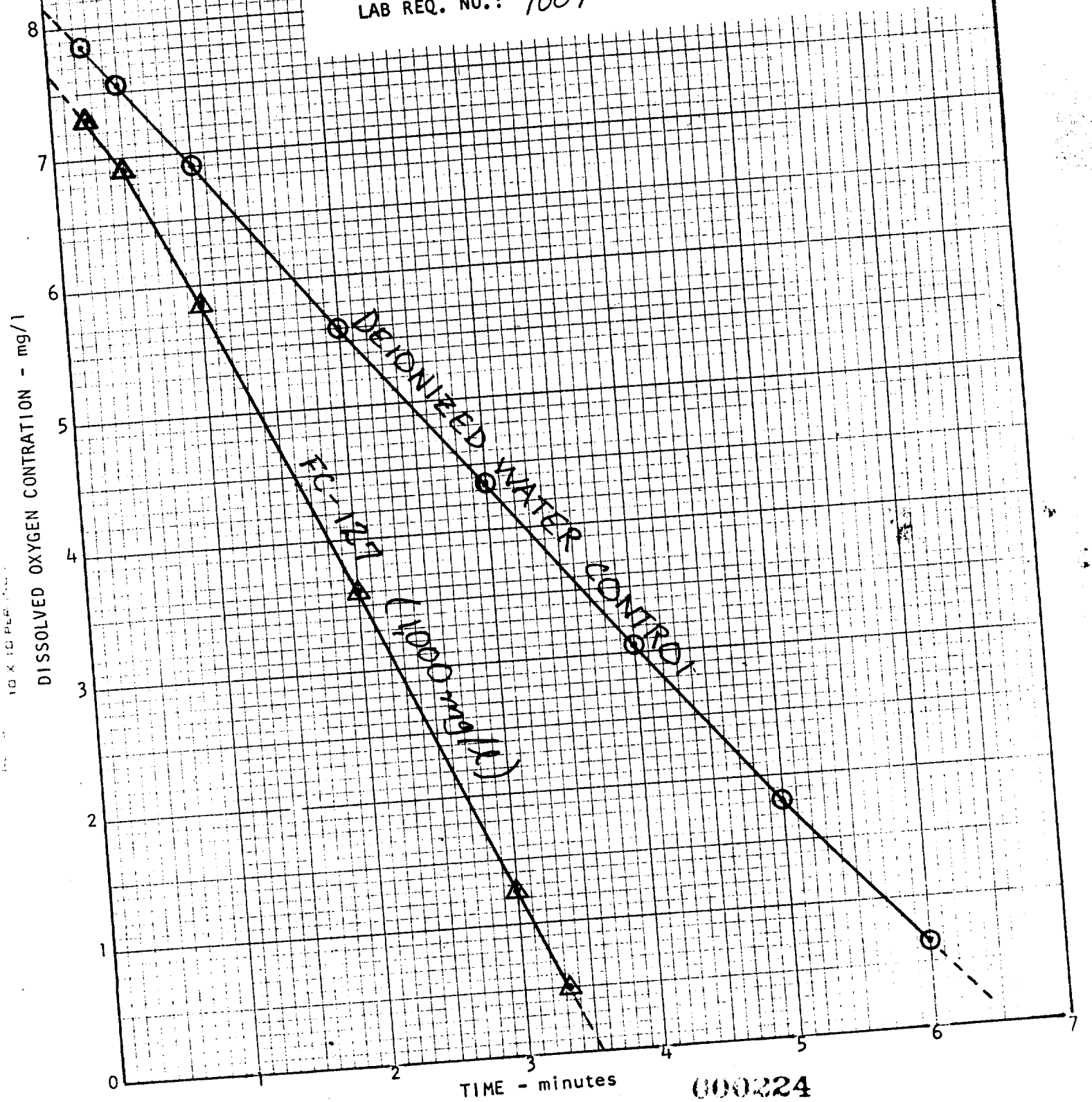
TEST: Acute Toxicity of a Compound to Activated Sludge Mixed Liquor.

SAMPLE DESCRIPT.: FC-127, F-6390, LOT-1

ANALYST: WADE A. SCHEIL

DATE ANALYZED: 8-14-81

LAB REQ. NO.: 7009



ACUTE TOXICITY TO FISH (FATHEAD MINNOW)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, or FC-109-X. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-127 (F-6390, Lot 1). Current information indicates it is a mixture of 40-44% test substance, 32% water, 14% isopropanol, 4% ethanol, and 6-10% C_4-C_7 fluoroalkyl carboxylate compounds.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD:

Methods: EPA protocol.
Test type: Static acute
GLP: No
Year Completed: 1981
Species: *Pimephales promelas*
Analytical monitoring: Temperature, pH, DO, and conductivity

RESULTS

Nominal concentrations: Bk control, 650, 870, 1150, 1550, 2100 mg/L
Element value: 96-hour LC_{50} = 863 (796-950) mg/L

Element value based on nominal concentrations.

Remarks: There was a slight separation in test concentrations after 24 hours (surface film formation).Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The value reported applies to that mixture and not the test substance. No attempt was made to determine the impact of the presence of the organic solvents or what portion of the toxicity can be contributed to the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

The FC-127 96-hour LC_{50} for *Pimephales promelas* was determined to be 863 mg/L with a 95% confidence interval of 796-950 mg/L by moving average angle method.

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, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample.

REFERENCES

This study was conducted at 3M Company, Environmental Laboratory, St. Paul, MN, Lab Request number 7009.

OTHER

Last changed: 5/18/00

ENVIRONMENTAL LABORATORY
AQUATIC TOXICITY DATA SHEET
EXPLORATORY (RANGE-FINDING TEST)

LR 7009

Type Test 96HR. ACUTE STATIC Material Tested EC-127 E-6390 Lot 1

Test Organism FATHEAD MINNOW Source KURTZ'S, EMERSON, PA. Date Rec'd. 4/28/81 Batch No. 5-8

Avg. Wt. 0.205 grams Avg. Size 3.0 CM No. Organisms per dose 5

Test Solution Volume 3 Liters/All-Glass Tank Tank Dimensions: WIDE-MOUTH 1 GALLON GLASS JA

Time Started 10:00AM Date Started 7/20/81 Date Completed 7/24/81

Dilution Water Carbon-filtered well water (Temp. 19 °C., n=2 D.O. 10.0 ppm, pH 8.2)

Investigator [illegible] (Full Signature) LC$_{50}$ (95% Confidence Limits)

Tank No.	Init. Conc. mg/l	Total Amount Added	24 Hours Total No. Dead	24 Hours pH	24 Hours D.O. ppm	48 Hours Total No. Dead	48 Hours pH	48 Hours D.O. ppm	72 Hours Total No. Dead	72 Hours pH	72 Hours D.O. ppm	96 Hours Total No. Dead	96 Hours pH	96 Hours D.O. ppm
CI	Control	— mg	0	8.1	7.5	0	8.1	6.1	0	8.0	6.1	0	8.0	6.[illegible]
CII	CONTROL	—	0	8.3	7.8	0	8.2	6.6	0	8.2	6.6	0	8.2	6.[illegible]
1	10	30*	0	8.3	7.7	0	8.2	6.1	0	8.2	6.1	0	8.2	6.[illegible]
2	100	300	0	8.3	7.8	0	8.2	4.8	0	8.2	5.0	0	8.2	5.[illegible]
3	500	1500	0	8.4	7.8	0	8.3	5.2	0	8.0	1.8	0	8.0	[illegible]
4	1000	3000	0	8.4	8.0	1/5	8.3	5.2	2/5	7.9	0.5	3/5	7.9	[illegible]
5	2000	6000	2/5	8.5	8.0	3/5	8.3	4.3	4/5	7.9	0.3	5/5	7.9	0.[illegible]
Test Temp. °C.			19°C			20°C			20°C			20°C		
Initials & Date			[illegible] 7/21/81			[illegible] 7/22/81			[illegible] 7/23/81			[illegible] 7/24/[illegible]		

Comments: * INDIVIDUAL WEIGHTS. TEST MATERIAL IS A CLEAR, AMBER LIQUID. EASILY DISPENSED IN WATER AT AMBIENT ROOM TEMPERATURE. SLIGHT SEPARATION AFTER 24 HOURS. SURFACE FILM.

Effects other than Mortality LC$_{50}$

Tank No.	Symptoms or Reactions	Total No. Affected 24-Hr.	48 Hr.	72-Hr.	96-Hr.
Control	I + CONTROL II				
1					
2					
3	SLIGHT SURFACING			5	5
4	SLIGHT SURFACING	3	3	3	2
5	SLIGHT SURFACING	3	2	1	—

Comments: (1) NOTEABLE INCREASE IN D.O. CONCENTRATION BETWEEN 72HR AND 96HR.

000227

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ENVIRONMENTAL LABORATORY
AQUATIC TOXICITY DATA SHEET
DEFINITIVE (FULL-SCALE TEST)

LR 7009

Type Test 96HR. ACUTE STATIC Material Tested FC-127 F-6390 Lot 1

Test Organism FATHEAD MINNOWS Source KURTZ, ELVERSON, PA. Date Rec'd 4/28/81 Batch # 5-81

Avg.Wt. 0.251 grams Avg. Size 3.0 cm No. Organisms per dose 20 Loading Factor 0.3 g/l

Fish Disease Treatment: 25 mg/l TETRACYCLINE HCl 6/8/81

Test Solution Volume 16 Liters/All-Glass Tank Tank Dimensions: 35x20 cm and 25 cm deep

Time Started 10:40 AM Date Started 7/27/81 Date Completed 7/31/81

Dilution Water Carbon-filtered well water (Temp. 20 °C., D.O. 8.9 ppm, pH 8.5)

Investigator [signature] LC50 (95% Confidence Limits) 833 mg/l (634-984)
(Full Signature) COMBINED 824 mg/l
USED PROBIT ANALYSIS → Calculated c̄ moving angle See attached 862 (796-950 mg/l)

Tank No.	Initial Conc. mg/l	Total Amount Added	24 Hours Total No. Dead	24 Hours pH	24 Hours D.O. ppm	48 Hours Total No. Dead	48 Hours pH	48 Hours D.O. ppm	72 Hours Total No. Dead	72 Hours pH	72 Hours D.O. ppm	96 Hours Total No. Dead	96 Hours pH	96 Hours D.O. ppm
CI	Control	— mg	0	8.3	7.4	1	8.2	6.8	1	8.1	6.2	1	8.0	6.2
CII	Control	—	0	8.2	7.4	2	8.2	7.0	2	8.2	6.6	2	8.0	6.4
1B	650	10400*	3	8.2	4.5	6	8.2	0.8	10	8.0	0.5	10	7.9	0.5
2B	870	13920	1	8.2	5.8	3	8.2	0.5	5	8.0	0.4	7	8.0	0.4
3B	1150	18400	0	8.3	4.5	5	8.2	1.5	6	8.0	0.4	13	8.0	0.4
4B	1550	24800	3	8.4	5.0	5	8.2	1.0	11	8.0	0.4	17	8.0	0.3
5B	2100	33600	5	8.4	1.0	16	8.2	0.4	20	8.0	0.3	20	—	—
Test Temp. °C			21°C			20°C			20°C			20°C		
Initials & Date			[illegible] 7/28/81			[illegible] 7/29/81			[illegible] 7/30/81			[illegible] 7/31/81		

Comments: * INDIVIDUAL WEIGHTS. TEST MATERIAL IS A CLEAR, AMBER LIQUID. EASILY DISPERSED IN WATER AT AMBIENT ROOM TEMPERATURE. SLIGHT SEPARATION AFTER 24 HR SURFACE FILM.

Secondary Effects (other than Mortality): EC50

Tank No.	Symptoms or Reactions	Total Number Affected 24-Hr.	48-Hr.	72-Hr.	96-Hr.
Control	I				000228
Control	II				
1B	SURFACING	ALL	ALL	ALL	ALL
2B	SURFACING	ALL	ALL	ALL	ALL
3B	SURFACING	ALL	ALL	ALL	ALL
4B	SURFACING	ALL	ALL	ALL	ALL
5B	SURFACING	ALL	ALL	—	—

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ENVIRONMENTAL LABORATORY
AQUATIC TOXICITY DATA SHEET
DEFINITIVE (FULL-SCALE TEST)

LR 4009

Type Test 96HR. ACUTE STATIC Material Tested FC-127 F-6390 Lot 1

Test Organism FATHEAD MINNOWS Source KURTZ, ELVERSON, PA Date Rec'd 4/28/81 Batch # 5-81

Avg. Wt. 0.251 grams Avg. Size 3.0 cm No. Organisms per dose 20 Loading Factor 0.3

Fish Disease Treatment: 25 mg/l TETRACYCLINE HCl 6/8/81

Test Solution Volume 16 Liters/All-Glass Tank Tank Dimensions: 35x20 cm and 25 cm deep

Time Started 10:40AM Date Started 7/27/81 Date Completed 7/31/81

Dilution Water Carbon-filtered well water (Temp. 20 °C., D.O. 8.9 ppm, pH 8.5)

Investigator [signature] LC_{50} (95% Confidence Limits) 816 mg/l n=2
(Full Signature) COMBINED 824 mg/l
USED PROBIT ANALYSIS

Tank No.	Initial Conc. mg/l	Total Amount Added	24 Hours Total No. Dead	24 Hours pH	24 Hours D.O. ppm	48 Hours Total No. Dead	48 Hours pH	48 Hours D.O. ppm	72 Hours Total No. Dead	72 Hours pH	72 Hours D.O. ppm	96 Hours Total No. Dead	96 Hours pH	96 Hours D.O. ppm
CI	Control	—	0	8.3	7.4	1	8.2	6.8	1	8.1	6.2	1	8.0	6.2
CII	Control	—	0	8.2	7.4	2	8.2	7.0	2	8.2	6.6	2	8.0	6.4
1C	650	10400*	3	8.2	4.0	6	8.2	0.8	9	8.0	0.6	10	7.9	0.6
2C	870	13920	0	8.2	4.2	2	8.2	1.2	5	8.0	0.4	7	8.0	0.4
3C	1150	18400	0	8.3	4.5	3	8.2	1.0	5	8.0	0.5	13	8.0	0.3
4C	1550	24800	4	8.4	4.5	7	8.2	0.5	15	8.0	0.4	20	8.0	0.3
5C	2100	33600	3	8.5	3.2	10	8.2	0.5	19	8.0	0.3	20	8.0	0.3
Test Temp. °C			21°C			20°C			20°C			20°C		
Initials & Date			[initials] 7/28/81			[initials] 7/29/81			[initials] 7/30/81			[initials] 7/31/81		

Comments: INDIVIDUAL WEIGHTS. TEST MATERIAL IS A CLEAR, AMBER LIQUID. EASILY DISPENSED IN WATER AT AMBIENT ROOM TEMPERATURE. SLIGHT SEPARATION AFTER 24 HOURS. SURFACE FILM.

Secondary Effects (other than Mortality): EC_{50}

Tank No.	Symptoms or Reactions	Total Number Affected 24-Hr.	48-Hr.	72-Hr.	96-Hr.
Control I					
Control II					
1C	SURFACING	ALL	ALL	ALL	ALL
2C	SURFACING	ALL	ALL	ALL	ALL
3C	SURFACING	ALL	ALL	ALL	ALL
4C	SURFACING	ALL	ALL	ALL	—
5C	SURFACING	ALL	ALL	1	—

000229

BIODEGRADATION

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, or FC-109-X. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-109-X. Current information indicates it is a mixture of 19-23% test substance, 61% water, 12% 2-methoxymethylethoxypropanol, 2% ethanol, and 2-6% C_4-C_7 fluoroalkyl carboxylate compounds. Test sample was from 3M production lot 2.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data will not accurately relate biodegradation of the test sample with that of the test substance.

METHOD:

Method: Not noted.
Type: Aerobic Carbonaceous BOD with Dichromate reflux determination of COD
GLP: No
Year completed: 1987
Contact time: 20 days
Innoculum: Not noted

RESULTS

Chemical Oxygen Demand: 375,000 mg O_2 / kg product
Biochemical Oxygen Demand:

5-Day:	31,000 mg/L
10-Day:	61,000 mg/L
20-Day:	69,000 mg/L

Remarks: The BOD and COD values are expected to be the result of biodegradation of the organic solvent components of the test sample. These values are not expected to be representative of the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

This testing indicated that some portion of the test sample biodegraded in aerobic environments capable of supporting biodegradation.

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

DATA QUALITY

Reliability: Klimisch ranking 3. This study lacks information on the methodology, characterization of the test substance purity and analytical measurement of test concentrations. Testing cannot conclude what portions of the test sample were responsible for the apparent biodegradation values.

REFERENCES

This study was conducted by Pace Incorporated of Minneapolis, MN at the request of 3M Company, Lab Request number E2113, 12/31/87.

OTHER

Last changed: 5/18/00

ARCHIVED FINAL REPORT

LAB REQUEST NO. E2113
REQUESTOR NAME: EAR/MTE
DEPARTMENT: 0535
PROJECT NO: 9970013000
DATE RECEIVED: 09/15/1987
DESC: FC-109-X ENVIRONMENTAL SCREENS

CONTRACT LAB(S): PACE
EXP COMP DATE: 12/31/1987
DATE COMPLETED: 12/31/1987
PROJECT LEAD: SA BEACH
PHONE NO: 651-778-7452
3M FAX NO: 651-778-6176

41-2601-0414-0 LOT 2
SAMPLE(S) SENT TO ABC LABS FOR FISH AND DAPHNIA WORK - SEE LR# E2566.

SAMPLE	DATE	CODE	DESCRIPTION	RESULT	MDL or (95% C.I.)
1		FC-109-X	----------		
	96-HR-LC50	FISH ACUTE TEST - 96 HOUR		SEE COMMENT ABOVE	
PACE	C-BOD-05	*BOD-05 (CARBONACEOUS)		31000 MG/KG	
PACE	C-BOD-10	*BOD-10 (CARBONACEOUS)		61000 MG/KG	
PACE	C-BOD-20	*BOD-20 (CARBONACEOUS)		69000 MG/KG	
PACE	COD-RFX	*COD (DICHROMATE REFLUX)		360000 MG/KG	
	MICROTOX	MICROTOX - EC50		COMPLETE	
PACE	REP-INORG	*INORGANIC TESTS REPORT		SEE ATTACHED REPORT	
	SLUDG-RESP	SLUDGE RESPIRATION - EC50		COMPLETE	
2			DUP OF #1		
PACE	COD-RFX	*COD (DICHROMATE REFLUX)		390000 MG/KG	

ACUTE TOXICITY TO FISH (FATHEAD MINNOW)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, or FC-109-X. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-109-X referred to by the test laboratory as E2566-2. Current information indicates it is a mixture of 19-23% test substance, 61% water, 12% 2-methoxymethylethoxypropanol, 2% ethanol, and 2-6% C_4-C_7 fluoroalkyl carboxylate compounds. Test material was from 3M production lot 2.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD:

Methods: OECD 203
Test type: Static acute
GLP: Yes
Year Completed: 1988
Species: *Pimephales promelas*
Analytical monitoring: Temperature, pH, DO
Statistical analysis: LC_{50} values were calculated using the program developed by Stephan et al.

RESULTS

Nominal concentrations: Bk control, 10, 18, 32, 56, 100, 180, 320 mg/L
Element values: 96-hour LC_{50} = 57 (36-93) mg/L
96-hour NOEL = <10 mg/L

All element values based on nominal concentrations.

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The value reported applies to that mixture and not the test substance. No attempt was made to determine the impact of the presence of the organic solvents or what portion of the toxicity can be contributed to the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

The FC-109-X 96-hour LC_{50} for *Pimephales promelas* was determined to be 57 mg/L with a 95% confidence interval of 36-93 mg/L. The 96-hour no observed effect level was <10 mg/L.

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, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample.

REFERENCES

This study was conducted at Analytical Bio-Chemistry Laboratories, Inc. of Columbia, Missouri at the request of the 3M Company.

OTHER

Last changed: 5/17/00

SPONSOR

3M Company
Attn: Mr. Mohamed Elnabarawy
EE & PC Environmental Labs
Building 21-2W-05, 900 Bush Avenue
St. Paul, Minnesota 55106

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STUDY TITLE

Acute Toxicity of E2566-2
to Fathead Minnow (*Pimephales promelas*)

DATA REQUIREMENT

OECD Guideline #203

AUTHOR

Jane H. Bowman
Biologist II

STUDY COMPLETED ON

January 12, 1988

PERFORMING LABORATORY

Analytical Bio-Chemistry Laboratories, Inc.
Aquatic Toxicology Division
7200 East ABC Lane
P.O. Box 1097
Columbia, Missouri 65205

ABC LABORATORY PROJECT ID

#36617

Submitted By: Analytical Bio-Chemistry Laboratories, Inc.
7200 East ABC Lane
P. O. Box 1097
Columbia, Missouri 65205
(314) 474-8579

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Prepared By:

Jane H. Bowman — Date
Biologist II/Study Director

Laura Mullen — Date
Biologist I

Approved By:

James P. Swigert, Ph.D. — 1-25-88 Date
Supervisor, Fish Acute/
Chronic Investigations

Phillip M. Buckler — 1/26/88 Date
Quality Assurance Supervisor

Carl M. Thompson — 1-29-88 Date
Aquatic Toxicology Manager

:dab31

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PROJECT PERSONNEL

The study director of the project for Analytical Bio-Chemistry Laboratories, Inc. was Ms. Jane H. Bowman, Biologist II. Supervising the conduct of the study for 3M Company was Mr. Mohamed Elnabarawy. The following ABC personnel assisted with various phases of the study.

Name	Title	Initials
Jane H. Bowman	Biologist II	JHB
Laura Mullen	Biologist I	LMM
Chris Brenneke	Biological Technician I	CAB
James P. Swigert, Ph.D.	Supervisor, Fish Acute/ Chronic Investigations	JPS

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FISH ACUTE TOXICITY COMPENDIUM

Subject: "Acute Toxicity of E2566-2 to Fathead Minnow (Pimephales promelas)," ABC Study Number 36617.

Sponsor: 3M Company

Testing Facility: Analytical Bio-Chemistry Laboratories, Inc.
Aquatic Toxicology Division
P.O. Box 1097
Columbia, Missouri 65205 (314-474-8579)

Location of Original Raw Data and Final Report:

3M Company
St. Paul, Minnesota 55106

Test Material: E2566-2

Test Concentrations: Control, 10, 18, 32, 56, 100, 180 and 320 mg/l

Dilution Water: 42 mg/l (hardness), 32 mg/l (alkalinity), 7.4 (pH)

Test Dates: Initiation - January 8, 1988

Termination - January 12, 1988

Length of Study: 96-hours

Results: 96-hour LC_{50} = 57 mg/l (95% C.I. = 36-93 mg/l)

96-hour NOEL = <10 mg/l

96-hour Slope of the Dose-Response Line was 16

Test Species: Fathead Minnow (Pimephales promelas)

Source of Organisms: ABC Laboratories in-house culture

Mean Weight: 0.061 g (±0.037 g)

Mean Length: 17 mm (±2.2 mm)

FISH ACUTE TOXICITY COMPENDIUM

Subject: "Acute Toxicity of E2566-2 to Fathead Minnow (Pimephales promelas)," ABC Study Number 36617.

Sponsor: 3M Company

Testing Facility: Analytical Bio-Chemistry Laboratories, Inc.
Aquatic Toxicology Division
P.O. Box 1097
Columbia, Missouri 65205 (314-474-8579)

Location of Original Raw Data and Final Report:

3M Company
St. Paul, Minnesota 55106

Test Material: E2566-2

Test Concentrations: Control, 10, 18, 32, 56, 100, 180 and 320 mg/l

Dilution Water: 42 mg/l (hardness), 32 mg/l (alkalinity), 7.4 (pH)

Test Dates: Initiation - January 8, 1988

Termination - January 12, 1988

Length of Study: 96-hours

Results: 96-hour LC_{50} = 57 mg/l (95% C.I. = 36-93 mg/l)

96-hour NOEL = <10 mg/l

96-hour Slope of the Dose-Response Line was 16

Test Species: Fathead Minnow (Pimephales promelas)

Source of Organisms: ABC Laboratories in-house culture

Mean Weight: 0.061 g (±0.037 g)

Mean Length: 17 mm (±2.2 mm)

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SUMMARY

The acute toxicity of E2566-2 to fathead minnow (Pimephales promelas) was assessed using the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms. Water quality parameters of temperature, dissolved oxygen and pH were measured throughout the test and were within acceptable limits. Culture and acclimation records indicated the fish were in good condition for testing.

The study was conducted at the following nominal concentrations of E2566-2: 10, 18, 32, 56, 100, 180 and 320 mg/l. At 0-hour, the 18, 32, 56, 100, 180 and 320 mg/l solutions had a white surface film. The amount of surface film increased with the concentration from a very slight amount in the 18 mg/l solution to a thicker, nearly complete covering over the 320 mg/l solution. After 24 hours, a slight surface film also appeared over the 10 mg/l solution while the other solutions had a surface film increasing with the concentration. At the 72- and 96-hour observations the 100, 180 and 320 mg/l solutions were cloudy and all solutions had a surface film that increased in proportion to the concentration. Ten fish, with a mean weight of 0.061 (±0.037) g and a mean standard length of 17 (±2.2) mm, were exposed to each test concentration and control.

The results of the four day static fish toxicity study using E2566-2 are summarized below. The 24-, 48- and 72-hour LC_{50} values were also determined. The 96-hour slope of the dose response line was 16.

Compound	96-Hour LC_{50} (95% C.I.)
E2566-2	57 mg/l (36-93 mg/l)

Also, the results indicated a 96-hour, no-observed effect concentration could be estimated at <10 mg/l, the lowest concentration tested. Abnormal effects of mortality, surfacing, loss of equilibrium, dark discoloration, fish on the bottom of test chamber, quiescence and/or excitability were observed during the 96-hour exposure period in the 10, 18, 56, 100, 180 and 320 mg/l test concentrations. One fish in the control chamber was quiescent and on the bottom of the test chamber at the 96-hour observation.

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INTRODUCTION

This static bioassay was performed by the Aquatic Toxicology Division of Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri, for 3M Company, from January 8, 1988 to January 12, 1988. The purpose of this test was to determine the 24-, 48- and 96-hour LC_{50} levels for E2566-2 to fathead minnow (*Pimephales promelas*). A preliminary range-finding study was conducted from December 31, 1987 to January 4, 1988 to determine the concentration range for the definitive bioassay. The study was conducted following the procedures outlined in ABC Protocol Number 7601 (Raw Data), as approved by Mr. Mohamed Elnabarawy, 3M Company, on January 7, 1988

METHODS AND MATERIALS

The procedures for static bioassay, as described in *Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians* (1) and *Standard Methods for Examination of Water and Wastewater* (2), were used in this experiment.

I. Test Fish

The fathead minnow (ABC Lot #2287 FHM) used in the test were obtained from an ABC Labs in-house culture. All test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least fourteen days prior to testing. Fish culture techniques used were basically those described by Brauhn et al. (3). A daily record of fish observations during the holding period, along with any prophylactic or therapeutic disease treatments, is included in the Raw Data. During this period, the fish received a standard commercial fish food occasionally supplemented with brine shrimp nauplii (*Artemia sp.*) daily until 48-96 hours prior to testing at which time feeding was discontinued. The fathead minnow used for this experiment had a mean weight of 0.061 (±0.037) g and a mean standard length of 17 (±2.2) mm. This gave a test chamber loading biomass of 0.041 g/l for the definitive study. Weight and length measurements were made on the control group of fish at the termination of the test and are included in the Raw Data.

II. Test Material

The E2566-2 sample was received on December 29, 1987, in good condition. The sample upon receipt was observed to be a foam yellow liquid and was stored at room temperature. Sample purity was not specified. The definitive test concentrations were obtained by transferring appropriate weights of test compound directly to the test chambers. All test concentrations were based on the total compound, i.e. not corrected for sample purity. All standard weights and dilution values are listed in the Raw Data.

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III. Test Water

The static fish bioassay was conducted in five gallon glass vessels containing 15 liters of soft reconstituted water composed of the following compounds in the amounts stated per liter of deionized water:

48 mg $NaHCO_3$
30 mg $CaSO_4 \cdot 2\ H_2O$
30 mg $MgSO_4$
2 mg KCl

This reconstituted water was prepared to yield a total hardness of 40-48 mg/l as $CaCO_3$, a total alkalinity of 25-35 mg/l as $CaCO_3$ and an initial pH of 7.2 to 7.6. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 9.3 mg/l and pH 7.4. The well water source from which this dilution water was prepared had the characteristics shown in Table 1.

The test vessels were kept in a water bath at 22°C (±1.0). The test fish were acclimated to the dilution water and test temperature and held without food for 48-96 hours prior to testing. An acclimation record is included in the Raw Data.

IV. Biological Test Procedure

A range-finding test of 96-hours duration was conducted to determine the concentration range for the definitive study. The preliminary test concentrations were set at 30, 100 and 320 mg/l. Based on the results of preliminary testing, seven concentrations of the test compound, ranging in a logarithmic series from 10 to 320 mg/l, with ten fish per concentration were selected for the definitive bioassay. Also included was a dilution water control. The fish were added to the test chambers by random assignment within 30 minutes after addition of test material. All test organisms were observed once every 24 hours for mortality and abnormal (sub-lethal) effects. Any dead individuals were removed from the test chambers after each 24-hour observation.

V. Biological Data Analysis

Statistical analysis of the concentration vs. effect data (generally mortality) was obtained by employing a computerized LC_{50} program developed by Stephan et al. (4). This program calculated the LC_{50} statistic and its 95 percent confidence limits using the binomial, the moving average and the probit tests. However, if no mortality occurred or if a dose response could not be demonstrated over a reasonable range (<37 to >63%), an LC_{50} and/or its 95 percent confidence limits

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could not be calculated. Three different methods of analyzing the data were used since no one method of analysis is appropriate for all possible sets of data that may be obtained. The method of calculation selected for presentation in this report was that which gave the narrowest confidence limits for the LC_{50} (4, 5) although all three models are valid. The concentration-mortality curve was constructed for the 96-hour mortality data. The slope of the dose-response line was reported as calculated by least squares regression analysis (log of concentration vs. probit transformation of percent mortality).

RESULTS

The results of the 96-hour static toxicity test with fathead minnow (Pimephales promelas) exposed to E2566-2 are presented in Table 2. The 24-, 48- and 96-hour LC_{50} values for E2566-2 were >320, 160 and 57 mg/l, respectively. The 96-hour slope of the dose-response curve was 16 (Figure 1). All results were based on the nominal concentrations of 10, 18, 32, 56, 100, 180 and 320 mg/l. At 0-hour, the 18, 32, 56, 100, 180 and 320 mg/l solutions had a white surface film. The amount of surface film increased with the concentration from a very slight amount in the 18 mg/l solution to a thicker, nearly complete covering over the 320 mg/l solution. After 24 hours a slight surface film also appeared over the 10 mg/l solution while the other solutions had a surface film increasing with the concentration. At the 72- and 96-hour observations the 100, 180 and 320 mg/l solutions were cloudy and all solutions had a surface film that increased in proportion to the concentration.

The 96-hour no-observed effect concentration was estimated to be <10 mg/l, the lowest concentration tested. The abnormal effects of mortality, surfacing, loss of equilibrium, dark discoloration, fish on the bottom of test chamber, quiescence and/or excitability were observed in the 10, 18, 56, 100, 180 and 320 mg/l test concentrations during the 96-hour exposure period. One fish in the control chamber was quiescent and on the bottom of the test chamber at the 96-hour observation. The 10 mg/l chamber also had 20% mortality at 96 hours while the 18 and 32 mg/l chambers had 0% mortality. An examination of the fish culture and acclimation records for this test indicated that the fish were in good condition for testing (Raw Data).

Nominal test concentrations, mortality rates and water quality data are presented in Table 3. The dissolved oxygen concentrations ranged from 6.8 to 9.8 mg/l during the test. These values represented 81 and 117% saturation at 22°C, respectively, and were considered adequate for testing (1), therefore, the test chambers were not aerated during testing. The pH values ranged from 7.2 to 7.9.

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The study was conducted following the intent of the Good Laboratory Practice Regulations (6) and the final report was reviewed by Analytical Bio-Chemistry Laboratories' Quality Assurance Unit. All original raw data was provided to 3M Company, with a copy retained at Analytical Bio-Chemistry Laboratories.

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TABLE 1

Chemical Characteristics of Well Water Used by ABC's Aquatic Toxicology Division.

Parameter	Concentration
Temperature	17 - 22°C[b]
Dissolved Oxygen[a]	7.4 - 9.1 ppm[b]
pH	7.6 - 8.4[b]
Hardness ($CaCO_3$)	206 - 275 ppm[b]
Alkalinity ($CaCO_3$)	224 - 336 ppm[b]
Conductivity	500 - 650 µmhos/cm[b]
Total Organic Carbon	1.4 ppm[c] (0.9 - 2.8 ppm)[d]
Suspended Solids	0 - 1.3 ppm[b]
Aluminum	<10 ppb
Arsenic	<1 ppb
Boron	2.25 ppm
Cadmium	<3 ppb
Chromium	<7 ppb
Cobalt	<3 ppb
Copper	<3 ppb
Iron	14 ppb
Lead	<30 ppb
Mercury	0.43 ppb
Nickel	<8 ppb
Silver	<4 ppb
Zinc	2 ppb
Measured organophosphorus pesticides	<0.14 ppb
Measured organochlorine pesticides plus PCB's	<1.0 ppb

[a]After aeration

[b]Represents seasonal variation, difference from one month to the next, will not exceed 10%

[c]Average of all well water values from January-September, 1987

[d]Total monthly range of organic carbon

Sample dates: Trace elements = June 3, 1987, organophosphate and organochlorine analyses = May 28, 1987.

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TABLE 2

Acute Toxicity of E2566-2 to Fathead Minnow
(*Pimephales promelas*)

Compound	LC_{50} in milligrams/liter (ppm)		
	24 hours	48 hours	96 hours
E2566-2[a]	>320[(b)]	160[(3)]	57[(2)]
		(120-210)[c]	(36-93)[c]

N = 10 fish per concentration.

[a]Bioassay as conducted at 22°C (±1.0), mean fish weight and length, 0.061 (±0.037)g and 17 (±2.7)mm.

[b]LC_{50} could not be calculated due to insufficient mortality data.

[c]95% confidence limits.

The 96-hour no-observed effect concentration could be estimated at <10 mg/l.

The 96-hour slope of the dose-response line was 16 as calculated by least squares regression analysis.

All values were rounded to two significant figures following ABC S.O.P. #8.7.

LC_{50} calculated using:

(1) Binomial Method

(2) Moving Average Method

(3) Probit Method

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TABLE 3

Mortality Rates and Water Quality Measurements During the Acute Toxicity Test of E2566-2 to Fathead Minnow (*Pimephales promelas*)

Nominal Concentration (mg/l)	Percent Mortality Hours			Water Quality								
				0-hours			48-hours			96-hours		
	24	48	96	Temp. °C	D.O.[a] mg/l	pH[b]	Temp. °C	D.O. mg/l	pH	Temp. °C	D.O. mg/l	pH
Control	0	0	0	22	9.3	7.4	22	8.2	7.4	22	8.1	7.5
10	0	0	20	22	9.2	7.5	22	8.2	7.4	22	7.4	7.4
18	0	0	0							22	7.5	7.4
32	0	0	0	22	9.3	7.5	22	7.5	7.3	22	6.8	7.2
56	0	10	30							22	7.0	7.3
100	0	10	100	22	9.5	7.6	22	7.5	7.4			
180	0	50	100				22	7.4	7.3			
320	10	100	100	22	9.8	7.9						

[a] Dissolved oxygen concentrations - Dissolved Oxygen Probe (YSI Model 54).

[b] pH - pH Probe (Corning Model 476182) used with a Corning Model 125 pH and mV meter.

NOTE: Dissolved oxygen saturation corrected for altitude at the test temperature of 22°C is 8.4 mg/l.

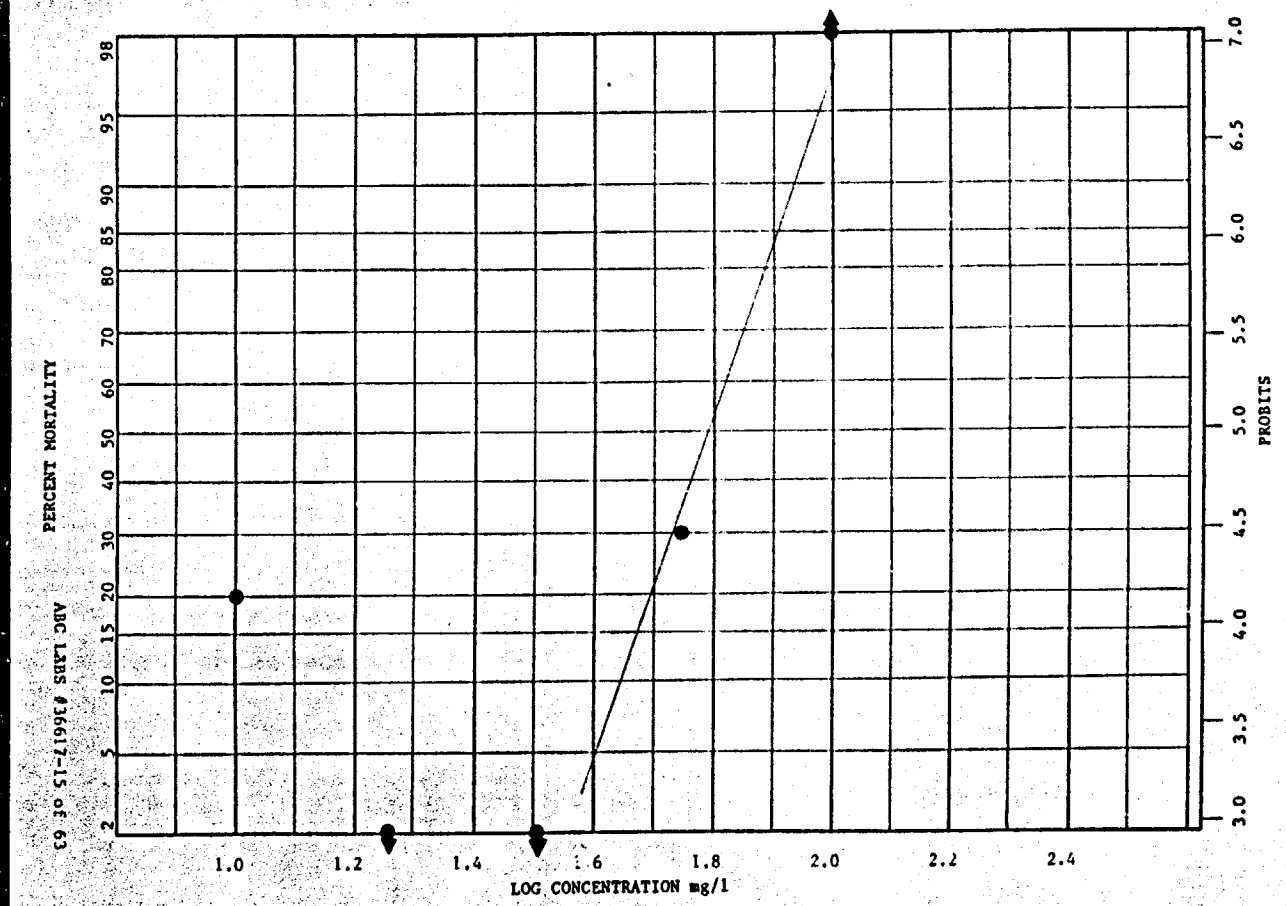
PERCENT MORTALITY

2 5 10 15 20 30 40 50 60 70 80 85 90 95 98

0002249

Figure 1

Slope of Dose-Response Line at 96 Hours for E2566-2 to Fathead Minnow (*Pimephales promelas*)



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(1) Committee on Methods for Toxicity Tests with Aquatic Organisms (C. E. Stephan, Chairman). 1975. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, April, 1975. 61 p.

(2) American Public Health Association. 1980. Standard Methods for the Examination of Water and Wastewater. 15th ed. Washington, DC. 1134 p.

(3) Brauhn, J. L. and R. A. Schoettger. 1975. Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegills. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-011, May, 1975. 45 p.

(4) Stephan, C. E., K. A. Busch, R. Smith, J. Burke and R. W. Andrew. 1978. A computer program for calculating an LC_{50}. U. S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript, August, 1978.

(5) Stephan, C. 1977. Methods for calculating an LC_{50}, p. 65-84. In F. L. Mayer and J. L. Hamelink (eds.). Aquatic Toxicology and Hazard Evaluation. ASTM Special Technical Publication 634. ASTM. Philadelphia.

(6) (a) U. S. Food and Drug Administration. 1978. Nonclinical Laboratory Studies, Good Laboratory Practice Regulations (21 CFR, Part 58). Federal Register, Vol. 43, No. 247:59986-60025.

(b) U. S. Environmental Protection Agency. 1983. Pesticide Programs; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). Federal Register, Vol. 48, No. 230: 53946-53969.

(c) U. S. Environmental Protection Agency. 1983. Toxic Substances Control; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 792). Federal Register, Vol. 48; No. 230:53922-53944.

(d) Organization for Economic Cooperation and Development. 1981. OECD Guidelines for Testing of Chemicals, Principles of Good Laboratory Practice Annex 2, C(81) 30(Final):7-28.

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QUALITY ASSURANCE STATEMENT

Quality Assurance Statement for final report #36617 entitled, "Acute Toxicity of E2566-2 to Fathead Minnow (Pimephales promelas)," for Mr. Mohamed Elnabarawy, 3M Company, St. Paul, Minnesota.

In accordance with ABC Laboratories' intent that all studies conducted at our facilities are designed and function in conformance with good laboratory practice regulations and the protocols for individual laboratory studies, an inspection of the final report for E2566-2 was conducted and found to be in an acceptable form by a member of our Quality Assurance Unit. An inspection of the daily mortality rate of the test organisms prior to the initiation of the study indicated they were in good health and should not bias the observed mortality in the study. A procedure audit was conducted on January 8, 1988. Results were reported to the study director on January 8, 1988. Results were reported to management on January 20, 1988. A final inspection of all data and records on January 20, 1988 indicated that the report submitted to you is an accurate reflection of the study as it was conducted by ABC Laboratories.

Should you have any questions relating to the information provided in this statement or the function of our Quality Assurance Unit, please contact the Quality Assurance Unit at your convenience.

Phillip M. Buckler 1/29/88

Phillip M. Buckler Date
Quality Assurance Supervisor

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STUDY COMPLIANCE STATEMENT

Study Compliance Statement for ABC report #36617 entitled, "Acute Toxicity of E2566-2 to Fathead Minnow (*Pimephales promelas*)," for Mr. Mohamed Elnabarawy, 3M Company, St. Paul, Minnesota.

In accordance with ABC Laboratories' intent that all aquatic toxicity tests conducted by our facility follow good laboratory practices, ABC's study director for the above test herein confirms that the study was conducted in compliance with the OECD Principles of Good Laboratory Practice Annex 2, C(81), OECD Guidelines for Testing of Chemicals.

All original raw data were provided to 3M Company, with a copy retained at Analytical Bio-Chemistry Laboratories.

Jane H. Bowman — Date
ABC Study Director

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Raw Data Report
#36617

Acute Toxicity of E2566-2 to Fathead
Minnow (Pimephales promelas)

NOTE: Some of the records which appear in this raw data appendix have been provided as photocopies of original records on file at ABC. This has been done by necessity for certain data which are used commonly in several studies at ABC. Such records include acclimation records, organism culture logs and well water chemical screen results.

ACUTE TOXICITY TO AQUATIC INVERTABRATES (DAPHNIA MAGNA)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, or FC-109-X. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-109-X referred to by the test laboratory as E2566-2. Current information indicates it is a mixture of 19-23% test substance, 61% water, 12% 2-methoxymethylethoxypropanol, 2% ethanol, and 2-6% C_4-C_7 fluoroalkyl carboxylate compounds. Test sample was from 3M production lot 2.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD:

Methods: OECD 202
Test type: Static acute
GLP: Yes
Year Completed: 1988
Species: *Daphnia magna*
Analytical monitoring: Temperature, pH, and DO

RESULTS

Nominal concentrations: Bk control, 1.0, 1.8, 3.2, 5.6, 10, 18, 32 mg/L
Element values: 24-hour LC_{50} = >32 mg/L (estimated)
24-hour NOEL = 32 mg/L
48-hour LC_{50} = 1.5 (1.0-1.8) mg/L
48-hour NOEL = 1.0 mg/L

All element values based on nominal concentrations.

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The value reported applies to that mixture and not the test substance. No attempt was made to determine the impact of the presence of the organic solvents or what portion of the toxicity can be contributed to the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

The FC-109-X 48-hour LC_{50} for *Daphnia magna* was determined to be 1.5 mg/L with a 95% confidence interval of 1.0-1.8 mg/L. The 48-hour no observed effect level was 1.0 mg/L.

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, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample.

REFERENCES

This study was conducted at Analytical Bio-Chemistry Laboratories, Inc. of Columbia, Missouri at the request of the 3M Company.

OTHER

Last changed: 5/18/00

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SPONSOR

3M Company
Attn: Mr. Mohamed Elnabarawy
EE & PC Environmental Labs
Building 21-2W-05, 900 Bush Avenue
St. Paul, Minnesota 55106

STUDY TITLE

Acute Toxicity of E2566-2 to
Daphnia magna

DATA REQUIREMENT

OECD Guideline 202

AUTHOR

Scott Frazier
Biologist II

STUDY COMPLETED ON

January 9, 1988

PERFORMING LABORATORY

Analytical Bio-Chemistry Laboratories, Inc.
Aquatic Toxicology Division
7200 East ABC Lane
P.O. Box 1097
Columbia, Missouri 65205

ABC LABORATORY PROJECT ID

#36616

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Submitted By: Analytical Bio-Chemistry Laboratories, Inc.
7200 East ABC Lane
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Prepared By:

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Michael Ruddy — Date
Biological Technician II

Scott Frazier — 1/15/88
Scott Frazier — Date
Biologist II/Study Director

Approved By:

Alan D. Forbis — 1/15/88
Alan D. Forbis — Date
Supervisor, Invertebrate/
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Kathy Landholt — 1/18/88
Kathy Landholt — Date
Quality Assurance Officer

Carl M. Thompson — 1-18-88
Carl M. Thompson — Date
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:dab28

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PROJECT PERSONNEL

The Study Director of the project for Analytical Bio-Chemistry Laboratories, Inc. was Scott Frazier, Biologist II. Supervising the conduct of the study for 3M Company was Mr. Mohamed Elnabarawy. The following ABC personnel assisted with various phases of the study.

Name	Title	Initials
Michael Ruddy	Biological Technician II	MR
Scott Frazier	Biologist II	SF
Jeffrey Latham	Biological Technician I	JL

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INVERTEBRATE ACUTE TOXICITY COMPENDIUM

Subject: "Acute Toxicity of E2566-2 to *Daphnia magna*," ABC Study Number 36616.

Sponsor: 3M Company

Testing Facility: Analytical Bio-Chemistry Laboratories, Inc.
Aquatic Toxicology Division
P.O. Box 1097
Columbia, Missouri 65205 (314-474-8579)

Location of Original Raw Data and Final Report:

3M Company
EE & PC Environmental Labs
Building 21-2W-05, 900 Bush Avenue
St. Paul, Minnesota

Test Material: E2566-2

Test Concentrations: Control, 1.0, 1.8, 3.2, 5.6, 10, 18 and 32 mg/l.

Dilution Water: 246 mg/l (hardness), 178 mg/l (alkalinity), 8.0 (pH).

Test Dates: Initiation - January 7, 1988

Termination - January 9, 1988

Length of Study: 48 hours

Results: 24-hour LC_{50} = >32 mg/l (estimated)

24-hour NOEL = 32 mg/l

48-hour LC_{50} = 1.5 mg/l (95% C.I. - 1.0-1.8 mg/l)

48-hour NOEL = 1.0 mg/l

Test Species: *Daphnia magna*

Source of Organisms: ABC Laboratories in-house culture.

Age of Organisms at Study Initiation: First-instar (≤24 hours old)

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SUMMARY

The acute toxicity of E2566-2 to *Daphnia magna* was assessed using the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms. Water quality parameters of temperature, dissolved oxygen and pH were measured at the termination of the test and were within acceptable limits.

The results of the 48-hour static *Daphnia magna* toxicity study are summarized below. All reported values were based upon nominal concentrations.

Compound	24-Hour LC_{50}	48-Hour LC_{50} (95% C.I.)
E2566-2	>32 mg/l (estimated)	1.5 mg/l (1.0-1.8 mg/l)

The 24 and 48 hour no-effect levels of E2566-2 based on the absence of mortality and abnormal effects were 32 and 1.0 mg/l, respectively.

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INTRODUCTION

This definitive static bioassay was performed by the Aquatic Toxicology Division of Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri, for 3M Company, from January 7, 1988 to January 9, 1988. The purpose of this test was to determine the 24- and 48-hour LC_{50} levels for E2566-2 to Daphnia magna. The study was performed using ABC Protocol #7806 as approved by Mr. Mohamed Elnabarawy, 3M Company, on January 7, 1988.

METHODS AND MATERIALS

The procedures for static bioassay, as described in Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1) and Standard Methods for Examination of Water and Wastewater (2), were used in this experiment.

I. Test Organisms

The Daphnia magna used in the test were cultured at the ABC facilities in well water (Table 1). The adult daphnids were fed algae (Selenastrum capricornutum) at least every three days prior to testing and supplemented with a suspension of Tetramin®, cereal leaves, vitamin solution and yeast. The daphnids were identified to species using the taxonomic key presented by Pennak (3).

II. Test Procedure

The static Daphnia bioassay was conducted in 250-ml glass beakers containing 200 ml of daphnid culture/test water. All test vessels were covered with loose-fitting petri dish covers to minimize evaporation and protect against contamination during the study. These vessels were kept at 20 (±2.0) °C in a temperature controlled area. The lighting was maintained at 50-70 footcandles on a 16-hour daylight photoperiod with 30-minute simulated dawn and dusk periods.

An initial range-finding experiment was conducted using ten Daphnia each in exposure concentrations of 1.0, 10, and 100 mg/l. From these results, seven treatment concentrations in duplicate with ten Daphnia (first instar less than 24 hours old) per beaker were selected for the definitive bioassay. These concentrations were a logarithmic series ranging from 1.0 to 32 mg/l and included a control. All concentrations were observed once every 24 hours for mortality and abnormal effects such as surfacing, clumping of the daphnids together and daphnids tending to the bottom of test chambers. Water chemistry parameters of temperature, dissolved oxygen and pH were measured in the control, low, middle and high concentrations at 0 and 48 hours.

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III. Test Compound

The E2566-2 sample was received on December 29, 1987 as a foamy, yellow liquid and was stored at room temperature in darkness. Sample purity was unspecified. Test concentrations were prepared based on total product. All standard weights and dilutions can be found in the Appendix. Daphnid culture/test water was used in the preparation of all working stock solutions.

IV. Statistics

Statistical analysis of the concentration vs. effect data (generally mortality) was obtained by employing a computerized LC_{50} program developed by Stephan et al. (4). This program calculated the LC_{50} statistic and its 95 percent confidence limits using the binomial, the moving average and the probit tests. Three different methods of analyzing the data were used since no one method of analysis is appropriate for all possible sets of data that may be obtained. The method of calculation selected for presentation in this report was that which gave the narrowest confidence limits for the LC_{50} (4, 5) although all three models are valid. However, if no mortality occurred or if a dose response could not be demonstrated over a reasonable range (<37 to >63%) an LC_{50} and/or its 95 percent confidence limits could not be calculated.

RESULTS

Table 2 presents the estimated and calculated LC_{50} values and 95-percent confidence intervals for E2566-2. The 24- and 48-hour LC_{50} values were >32 (estimated) and 1.5 mg/l, respectively. All results were based on the nominal concentrations of 1.0, 1.8, 3.2, 5.6, 10, 18 and 32 mg/l. The no-effect concentration based on the absence of mortality and abnormal effects was 32 mg/l at 24 hours. At 48 hours the no-effect level was 1.0 mg/l. The abnormal effect of mortality was observed in the 1.8, 3.2, 5.6, 10, 18 and 32 mg/l test concentrations. The 10, 18 and 32 mg/l test concentrations were observed at 24 and 48 hours to have a surface film and precipitate present.

Table 3 presents the percent mortality and water quality parameters measured during the test. The dissolved oxygen concentrations ranged between 8.7 and 9.5 mg/l. These values represented 100 and 107-percent saturation at 20 and 19 °C (corrected for local altitudinal pressure), respectively, and were considered adequate for testing (1). The pH values of the test solutions ranged from 8.2 to 8.4 and were consistent with the control.

The study was conducted following the intent of the Good Laboratory Practice Regulations (6) and the final report was reviewed by Analytical Bio-Chemistry Laboratories' Quality Assurance Unit. All original raw data were provided to 3M Company, with a copy retained at Analytical Bio-Chemistry Laboratories.

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TABLE 1

Chemical Characteristics of Well Water Used by ABC's Aquatic Toxicology Division.

Parameter	Concentration
Temperature	17 - 22°C[b]
Dissolved Oxygen[a]	7.4 - 9.1 ppm[b]
pH	7.6 - 8.4[b]
Hardness ($CaCO_3$)	206 - 275 ppm[b]
Alkalinity ($CaCO_3$)	224 - 336 ppm[b]
Conductivity	500 - 650 µmhos/cm[b]
Total Organic Carbon	1.4 ppm[c] (0.9 - 2.8 ppm)[d]
Suspended Solids	0 - 1.3 ppm[b]
Aluminum	<10 ppb
Arsenic	<1 ppb
Boron	2.25 ppm
Cadmium	<3 ppb
Chromium	<7 ppb
Cobalt	<3 ppb
Copper	<3 ppb
Iron	14 ppb
Lead	<30 ppb
Mercury	0.43 ppb
Nickel	<8 ppb
Silver	<4 ppb
Zinc	2 ppb
Measured organophosphorus pesticides	<0.14 ppb
Measured organochlorine pesticides plus PCB's	<1.0 ppb

[a]After aeration

[b]Represents seasonal variation, difference from one month to the next, will not exceed 10%

[c]Average of all well water values from January-September, 1987

[d]Total monthly range of organic carbon

Sample dates: Trace elements = June 3, 1987, organophosphate and organochlorine analyses = May 28, 1987.

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TABLE 2

Acute Toxicity of E2566-2
to *Daphnia magna*[a]

Compound	LC_{50} (mg/l) 24 hours	LC_{50} (mg/l) 48 hours
E2566-2	>32 (estimated)	1.5 (1)
	b	(1.0-1.8)[c]

[a]Bioassay conducted at 20°C (±2.0).

[b]95% confidence limits could not be established.

[c]95% confidence limits.

The 24- and 48-hour no-effect concentrations were 32 and 1.0 mg/l, respectively, based on the absence of mortality and abnormal effects.

All values were rounded to two significant figures following the technique provided in ABC S.O.P. #8.7.

LC_{50} calculated using:

(1) Binomial Method

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TABLE 3

Percent Mortality and Water Quality Measurements During the Acute Toxicity Test of E2566-2 to *Daphnia magna*.

Nominal Concentration (mg/l)	Percent Mortality		Water Quality					
			0 hours			48 hours		
	24 hr	48 hr	Temp.[a] °C	D.O.[b] mg/l	pH[c]	Temp. °C	D.O. mg/l	pH
Control	0	0	19	9.3	8.2	20	8.7	8.3
1.0	0	0	19	9.4	8.2	20	8.7	8.3
1.8	0	80						
3.2	0	100						
5.6	0	100	19	9.4	8.2	20	8.7	8.3
10	0	100						
18	0	100						
32	0	100	19	9.5	8.2	20	8.7	8.4

[a]Temperature - mercury thermometer.

[b]Dissolved oxygen concentrations - YSI 54 ARC dissolved oxygen meter.

[c]pH - Corning 140 pH/mV meter with Beckman 39831 electrode.

NOTE: Dissolved oxygen saturations at the test temperatures of 19 and 20°C are 8.9 and 8.7 mg/l, respectively (corrected for altitudinal pressure at ABC Labs).

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LITERATURE CITED

(1) Committee on Methods for Toxicity Tests with Aquatic Organisms. Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. 1975. U.S. EPA, Ecol. Res. Ser. 660/3-75-009.

(2) American Public Health Association. 1980. Standard Methods for the Examination of Water and Wastewater. 15th ed. Washington, DC. 1134 p.

(3) Pennak, R. W. 1978. Freshwater Invertebrates of the United States. John Wiley & Sons, New York, 2nd ed. 803 p.

(4) Stephan, C. E., K. A. Busch, R. Smith, J. Burke and R. W. Andrews. 1978. A Computer Program for Calculating an LC_{50}. U. S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript, August, 1978.

(5) Stephan, C. 1977. Methods for Calculating an LC_{50}, p. 65-84. In F. L. Mayer and J. L. Hamelink (eds.). Aquatic Toxicology and Hazard Evaluation. ASTM Special Technical Publication 634. ASTM. Philadelphia.

(6) Organization for Economic Cooperation and Development. 1981. OECD Guidelines for Testing of Chemicals, Principles of Good Laboratory Practice Annex 2, C(81) 30(Final):7-28.

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QUALITY ASSURANCE STATEMENT

Quality Assurance Statement for report #36616 entitled, "Acute Toxicity of E2566-2 to Daphnia magna," for Mr. Mohamed Elnabarawy, 3M Company, St. Paul, Minnesota.

In accordance with ABC Laboratories' intent that all studies conducted at our facilities are designed and function in conformance with good laboratory practice regulations and the protocols for individual laboratory studies, an inspection of the final report for E2566-2 was conducted and found to be in an acceptable form by a member of our Quality Assurance Unit. A procedure audit was conducted on December 14, 1987. The study director was notified of the results on the same day. The results of this audit were reported to ABC Laboratories' management on December 30, 1987. A final inspection of all data and records on January 14, 1988, indicated that the report submitted to you is an accurate reflection of the study as it was conducted by ABC Laboratories.

Should you have any questions relating to the information provided in this statement or the function of our Quality Assurance Unit, please contact the Quality Assurance Unit at your convenience.

Kathy Landholt 1/18/88
Kathy Landholt Date
Quality Assurance Officer

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STUDY COMPLIANCE STATEMENT

Study Compliance Statement for ABC report #36616 entitled, "Acute Toxicity of E2566-2 to *Daphnia magna*," for Mr. Mohamed Elnabarawy, 3M Company, St. Paul, Minnesota.

In accordance with ABC Laboratories' intent that all aquatic toxicity tests conducted by our facility follow good laboratory practices, ABC's study director for the above test herein confirms that the study was conducted in compliance with the OECD Guidelines for Testing of Chemicals, Principles of Good Laboratory Practice Annex 2, C(81) 30(Final):7-28.

All original raw data were provided to 3M Company, and a copy retained at Analytical Bio-Chemistry Laboratories.

Scott Frazier — Date
ABC Study Director

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APPENDIX - RAW DATA

NOTE: Some of the records which appear in this raw data appendix have been provided as photocopies of original records on file at ABC. This has been done by necessity for certain data which are used commonly in several studies at ABC. Such records may include analytical standard solution preparation sheets, organism culture logs and well water chemical screen results.

ACTIVATED SLUDGE RESPIRATION INHIBITION

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, or FC-109-X. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-109-X. Current information indicates it is a mixture of 19-23% test substance, 61% water, 12% 2-methoxymethylethoxypropanol, 2% ethanol, and 2-6% C_4-C_7 fluoroalkyl carboxylate compounds. Test sample was from 3M production lot 2.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD:

Methods: OECD 209
Test type: Static acute
GLP: No
Year Completed: 1987
Inoculum: Activated sludge mixed liquor collected from the City of St. Paul, Minnesota Wastewater Treatment Facility.
Inoculum mixed liquor suspended solids: 2.9 g/L
Inoculum holding: None – used the day it was obtained
Analytical monitoring: Dissolved oxygen concentrations
Exposure period: 30-minutes and 3-hours
Measurements: Dissolved oxygen depletion rate after 30-minutes and 3-hours of exposure.

RESULTS

Nominal concentrations: 2 Blank controls, 100, 180, 320, 560, 1000 mg/L
Element values: 30-minute EC_{50} = >1000 mg/L
3-hour EC_{50} = >1000 mg/L (5.9% inhibition at 1000 mg/L)

All element values based on nominal concentrations.

Control response: The largest percent difference between control uptake rates was 4.3%; thus the two controls were within 15% of each other.
Reference substance results: 3,5-dichlorophenol at a concentration of 10 mg/L induced 45% inhibition in respiration rate. This was within the normal range seen in this laboratory with this inoculum.

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The value reported applies to that mixture and not the test substance. No attempt was made to determine the impact of the presence of the organic solvents or what portion of the toxicity can be contributed to the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

The FC-109-X 3-hour EC_{50} for activated sludge respiration inhibition was determined to be >1000 mg/L.

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, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample. Because a mixture was tested, care must be taken when interpreting the results.

REFERENCES

This study was conducted by the 3M Company Environmental Laboratory, St Paul, MN, Lab Request number E2113.

OTHER

Last changed: 5/18/00

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LR E 2113
Date 10-30-87
Analyst SAB

ENVIRONMENTAL LABORATORY

ACTIVATED SLUDGE MIXED LIQUOR

Type Test OECD Activated Sludge Respiration Inhibition #209

Source Metro Waste Treatment Plant St Paul, MN

Date Obtained 10-30-87 Date Used 10-30-87

Mixed Liquor Suspended Solids (MLSS) 2.9 g/L Adjustment None g/L

pH Initial = 7.9, Final = 8.1

Comments

Test Substance

Sample Description FC-109-X (41-2601-0414-0 Lot 2) FC Surfactant

Sample pH highest conc, 1000 mg/L = 7.3 Adjustment None

Comments

Reference Substance

Sample Description 3,5-Dichlorophenol

Sample pH 7.6 Adjustment None

Comments Stock Solution, 500 mg/L

Exposure Conditions

Test Duration 3-Hours

Test Temperatur 19-20 °C

Comments Test run with LR E 2112

000275

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Lab Request No. E2113

Page 1 of 3

Environmental Lab – Work Sheet

Date: 10-30-87

Analyst: SJB

Sample Description
FC-109-X
(91 2601-0414 0 to 2)
FC Surfactant
Yellow Liquid

OECD Activated Sludge
Respiration Inhibition Test #209

Test Solutions Preparation

Test Conc mg/L	mL Sample (1) Added/500ml		mL DI water Added/500ml		mL Synthetic Sewage Feed Added/500ml		mL Microbial Inoculum Added/500ml		Total Mixture Volume mL
100	50	+	284	+	16	+	200	=	500
180	90	+	284	+	16	+	200	=	500
320	160	+	284	+	16	+	200	=	500
560	280	+	284	+	16	+	200	=	500
1000	500	+	284	+	16	+	200	=	500
Blank (2) Controls	0		284	+	16	+	200	=	500

(1) Individual weights. Apparently miscible with DI water.
Initial pH of test solns. ranged from 7.1 (lowest conc, 100 mg/L) to 7.3 (highest conc, 1000 mg/L).
No pH adjustment was made

(2) Control I is shared with LR E2112, as Control II

DI water used: Distilled 10-23-87, Aeration started 10-27-87, pH = 6.6

000276

LR E 2113

pg 2 of 3

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: FC-109-X (41-2601-0414-0 Lot 3) FC Surfactant

Source of Activated Sludge Mixed Liquor: Metro Waste Treatment Plant St Paul, MN

Date Received: 10-30-87 Date Used: 10-30-87

Analyst: Susan A. Beach

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30 - MINUTE EXPOSURE

DISSOLVED OXYGEN CONCENTRATIONS mg/L

Test Substance Concentration (mg/L)	TIME-MINUTES 0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	TEMP C°
CONTROL I	7.4	7.2	6.7	6.2	5.7	5.2	4.7	4.2	3.7	3.2	2.7	20
100	7.0	6.8	6.3	5.8	5.3	4.8	4.3	3.8	3.3	2.8	2.3	20
180	7.2	7.0	6.5	6.0	5.5	5.0	4.5	4.0	3.5	3.0	2.5	20
320	6.7	6.5	6.0	5.5	5.0	4.4	3.9	3.4	2.9	2.3	1.8	20
560	6.9	6.6	6.2	5.7	5.2	4.7	4.3	3.8	3.3	2.8	2.3	20
1000	4.6	4.2	3.7	3.2	2.7	2.1	1.6	1.1	0.6	0.57 / 0.11		20
CONTROL II	7.1	6.8	6.4	5.9	5.5	5.0	4.5	4.1	3.6	3.2	2.7	19

3 - HOUR EXPOSURE

DISSOLVED OXYGEN CONCENTRATIONS mg/L

Test Substance Concentration (mg/L)	TIME-MINUTES 0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	TEMP C°
CONTROL I	7.0	6.8	6.2	5.7	5.1	4.6	4.0	3.5	2.9	2.4	1.9	20
100	7.4	7.2	6.7	6.2	5.7	5.2	4.7	4.1	3.6	3.1	2.6	20
180	7.5	7.4	7.0	6.5	6.0	5.6	5.1	4.6	4.1	3.6	3.1	20
320	7.6	7.5	7.1	6.6	6.1	5.5	5.0	4.5	4.0	3.4	3.0	20
560	7.2	6.9	6.5	5.9	5.4	4.9	4.3	3.8	3.3	2.7	2.2	20
1000	7.1	6.9	6.4	6.0	5.5	5.0	4.5	3.9	3.4	2.8	2.3	20
CONTROL II	7.5	7.2	6.7	6.1	5.6	5.1	4.5	4.0	3.4	2.8	2.3	20

pH of Activated Sludge Mixed Liquor: Initial 7.9 Final 8.1

pH of highest concentration tested: Initial 7.4 Final 7.8

Test Temperature: 19-20°C

000277

See pg 1 for test soln. prep

ENVIRONMENTAL LABORATORY

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
CALCULATION OF RESULTS

LR E2113
Date 10-30-87
Analyst SJB

Test Substance EC-109-X (41-2601-0414-C Lot 2) EC Surfactant

pg 3 of 3

30 - MINUTE EXPOSURE

Test Substance Concentration (mg/L)	Oxygen Uptake Rate R	R Corrected[1] to 20°C	% Inhibition in Respiration Rate[2]
CONTROL I	0.5000	0.5000	—
100	0.5000	0.5000	—
180	0.5000	0.5000	—
320	0.5242	0.5242	—
560	0.4794	0.4794	2.1
1000	0.5184	0.5184	—
CONTROL II	0.4576	0.4791	—

Percent Difference in Controls[3] 4.3%

EC_{50} (95% Confidence Limits) mg/L > 1000 mg/L

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3 - HOUR EXPOSURE

Test Substance Concentration (mg/L)	Oxygen Uptake Rate R	R Corrected[1] to 20°C	% Inhibition in Respiration Rate[2]
CONTROL I	0.5461	0.5461	—
100	0.5145	0.5145	5.9
180	0.4800	0.4800	12
320	0.5133	0.5133	6.1
560	0.5291	0.5291	3.2
1000	0.5145	0.5145	5.9
CONTROL II	0.5473	0.5473	—

Percent Difference in Controls[3] 0.2%

EC_{50} (95% Confidence Limits) mg/L > 1000 mg/L

(1) Temp. Correction $= \frac{R_{T,obs}}{\theta^x}$ (mg O_2/L-min.)

(2) % Inhibition $= \frac{\left(\frac{RC_1 + RC_2}{2}\right) - R_S}{\left(\frac{RC_1 + RC_2}{2}\right)} \times 100$

(3) % Difference $= \frac{|RC_1 - RC_2|}{\left(\frac{RC_1 + RC_2}{2}\right)} \times 100$

The two control respiration rates should be within 15% of each other

where:
R = Oxygen uptake rate (mg O_2/L-min.)
T,obs = Sample temp., C°
θ = 1.047, x = T,obs - 20°C
C = Control

See pg 1. for test Solution prep.

Form 10620 8-8 PWO

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Lab Request No. E2112 + E2113

Environmental Lab – Work Sheet

Page 1 of 3

CECD Activated Sludge Respiration Inhibition Test #209

Date: 10-30-87

Analyst: SAB

Sample Description
3,5-Dichlorophenol
Reference Substance
Aldrich Red Label
Lot
purple crystals

Stock + Test Solutions Preparation

Test Stock Solution: 500 mg/L Each 1ml = 0.5 mg

- Dissolved 500 mg 3,5-DCP in 10 mL 1.0 N NaOH
- Diluted to 300 mL with DI water
- Added, under stirring, 1.0 N H_2SO_4 dropwise until incipient ppt
- Diluted to 1 L with DI water.

Initial pH of stock solution = 7.6 After 24 Hrs, pH = 7.6

Test Solutions:

Test Conc mg/L	mg/500ml	ml Stock Solution Added/500ml		ml DI water Added/500ml		ml Synthetic Sewage Feed Added/500ml		ml Microbial Inoculum Added/500ml		Total Mixture Volume ml
10	5	10	+	274	+	16	+	200	=	500
Blank (1) Controls	0	0	+	284	+	16	+	200	=	500

(1) Controls Shared with LB E2113

DI water used: Distilled 10-23-87, Aeration Started 10-27-87 pH = 6.6

000279

LR E2112 + E2113

Pg 2 of 3

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: 3,5-Dichlorophenol Reference Substance

Source of Activated Sludge Mixed Liquor: Metro Waste Treatment Plant St Paul, MN

Date Received: 10-30-87 Date Used: 10-30-87

Analyst: Susan A Beach

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30 - MINUTE EXPOSURE

DISSOLVED OXYGEN CONCENTRATIONS mg/L

Test Substance Concentration (mg/L)	TIME-MINUTES 0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	TEMP C°
CONTROL I	7.4	7.2	6.7	6.2	5.7	5.2	4.7	4.2	3.7	3.2	2.7	20
10	7.3	7.1	6.8	6.4	6.0	5.7	5.3	4.9	4.5	4.2	3.8	20
CONTROL II	7.1	6.8	6.4	5.9	5.5	5.0	4.5	4.1	3.6	3.2	2.7	19

3 - HOUR EXPOSURE

DISSOLVED OXYGEN CONCENTRATIONS mg/L

Test Substance Concentration (mg/L)	TIME-MINUTES 0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	TEMP C°
CONTROL I	7.0	6.8	6.2	5.7	5.1	4.6	4.0	3.5	2.9	2.4	1.9	20
10	8.1	8.1	7.8	7.5	7.2	6.9	6.6	6.2	5.9	5.6	5.3	21
CONTROL II	7.5	7.2	6.7	6.1	5.6	5.1	4.5	4.0	3.4	2.8	2.3	20

pH of Activated Sludge Mixed Liquor: Initial 7.9 Final 8.1

pH of highest concentration tested: Initial 7.4 Final 7.9

Test Temperature: 19-21°C

000280

See pg 1 for Test Soln. prep.

ENVIRONMENTAL LABORATORY

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
CALCULATION OF RESULTS

LR 22113 + 22112
Date 10-30-87
Analyst SHB

Test Substance 3,5-Dichlorophenol Reference Toxicant

Pg 3 of 3

30 - MINUTE EXPOSURE

Test Substance Concentration (mg/L)	Oxygen Uptake Rate R	R Corrected[1] to 20°C	% Inhibition in Respiration Rate[2]
CONTROL I	0.5000	0.5000	—
10	0.3703	0.3703	24
CONTROL II	0.4576	0.4791	—

Percent Difference in Controls[3] 4.3%

EC_{50} (95% Confidence Limits) mg/L 10 mg/L induced 24% inhibition

3 - HOUR EXPOSURE

Test Substance Concentration (mg/L)	Oxygen Uptake Rate R	R Corrected[1] to 20°C	% Inhibition in Respiration Rate[2]
CONTROL I	0.5461	0.5461	—
10	0.3145	0.3004	45
CONTROL II	0.5473	0.5473	—

Percent Difference in Controls[3] 0.2%

EC_{50} (95% Confidence Limits) mg/L 10 mg/L induced 45% inhibition

(1) Temp. Correction $= \frac{R_{T,obs}}{\theta^x}$ (mg O_2/L-min.)

(2) % Inhibition $= \frac{\left(\frac{RC_1 + RC_2}{2}\right) - R_S}{\left(\frac{RC_1 + RC_2}{2}\right)} \times 100$

(3) % Difference $= \frac{|RC_1 - RC_2|}{\left(\frac{RC_1 + RC_2}{2}\right)} \times 100$

The two control respiration rates should be within 15% of each other

Where:
R = Oxygen uptake rate (mg O_2/L-min.)
T,obs = Sample temp., C°
θ = 1.047, x = T,obs - 20°C
C = Control

See pg 1 for stock + test soln. prep.

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MICROBICS MICROTOX® TOXICITY TEST

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, or FC-109-X. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-109-X. Current information indicates it is a mixture of 19-23% test substance, 61% water, 12% 2-methoxymethylethoxypropanol, 2% ethanol, and 2-6% C_4-C_7 fluoroalkyl carboxylate compounds. Test sample was from 3M production lot 2.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD:

Method: Microbics Microtox® "BASIC" Procedure
Test type: Static acute
GLP: No
Year Completed: 1987
Species: *Photobacterium phosphoreum*
Analytical monitoring: None
Statistical methods: EC_{20}, EC_{50}, and EC_{80} values calculated by statistical linear regression program provided for Microbics Microtox® system.
Test organism source: Microbics Microtox® reagent
Test conditions: As per Microbics Microtox method
Replicates: Two

RESULTS

Nominal concentrations: Blank control, 125, 250, 500, 1000 mg/L
Element value: 5-minute EC_{20} = 170 mg/L
5-minute EC_{50} = 650 (560-750) mg/L
5-minute EC_{80} = >1000 mg/L
15-minute EC_{20} = 140 mg/L
15-minute EC_{50} = 410 (320-530) mg/L
15-minute EC_{80} = >1000 mg/L

All element values based on nominal concentrations.

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The value reported applies to that mixture and not the test substance. No attempt was made to determine the impact of the presence of the organic solvents or what portion of the toxicity can be contributed to the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

The FC-109-X 15-minute EC_{50} for *Photobacterium phosphoreum* was determined to be 410 mg/L with a 95% Confidence Interval of 320-530 mg/L.

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, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample.

REFERENCES

This study was conducted by the 3M Company Environmental Laboratory, St Paul, MN, Lab Request number E2113.

OTHER

Last changed: 5/18/00

E2113

Toxicity of FC-109X (41-2601-0414-0 Lot 2) SAB

To Microbial Systems

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Microtox®

Test Conc. mg/L	Percent Light Loss 5-MIN EXP	15-MIN EXP
125	19	25
250	25	28
500	41	48
1000	63	79
EC_{20}	170	140
EC_{50} (95% CI)	650 (560-750)	410 (320-530)
EC_{80}	>1000	>1000

OECD Activated Sludge Respiration Inhibition Test (#209)

Test Conc. mg/L	Percent Inhibition 30-MIN EXP	3-HR EXP
100	—	5.9
180	—	12
320	—	6.1
560	2.1	3.2
1000	—	5.9
EC_{50} (95% CI)	>1000	>1000

LR E 2113

pg 1 of 2

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ENVIRONMENTAL LABORATORY

MICROBICS MICROTOX[R] SYSTEM

RAW DATA

TEST SUBSTANCE: FC-109-X (41-2601-0414-0 Lot 2) FC Surfactant

TEST DATE: 10-15-87

ANALYST: Susan A. Beach

SAMPLE CUVETTE	FINAL ASSAY CONCENTRATION mg/L	INITIAL READING I(0)	FINAL READINGS 5-MIN. I(5)	15-MIN. I(15)	30-MIN. I(30)
B1	BLANK CONTROL	90	68	55	
C1	DUPLICATE	99	76	61	
B2	125	99	62	46	
C2	Duplicate	100	62	46	
B3	250	97	57	44	
C3	Duplicate	96	54	41	
B4	500	94	43	31	
C4	Duplicate	100	44	31	
B5	1000	94	27	12	
C5	Duplicate	99	28	13	

Test Substance: (Yellow Liquid) Stock Solution, 500 mg/250 ml DI water. Each 1 ml = 2 mg. Apparently miscible with water. Stock solution has faint color; color not apparent at highest conc. tested, 1000 mg/L. Initial pH = 9.3, adjusted to pH 6.7 with 1N H_2SO_4. After 24 Hrs, pH = 6.7 Osmotic adjustment: Added 200 mg NaCl to 10 ml pH adjusted sample.

Diluent: Microbics 2% NaCl Solution

000285

Initial Dilutions: Initial concentrations were double the final assay concentrations mentioned above. The initial dilutions were introduced into the top row (Row A) and were then transfered (50/sc) into the middle and the lower rows (Rows B & C).

DI water used: Distilled 10-9-87, aeration started 10-11-87, pH = 6.6

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MICROBICS MICROTOX[R] SYSTEM

MICROTOX[R] DATA REDUCTION

FC-109-X (41-2601-0414-0 Lot 2)
FC Surfactant

LR E 2113
Date 10-15-87
Analyst SLB
pg 2 of 2
See pg 1 for stock solution prep.

BLANK RATIOS* = $\frac{\text{Final}}{\text{Initial}}$

BLANK CUVETTE	5-MIN. BLANK RATIO	5-MIN. DIFFERENCE	15-MIN. BLANK RATIO	15-MIN. DIFFERENCE	30-MIN. BLANK RATIO	30-MIN. DIFFERENCE
B1	68/90=0.756	0.012	55/90=0.611	0.005		
C1	76/99=0.768		61/99=0.616			

*The blank ratios for B1 and C1 should agree within 0.02.

MEAN BLANK RATIOS = $\frac{\text{B1 BK RATIO + C1 BK RATIO}}{2}$

5-MIN.	15-MIN.	30-MIN.
0.762	0.614	

NORMALIZED LIGHT LOSS (ΓEFFECT) = $\frac{\text{Mean Blank Ratio x Initial Sample Reading}}{\text{Final Sample Reading}}$ - 1

SAMPLE CUVETTE	5-MIN. Γ	15-MIN. Γ	30-MIN. Γ
Test Concentration	0.217	0.321	
B2 C2 125 mg/L	0.229	0.335	
B3 C3 250	0.297 0.355	0.354 0.438	
B4 C4 500	0.666 0.732	0.862 0.981	
B5 C5 1000	1.653 1.694	3.810 3.676	

% LIGHT LOSS (%Δ) = $\frac{\Gamma}{1+\Gamma}$ X 100

SAMPLE CUVETTE	5-MIN. %Δ	15-MIN. %Δ	30-MIN. %Δ
Test Concentration	18	24	
B2 C2 125 mg/L	19	25	
B3 C3 250	23 26	26 30	
B4 C4 500	40 42	46 50	
B5 C5 1000	62 63	79 79	

EFFECTIVE CONCENTRATIONS (EC's) mg/L (Calculated using "Γ,C" data pairs.)

EC'S	5-MIN.	15-MIN.	30-MIN.
EC_{20}	166	144	
EC_{50} (95% Confid. Limits)	649 (562-750)	413 (321-532)	
EC_{80}	>1000	>1000	

286

BIODEGRADATION (WARBURG)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, or FC-109-X. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-128. Current information indicates it is a mixture of 86% test substance and 14% C_4-C_7 fluoroalkyl carboxylate compounds.

METHOD:

Method: Warburg Determination.
Type: Aerobic
GLP: No
Year completed: 1976
Contact time (units): 7 hours
Inoculum: Activated sludge mixed liquor collected from the Metropolitan Wastewater Treatment Plant, St. Paul, Minnesota. Liquor was washed, suspended in a basal salts medium, and used at a concentration of 2000 mg of biological solids per liter.
Analytical monitoring: Oxygen uptake
Temperature: 30°C
Test Concentrations: 1667 mg/L glucose control, Endogenous control, 20 mg/L test substance and 1667 mg/L glucose combined solution, 20, 100 and 500 mg/L test substance solutions, and 500 mg/L and 1667 mg/L glucose combined solution,
Test Solution Preparation: Dissolved 1.5 grams of FC-128 in 500 mL of DI water to make a 3000 mg/L stock solution. A 5 mL aliquot of the stock solution was added to 20 mL of DI water to make a 600 mg/L sample. A 5 mL aliquote of the 600 mg/L sample was added to 20 mL of DI water to make a 120 mg/L sample. One half mL aliquots of one of the above 3000, 600 and 120 mg/L solutions is then added to 2 mL of the mixed liquor plus 0.5 mL DI water or 0.5 mL glucose control solution to make the final test substance concentrations of 500, 100, and 20 mg/L.

Remarks: Described method lacks specificity on test procedures.

RESULTS

Approximately 70% of the theoretical maximum oxygen uptake occurred within the 7 hour experimental period if biodegradation followed the theoretical degradation route:

$$C_8F_{17}SO_2N(C_2H_5)CH_2COO^- K^+ + 4.25O_2 + H^+ \rightarrow C_8F_{17}SO_2NH_2 + 4CO_2 + 3H_2O + K^+$$

Remarks: Oxygen uptake appeared to be continuing at the end of the experiment.

CONCLUSIONS

This testing indicated that the hydrocarbon moiety of the test substance potentially biodegraded in aerobic environments capable of supporting biodegradation.

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

DATA QUALITY

Reliability: Klimisch ranking 3. Testing indicated a high level of biodegradation, but only for a theoretical partial degradation of the compound. Sample purity was not properly characterized. Impurities may have significantly affected recorded values. Test method, raw data, and an accurate description of the procedure followed during testing are lacking specificity required to insure validity of the study.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, 1976.

OTHER

Last changed: 5/16/00

Form 0Y47

TO:
Technical Communications Center, 201-25

MICROFORM COPIES:

3M TECHNICAL REPORT SUMMARY

DATE: August 12, 1976

Environmental LABORATORY, DEPT. NUMBER 0222

Title
Biodegradation Studies of Fluorocarbons

Project:
Fate of Fluorochemicals in the Environmental

Project Number: 75-6398-29

Report No: (3 digits)

To:
R. L. Bohon

By:
E. A. Reiner

Employee Number: 47816

Objective:
To determine the biodegradability of selected 3M fluorocarbon compounds.

Notebook Reference:
40671
Pgs. 29-37, 41-50

No. of pages including coversheet: 12

SECURITY
- [ ] Company Confidential(Open)
- [ ] Special Authorization(Closed)

IF SUMMARY REPORT
Has information in this report been covered by other reports submitted to TCC?
- [X] No
- [ ] Partially
- [ ] Completely

Please keyword information not included in other reports and give page numbers of new material:

3M CHEMICAL REGISTRY
New chemicals reported?
- [X] No
- [ ] Yes

KEYWORDS
Select general, specific, and 3M product terms from 3M Thesaurus. Enclose suggested terms in parentheses.

EE & PC - Div.
Environ - Assess
Biology
Bacteria
Bioscreening
Fluorochemical
Biodegradable

ABSTRACT and Conclusions. (System can accommodate 200-250 words)

Biodegradation studies using a Warburg respirometer were conducted on FC-95, FM 3422, FC-128, and hydrogen analogs of FC-95 and FM 3422. No biodegradability was observed on FC-95, although an approximate hydrogen analog of FC-95 was readily degradable. FM 3422 and FC-128 both were demonstrated to undergo some biodegradation. Attempts to isolate degradation products of FM 3422, from the Warburg studies, and from a subsequent activated sludge study were unsuccessful.

SPECIFIC PROBLEMS remaining to reach objective.

Continued attempts will be made to isolate and identify the biodegradation products of FM 3422 and other 3M fluorocarbons. Work with radioactivity labeled FM 3422 is being considered.

Information Scientist
Initials

BIODEGRADATION STUDIES OF FLUOROCARBONS

SUMMARY AND RECOMMENDATION

No biodegradation was observed in Warburg studies on FC 95. Biodegradation of FC 95 is improbable because it is completely fluorinated. The resistance of this compound to biodegradation by an acclimated microbial culture, however, has not yet been demonstrated.

Warburg studies on FM 3422 and FC 128 both indicated that some biodegradation occurred. The products of this biodegradation are not known. Semicontinuous activated sludge studies on FM 3422 did not confirm or disprove the Warburg findings.

Future investigation of the biodegradability of the fluorocarbon compounds would be greatly facilitated by the development of an analytical procedure for FC 95. Warburg studies using purified FC 128 should be made to confirm the present findings.

Studies on the biodegradability of FM 3422 were hindered by its low water solubility. This problem could be overcome using FM 3422 radioactivity labeled on its hydrocarbon portion provided this material had a high specific activity (>5 mci/m mole) and purity. Biodegradation of a saturated solution of the labeled compound could be measured by detecting $^{14}CO_2$ evolution.

INTRODUCTION

The susceptibility to microbial modification is an important parameter in the study of the environmental fate of any class of compounds. It is the most important form of degradation for organic compounds.

A vast array of organic compounds can be completely degraded by microorganisms. So vast in fact that it was once believed by some that given enough time and the proper conditions, microorganisms could degrade any organic material. This doctrine of microbial infallibility is still a common misconception(1).

Perfluorinated compounds are extremely resistant to biodegradation (2). Although compounds with single fluorines have been shown to release fluoride ions as a result of biodegradation, perfluorinated compounds have rarely or never been shown to undergo natural degradation. For this reason, no modification of the perfluoro components of compounds in this study was anticipated. However, modification of its hydrocarbon components seemed possible.

An understanding of the partial degradation products is important since the environment will be exposed to these products in addition to the undegraded materials.

METHODS AND MATERIALS

Chemicals

The chemicals used in these experiments are shown in Table I.

TABLE I. CHEMICALS USED IN BIODEGRADATION EXPERIMENTS

FM 3422	$C_8F_{17}SO_2N(C_2H_5)C_2H_4OH$
Hydrogen Analog of FM 3422	$C_8H_{17}SO_2N(C_2H_5)C_2H_4OH$
FC 95	$C_8F_{17}SO_3K$
Sipex-ols	$C_8H_{17}OSO_3Na$
FC 128	$C_8F_{17}SO_2N(C_2H_5)CH_2COOK$

They were obtained from Don Ricker of the Commercial Chemical Division in September, 1975. FM 3422 (N-et Fose alcohol) was identified as 788 CC 745-2. The FC 95 used was from lot 583. Lot numbers were not given for the FM 3422 hydrogen analog or the sipex-ols (RM 26442).

These chemicals were selected for a number of reasons. FC 95 is essentially the fluorocarbon constituent of a large number of 3M fluorocarbon compounds. FM 3422 is an intermediate in the production of 3M fluorocarbons, and FC 128 is a finish fluorocarbon product. Sipex-ols and the Hydrogen Analog of FM 3422 were selected for comparison to the fluorocarbons. Sipex-ols is an approximate hydrogen analog of FC 95. These hydrogen analogs were tested because biologically labile fluorocarbons have frequently been found to be gratuitously defluorinated by enzymes which normally remove a hydrogen. Thus, it seemed probable that microbial growth on hydrogen analogs could select populations of organisms which could more completely degrade fluorocarbons.

WARBURG DETERMINATION

Warburg studies were conducted according to the attached standard procedures. (Attachment) Microogranisms were collected from the mixed liquor of the Pigs Eye treatment system,washed, and suspended in a basal salts medium and used at a concentration of 2000 mg of biological solids per liter.

Water insoluble substrates were emulsified in water prior to addition to the Warburg flasks. Emulsions were made using a Blackstone model EP-2 ultrasonic probe, base 1/2 inch, at 100% power. Logarithmic dilutions in water were made of the test substrates, and 1/2 ml was placed in the first side arm of the Warburg flasks. Controls contained 1/2 ml of 10 g/l glucose solution or deionized water in this side arm. The second side arm contained either glucose or deionized water.

Oxygen uptake was first observed in each flash for a period up to 1.5 hrs. with readings at 10-15 min. intervals to establish the endogenous activity. This was followed by addition of the first side arm and continued oxygen monitoring for approximately 2 hrs. Addition of the second side arm containing glucose, a readily degradable material, allowed a further evaluation of the toxicity of the previously added material.

Semicontinuous Activated Sludge Studies

A week-long semicontinuous activated sludge (SCAS) study was conducted on FM 3422. The microorganisms used were obtained, as before, from Pigs Eye Treatment Plant. One Hundred Fifty ml of activated sludge was added to 3 SCAS reactors and tap water as a control to a fourth. FM 3422 was added to 3 reactors below the water surface in 1/2 ml of absolute alcohol. Each addition increased the FM 3422 concentration by 33 mg/l. Pure ethanol was added to one sludge-containing reactor as a control. The operation of the semicontinuous reactors is shown in Figure 1.

The SCAS reactors were aerated for 23 hrs. with 500 ml/min. of air while the contents of each reactor were stirred with a magnetic stirrer to prevent settling. After the aeration period, the sludge was settled for an hour and one liter of supernatant was replaced with primary effluent from the Pigs Eye Plant.

FM 3422 was added at the beginning of the Test Cycles 1, 2, and 4. Samples were taken at the start and end of each test cycles and from the supernatant after settling.

The aeration chambers used in the SCAS studies were plexiglass cylinders 13" high with a 4" internal diameter. A side arm allowed drainage of the supernatant leaving the 500 ml with the settled sludge undisturbed.

Analytical

Samples taken at the termination of the first Warburg study on FM 3422 were evaluated by thin layer chromatography (Central Research analytical work req. No. A59412). The samples were extracted into dichloromethane, dried to a small volume, and separated on Woelm silica plates. The developing solvent system was 10:90 ethanol, chloroform (V:V). The developed plates were visualized by the iodine starch technique and compared to known standards with a detection limit of one μg of FM 3422.

Samples for the SCAS study were extracted into n-octanol and separated by gas chromatography with an electron capture detector. Extractions were performed in capped 50 ml polypropylene centrifuge tubes and phases separated by centrifuging at 26,700XG for 10 minutes.

RESULTS AND DISCUSSION

Warburg - FM 3422

Results from the Warburg study on FM 3422 are summarized in Figure 2. This experiment was performed by first sonicating FM 3422 and its analog in water to make emulsions of approx. 24,000 mg/l of the FM 3422 and 11,000 mg/l of the FM 3422 analog. Since FM 3422 and its hydrogen analog are not very soluble in water, it was felt that forming an emulsion would put more of these compounds in contact with the microorganisms in the Warburg study.

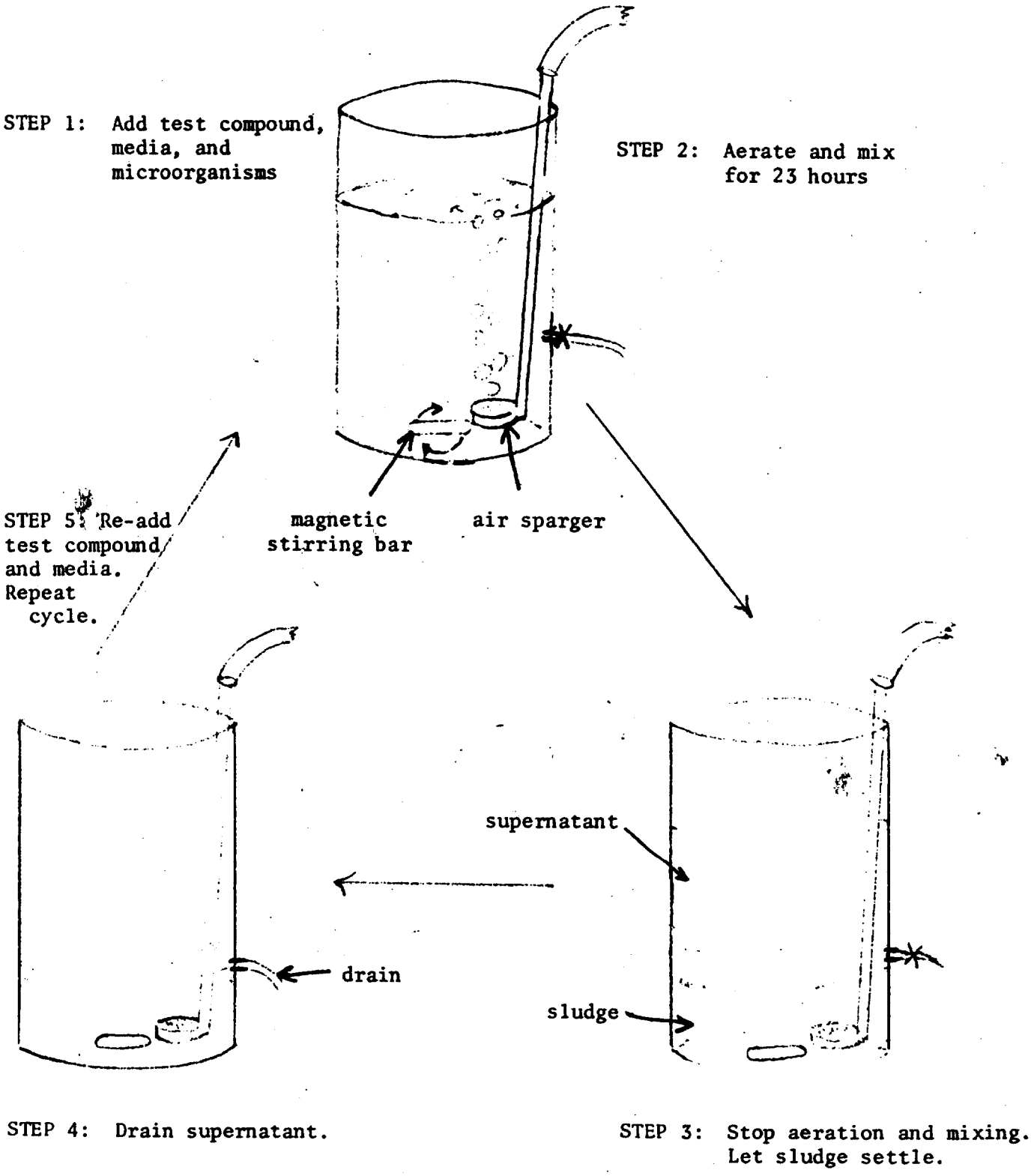

FIGURE 1: Test cycle for semicontinuous activated sludge reactor.

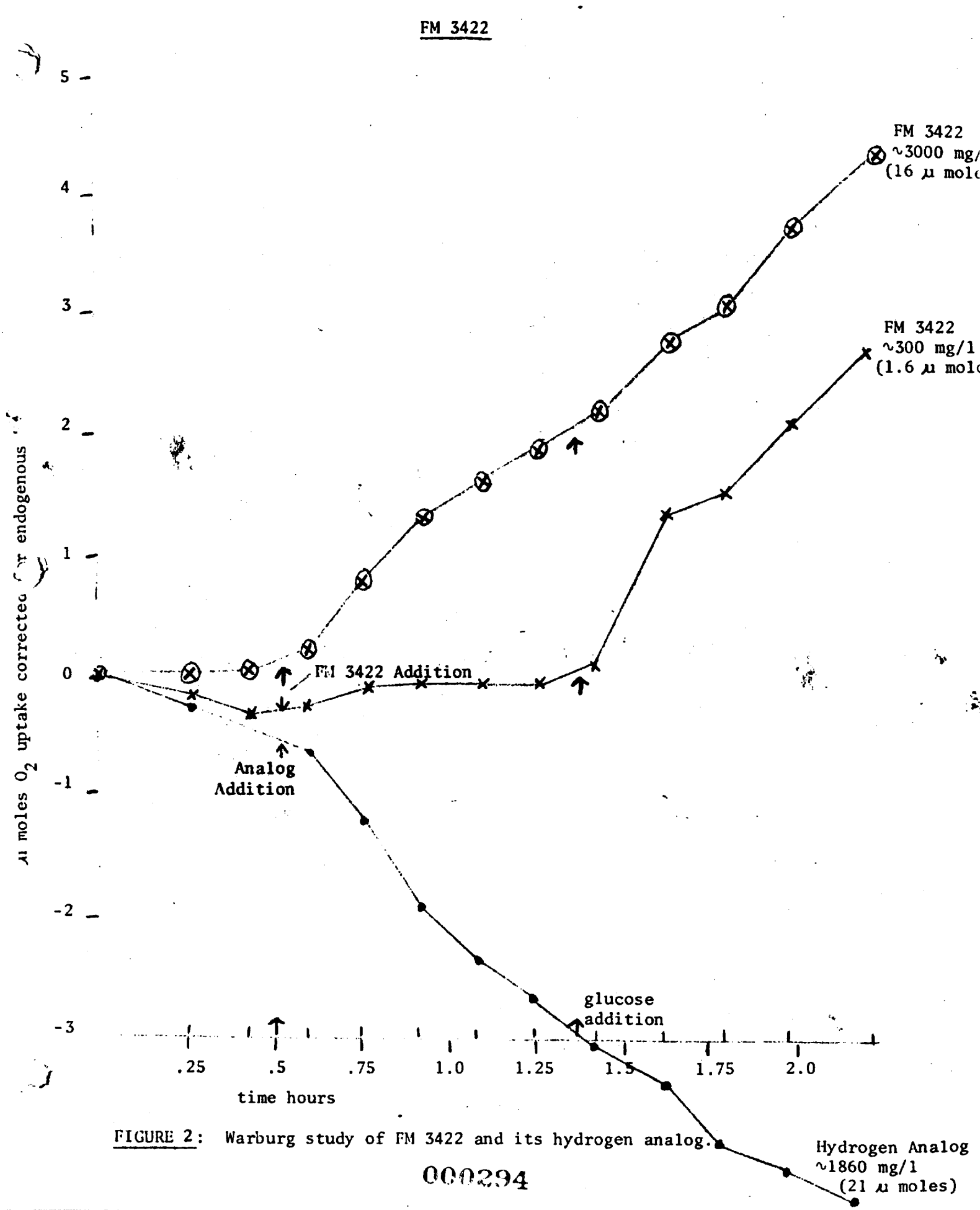

FIGURE 2: Warburg study of FM 3422 and its hydrogen analog.

While the FM 3422 analog was relatively easily emulsified and stable once emulsified, the FM 3422 was not. Approximately one hour was required to put 75% of the FM 3422 into emulsion, and this material proceeded to slowly come back out of emulsion. In about two to three hours, excess FM 3422 emulsion,which had not been used in the experiment, turned into a semi-solid gel.

Complete chemical oxidation of the hydrocarbon component of the FM 3422 at the highest concentration (∿16 μ moles) would require 87 μ moles of O_2 based on the following equation:

$$C_8F_{17}SO_2N(C_2H_5)C_2H_4OH + 5.5O_2 \rightarrow C_8F_{17}SO_2NH_2 + 4CO_2 + 4H_2O$$

Microbial oxidation rarely exceeds 60% of the chemical oxidation. In this experiment, only 2-3 micro moles of oxygen uptake was observed. However, oxygen uptake was continuing at the end of this experiment. Addition of glucose to the FM 3422 culture also produced increased oxygen uptake, confirming that the FM 3422 emulsion was not inhibitory to the microbial culture.

On the other hand, the hydrogen analog of FM 3422 showed significant toxicity. Upon addition of the most concentrated emulsion of the analog, endogenous oxygen uptake ceased and was not restored even after the addition of glucose to the culture. The negative slope of the hydrogen analog's oxygen uptake curve (Figure 2) is due to the endogenous correction and not oxygen evolution. Similar results were obtained in a second Warburg experiment with FM 3422 and its hydrogen analog.

Analysis of FM 3422 has shown it to be quite pure. The oxygen uptake observed was greater than would be expected from impurities in the compound. It is conceivable that sonication produced degradation products that were biodegradable, but not detectable by thin layer chromatography. It is also possible that some of the hydrocarbon components of FM 3422 molecule were degraded. However, using thin layer chromatography we were unable to detect any materials formed as a result of the biodegradation of FM 3422. It is not known if the hydrogen analog of FM 3422 itself is toxic. Thin layer and gas chromatography showed this material to be impure. Gas chromatograph* showed the analog to be 90% pure with two major contaminants. The contaminants may have been the cause of the observed toxicity.

SCAS - FM 3422

The semicontinuous activate sludge (SCAS) study was a second attempt to isolate the hypothesized degradation products of FM 3422. This study was conducted over a period of 1 week with samples taken at the initiation and end of each 24-hr. cycle. The FM 3422 samples added in an ethanol solution rapidly separated from the liquid phase, and as a result may have had too small a surface area to allow significant microbial degradation.

n-octanol extracts of the samples were analyzed by gas chromatography. No new leaks were formed as a result of exposure of the FM 3422 to the microorganisms. If some of the FM 3422 had been degraded to the sulfonic acid, it would not have been detected. The sulfonic acid is not sufficiently volatile to pass through the gas chromatography column.

* performed by Commercial Chemicals Division

The n-octanol extracts could not be separated by thin layer chromatography because of the low volatility of this solvent. Frozen nonextracted samples still exist at this date and could be extracted into a more volatile solvent for thin layer analysis.

Three additions of FM 3422 in 33 ppm increments were made during the SCAS experiment. The FM 3422 settled with the solids and for the most part remained in the reactor when the supernatant was withdrawn. The final concentration (although not in solution) was approximately 100 mg/l. This material was not homogeneously distributed and accumulated on the sides of the reactors.

WARBURG FC-95

The results of Warburg studies with FC-95 are graphed in Figure 3.

No oxygen uptake was observed as a result of the addition of FC-95. This material also caused no toxic effects. Sipel-ol, an approximate hydrogen analog of FC-95, was shown to be readily biodegradable and to have no toxic effects.

The Sipel-ols was soluble at all concentrations tested (as high as 1700 mg/l). FC-95 was incompletely soluble at 4000 mg/l, but was completely in solution at 400 mg/l. The lack of degradation with FC-95 was expected since perfluorinate compounds are characteristically nonbiodegradable.

WARBURG FC-128

Oxygen uptake curves from Warburg studies on FC-128 as shown in Figure 4. These results indicate that FC-128 is readily biodegradable. Assuming biodegradation occurs as is shown below, approximately 70% of the theoretical maximum oxygen uptake occurred within the 7-hr. experimental period. This oxygen uptake is greater than expected and appeared to be continuing at the end of the experiment. These results are somewhat in question since this FC-128 is known to be an impure chemical.

$$C_8F_{17}SO_2N(C_2H_5)CH_2COOK + 4.25O_2 + H^+ \rightarrow C_8F_{17}SO_2NH_2 + 4CO_2 + 3H_2O + K^+$$

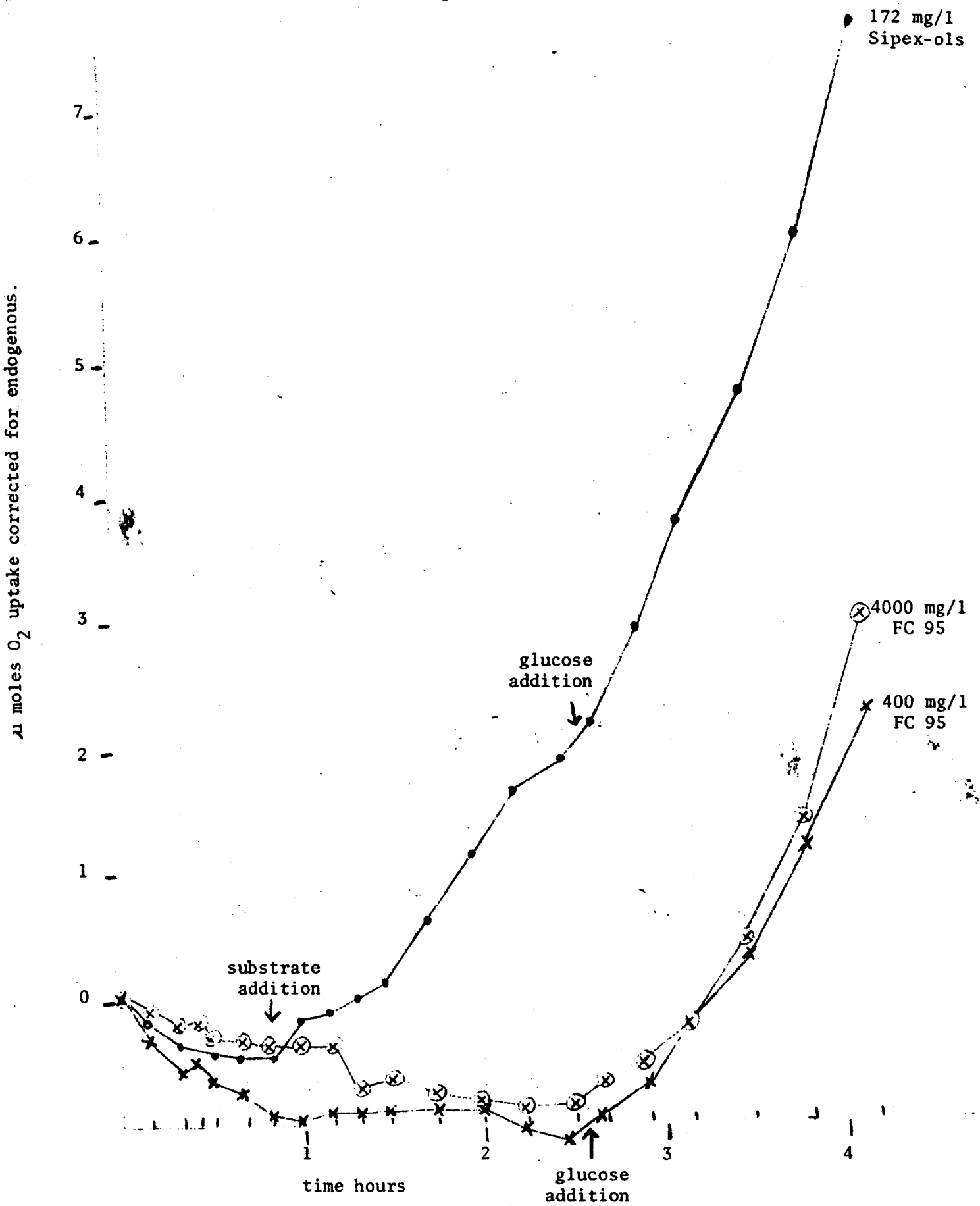

FIGURE 3: Warburg study of FC 95 and Sipex-ols.

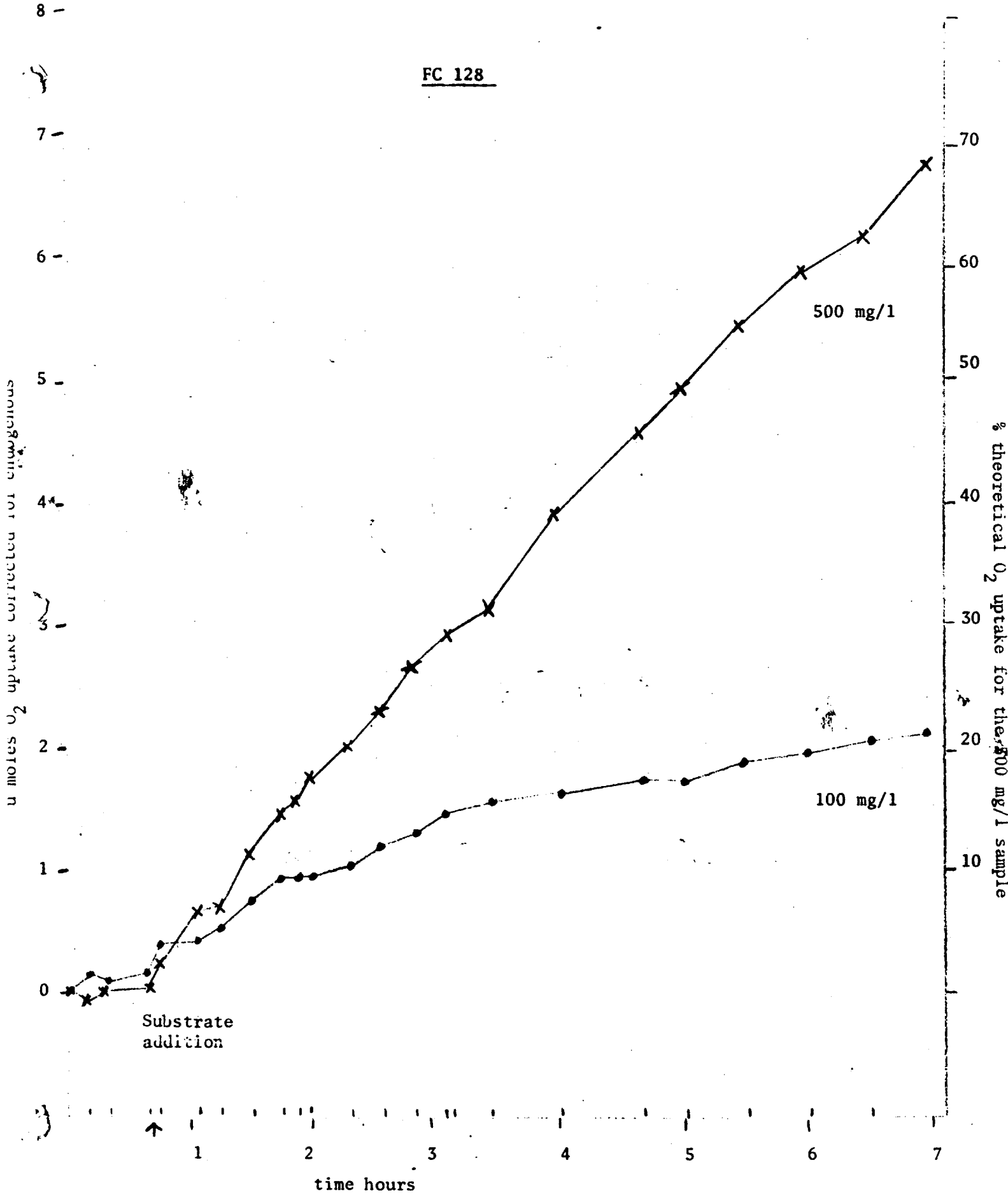

FIGURE 4: Warburg study of FC 128.

REFERENCES:

(1) Alexander, M.; Biodegradation: Problems of Molecular Recalcitrance and Microbial Fallibility. Adv. Appl. Microbial 7: 35-80, 1965.

(2) Chapman, P. J.; Department of Biochemistry, University of Minnesota, St. Paul, Minnesota, Personal Communications 2/24/76.

7/10/75
E. A. Reiner

ATTACHMENT I

STANDARD PROCEDURE FOR WARBURG DETERMINATIONS

1. Design experiment and calculate concentrations of materials to add.

2. Fill water bath (DI water if left in bath).

3. Adjust temp. of bath (several hrs. or overnight).

4. Place manometers in desired order.

5. Prepare thermobarometer. Add about 3ml of H_2O to 1 flask.

6. Set out glassware in desired order (to match the manometer with which they were calibrated).

7. Lightly grease center-well top with stopcock grease that can be removed with solvent. Add 0.2 ml 10% KOH.

8. Prepare samples in DI water (or according to request) to add to side arms. Keep refrigerated until used.

9. Prepare cells (keep cells cold at all times but avoid freezing).

   A. Centrifuge 0° C.
   B. Wash with cold BSM - centrifuge.
   C. Resuspend in cold BSM.
   D. Determine concentration of an aliquot with the spectronic 20 at 600 nm. Adjust remainder to desired conc. C basic salts medium. Refrigerate until use.
   E. Take sample of final adjusted sludge for standard MLSS analysis.

10. Add samples to side arms (usually 1 ml if one side arm, ½ ml to each side arm if 2 side arms).

11. Add 2 ml of washed cells to flasks.

12. Add filter paper strip to alkali in center cup.

13. Attach flasks to the correct manometer.

14. Retighten flasks after about 5 min. shaking in bath.

15. Leave stopcock open to atmosphere, and let temp. adjust for an additional 10 min.

16. Adjust level in manometer to 150 with stopcock open (close stopcock).

17. Begin readings (always adjust closed arm of manometer to 150 mm before reading).

18. Add contents of side arms according to experiment design requirements.

19. Take readings periodically (on open arms) throughout course of experiment.

20. Disconnect and clean flasks.

    A. Rinse with water.
    B. Wash off grease with acetone.
    C. Acid wash.
    D. Rinse with DI Water.

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WARBURG DATA FORM

Pg 1

Title: Warburg Experiment to Evaluate Toxicity & Biodegradability of FC-128 Run No.: 5 Date: 9-26-75

Cell Conc.: 2000 mg/l Temp.: 30°C Strokes/Min.: 115 Contents of Inner Well: 2 ml 10% KOH

No.	Flask No.	Time → 915	0	10	20	40	45	65	75	90	105	112	120	140	155	170	185		188	205	240	280 min
		+ Flask Contents +	Reading in mm.																			
1	8692 2576	Thermobarometer	151	150	150	150	150	151	151	151	151	150	150	150	150	153	158		151	153	157	162
2	9935 2599	Thermobarometer	150	150	150	149	150	150	149	150	149	150	150	150	150	149	*150		150	151	156	162
3	2287 1502	Endogenous control	151	148	144	136	139	127	124	121	117	113	111	108	104	102	98		150	148	143	137
4	2288 1604	Low glucose control	150	149	147	141	137	131	127	124	119	116	111	109	93	85	69		149	148	116	89
5	2288 1809	High glucose control	149	149	149	149	150	149	150	149	149	150	148	148	145	139	122		149	139	115	106
6	2297 2366	20 mg/l FC-128	150	148	144	138	139	129	124	120	117	114	113	111	107	105	101		150	148	145	141
7	2302 2377	20 mg/l FC-128 + glucose	151	149	145	140	137	132	127	123	119	116	112	106	96	88	71		150	141	117	85
8	2303 2378	100 mg/l FC-128	151	148	145	136	133	126	122	117	112	109	107	104	99	96	89		150	147	143	137
9	2385 2399	100 mg/l FC-128	151	146	143	134	129	122	118	112	106	102	100	96	90	87	81		149	146	140	133
10	2322 2401	100 mg/l FC-128 + glucose	149	143	136	121	116	104	98	86	74	70	64	53	37	23	-2		149	130	69	
11	2215 2417	500 mg/l FC-128	149	147	142	134	130	118	115	107	99	94	90	89	77	71	64		149	145	131	118
12	2288 2435	500 mg/l FC-128	150	148	146	142	138	134	131	125	116	111	108	103	96	91	82		149	144	129	116
13	2289 2459	500 mg/l FC-128 + glucose	149	148	145	140	137	129	128	122	115	112	108	101	92	85	70		150	138	104	61
14																						

Notes: Added side arm #1 (with FC-128 solutions) at 42 min. Added side arm #2 (glucose or DI) at 107 min. Readjusted all bath to 50° at 188 took 2 min

leaks →

may not have added FC 128 until 107 min →

* fixed leak

probably was dirty flask

160-2 set to 150

File FC-128

2

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WARBURG DATA FORM

Title: Warburg Experiment to evaluate toxicity + Biodegradability of FC-128 | Run No.: 5 | Date: 9-26-75

Cell Conc.: 2000 mg/l | Temp.: 30°C | Strokes/Min.: 115 | Contents of Inner Well: 2 ml 10% KOH

No.	Flask No. / man	Time → 9:15 / ↓ Flask Contents ↓	300	330	360	390	420
			Reading in mm.				
1	8692 2576	thermobarometer	165	167	170	174	174
2	9935 2599	thermobarometer	165	166	169	172	173
3	2287 1502	Endogenous Control	135	131	128	124	120
4	228820 1609	glucose control	72	46	18	—	—
5	228822 1809	glucose control	157	129	98	52	8
6	2297 2366	20 mg/l FC128	140	136	132	128	126
7	2302 2387	20 mg/l FC128 + glucose	165	33	-3	—	—
8	2303 2378	100 mg/l FC-128	134	128	123	119	115
9	2385 2399	100 mg/l FC128	131	123	121	116	111
10	2322 2401	100 mg/l FC128 + glucose	99	12	—	—	—
11	2215 2917	500 mg/l FC128	112	102	94	87	76
12	2288-790 2935	500 mg/l FC128	109	99	91	83	74
13	2299 2454	500 mg/l FC-128 + glucose	33	—	—	—	—
14							

Notes: 158-0

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Subject: Warburg experiment to evaluate toxicity & Biodegradability of FC-128

Date: 9-25-75

Object:

Reference: Will be Run on 9-26-75
Sludge will be Pigs Eye collected 9-23-75 adjusted to 2000 mg/l (when everything is added).

Sludge will be endogenized overnight to get cells in the resting phase with vent open on warburg. After endogenizing ~~air will be through the flask and the flask closed off~~ flasks will be opened at top to bring O_2 supply up to 20%

flask #	flask name	flask contents	side arm #1	side arm #2	Center well
1	thermobank	~3ml DI	—	—	—
2	thermobank	~3ml DP	—	—	—
3	(Endogenous control)	(2ml of 2000mg/l washed w. endogenous overnight cells)	½ ml DI	½ ml DI	2ml 10% KOH
Lost 4	"	"	"	"	"
4 5	(glucose control)	"	"	½ ml .5g/50ml glucose in DI	"
5 6	"	"	"	"	"
6 7	20mg/l FC-128	"	½ ml 120 mg/l FC-128	½ ml DI H_2O	"
7 8	20mg/l FC128 + glucose	"	"	½ ml .5g/50ml glucose in DI	"

Signature _____ 000304 Date: _____

Read and Understood _____ Date: _____

PROJECT NO.

3M TECHNICAL NOTEBOOK NO. 40671

PAGE 34

Subject: FC 128 cont

Date: 9-25-75

Object:

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Reference:

Flask #	Flask Name	Flask Contents	Stopper Side arm #1	Vent Side arm #2	Center well
8 (9)	100 mg/l FC-128	(2 ml of 3000 mg/l cells washed + enlarged overnight)	½ ml 600 mg/l FC-128	½ ml DI	0.2 ml 10% KOH
9 (10)	100 mg/l FC-128	"	"	"	"
10 (11)	100 mg/l FC-128 + glucose	"	½ ml 600 mg/l FC-128	½ ml 0.5 g/50 ml glucose	0.2 ml 10% KOH
11 (12)	500 mg/l FC-128	"	½ ml 3000 mg/l FC-128	½ ml DI	"
12 (13)	500 mg/l FC-128	"	"	"	"
13 (14)	500 mg/l FC-128 + glucose	"	"	½ ml 0.5 g/50 ml glucose in DI	"

Make solution as follows dissolve 1.5 grams of FC-128 in 500 ml of of DI water ⇒ 3000 mg/l
add 5 ml of this to 20 ml DI ⇒ 600 mg/l
add add 5 ml of the 600 mg/l solution to 20 ml DI ⇒ 120 mg/l.

Signature

000305

Date:

Read and understood

FC-128

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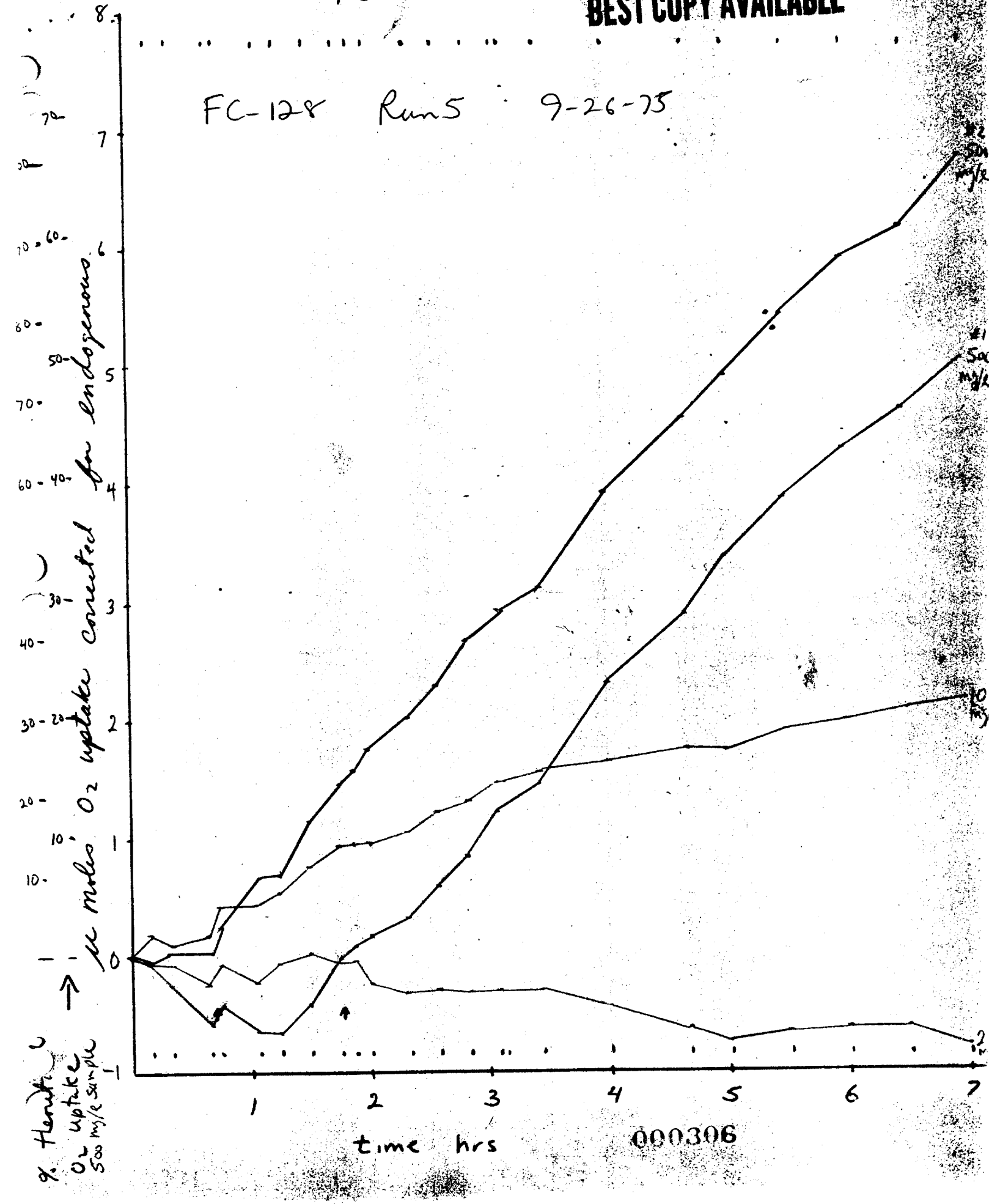

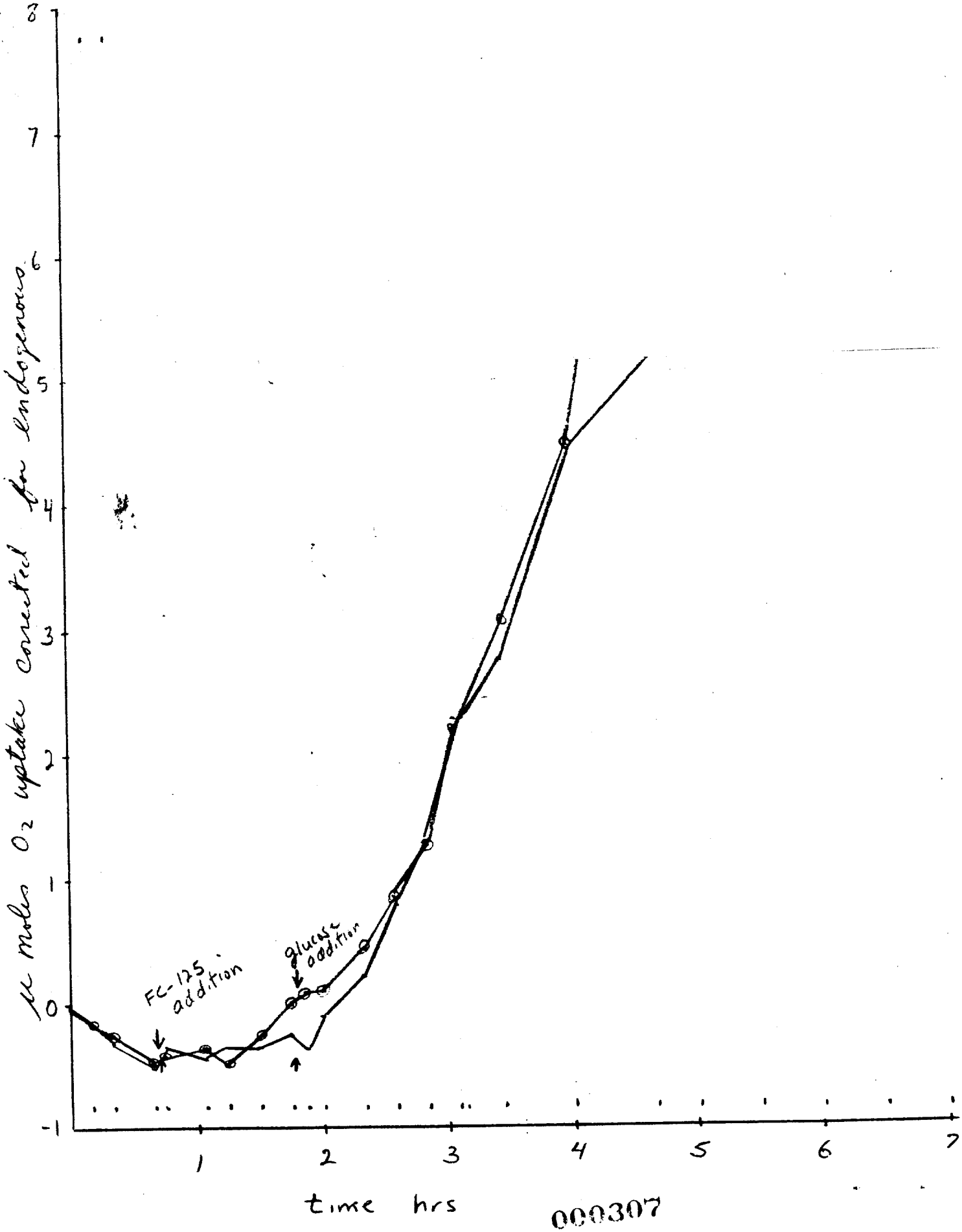
μ moles O2 uptake corrected for endogenous
8
7
6
5
4
3
2
1
0
-1
FC-125 addition
glucose addition
1
2
3
4
5
6
7
time hrs

000307

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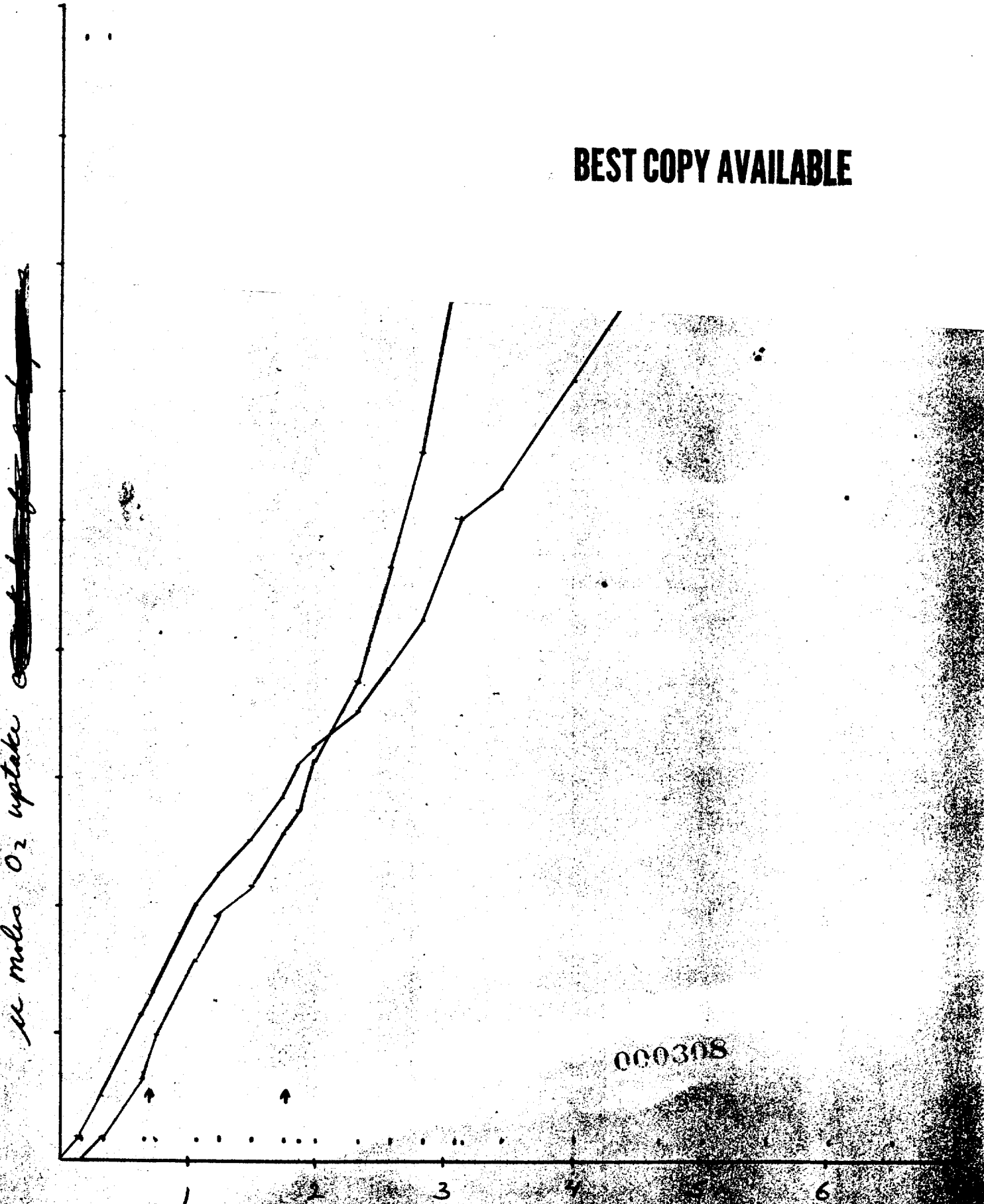

000308

FC-128

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$500\ mg/\ell \times 3\times10^{-3}\ \ell = 1500\ mg\ FC\ 128 \times 144\ \mu g\ O_2/mg\ FC$

$216\ \mu g$

Assume theoretical for graph

$$C_8F_{17}SO_2N(C_2H_5)CH_2COOK + 4.25\ O_2 \rightarrow$$

MW = 623

$$C_8F_{17}SO_2NH_2 + 4CO_2 + 3\ H_2O$$

$\frac{136\ mg\ O_2}{623\ mg\ FC\ 128}$

$\boxed{327\ \mu g\ O_2\ \text{theoretical}}$

COD

$\boxed{144\ mg/g\ \text{average}}$ for FC-128

#1 124 mg/g

#2 141 mg/g

#3 167 mg/g

used 50 ml of a 3 g/ℓ solution

Flask Constant .08399

Page 1

WARBURG O_2 UPTAKE CALCULATION SHEET

Title: FC-128 Toxicity + Biodegradability Date: 9-26-75 Flask No.: 3 Flask Contents: Endogenous Control

Time hrs	Elapsed Time min	Reading T.B.[1] (mm)	Reading Flask (mm)	Change[2] in mm. (mm)	T.B. Correction[3]	Actual Change[4] (mm)	O_2 Uptake[5] (μmoles)	Endogenous O_2 Uptake (μmoles)	Corrected O_2 Uptake[6] (μmoles)
0	0	151	151	0	0	0	0		
.167	10	150	148	-3	-1	-2	0.17		
.33	20	150	144	-7	-1	-6	0.50		
.67	40	150	136	-15	-1	-14	1.18		
.75	45	150	134	-17	-1	-16	1.34		
1.08	65	151	127	-24	0	-24	2.02		
1.25	75	151	124	-27	0	-27	2.27		
1.5	90	151	121	-30	0	-30	2.52		
1.75	105	151	117	-34	0	-34	2.86		
1.87	112	150	113	-38	-1	-37	3.11		
2	120	150	111	-40	-1	-39	3.28		
2.33	140	150	108	-43	-1	-42	3.53		
2.58	155	150	104	-47	-1	-46	3.86		
2.83	170	153	102	-49	+2	-51	4.28		
3.08	185	158	98	-53	+7	-60	5.04		
3.13	188	150	150	-53	+7	-60	5.05		
3.42	205	151	148	-55	+8	-63	5.29		
4	240	156	143	-60	+13	-73	6.13		
4.67	280	162	137	-66	+19	-85	7.14		
5	300	165	135	-68	+22	-90	7.56		

Footnotes over.

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WARBURG O_2 UPTAKE CALCULATION SHEET

Title: FC-128 Toxicity & Biodegradability Date: 9-26-75 Flask No.: 3 Flask Contents: Endogenous control

Time hrs	Elapsed Time Min	Reading T.B.[1] (mm)	Reading Flask (mm)	Change[2] in mm. (mm)	T.B. Correction[3]	Actual Change[4] (mm)	O_2 Uptake[5] (µmoles)	Endogenous O_2 Uptake (µmoles)	Corrected O_2 Uptake[6] (µmoles)
5.5	330	166	131	-72	+23	-95	7.98		
6	360	169	128	-75	+26	-101	8.48		
6.5	390	172	124	-79	+29	-108	9.07		
7	420	173	120	-83	+30	-113	9.49		

Footnotes over.

flask constant
.08315

Page 1

WARBURG O_2 UPTAKE CALCULATION SHEET

Title: FC-128 Toxicity + Biodegradability Date: 9-26-75 Flask No.: 4 Flask Contents: glucose control

Time hrs	Elapsed Time min	Reading T.B.[1] (mm)	Reading Flask (mm)	Change[2] in mm. (mm)	T.B. Correction[3]	Actual Change[4] (mm)	O_2 Uptake[5] (μmoles)	Endogenous O_2 Uptake (μmoles)	Corrected O_2 Uptake[6] (μmoles)
0	0	151	150	0	0	0	0	0	0
.167	10	150	149	-1	-1	0	0	0.17	-0.17
.33	20	150	147	-3	-1	-2	.17	0.50	-.33
.67	40	150	141	-9	-1	-8	.665	1.18	-.52
.75	45	150	137	-13	-1	-12	1.00	1.34	-.34
1.08	65	151	131	-19	0	-19	1.58	2.02	-.44
1.25	75	151	127	-23	0	-23	1.91	2.27	-.36
1.5	90	151	124	-26	0	-26	2.16	2.52	-.36
1.75	105	151	119	-31	0	-31	2.58	2.86	-.28
1.87	112	150	116	-34	-1	-33	2.74	3.11	-.37
2	120	150	111	-39	-1	-38	3.16	3.28	-.12
2.33	140	150	104	-46	-1	-45	3.74	3.53	.21
2.58	155	150	93	-57	-1	-56	4.66	3.86	.80
2.83	170	153	85	-65	+2	-67	5.57	4.28	1.29
3.08	185	158	69	-81	+7	-88	7.32	5.04	2.28
3.13	188	150	149	-81	+7	-88	7.32	5.05	2.27
3.42	205	151	141	-89	+8	-97	8.07	5.29	2.78
4	240	156	116	-114	+13	-127	10.56	6.13	4.43
4.67	280	162	89	-141	+19	-160	13.30	7.14	6.16
5	300	165	72	-158	+22	-180	14.97	7.56	7.41

adjusted 150 →

Footnotes over.

WARBURG O_2 UPTAKE CALCULATION SHEET

Title: FC-128 Toxicity & Biodegradability Date: 9-26-75 Flask No.: 4 Flask Contents: Glucose control

Time hrs	Elapsed Time min	Reading T.B.[1] (mm)	Reading Flask (mm)	Change[2] in mm. (mm)	T.B. Correction[3]	Actual Change[4] (mm)	O_2 Uptake[5] (µmoles)	Endogenous O_2 Uptake (µmoles)	Corrected O_2 Uptake[6] (µmoles)
5.5	330	166	46	-184	+23	-207	17.21	7.98	9.23
6	360	169	18	-212	+26	-238	19.79	8.48	11.31
6.5	390	172			+29			9.07	
7	420	173			+30			9.49	

Footnotes over.

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flask constant .08443

Page 1

WARBURG O_2 UPTAKE CALCULATION SHEET

Title: FC-128 Toxicity + Biodegradability Date: 9-26-75 Flask No.: 6 Flask Contents: 20 mg/l FC-128

Time hrs	Elapsed Time min	Reading T.B.[1] (mm)	Reading Flask (mm)	Change[2] in mm. (mm)	T.B. Correction[3]	Actual Change[4] (mm)	O_2 Uptake[5] (µmoles)	Endogenous O_2 Uptake (µmoles)	Corrected O_2 Uptake[6] (µmoles)
0	0	151	150	0	0	0	0	0	
.167	10	150	148	-2	-1	-1	.08	0.17	-.09
.33	20	150	144	-6	-1	-5	.42	0.50	-.08
.67	40	150	138	-12	-1	-11	.93	1.18	-.25
.75	45	150	134	-16	-1	-15	1.27	1.34	-.07
1.08	65	151	129	-21	0	-21	1.77	2.02	-.25
1.25	75	151	124	-26	0	-26	2.20	2.27	-.07
1.5	90	151	120	-30	0	-30	2.53	2.52	+.01
1.75	105	151	117	-33	0	-33	2.79	2.86	-.07
1.87	112	150	114	-36	-1	-35	2.96	3.11	-.05
2	120	150	113	-37	-1	-36	3.04	3.28	-.24
2.33	140	150	111	-39	-1	-38	3.21	3.53	-.32
2.58	155	150	107	-43	-1	-42	3.55	3.86	-.31
2.83	170	153	105	-45	+2	-47	3.96	4.28	-.32
3.08	185	158	101	-49	+7	-56	4.73	5.04	-.31
3.13	188	150	150	-49	+7	-56	4.73	5.05	-.32
3.42	205	151	148	-51	+8	-59	4.98	5.29	-.31
4	240	156	145	-54	+13	-67	5.66	6.13	-.47
4.67	280	162	141	-58	+19	-77	6.50	7.14	-.64
5	300	165	140	-59	+22	-81	6.84	7.56	-.72

adjusted 150

Footnotes over.

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WARBURG O_2 UPTAKE CALCULATION SHEET

Page 2

Title: FC-128 Toxicity & Biodegradability Date: 9-26-75 Flask No.: 6 Flask Contents: 20 mg/l FC-128

Time hrs	Elapsed Time min	Reading T.B.[1] (mm)	Reading Flask (mm)	Change[2] in mm. (mm)	T.B. Correction[3]	Actual Change[4] (mm)	O_2 Uptake[5] (µmoles)	Endogenous O_2 Uptake (µmoles)	Corrected O_2 Uptake[6] (µmoles)
5.5	330	166	136	-63	+23	-86	7.26	7.98	-.69
6	360	169	132	-67	+26	-93	7.85	8.48	-.63
6.5	390	172	128	-71	+29	-100	8.44	9.07	-.63
7	420	173	126	-73	+30	-103	8.69	9.49	-.80

Footnotes over.

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WARBURG O_2 UPTAKE CALCULATION SHEET

Page 1

Title: FC-128 Toxicity + Biodegradability Date: 9-26-75 Flask No.: 7 Flask Contents: 20 mg/l FC-128 + glucose

Time hrs	Elapsed Time min	Reading T.B.[1] (mm)	Reading Flask (mm)	Change[2] in mm. (mm)	T.B. Correction[3]	Actual Change[4] (mm)	O_2 Uptake[5] (µmoles)	Endogenous O_2 Uptake (µmoles)	Corrected O_2 Uptake[6] (µmoles)
0	0	151	151		0			0	
.167	10	150	149	2	-1	1	.08	0.17	-.09
.33	20	150	145	6	-1	5	.42	0.50	-.08
.67	40	150	140	11	-1	10	.85	1.18	-.33
.75	45	150	137	14	-1	15	1.27	1.34	-.07
1.08	65	151	132	19	0	19	1.61	2.02	-.41
1.25	75	151	127	24	0	24	2.03	2.27	-.24
1.5	90	151	123	28	0	28	2.37	2.52	-.15
1.75	105	151	119	32	0	32	2.71	2.86	-.15
1.87	112	150	116	35	-1	34	2.88	3.11	-.23
2	120	150	112	39	-1	39	3.30	3.28	-.02
2.33	140	150	106	45	-1	44	3.73	3.53	+.20
2.58	155	150	96	55	-1	54	4.57	3.86	.71
2.83	170	153	88	63	+2	65	5.50	4.28	1.22
3.08	185	158	71	80	+7	87	7.37	5.04	2.33
3.13	188	150	150	80	+7	87	7.37	5.05	2.32
3.42	205	151	141	89	+8	97	8.21	5.29	2.92
4	240	156	117	113	+13	126	10.67	6.13	4.54
4.67	280	162	85	145	+19	164	13.89	7.14	6.75
5	300	165	65	165	+22	187	15.83	7.56	8.27

adjusted 130 →

Footnotes over.

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WARBURG O_2 UPTAKE CALCULATION SHEET

Page 2

Title: FC-128 Toxicity + Biodegradability Date: 9-26-75 Flask No.: 7 Flask Contents: 20 mg/l FC-128 + Sludge

Time hrs	Elapsed Time min	Reading T.B.[1] (mm)	Reading Flask (mm)	Change[2] in mm. (mm)	T.B. Correction[3]	Actual Change[4] (mm)	O_2 Uptake[5] (µmoles)	Endogenous O_2 Uptake (µmoles)	Corrected O_2 Uptake[6] (µmoles)
5.5	330	166	33	197	+23	220	18.63	7.98	10.65
6	360	169	-3	233	+26	259	21.93	8.48	13.45
6.5	390	172			+29			9.07	
7	420	173			+30			9.49	

Footnotes over.

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WARBURG O_2 UPTAKE CALCULATION SHEET

Title: FC-128 Toxicity + Biodegradability Date: 9-26-75 Flask No.: 9 Flask Contents: 100 mg/l FC-128

Time hrs	Elapsed Time min	Reading T.B.[1] (mm)	Reading Flask (mm)	Change[2] in mm. (mm)	T.B. Correction[3]	Actual Change[4] (mm)	O_2 Uptake[5] (µmoles)	Endogenous O_2 Uptake (µmoles)	Corrected O_2 Uptake[6] (µmoles)
0	0	151	151	0	0			0	0
.167	10	150	146	5	-1	4	.34	0.17	.17
.33	20	150	143	8	-1	7	.59	0.50	.09
.67	40	150	134	17	-1	16	1.35	1.18	.17
.75	45	150	129	22	-1	21	1.77	1.34	.43
1.08	65	151	122	29	0	29	2.45	2.02	.43
1.25	75	151	118	33	0	33	2.79	2.27	.52
1.5	90	151	112	39	0	39	3.29	2.52	.77
1.75	105	151	106	45	0	45	3.80	2.86	.94
1.87	112	150	102	49	-1	48	4.05	3.11	0.94
2	120	150	100	51	-1	50	4.22	3.28	0.94
2.33	140	150	96	55	-1	54	4.56	3.53	1.03
2.58	155	150	90	61	-1	60	5.07	3.86	1.21
2.83	170	153	87	64	+2	66	5.58	4.28	1.30
3.08	185	158	81	70	+7	77	6.50	5.04	1.46
3.13	188	150	149	70	+7	77	6.50	5.05	1.45
3.42	205	151	146	73	+8	81	6.84	5.29	1.55
4	240	156	140	79	+13	92	7.77	6.13	1.64
4.67	280	162	133	86	+19	105	8.87	7.14	1.73
5	300	165	131	88	+22	110	9.29	7.56	1.73

Footnotes over.

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WARBURG O_2 UPTAKE CALCULATION SHEET

Page 2

Title: FC-128 Toxicity + Biodegradability Date: 9-26-75 Flask No.: 9 Flask Contents: 100 mg/l FC-128

Time hrs	Elapsed Time Min	Reading T.B.[1] (mm)	Reading Flask (mm)	Change[2] in mm. (mm)	T.B. Correction[3]	Actual Change[4] (mm)	O_2 Uptake[5] (μmoles)	Endogenous O_2 Uptake (μmoles)	Corrected O_2 Uptake[6] (μmoles)
5.5	330	166	125	94	+23	117	9.88	7.98	1.90
6	360	169	121	98	+26	124	10.47	8.48	1.99
6.5	390	172	116	103	+29	132	11.15	9.07	2.08
7	420	173	111	108	+30	138	11.66	9.49	2.17

Footnotes over.

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Flask contents 08066 #2

Page 1

WARBURG O_2 UPTAKE CALCULATION SHEET

Title: FC-128 Toxicity + Biodegradability Date: 9-26-75 Flask No.: 11 Flask Contents: 500 mg/l FC-128

Time hrs	Elapsed Time min	Reading T.B.[1] (mm)	Reading Flask (mm)	Change[2] in mm. (mm)	T.B. Correction[3]	Actual Change[4] (mm)	O_2 Uptake[5] (μmoles)	Endogenous O_2 Uptake (μmoles)	Corrected O_2 Uptake[6] (μmoles)
0	0	151	149	0	0	0	0	0	0
.167	10	150	147	-2	-1	-1	.09	0.17	-.08
.33	20	150	142	-7	-1	-6	.52	0.50	.02
.67	40	150	134	-15	-1	-14	1.21	1.18	.03
.75	45	150	130	-19	-1	-18	1.56	1.34	.22
1.08	65	151	118	-31	0	-31	2.68	2.02	.66
1.25	75	151	115	-34	0	-34	2.94	2.27	.67
1.5	90	151	107	-42	0	-42	3.64	2.52	1.12
1.75	105	151	99	-50	0	-50	4.33	2.86	1.47
1.87	112	150	94	-55	-1	-54	4.68	3.11	1.57
2	120	150	90	-59	-1	-58	5.02	3.28	1.74
2.33	140	150	84	-65	-1	-64	5.54	3.53	2.01
2.58	155	150	77	-72	-1	-71	6.15	3.86	2.29
2.83	170	153	71	-78	+2	-80	6.93	4.28	2.65
3.08	185	158	64	-85	+7	-92	7.97	5.04	2.93
3.13	188	150	149	-85	+7	-92	7.97	5.05	2.92
3.42	205	151	145	-89	+8	-97	8.40	5.29	3.11
4	240	156	131	-103	+13	-116	10.05	6.13	3.92
4.67	280	162	118	-116	+19	-135	11.69	7.14	4.55
5	300	165	112	-122	+22	-144	12.47	7.56	4.91

Footnotes over.

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WARBURG O_2 UPTAKE CALCULATION SHEET

Title: FC-128 Toxicity + Biodegradability

Date: 9-26-75 Flask No.: 11 Flask Contents: 500 mg/l FC-128

Time hrs	Elapsed Time min	Reading T.B.[1] (mm)	Reading Flask (mm)	Change[2] in mm. (mm)	T.B. Correction[3]	Actual Change[4] (mm)	O_2 Uptake[5] (µmoles)	Endogenous O_2 Uptake (µmoles)	Corrected O_2 Uptake[6] (µmoles)
5.5	330	166	102	132	+23	-155	13.42	7.98	5.44
6	360	169	94	140	+26	-166	14.38	8.48	5.90
6.5	390	172	87	147	+29	-176	15.24	9.07	6.17
7	420	173	76	158	+30	-188	16.28	9.49	6.79

Footnotes over.

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Flask constant 0.08348 #1 Page 1

WARBURG O_2 UPTAKE CALCULATION SHEET

Title: FC-128 Toxicity + Biodegradability Date: 9-26-75 Flask No.: 12 Flask Contents: 500 mg/l FC-128

Time hrs	Elapsed Time min	Reading T.B.[1] (mm)	Reading Flask (mm)	Change[2] in mm. (mm)	T.B. Correction[3]	Actual Change[4] (mm)	O_2 Uptake[5] (µmoles)	Endogenous O_2 Uptake (µmoles)	Corrected O_2 Uptake[6] (µmoles)
0	0	151	150	0	0	0	0	0	0
.167	10	150	148	-2	-1	-1	.08	0.17	-.09
.33	20	150	146	-4	-1	-3	.25	0.50	-.25
.67	40	150	142	-8	-1	-7	.58	1.18	-.60
.75	45	150	138	-12	-1	-11	.92	1.34	-.42
1.08	65	151	134	-16	0	-16	1.34	2.02	-.68
1.25	75	151	131	-19	0	-19	1.59	2.27	-.68
1.5	90	151	125	-25	0	-25	2.09	2.52	-.43
1.75	105	151	116	-34	0	-34	2.84	2.86	-.02
1.87	112	150	111	-39	-1	-38	3.17	3.11	+.06
2	120	150	108	-42	-1	-41	3.42	3.28	.14
2.33	140	150	103	-47	-1	-46	3.84	3.53	.31
2.58	155	150	96	-54	-1	-53	4.42	3.86	.56
2.83	170	153	91	-59	+2	-61	5.09	4.28	.81
3.08	185	158	82	-68	+7	-75	6.26	5.04	1.22
3.13	188	150	149	-68	+7	-75	6.26	5.05	1.21
3.42	205	151	144	-73	+8	-81	6.76	5.29	1.47
4	240	156	129	-88	+13	-101	8.43	6.13	2.30
4.67	280	162	116	-101	+19	-120	10.02	7.14	2.88
5	300	165	109	-121	+22	-143	11.93	7.56	4.37

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WARBURG O_2 UPTAKE CALCULATION SHEET

Title: FC-128 Toxicity & Biodegradability Date: 9-26-75 Flask No.: 12 Flask Contents: 500 mg/l FC-128

Time hrs	Elapsed Time min	Reading T.B.[1] (mm)	Reading Flask (mm)	Change[2] in mm. (mm)	T.B. Correction[3]	Actual Change[4] (mm)	O_2 Uptake[5] (µmoles)	Endogenous O_2 Uptake (µmoles)	Corrected O_2 Uptake[6] (µmoles)
5.5	330	166	99	-131	+23	-154	12.86	7.98	4.88
6	360	169	91	-139	+26	-165	13.77	8.48	5.29
6.5	390	172	83	-147	+29	-176	14.69	9.07	5.62
7	420	173	74	-156	+30	-186	15.52	9.49	6.03

Footnotes over.

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Flask constant 0.08424

WARBURG O_2 UPTAKE CALCULATION SHEET

Title: FC-128 Toxicity + Biodegradability Date: 9-26-75 Flask No.: 13 Flask Contents: 500 mg/l FC128 + glucose

Time hrs	Elapsed Time min	Reading T.B.[1] (mm)	Reading Flask (mm)	Change[2] in mm. (mm)	T.B. Correction[3]	Actual Change[4] (mm)	O_2 Uptake[5] (µmoles)	Endogenous O_2 Uptake (µmoles)	Corrected O_2 Uptake[6] (µmoles)
0	0	151	149	0	0	0	0	0	0
.167	10	150	148	-1	-1	0	0	0.17	-.17
.33	20	150	145	-4	-1	-3	.25	0.50	-.25
.67	40	150	140	-9	-1	-8	.67	1.18	-0.51
.75	45	150	137	-12	-1	-11	.92	1.34	-.42
1.08	65	151	129	-20	0	-20	1.68	2.02	-.34
1.25	75	151	128	-21	0	-21	1.77	2.27	-.50
1.5	90	151	122	-27	0	-27	2.27	2.52	-.25
1.75	105	151	115	-34	0	-34	2.86	2.86	0
1.87	112	150	112	-37	-1	-36	3.03	3.11	0.08
2	120	150	108	-41	-1	-40	3.37	3.28	0.09
2.33	140	150	101	-48	-1	-47	3.96	3.53	.43
2.58	155	150	92	-57	-1	-56	4.71	3.86	.85
2.83	170	153	85	-64	+2	-66	5.56	4.28	1.28
3.08	185	158	70	-79	+7	-86	7.24	5.04	2.20
3.13	188	150	150	-79	+7	-86	7.24	5.05	2.20
3.42	205	151	138	-91	+8	-99	8.34	5.29	3.05
4	240	156	104	-125	+13	-138	11.62	6.13	5.49
4.67	280	162	61	-168	+19	-187	15.75	7.14	8.61
5	300	165	33	-196	+22	-218	18.36	7.56	10.80

FC-128 →

glucose →

adjusted 150 →

Footnotes over.

FORM 10620-8-PWO

File: Page No. _____

ENVIRONMENTAL ENGR. LAB – WORK SHEET

SAMPLE DESCRIPTION
FC - 128

Date: 10-6-75
Analyst: JP
Hrs.:

COD Strong 3[illegible] 25.2

#1	5ml			
#2	10ml	23.4		360
			25.4	
1	50 .5g/l	23.1	92	184
2	50 .5g/l	23.4	80	160
3	50 3g/l	14.8	424 mg/l	141
4	50 3g/l	16.1	372 mg/l	124
5	50 3g/l	12.9	500 mg/l	167
				144 mg/g

000325

Reviewed by: _____

BIODEGRADATION (BOD/COD/TOC)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127 or FC-129. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-128. Current information indicates it is a mixture of 86% test substance and 14% C_4-C_7 fluoroalkyl carboxylate compounds. 3M production lot number 751.

METHOD:

Method: Not noted.
Type: Aerobic
GLP: No
Year completed: 1977
Contact time: NA
Innoculum: Not noted

RESULTS

BOD20: <7600 mg/kg
COD: 140,000 mg/kg
TOC: 162,000 mg/kg

Remarks: The 5-, 7-, 11-, and 14-day BOD values could not be calculated because of insufficient dissolved oxygen depletion.

CONCLUSIONS

No reliable conclusions can be derived from this study.

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

DATA QUALITY

Reliability: Klimisch ranking 4. Summary was created using only a listing of test values. The original study procedure and raw data were not found.

REFERENCES

The COD and BOD studies were conducted by the 3M Company, Environmental Laboratory, St. Paul, MN, Lab Request number 3844, 7/8/77.

The TOC study was conducted by ARRO Laboratories at the request of 3M, Lab Request number 3849, 1977

OTHER

Last changed: 5/16/00

Form 10620-5-PWO
cc: LAB REQUEST BOOK – 42-5W

3M "Action" 200 Paper

PRODUCT ECOLOGICAL ASSESSMENT
REQUEST FOR LABORATORY WORK

Ref: ENVIR. ASSESS. INQ. # _____ DIVISION

LAB REQUEST NO. 3844

PRODUCT: _____

REQUESTER		LABORATORY
Name: Dale Bacon	Phone No. 5453	Date Received Sample:
Request Date: 7/8/77		Analyst:
Project No.: ~~[illegible]~~	Prob. No.	Date Completed:
Code No.:		Hours Spent:
Sample Date:	Date Needed:	Y ____ B ____ P ____
Description:		Please Call Requester If Delays Are Encountered.
Purpose of Tests: Report COD/BOD/TOC on MY/KG		

PHYSICAL & CHEMICAL PROPERTIES								
Heat of Combustion Cal/G (BTU/Lb.)		FC 805 Lt 540		FC 214-30 Lt 505		FC 128 Lt 751		FC 807 Lt 900
Density								
Ash								
Other								
WATER POLLUTION								
pH	✓	<0.0						
COD	✓	1,100,000	✓	440,000	✓	140,000	✓	420,000
BOD_5	✓		✓		✓	Nil	✓	
BOD, Ultimate	✓		✓		✓	<7600	✓	
TOC	✓		✓		✓	162,000	✓	
GC								
Oxygen Uptake								
Dehydrogenase Activity (TTC)								
Other								
Cr^{+6}	✓							
Cr^{+3}	✓							
BIOASSAY								
Specify Organism and Method: Fathead -96 hr.	✓		✓		✓		✓	
LEACHATE FROM LEACHING TEST								
pH								
COD								
BOD_5								
BOD, Ultimate								
TOC								
GC								
Bioassay								
Other								
OTHER								
Bioaccumulation								
Persistence								
Degradation Products								

000328

White - Return to Requester Canary - Environmental Engrg. Office File Pink - Environmental Engrg. Lab Goldenrod - Return to Requester

* ALL RESULTS EXPRESSED AS mg/Kg – EXCEPT pH

ANALYSIS FORM 1038-A

FILE
SEC.
DATE
SHEET

ENVIRONMENTAL LAB DATA SHEET

RE: LR. NOS. 3844 AND 3849

			A	B pH	C C.O.D	D T.O.C.	E HEX. CHROM.	F T. CHROMIUM	G ULTIMATE BOD	H BOD3	J BOD4	K BOD5	L BOD7	M BOD11	N BOD14	O BOD20	P
1	FC 43	LOT 253		< 180	‡												
2	FC 95	LOT 583		<4,000	78,000												
3	FC 98	LOT 315		<4,000	119,000					*	–	*	*	*	*	<7,800	
4	FC 128	LOT 751		140,000	162,000					*	–	*	*	*	*	<3,800	
5	FC 143	LOT 261		<4,000	186,000 238,000					*	–	*	*	*	*	<7,600	
6	FC 171	LOT 1		600,000	206,000					*	–	*	*	*	<10,200	–	
7	FC 207	LOT 2		420,000	91,000					–	*	*	*	28,000	36,000	53,000	
8	FC 207B	LOT 2		420,000	195,000					–	72,000	120,000	160,000	300,000	340,000	320,000	
9	FC 214-30	LOT 508		360,000	182,000					–	66,000	88,000	140,000	260,000	350,000	340,000	
10	FC 217	LOT 517		280,000	84,000					140,000	–	140,000	180,000	160,000	170,000	160,000	
11	FC 218	LOT 687	3.3	500,000	194,000					–	95,000	130,000	120,000	130,000	140,000	120,000	
12	FC 232	LOT 539	5.5	460,000	171,000					110,000	–	110,000	120,000	120,000	110,000	100,000	
13	FC 321	LOT 572	7.9	290,000	‡				‡	110,000	–	130,000	150,000	140,000	130,000	120,000	
14	FC 379	LOT 525		280,000	240,000												
15	FC 387	LOT 100		>510,000	‡				‡	34,000	–	84,000	110,000	120,000	120,000	86,000	
16	FC 430	LOT 24		1,000,000 1,300,000	456,000												
17	FC 431	LOT 8		1,100,000	338,000					*	–	*	*	*	*	<39,000	
18	FC 504	LOT 1		>990,000	‡				‡	378,000	–	405,000	431,000	446,000	495,000	480,000	
19	FC 650	LOT 2		730,000	150,000												
20	FC 805	LOT 540	<0.0	1,100,000	203,000	<10	50,000			*	–	*	*	*	*	28,000	
21	FC 807	LOT 900		420,000	84,000					*	–	330,000	740,000	740,000	720,000	740,000	
22	FC 859	LOT 715		130,000	‡				‡	*	–	*	140,000	250,000	210,000	170,000	
23	FC 3019N			160,000	‡				‡								
24	FM 3422	LOT 716		260,000	‡				‡								
25																	
26	~~FC 77 (HOLDING)~~																
27																	
28	~~FC 214-30~~	SAMPLES															
29	~~FC 218~~	FOR															
30	~~FC 232~~	HYDROLYSIS															
31	~~FC 321~~	STUDIES															
32	~~FC 805~~																
33	~~FC 807~~																
34																	
35																	

NOTES: – SOLIDS FORMED DURING COD TEST AFTER (1) HOUR OF REFLUXING OF SAMPLES #13, #14 AND #23.

– TOC ANALYSES WERE PERFORMED BY ARRO LABORATORIES.

* INDICATES AN INSUFFICIENT DISSOLVED OXYGEN DEPLETION NECESSARY TO CALCULATE RELIABLE BOD VALUES.

‡ INDICATES THE SAMPLE IS WATER INSOLUBLE – TESTS NOT PERFORMED.

000329

ACUTE TOXICITY TO FISH

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, or FC-109-X. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-128. Current information indicates it is a mixture of 86% test substance and 14% C_4-C_7 fluoroalkyl carboxylate compounds. 3M production lot number 664.

METHOD:

Method: Not noted.
Type: Static acute
GLP: No
Year completed: 1974
Species: *Pimephales promelas*
Supplier: Not noted.
Analytical monitoring: Temperature, pH and DO
Exposure period: 96-hours
Statistical methods: Graphed
Test fish age: Not noted.
Length and weight: Average length = 2 inches
Average weight = 1.6 g
Pretreatment: Not noted.

Test conditions:
Dilution water: Carbon Filtered city water
Dilution water chemistry: Not noted.

Stock and test solution preparation: Individual weights
Concentrations dosing rate: Once
Stability of the test chemical solutions: Not noted
Exposure vessels: Type not noted. 16L volume.
Number of replicates: one
Number of fish per replicate: Not noted
Number of concentrations: five plus a negative control
Water chemistry during the study:
Final Dissolved oxygen *:
4.7 mg/L (control exposure)
3.1 mg/L (40 mg/L exposure)**
Final pH:*

7.1 (control exposure)
6.7 (40 mg/L exposure)**

Final Test temperature:* 70°F

* Copy of study is not legible for the readings taken at 24 hours. Therefore, only final values are noted.
** 40 mg/L test group data given because total mortality occurred in the 50 mg/L test group by 72 hours and all data was not collected.

RESULTS

Nominal concentrations: Bk control, 10, 20, 30, 40, and 50 mg/L
Element value: 96-hour LC50 = 30 mg/L (C.I. not calculated)

All element values based on nominal concentrations

CONCLUSIONS

The test sample 96-hour LC_{50} for fathead minnow was determined to be 30 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking 3. This study lacks documentation and information on the methodology. Sample purity was not properly characterized.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, St. Paul, MN, completed on June 29, 1974.

OTHER

Last changed: 5/16/00

FORM 10620-PWO

cc: LAB REQUEST BOOK – 42-5W

File: _____

BEST COPY AVAILABLE

Water and Wastewater

REQUEST FOR LABORATORY WORK

Lab Request No. 2340

TO: ENVIRONMENTAL ENGINEERING LABORATORY – BUILDING 53-6N

Requestor	Lab Personnel
Name: D. Bacon Request Date _____	Date Rec'd. Sample: _____
Project No. _____ Prob. No. _____ Code # _____	Analyst: MTE
Description: FC - Surfactants	Y _____ B _____ P Bio-Assay
Sample Date: _____ Date Needed: _____	Date Completed: 6-29-74
Hold Sample For _____ Days Est. Time: _____ Hrs.	Hrs. Spent: 20

Sample Description	(1) FC-95	(2) FC-128	(3) FC-134	(4) FC-143
Total Hardness, mg/l as $CaCO_3$				
Calcium Hardness, mg/l as $CaCO_3$				
"P" Alkalinity, mg/l as $CaCO_3$				
"M" Alkalinity, mg/l as $CaCO_3$				
pH				
Sulfate, mg/l as SO_4				
Nitrate, mg/l as NO_3				
Chloride, mg/l as Cl				
Silica, mg/l as SiO_2				
Fluoride, mg/l as F				
Ortho Phosphate, mg/l as P				
Total Phosphate, mg/l as P				
Total Chromium, mg/l as Cr				
Hex. Chromium, mg/l as Cr				
Total Cyanide, mg/l as CN				
Ferrocyanide, mg/l as CN				
Iron, mg/l as Fe				
Manganese, mg/l as Mn				
Surfactant, mg/l as LAS				
Phenol, mg/l				
BOD, mg/l				
COD, mg/l (Short, Long, Dilute)				
Total Suspended Solids, mg/l				
Volatile Suspended Solids, mg/l				
Turbidity, mg/l Silica				
Total Solids, mg/l				
Total Volatile Solids, mg/l				
Conductivity, mhos				
Total Bacteria, No./100 ml				
Total Coliform, No./100 ml				
Fecal Coliform, No./100 ml				
Fecal Streptococcus, No./100 ml				
Ammonia Nitrogen, mg/l as N				
Kjeldahl Nitrogen, mg/l as N				
Oil and Grease, mg/l				
Bioassay 96 hour static LC50	29 mg/l	30 mg/l	20 mg/l	70 mg/l

Comments: Lot # 542 664 510 83

Previous study: FC-95 → 32 mg/l
FC-128 → 34 mg/l
FC-134 → 32 mg/l

000372

3M Carbonless Paper

White – Environmental Engrg. Lab Canary – Environmental Engrg. Lab Pink – Environmental Engrg. Lab Goldenrod – Requesters Copy

740101

ENVIRONMENTAL ENGINEERING LABORATORY
AQUATIC TOXICITY WORK SHEET

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Type Test 96 hour Static

Material Tested FC-128 Lot #664

Test Organism Fathead Minnow

Avg. Weight 1.6 g

Avg. Size 2 Inches

Date Started 6-[illegible]-74

Date Completed 6-29-74

Time Started [illegible] PM

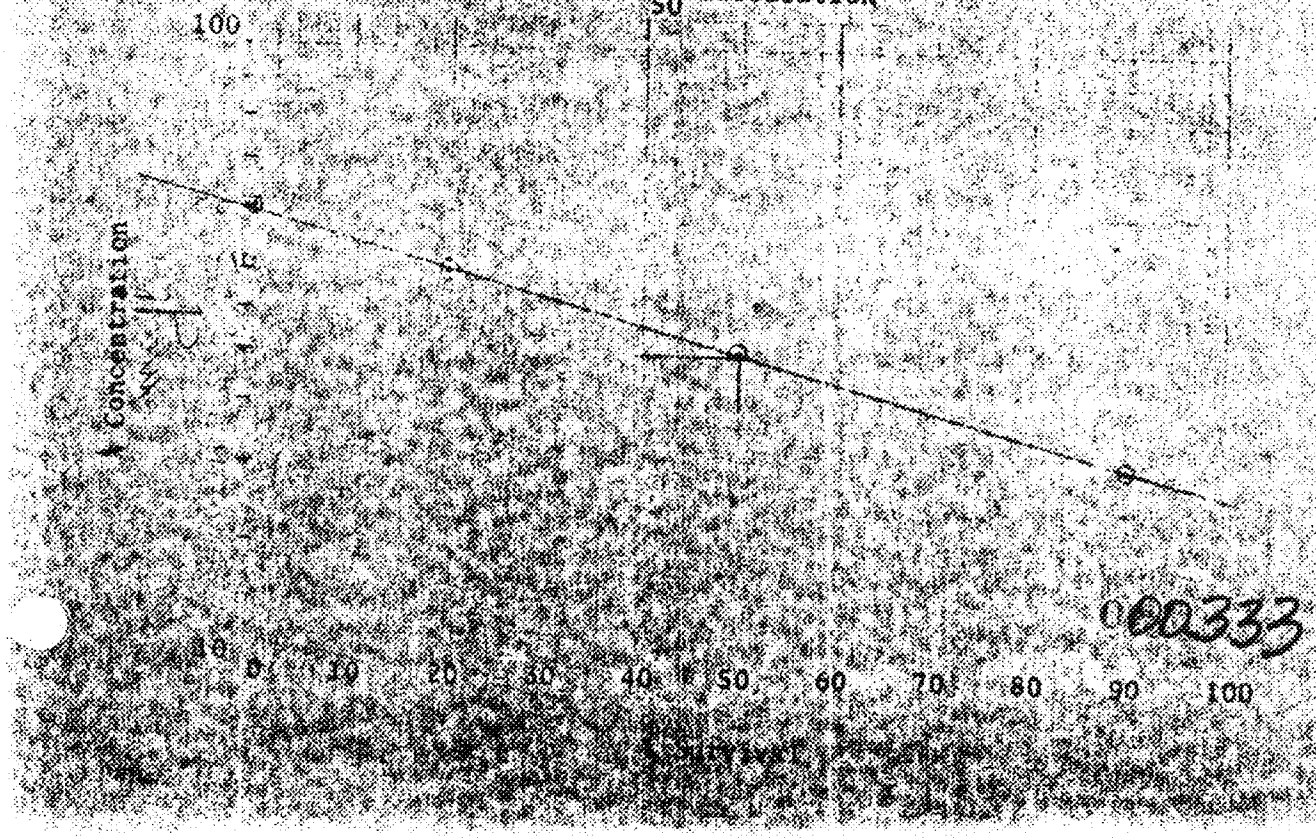
Plotted LC_{50} [illegible] g/l

Dilution Water Calcium-treated-city water

Conc.	24 hrs % Survival	pH	D.O ppm	48 hrs % Survival	pH	D.O ppm	72 hrs % Survival	pH	D.O ppm	96 hrs % Survival	pH	D.O ppm
Control	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	90	7.0	5.0	90	7.1	4.7
[illegible]		[illegible]	[illegible]		[illegible]	[illegible]	100	7.0	4.0	100	7.0	4.2
[illegible]		7.0	[illegible]		[illegible]		90	7.0	4.5	90	7.0	4.0
[illegible]		6.9	[illegible]	70	[illegible]	[illegible]	50	6.9	4.2	[illegible]	6.9	4.0
[illegible]		6.7	[illegible]	40	[illegible]	[illegible]	20	6.7	4.0	20	6.7	3.1
[illegible]	90	6.5	[illegible]		[illegible]	[illegible]	0	—	—	0	—	—
Average Temperature							70			70		

Comments: [illegible] 780 640

LC_{50} Calculation



000333

ACUTE TOXICITY TO FISH

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, or FC-109-X. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-128. Current information indicates it is a mixture of 86% test substance and 14% C_4-C_7 fluoroalkyl carboxylate compounds.

METHOD:

Method: Not noted.
Type: Static acute
GLP: No
Year completed: 1974
Species: *Pimephales promelas*
Supplier: Not noted.
Analytical monitoring: pH and DO
Exposure period: 96-hours
Statistical methods: Graphed
Test fish age: Not noted.
Length and weight: Average length = 2 inches
Average weight = 1.5 g
Pretreatment: Not noted.

Test conditions:
Dilution water: Carbon Filtered city water
Dilution water chemistry: Not noted.

Stock and test solution preparation: Individual weights
Concentrations dosing rate: Once
Stability of the test chemical solutions: Not noted
Exposure vessels: Type not noted. 16L volume.
Number of replicates: one
Number of fish per replicate: Not noted
Number of concentrations: five plus a negative control
Water chemistry during the study:
Dissolved oxygen range (24 – 96 hours):
5.0 – 6.1 mg/L (control exposure)
4.0 – 5.7 mg/L (40 mg/L exposure)*
pH range (24 – 96 hours):

7.0 – 7.1 (control exposure)
6.7 – 7.0 (40 mg/L exposure)*

* 40 mg/L test group data given because total mortality occurred in the 50 mg/L test group by 72 hours and all data was not collected.

Test temperature (24 – 96 hours): 72 to 74°F

RESULTS

Nominal concentrations: Bk control, 10, 20, 30, 40, and 50 mg/L
Element value: 96-hour LC50 = 34 mg/L (C.I. not calculated)

All element values based on nominal concentrations

CONCLUSIONS

The test sample 96-hour LC_{50} for fathead minnow was determined to be 34 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking 3. This study lacks documentation and information on the methodology. Sample purity was not properly characterized.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, St. Paul, MN, completed on May 18, 1974.

OTHER

Last changed: 5/18/00

FORM 10620-PWO

cc: LAB REQUEST BOOK – 42-5W

File: _____

Water and Wastewater

REQUEST FOR LABORATORY WORK Lab Request No. 2254

TO: ENVIRONMENTAL ENGINEERING LABORATORY – BUILDING 53-6N

Requestor	Lab Personnel
Name: D. Bacon Request Date _____	Date Rec'd. Sample: _____
Project No. _____ Prob. No. _____ Code # _____	Analyst: MTE
Description: FC-128 powder	Y _____ B _____ P Bio-Assay
Sample Date: _____ Date Needed: _____	Date Completed: 5-18-74
Hold Sample For _____ Days Est. Time: _____ Hrs.	Hrs. Spent: 10

Sample Description	FC-128					
Total Hardness, mg/l as $CaCO_3$						
Calcium Hardness, mg/l as $CaCO_3$						
"P" Alkalinity, mg/l as $CaCO_3$						
"M" Alkalinity, mg/l as $CaCO_3$						
pH						
Sulfate, mg/l as SO_4						
Nitrate, mg/l as NO_3						
Chloride, mg/l as Cl						
Silica, mg/l as SiO_2						
Fluoride, mg/l as F						
Ortho Phosphate, mg/l as P						
Total Phosphate, mg/l as P						
Total Chromium, mg/l as Cr						
Hex. Chromium, mg/l as Cr						
Total Cyanide, mg/l as CN						
Ferrocyanide, mg/l as CN						
Iron, mg/l as Fe						
Manganese, mg/l as Mn						
Surfactant, mg/l as LAS						
Phenol, mg/l						
BOD, mg/l						
COD, mg/l (Short, Long, Dilute)						
Total Suspended Solids, mg/l						
Volatile Suspended Solids, mg/l						
Turbidity, mg/l Silica						
Total Solids, mg/l						
Total Volatile Solids, mg/l						
Conductivity, mhos						
Total Bacteria, No./100 ml						
Total Coliform, No./100 ml						
Fecal Coliform, No./100 ml						
Fecal Streptococcus, No./100 ml						
Ammonia Nitrogen, mg/l as N						
Kjedldahl Nitrogen, mg/l as N						
Oil and Grease, mg/l						
Bioassay, 96 hour static LC_{50}	34 mg/l					

Comments: _____

000336

3M Carbonless Paper

White – Environmental Engrg. Lab Canary – Environmental Engrg. Lab Pink – Environmental Engrg. Lab Goldenrod – Requesters Copy

740101

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ENVIRONMENTAL ENGINEERING LABORATORY
AQUATIC TOXICITY WORK SHEET

Type Test 96 hour, static

Material Tested FC-128 powder

Test Organism Fathead Minnows

Avg. Weight 1.5g

Avg. Size 2 Inches

Date Started 5-14-7

Date Completed 5-18-74

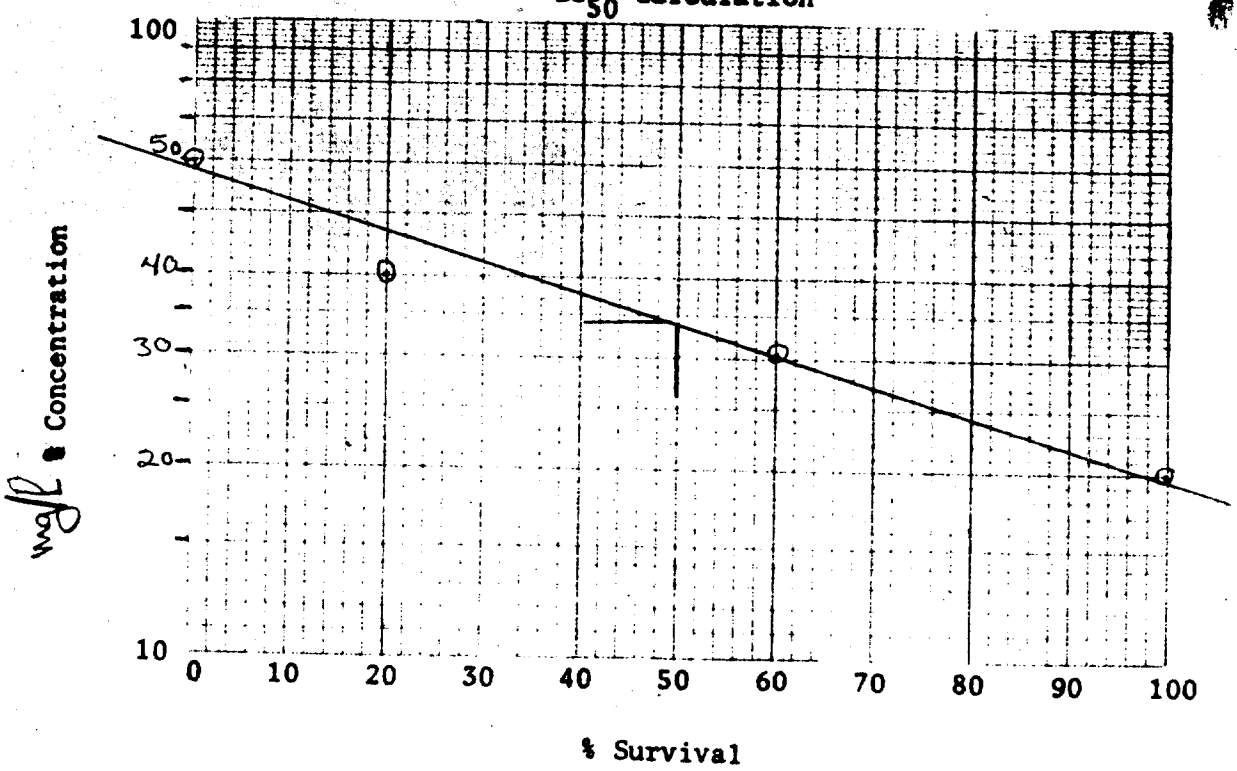
Time Started 2:00PM

Plotted LC_{50} 34 mg/l

Dilution Water carbon filtered - city water

Conc.	24 hrs % Survival	pH	D.O ppm	48 hrs % Survival	pH	D.O ppm	72 hrs % Survival	pH	D.O ppm	96 hrs % Survival	pH	D.O ppm
Control	100	7.0	6.1	100	7.0	5.7	100	7.1	5.2	100	7.0	5.0
10mg/l	100	7.0	5.9	100	7.0	5.5	100	6.9	5.0	100	6.9	4.5
20	100	6.9	5.7	100	7.0	5.2	100	6.9	4.7	100	6.9	4.5
30	100	6.9	5.7	100	7.0	5.2	70	6.9	4.5	60	6.9	4.0
40	90	6.7	5.7	70	6.9	5.0	30	7.0	4.2	20	6.9	4.0
50	70	6.7	5.5	20	6.7	4.5	0	6.5	4.0	0	—	—
Average Temperature			72		72			72			74	
Comments: Mg/lbl			160		320			480			640	800

LC_{50} Calculation



000337

ACUTE TOXICITY TO FISH

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, or FC-109-X. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-128. Current information indicates it is a mixture of 86% test substance and 14% C_4-C_7 fluoroalkyl carboxylate compounds. 3M production lot number 751.

METHOD:

Method: Not noted.
Type: Static acute
GLP: No
Year completed: 1977
Species: *Pimephales promelas*
Supplier: Not noted.
Analytical monitoring: Temperature, pH and DO
Exposure period: 96-hours
Statistical methods: Probit analysis.
Test fish age: Not noted.
Length and weight: Average length = Not noted
Average weight = 1.1 g
Pretreatment: Not noted.

Test conditions:
Dilution water: Aerated carbon-filtered well water
Dilution water chemistry:
Initial Dissolved Oxygen: >9.0 mg/L
hardness: Not noted
alkalinity: Not noted
pH: Not noted
Conductivity: Not noted

Stock and test solution preparation: Not noted
Concentrations dosing rate: Once
Stability of the test chemical solutions: Not noted
Exposure vessels: Not noted.
Number of replicates: one
Number of fish per replicate: 10
Number of concentrations: five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

5.7 – >9.0 mg/L (control exposure)

5.1 - >9.0 mg/L (180 mg/L exposure)*

pH range (24 – 96 hours):

7.6 – 7.8 (control exposure)

7.5 – 7.9 (180 mg/L exposure)*

* 180 mg/L test group data given because total mortality occurred in the 320 mg/L test group by 72 hours and all data was not collected.

Test temperature (0 – 96 hours): 62 to 67°F

RESULTS

Nominal concentrations: Bk control, 32, 56, 100, 180, and 320 mg/L
Element value: 96-hour LC50 = 100 (70 - 142) mg/L

All element values based on nominal concentrations

Statistical Evaluation of Mortality:
Probit analysis was used to calculate LC50 value and the corresponding confidence limits.

CONCLUSIONS

The test sample 96-hour LC_{50} for fathead minnow was determined to be 100 mg/L with a 95% confidence interval of 70 - 142 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking 3. This study lacks documentation and information on the methodology. Sample purity was not properly characterized. Test concentrations were not characterized.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, St. Paul, MN, completed on August 26, 1977.

OTHER

Last changed: 5/18/00

740101

(3)

ENVIRONMENTAL ENGINEERING LABORATORY
AQUATIC TOXICITY WORK SHEET

Type Test 96 Hours - STATIC

Material Tested FC-128 LOT 751

Test Organism Fathead Minnow

Avg. Weight 1.1 g

Avg. Size

Date Started 8-22-77

Date Completed 8-26-77

Time Started 2:00 PM

Plotted LC_{50} 100 mg/l

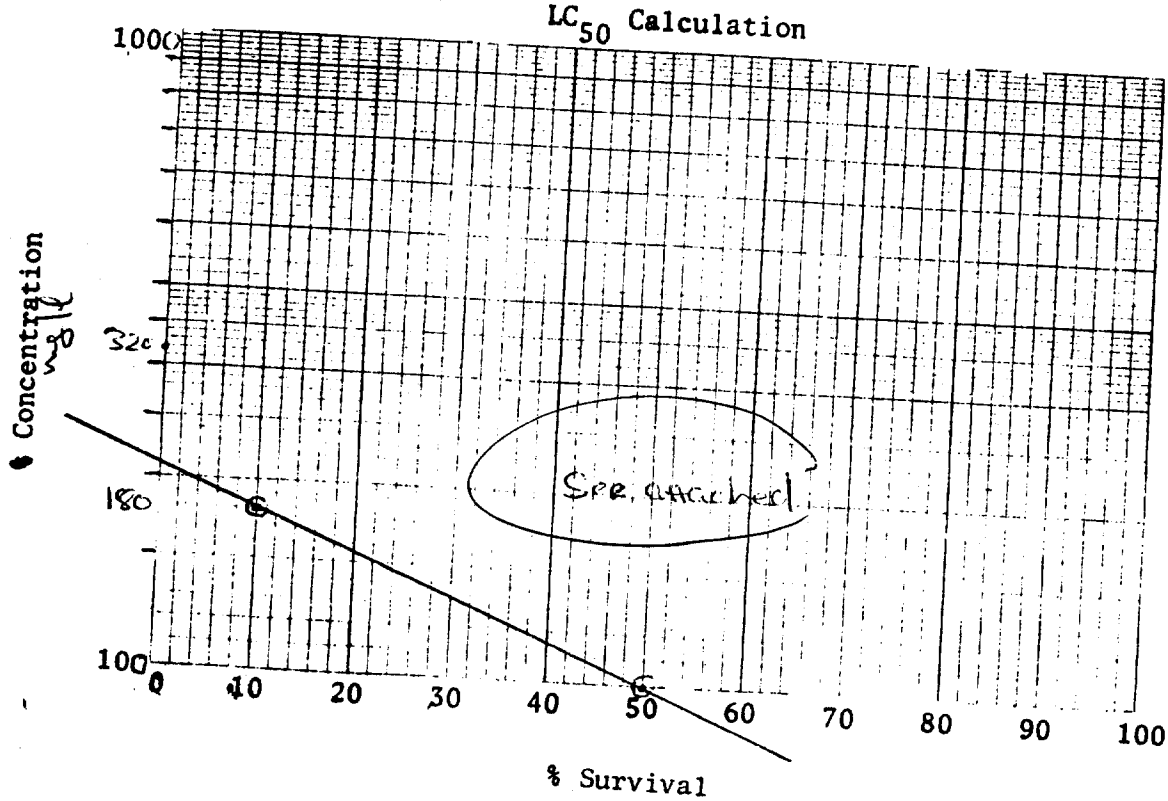
Dilution Water Carbon Filtered - Well Water (Aerated)

	Conc.	24 hrs % Survival	pH	D.O ppm	48 hrs % Survival	pH	D.O ppm	72 hrs % Survival	pH	D.O ppm	96 hrs % Survival	pH	D.O ppm
	Control	100	7.7	6.8	100	7.8	6.2	100		5.8	100	7.6	5.7
B	mg/l 32	100	7.8		100	7.8	7.3	100		6.0	100	7.3	5.4
B	56	100	7.9	5.1	100	7.8	7.5	100		6.2	90	7.3	5.3
B	100	100	7.9	5.4	100	7.8	7.8	100		6.3	50	7.5	5.0
B	180	100	7.9	5.4	100	7.7	7.5	70		6.0	10	7.5	5.1
SB	320	80	7.9	5.7	30	7.8	7.8	0		6.0	0	—	—

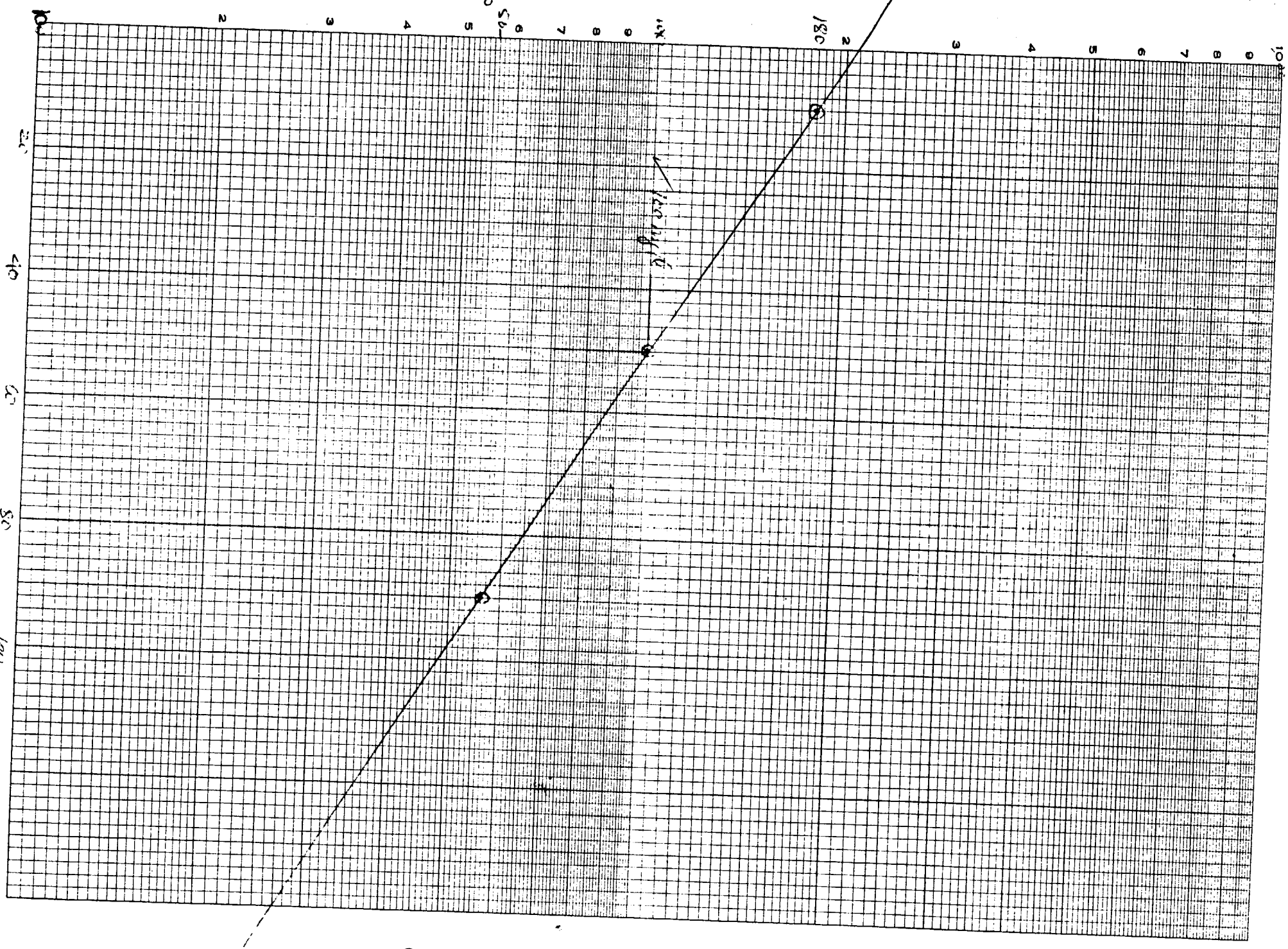
Average Temperature 62 | 65 | 65 | 67

Comments: INITIAL D.O. >9.0 mg/l
" TEMP. 62°F

LC_{50} Calculation



000040


SEMI-LOGARITHMIC
2 CYCLES X 10 DIVISIONS PER INCH
Conc. mg/l
DIETZGEN CORPORATION
MADE IN U.S.A.
T/L
100
180
20
40
60
80
100

AQUATIC TOXICITY - PROBIT ANALYSIS WORK SHEET

Date Started: _____ Material Tested: FC-128

Time Started: _____ (AM) (PM) Test Organism (Fathead minnow)

Type Test: (Continuous Flow) (Static) Avg. Wt. _____ (gram) Avg. Size _____ (inch)

Exposure Period: _____ -Hour Date Completed: _____

Diluent: (carbon-filt.-St.P. City Wtr.) Analysis by: _____

(1) K No. of Doses Plotted	mg/l Conc.	Observed % Mortality	(2) Expected % Mortality	(3) Corrected Values	O - E Observed-Expected	Contribution to chi (from Nomograph #
1	32	0	.6	.3		
2	56	10				
3	100	50				
4	180	90				
5	320	100	99.4	99.7		
6						
7						

K = 10 Animals/Dose = _____ Total contribution to chi = _____

Total Animals = _____ (chi^2) = contrib. to chi x Tot. Animals / K = _____

chi^2 (P-.05) for (K-2) _____ degree of freedom (from Table 2) = _____

_____ is less than _____; therefore, the data are not significant heterogenous.

95% Confidence Limits for LC_{50}*

LC_{84} = 150 mg/l LC_{50} = 100 mg/l LC_{16} = 68 mg/l

Slope function (S) = $LC_{84}/LC_{50} + LC_{50}/LC_{16}$ = $\frac{1 + 1}{2}$ = $\frac{1.5 + 1.47}{2}$ = 1.49

Total number of animals used between 16% and 84% expected effects (N') = 10

$fLC_{50} = (S)^{2.77/\sqrt{N'}}$ = $(1.49)^{.88}$ = (from Nomograph #2) 1.42

LC_{50}/fLC_{50} = lower limit = 100 / 1.42 = 70.4

LC_{50} X fLC_{50} = upper limit = 100 X 1.42 = 142

LC_{50} = 100 mg/l (95% confidence limits 70.4 to 142)

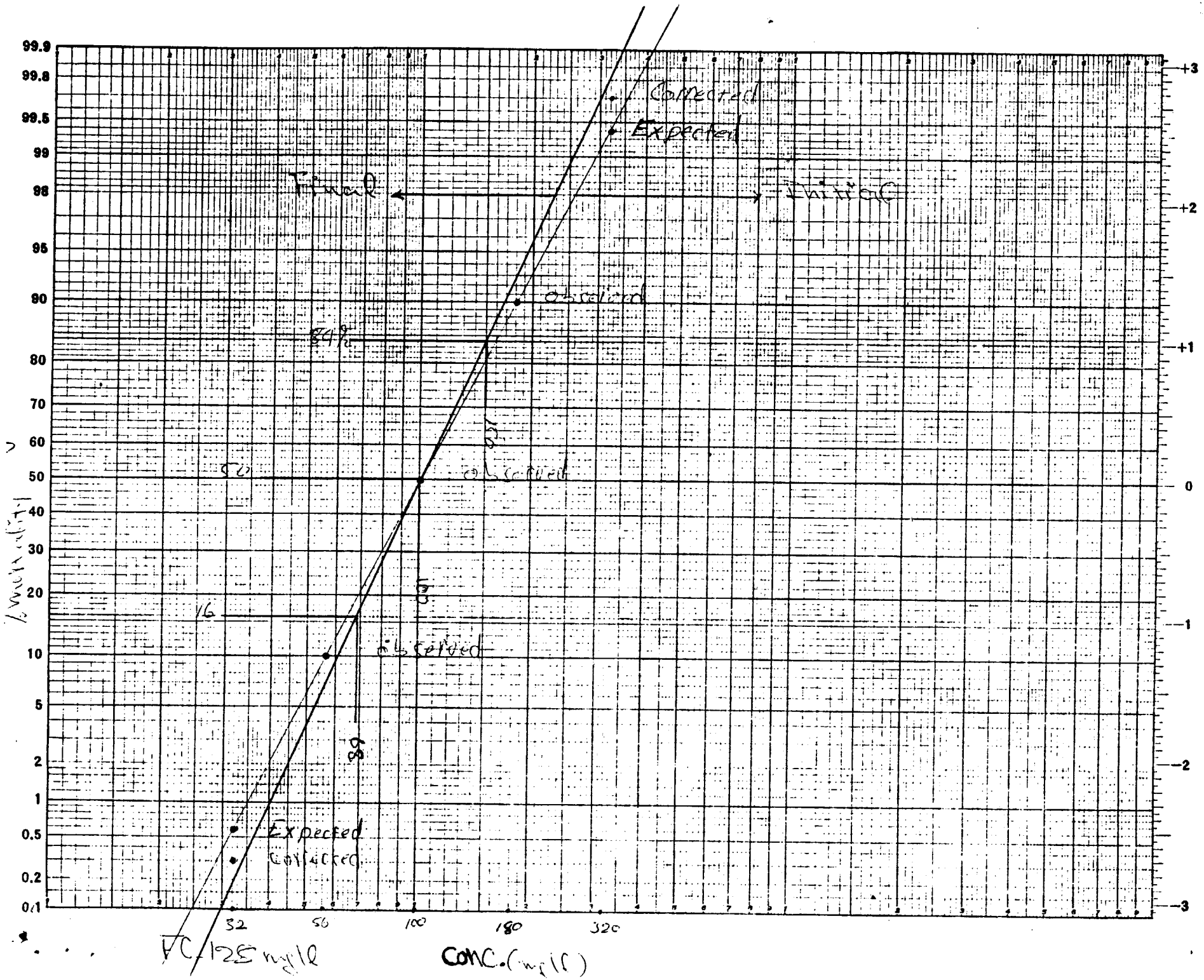
70 – 142

(1) Do not list more than two consecutive 0% effects or more than two consecutive 100% effects.

(2) Expected value for any dose should be greater than 0.01% or less than 99.99%.

(3) Corrected value for each 0% or 100% effect (from Table 1).

* There is a 95% chance that the true value of LC_{50} lies within these limits.



Corrected
Expected
Final
Initial
observed
84%
50
observed
16
observed
Expected
corrected
32
56
100
180
320
FC 125 mg/l
Conc. (mg/l)
% mortality
99.9
99.8
99.5
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
+3
+2
+1
0
-1
-2
-3

000343

BIOACCUMULATION (ANALYTICAL ONLY)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, or FC-109-X. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-128. Current information indicates it is a mixture of 86% test substance and 14% C_4-C_7 fluoroalkyl carboxylate compounds.

METHOD:

Method: Devised by 3M Company to determine if bluegill sunfish bioaccumulate fluorochemicals from 3M's Decatur, AL plant effluent (See Remarks)
GLP (Y/N): No
Year completed: 1978
Remarks: The following method was devised and used:

1. Two lots of 30 fish each were used.
2. One lot was exposed to Decatur plant effluent from Oct. 5 to Oct. 25. The other lot was exposed to Tennessee River water only from Oct. 3 to Oct. 25.
3. Twelve fish from the effluent exposed lot were collected dead and the remaining eighteen fish were sacrificed in Decatur. The river water exposed fish survived through the exposure period and all were sacrificed in Decatur.
4. Effluent-exposed and river water-exposed fish were all preserved and shipped in dry ice to the 3M Environmental Laboratory in St. Paul, MN for analysis.
5. Fish were homogenized using a Tekmar Model SDT tissuemizer.
6. Two 2 g. portions were weighed and extracted using ethylacetate on one and toluene on the other.
7. Samples were analyzed by GC, TLC, and GC/MS.

RESULTS

Analytical Observations:

The effluent-exposed fish weighed 25.7 grams. The river water-exposed fish weighed 33.0 grams.

GC/MS analysis of ethyl acetate extracted fish exposed to plant effluent did not detect the presence of FC-128.

No fluorochemicals were detected in the river water-exposed fish.

Remarks: Study was conducted because 3M's Decatur plant manufactures fluorochemicals. Water used in manufacturing is later treated and the treated effluent discharges eventually into the Tennessee River.

CONCLUSIONS

No reliable conclusions can be derived from this study.

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

DATA QUALITY

Reliability: Klimisch ranking 3. Study was conducted at the manufacturing facility rather than at 3M's Environmental Laboratory. Effluent concentrations of subject fluorochemicals were not characterized and the specific protocol for exposure of the fish was not found. There was also no information on analysis of the Tennessee River water or effluent used in the study (e.g., NH_3-N in effluent is known to cause death during NPDES testing, or low DO, high TDS). Additionally, it is not known if there was any opportunity for depuration of the fish prior to sacrifice. Presence of the test substance could have been a normal function of the fish concentrations homogenizing with the effluent concentrations. No explanation is attempted as to what was the cause of the 12 dead fish in the effluent-exposed group. No differentiation was given to whether the subject chemical was actually accumulated in the fish or simply sorbed on the surface of the fish body. Sampling locations were not described.

REFERENCES

3M Technical Report "Analysis for Fluorochemicals in Bluegill Fish" G. A. Vraspir / Arthur Mendel, Project 9970612600, Fate of Fluorochemicals, Report Number 14, May 1, 1979

3M requested expert overview, "Bioaccumulation Studies", Dr. James Gillett, Cornell University, March 8, 1993

OTHER

Last changed: 5/18/00

3M Company
Form 6747-11-A

TECHNICAL REPORT SUMMARY

Date: 5/1/79

TO: TECHNICAL COMMUNICATIONS CENTER – 201-2CN

(Important – If report is printed on both sides of paper, send two copies to TCC.)

Division: Environmental Laboratory (EE & PC) Req. No. 48765	Dept. Number: 0535
Project: Fate of Fluorochemicals	Project Number: 9970612600
Report Title: Analysis for Fluorochemicals in Bluegill Fish	Report Number: 014
To: D. L. Bacon	
Author(s): G. A. Vraspir/Arthur Mendel	Employee Number(s): 76416/43939
Notebook Reference: 48838-18	No. of Pages Including Coversheet: 7

SECURITY ▶ ☐ Open (Company Confidential) ☐ Closed (Special Authorization)

3M CHEMICAL REGISTRY ▶ New Chemicals Reported ☐ Yes ☒ No

KEYWORDS: (Select terms from 3M Thesaurus. Suggest other applicable terms.)

CURRENT OBJECTIVE:

Status Report

REPORT ABSTRACT: (200-250 words) This abstract information is distributed by the Technical Communications Center to alert 3M'ers to Company R&D. It is Company confidential material.

c: R.A.Prokop-236-3B
V.Pothapragada-236-3A
A.N.Welter-21-2W
R.L.Bohon-21-2W

000346

Information Liaison Initials: [illegible]

OBJECTIVE

The purpose of this investigation was to determine if bluegill fish, after exposure to our Decatur plant effluent, bioaccumulated fluorochemicals.

SUMMARY

Extracts from bluegill fish exposed to Decatur plant effluent (PE) contained 10 ppm (μg/g) of FM 3923 and 7 ppm of FM 3422. TLC indicated the presence of FC-95, while FC-143 and FC-128 were not detected.

Extracts from bluegill fish exposed to river water (RW) contained ethanol, ethyl ether, ethoxyethyl acetate, toluene, and xylene. No fluorochemicals were detected.

A copy is attached of CRL report 7124 which detailed GC/MS experiments.

INTRODUCTION

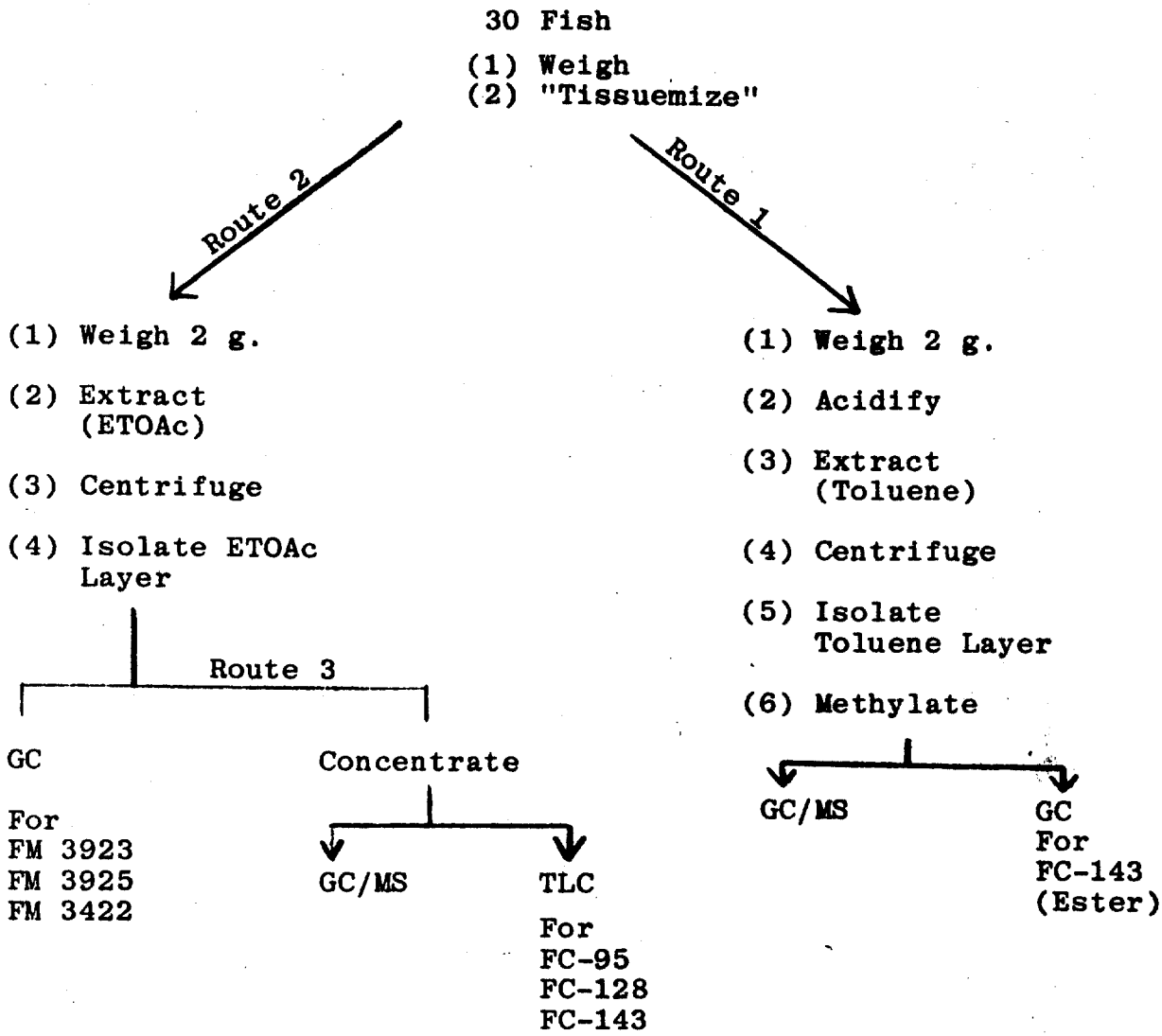
The uptake and clearance of specific radiolabeled fluorochemicals by fish were reported (1). The current report details the analyses for fluorochemicals in fish previously exposed to our Decatur plant effluent and in fish exposed only to Tennessee River water (2). Thin-layer chromatography (TLC), gas chromatography, and gas chromatography/mass spectroscopy (GC/MS) were used.

DISCUSSION

Our Decatur plant manufactures fluorochemicals. Water used in manufacturing is later treated and the treated effluent discharges eventually into the Tennessee River. Bluegill fish, chosen for these bioassay experiments, were exposed to P.E. Another lot of fish were exposed only to the Tennessee River water (2). The fish were then analyzed for fluorochemicals by an involved three-route procedure as outlined in Flow Diagram 1 and detailed in the experimental section. Briefly, fish were homogenized, and some of the homogenate was extracted with toluene (Route 1), while some of the homogenate was extracted with ethyl acetate (Route 2). A portion of the latter extract was concentrated Route 3) for GC/MS and TLC. Results are given in the summary section.

FLOW DIAGRAM 1

ANALYSIS OF BLUEGILL FISH



EXPERIMENTAL

Two lots of thirty fish each, wrapped in aluminum foil, respectively, and shipped in dry ice, were received on November 3, 1978, by the Environmental Laboratory. The fish were kept frozen until analyzed (Nov. 3, 1978). The effluent-exposed lot (Oct. 5 to Oct. 25) weighed 25.7 g. Twelve fish were collected dead and eighteen fish were sacrificed in Decatur. The river-exposed lot (Oct. 3 to Oct. 25) weighed 33.0 grams and were all sacrificed at Decatur.

To simplify, the experimental one lot is discussed. The other was analyzed in exactly the same manner. The following codes were used:

River Water Holding Tank Exposed Fish - ethylacetate extract (R.W.-EtAc)
River Water Holding Tank Exposed Fish - toluene extract (R.W.-Tol.)
Plant Effluent S-1 Exposed Fish - ethylacetate extract (P.E.-EtAc)
Plant Effluent S-1 Exposed Fish - toluene extract (P.E.-Tol.)

The fish were homogenized using a Tekmar Model SDT tissuemizer. Two 2 g. portions were weighed into separate polyethylene centrifuge tubes. Two ml's of pesticide grade ethylacetate were added to one and 2 ml conc. HCl plus 2 ml of AR grade toluene were added to the other. Each sample was mixed approximately 1 min. on a Vortex Genie mixer. The samples were centrifuged for 15 min. at 10,000 rpm's. The ethylacetate and toluene extracts were drawn off and placed in glass vials, respectively. The ethylacetate extract was examined using the following GC conditions: Instr. - HP 5713 with Ni^{63} electron capture detector. Column - 6'x 1/8" S.S. 10% Carbowax 20 M on 60/80 mesh Chromosorb W-AW. Inj. Port - Temp - 200° C., Def. Temp - 300° C., Oven Temp - 150° C isothermal, Flow - 40 cc/min. Argon/Methane (95/5).

After GC analysis of EtAc extract, a portion was concentrated under N_2 purge for two dimensional TLC analyses. The following TLC conditions were used. The concentrated EtAc extracts were spotted alongside standards of FC-95, FC-143, and FC-128 on E. Merck silica gel plates. They were first developed in 10% EtOH in EtAc and dried. Then the plates were turned 90° and developed in 50% EtOH in EtAc. The dried plates were visualized by spraying with 0.01% New Fuchsin in water (3).

The toluene extract was methylated with diazomethane and then examined using the following GC conditions: Instr. HP 5713 with Ni^{63} electron capture detector. Column - 12'x 1/8" S.S. 20% DC-200 and 10% Bentone 34 or 80/90 mesh Anakrom PA. Inj. Port - 200° C. Det. temp - 300° C., Oven - 110° C Isothermal, Flow - 40 cc/min. Argon/Methane (95/5). The ethylacetate and methylated toluene extracts were submitted to Central Research Analytical for GC/MS analysis (Req. No. C-50687). See attached CR Report No. 7124 for GC/MS conditions.

PE/EtAc extract showed approximately 10 μg/g of FM 3422. RW & PE-Tol. showed no difference. No FC-143 or FC-128 was detected by TLC of the PE EtAc, but a spot was noticed whose R_f was that of reference FC-95.

REFERENCES

(1) a) M. T. Elnabarawy, "Bioconcentration of FM 3422 in Bluegill Sunfish and In Channel Catfish," EE & PC Report, 5/17/77.

b) A. N. Welter, "Aquatic Fate of A Fluorochemical, FM 3422," EE & PC Report, 10/14/77.

(2) Decatur Effluent Fish Toxicity Test Protocols, dated Oct. 18, 1978 and Feb. 5, 1979; M. T. Elnabarawy.

(3) J. Sliwiok and A. Macioszczyk, Microchemical Journal, 23, 121 (1978).

J. A. Vrazpir 5/21/79

5/24/79

GAV/AM/cen

Attachment

Form 2643-1 rev

CENTRAL ANALYTICAL LABORATORY

Report No. 7124

Date March 28, 1979

Subject: Decatur Fish Bioassy.

Requestor: G. Vraspir, A. Mendel Dept. Name EE & PC Proj. No. 915016190404

Request No. C50687 Dated November 16, 1978

Report:

INTRODUCTION

Samples were received from the requestors from a fish bioassy taken at the Decatur Plant. Fish were exposed to two different water systems: the Tennessee River which flows just outside the plant and the plant effluent running into the river. Ethyl acetate extracts of the fish were analyzed using the GC/MS system. Toluene extracts of the fish were methylated with diazomethane and analyzed.

DISUCSSION

The samples were labeled R.W. #1 and P.E. #1 (methylated toluene extracts) and R.W. #2 and P.E. #2 (ethyl acetate extracts). Samples R.W. #1 and P.E. #1 were analyzed using conditions for column A below and samples R.W. #2 and P.E. #2 were analyzed using conditions for column B below.

Instrument: Varian 2740 gas chromatograph
Column A: 12' S.S. 20% DC-200 + 10% Bentone 34 on Anakrom PA
Oven: 110°C isothermal for 11 minutes then 110-170°C at 10°C/min.
Injector: 165°C

Column B: 6' S.S. CW 20M 15% on Chromosorb W
Oven: 50 - 225°C at 10°C/min.
Injector: 220°C

Detector: Varian flame ionization at 290°C
Carrier Gas: Helium at 25 cc/min.

Instrument: duPont 21-491B mass spectrometer
Source: Chemical Ionization
Mode: all samples were run in Electron Ionization, sample P.E. #2 was also run in Chemical Ionization with NH_3 gas
Mass Range: 18 - 617 m/e
Helium Separator: Single Stage Jet
Multiplier: Bendix Channeltron®

RESULTS

Samples R.W. #1 and P. E. #1 showed by GC/MS only the solvents used in extracting and methylating: ethanol, ethyl ether, ethoxy ethyl acetate toluene and xylenes.

It was necessary to air concentrate the ethyl acetate extracts in order to obtain sufficient data to interpret the mass spectra.

The Tennessee River exposed fish ethyl acetate extract contained numerous G.C. peaks none of which appeared to contain fluorine. The components appear to correspond to references of 2-methyl-3-octanone, acetic acid, a phthalate, and high molecular alkanes from about C_{15} to at least C_{24}.

The plant effluent exposed fish ethyl acetate extract contained compounds which appear to correspond to references of 2-methyl-3-octanone, acetic acid, fluorochemicals whose highest mass fragments seen were 483 m/e and 582 m/e, nitrogen containing fluorocehmicals (whose highest mass fragments seen were 448 m/e and 584 m/e), FM 3923 (FOSE amide) and FM 3422 (FOSE alcohol).

SUMMARY

This fish bioassy showed that extracts from the fish exposed to the Tennessee River water contained such compounds as ethanol, ethyl ether, ethoxy ethyl acetate, toluene and xylene. The extracts from the fish exposed to the Decatur plant effluent contained FOSE amide, alcohol, other related fluorochemicals, 2-methyl-3-octanone, and acetic acid.

J. N. Schroepfer

J. N. Schroepfer

/ch

BIODEGRADATION

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, FC-109-X or R1904. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-129 referred to by the test laboratory as R1904. Current information indicates it is a mixture of 42% test substance, 32% water, 14% 2-butoxyethanol, 4% ethanol, and 8% C_4-C_7 fluoroalkyl carboxylate compounds. Test material was from 3M production lot 930014, mfg. date 3/97.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data does not accurately relate biodegradation of the test sample with that of the test substance.

METHOD:

Method: Not noted.
Type: Aerobic
GLP: No
Year completed: 1997
Contact time: 28 days
Inoculum: Polyseed NX, lot D257

RESULTS

COD: 462,000 mg O_2 / kg chemical
BOD

Day	First Replicate BOD Mean Value (mg/kg)	Second Replicate BOD Mean Value (mg/kg)
5	<39,800	<39,800
10	186,900	156,750
20	82,700	179,400
28	289,000	289,000

Remarks: The BOD and COD values are expected to be the result of biodegradation of the organic solvent components of the test sample. These values are not expected to be representative of the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

This testing indicated that some portion of the test sample biodegraded in aerobic environments capable of supporting biodegradation.

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

DATA QUALITY

Reliability: Klimisch ranking 3. This study lacks information on the methodology. Testing cannot conclude what portions of the test sample were responsible for the apparent biodegradation values. Comparison with the COD value for determination of the level of biodegradation is not valuable without a description of how the COD value was obtained.

REFERENCES

This study was conducted at AScl Corporation of Duluth, Minnesota at the request of the 3M Company.

OTHER

Last changed: 5/17/00

R1904 BOD REPORT FOR 3M COMPANY

Submitted to

3M Environmental Technology and Safety Services
935 Bush Avenue
Building 2-3E-09
PO Box 33331
St. Paul, MN 55133-3331

Prepared by

AScI Corporation/AScI-Duluth
Environmental Testing Division
4444 Airpark Boulevard
Duluth, Minnesota 55811

JULY 1997

Sponsor: 3M Company
Sponsor Study ID# R1904

COD = 462,000 mg O_2 / kg chemical.

Dates of BOD test: 6/11/97 - 7/9/97.

Water bath temperature range during the test: 19.0 - 20.0°C.

Lot number of Polyseed NX: D257.

TABLE 1. BOD Dilution Information for R1904

Sample ID (mg/L)	Stock Solution Concentration (mg/L)	Sample Volume (ml)	Sample Concentration (mg/L)
10	1005.04	3.0	10.1
18	1005.04	5.5	18.4
25	1005.04	7.5	25.1
50	1005.04	15.0	50.3

TABLE 2. BOD Blank Calculations for R1904

Sample ID	Day	FIRST REPLICATE			SECOND REPLICATE		
		Initial D.O.	Final D.O.	D.O. Drop	Initial D.O.	Final D.O.	D.O. Drop
Blank	5	8.89	8.90	-0.01	8.84	8.84	0
Blank	10	8.89	8.78	0.11	8.84	8.64	0.20
Blank	20	8.89	8.52	0.37	8.89	8.50	0.34
Blank	28	8.89	8.40	0.49	8.84	8.52	0.32

TABLE 3. BOD Seed Correction Factor Calculations for R1904

Seed Conc. ml/Bottle	Day	FIRST REPLICATE				SECOND REPLICATE				Average SCF
		Initial D.O.	Final D.O.	D.O. Drop	SCF[a]	Initial D.O.	Final D.O.	D.O. Drop	SCF	
10	5	8.90	6.01	2.89	0.58	8.91	5.63	3.28	0.66	0.62
10	10	8.90	5.87	3.03	0.61	8.91	6.02	2.89	0.58	0.60
10	20	8.90	4.46	4.44	0.89	8.91	4.05	4.85	0.97	0.93
10	28	8.90	3.52	5.38	1.08	8.91	3.56	5.35	1.07	1.08

[a] SCF = (D.O. drop * ml seed in test) / ml seed in seed blank.

TABLE 4. BOD Reference[a] and Tox Blanks Calculations for R1904

Conc. mg/L	Day	SCF	FIRST REPLICATE					SECOND REPLICATE				
			Initial D.O.	Final D.O.	D.O. Drop	Corrected Drop	BOD mg/L[b]	Initial D.O.	Final D.O.	D.O. Drop	Corrected Drop	BOD mg/L
0	5	0.62	8.90	4.80	4.72	4.10	205	8.89	4.64	4.25	3.63	182
50.3	5	0.62	8.91	2.34	6.57	5.95	298	8.90	3.62	5.28	4.66	233

[a] Reference = 150 mg glucose and 150 mg glutamic acid in 1 liter BOD dilution water, tested at 2% dilution.

[b] BOD mg/L = (corrected D.O. drop) * 300 ml BOD volume / 6 ml reference.

TABLE 5. BOD Calculations for R1904

Conc. mg/L	Day	SCF	FIRST REPLICATE					SECOND REPLICATE				
			Initial D.O.	Final D.O.	D.O. Drop	Corrected Drop	BOD mg/kg[a]	Initial D.O.	Final D.O.	D.O. Drop	Corrected Drop	BOD mg/kg
10.1	5	0.62	8.90	8.00	0.90	0.28	--	8.91	8.32	0.59	-0.03	--
18.4	5	0.62	8.89	7.96	0.93	0.31	--	8.90	7.83	1.07	0.45	--
25.1	5	0.62	8.90	7.74	1.16	0.54	--	8.90	7.72	1.18	0.56	--
50.3	5	0.62	8.88	6.61	2.27	1.65	32,800	8.90	6.70	2.20	1.58	31,400
							Mean <39,800					Mean <39,800
10.1	10	0.60	8.90	6.28	2.62	2.02	200,000	8.91	7.60	1.31	0.71	--
18.4	10	0.60	8.89	7.20	1.69	1.09	--	8.90	6.02	2.88	2.28	123,900
25.1	10	0.60	8.90	2.74	6.16	5.56	222,000	8.90	3.54	5.36	4.76	189,600
50.3	10	0.60	8.88	1.31	7.57	6.97	138,600	8.90	0.05	8.85	8.25	--
							Mean 186,900					Mean 156,750
10.1	20	0.93	8.90	7.02	1.88	0.95	--	8.91	7.28	1.63	0.70	--
18.4	20	0.93	8.89	6.00	2.89	1.96	--	8.90	6.04	2.86	1.93	--
25.1	20	0.93	8.90	5.69	3.21	2.28	90,800	8.90	1.48	7.42	6.49	258,600
50.3	20	0.93	8.88	4.20	4.68	3.75	74,600	8.90	2.95	5.95	5.02	100,200
							Mean 82,700					Mean 179,400
10.1	28	1.08	8.90	4.96	3.94	2.86	283,000	8.91	5.06	3.85	2.77	274,000
18.4	28	1.08	8.89	2.40	6.49	5.41	294,000	8.90	2.25	6.65	5.57	303,000
25.1	28	1.08	8.90	0.48	8.42	7.34	--	8.90	0.12	8.78	7.70	--
50.3	28	1.08	8.88	0	--	--	--	8.90	0	--	--	--
							Mean 289,000					Mean 289,000

[a] BOD = corrected drop x 1,000,000 / test concentration.

Omitted data points: D.O. drop corrected for SCF too low (<2.0).
Final D.O. <0.50 mg/L.

APPENDIX A

-- Raw Data Sheets

BIODEGRADATION (METHYLENE BLUE ACTIVE SUBSTANCE)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, FC-109-X or R1904. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-129 referred to by the test laboratory as sample G1208-1 and replicate analysis as sample G1208-4. Current information indicates it is a mixture of 42% test substance, 32% water, 14% 2-butoxyethanol, 4% ethanol, and 8% C_4-C_7 fluoroalkyl carboxylate compounds. Test material was from 3M production lot 564.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data does not accurately relate biodegradation of the test sample with that of the test substance.

METHOD:

Method: EPA Method 425.1
Type: Methylene Blue Active Substance
GLP: No
Year completed: 1989

RESULTS

G1208-1: 2200 mg/kg
G1208-4: 2800 mg/kg (duplicate of G1208-1)
Duplicate EPA LAS Reference samples: 60,000 mg/kg
60,000 mg/kg

CONCLUSIONS

This testing indicated that the test sample exhibited minimal activity in this MBAS study.

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

DATA QUALITY

Reliability: Klimisch ranking 2. This study lacks information on the methodology and testing parameters. Test substance purity within the test sample was not sufficiently characterized.

REFERENCES

This study was conducted at Pace Analytical of Minneapolis, Minnesota at the request of the 3M Company.

OTHER

Last changed: 5/17/00

*** REVISED ***

LAB REQUEST NO. G1208

REQUESTOR NAME: EA REINER
DEPARTMENT: 0535
PROJECT NO: 9970010100
DATE RECEIVED: 02/03/1989
DESC: MBAS OF FC-129, TRITON H66

CONTRACT LAB: PACE
CONTRACT LAB COST: 162
3M E-L HOURS: 0
EXP COMP DATE: 02/17/1989
DATE COMPLETED: 02/15/1989

SAMPLES SHOULD BE DELIVERED IN MY NAME TO THE LAB. RUN A MEASUREMENT ON THE STANDARD LAS SOLUTION FOR COMPARISON. DO ALL EXTRACTIONS AND BACK WASH CALLED FOR IN PROCEDURE. FC-129 IS 50% SOLIDS. ITS MW IS 623. THE MW OF THE LAS STANDARD IS PROBABLE ABOUT 325. TEST FC-129 AT A MOLAR CONCENTRATION EQUAL TO THAT OF LAS.
G1208-3 IS AN EPA LAS REFERENCE MATERIAL: LOT #1138, AVE. M.W. 318, ACTIVE LAS 6.00 %, PREPARED BY EMSL CINCINNATI US EPA IN COOPERATION WITH SOAP AND DETERGENT ASSOCIATION, DATE 985.

Revised report shows correction in project description above.

SAMPLE	DATE	CODE	DESCRIPTION	RESULT	MIN DET LIMIT
1		FC-129	LOT 564		
		*SURFACTANTS -	MBAS	2200 MG/KG	5
		*INORGANIC TESTS	REPORT	SEE ATTACHED REPORT	
2		TRITON H66	RH-RM 6-1536		
		*SURFACTANTS -	MBAS	1000 MG/KG	5
3		LAS STD.	EPA LOT 1138		
		*SURFACTANTS -	MBAS	60000 MG/KG	5
4			DUPLICATE #1		
		*SURFACTANTS -	MBAS	2800 MG/KG	5
5		DUPLICATE #2	~~~~~~~~~~~~		
		*SURFACTANTS -	MBAS	1200 MG/KG	5
6			DUPLICATE #3		
		*SURFACTANTS -	MBAS	60000 MG/KG	5

* = CONTRACT LAB

SUBMITTED BY: K Hoffmann DATED: 2-23-89

pace laboratories, inc.

REPORT OF LABORATORY ANALYSIS

Offices:
Minneapolis, Minnesota
Tampa, Florida
Coralville, Iowa
Novato, California

G1208

February 10, 1989

Rec 2/14/88
WMS

Mr. Wade Scheil
3M Environmental Laboratory
935 Bush Avenue
Bldg. 2-3E
St. Paul, MN 55133

Dear Mr. Scheil:

Enclosed is the report of laboratory analyses for samples received 02/03/89.

If you have any questions concerning this report, please feel free to contact Tom Halverson or me.

Sincerely,

Peggy F. Gaskill for
Roger C. Splinter, Ph.D.
Director, Laboratory Services

Enclosures

000363

1710 Douglas Drive North □ Minneapolis, MN 55422 □ Phone (612) 544-5543
an equal opportunity employer

pace laboratories, inc.

REPORT OF LABORATORY ANALYSIS

Offices:
Minneapolis, Minnesota
Tampa, Florida
Coralville, Iowa
Novato, California

3M Environmental Laboratory
935 Bush Avenue
Bldg. 2-3E
St. Paul, MN 55133

February 10, 1989
PACE Project Number: 890203509

Attn: Mr. Wade Schell

G1208

Date Sample(s) Collected: By Client
Date Sample(s) Received: 02/03/89

PACE Sample Number:			032110	032120	032130
Parameter	Units	MDL	G1208-1	G1208-2	G1208-3
INORGANIC ANALYSIS					
INDIVIDUAL PARAMETERS					
Surfactants (1)	mg/kg	5	2200	1000	60000

MDL Method Detection Limit

000364

pace laboratories, inc.

REPORT OF LABORATORY ANALYSIS

Offices:
Minneapolis, Minnesota
Tampa, Florida
Coralville, Iowa
Novato, California

Mr. Wade Scheil
Page 2

February 10, 1989
PACE Project Number: 890203509

PACE Sample Number:			032140	032150	032160
Parameter	Units	MDL	G1208-4	G1208-5	G1208-6
INORGANIC ANALYSIS					
INDIVIDUAL PARAMETERS					
Surfactants (1)	mg/kg	5	2800	1200	60000

MDL Method Detection Limit
(1) Analysis performed by EPA Method 425.1.

The data contained in this report were obtained using EPA or other approved methodologies. All analyses were performed by me or under my direct supervision.

Thomas L. Halverson
Inorganic Chemistry Manager

000365

1710 Douglas Drive North □ Minneapolis, MN 55422 □ Phone (612) 544-5543
an equal opportunity employer

ANALYSIS:

FOR CLP USE ONLY? [ ]

MBAS X

DATE ANALYZED: 2/6/89
ANALYZED BY: BT
TIME:
DATA REVIEWED BY: TCB 2/7
ENTERED BY: BT

CLIENT NAME:
PROJECT NUMBER:
PROJECT NAME:
FILE #:
DATE RECEIVED:
DATE COLLECTED:

SAMPLE	ANALYSIS RESULTS	UNITS	COMMENTS	ml Sample	ABS	µg LAS
BLK	<0.05	mg/L		100	0	< 5
Std	100	µg	10ml @ 10µg/ml TV=100µg 100%	100	.255	100
2879	<0.5	mg/L		10	<.005	<5
3211	2200	mg/Kg	ig → 100 ml G1208-1	1 5	0.05	22
3212	1,000	↓	" -2	1 5	0.02	10
3213	60,000	↓	" -3	0.2 5	0.305	119
3214	2600	↓	Ave = 2800 " -4	1 5	0.06	26
↓ (B)	3100	↓		1 5	0.075	31
3215	1200	↓	" -5	1 5	0.025	12
3216	60,000	↓	" -6	0.2 5	0.305	119

BIODEGRADATION

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, FC-109-X or R1904. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-129. The study-defined composition is 50% Potassium fluoro alkyl carboxylate, 18% butyl carbitol, 28% water, and 4% ethanol. This is a minor contradiction to the formulation available from proprietary 3M databases indicating it is a mixture of 42% test substance, 32% water, 14% 2-butoxyethanol, 4% ethanol, and 8% C_4-C_7 fluoroalkyl carboxylate compounds. Test material was from 3M Deutschland production batch 2304.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data does not accurately relate biodegradation of the test sample with that of the test substance.

METHOD:

Method: OECD 301E
Type: Aerobic
GLP: No
Year completed: 1984
Contact time: 28 days
Inoculum: Secondary Effluent of a domestic sewage plant

RESULTS

Mean DOC removal after 28 days: 90%

Remarks: Organic carbon content of the test material was determined to be 13.5 mg/100 mg sample by a carbon analyzer.

CONCLUSIONS

No reliable conclusions can be derived from this study.

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

DATA QUALITY

Reliability: Klimisch ranking 3. The digestive procedure of the instrument used in this study to determine TOC is not aggressive enough to break the carbon-fluorine bonds. The TOC is therefore inaccurate. The calculated TOC is 22% compared to the 13.5% obtained by analysis. These results are expected to be representative of the organic solvents present and not of the Perfluorooctanesulfonylamido(ethyl)acetate. Lack characterization of test substance and confirmation of test concentrations.

REFERENCES

This study was conducted at Research and Consulting Company AG, Ch-4452 Itingen / Switzerland at the request of the 3M Company.

OTHER

Last changed: 5/17/00

Internal Correspondence

cc: R. L. Bohon - 21-2W-05

To: D. R. RICKER - COMMERCIAL CHEMICALS DIV. - 236-1B-10

From: E. A. REINER - ENVIRONMENTAL LABORATORY/EE & PC - 21-2W-5

Subject: OECD BIODEGRADATION TESTS ON FC-129 AND FC-170C

Date: OCTOBER 24, 1984

3M

We have received copies of the OECD Screening Tests on FC-129 and FC-170C from D. Reese of 3M Germany.

The results of the FC-129 study incorrectly indicate that this product is readily degradable. This result does not accurately represent the actual susceptibility of this product to biodegradation because the instrument used to measure total organic carbon (TOC) uses a UV-persulfate digestion method. The OECD Screening test does not require a specific TOC method, but the digestive procedure of the instrument used in this study is not strong enough to break fluorine-carbon bonds. Therefore, these results, really only show that the butyl Carbitol® and ethanol in this product are biodegradable.

The results of the FC-170C study are more useful but still inaccurate. These results confirm our previous BOD tests that suggested partial degradation of the polyethylene glycol portion of this molecule. Again, however, the results obscure the fact that the perfluoro portion of the FC-170C molecule is almost certainly not biodegraded.

The data in these reports confirm the inability of the UV-persulfate digestion method used to degrade the perfluorocarbon portion of these products. The measured TOC for FC-170C was 24 mg C per 100 mg product while the calculated TOC based on formulation information is 38 mg C per 100 mg product. The measured TOC for FC-129 was 13.5%; its calculated TOC is about 22%.

Because of the weak and inappropriate TOC method used, we are not going to add these results to our Product Environmental Data Sheets. Customers and regulatory agencies would likely misinterpret these results and think that these products are more biodegradable than they actually are.

Eric Reiner

EAR/mb

Attachment: OECD Screening Tests on FC-170C and FC-129

FC-129 INF

9/14/84

R C C

RESEARCH & CONSULTING COMPANY AG

These results are not appropriate for use because the persulfate oxidation method

PROJECT 034244

READY BIODEGRADABILITY:

"MODIFIED OECD SCREENING TEST"

FOR FLUORAD FC-129

REPORT

P.O. Box CH-4452 Itingen Switzerland
Phone 061 / 98 52 52 Telex 966 136 RCC CH

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PREFACE

Project	034244
Sponsor	3M Deutschland GmbH Postfach 643 D 4040 Neuss 1
Study monitor	D. Reese
Testing facility	R C C Research and Consulting Company AG CH-4452 Itingen/Switzerland
Study director	Dr. H. Ellgehausen H. Ellgehausen Date: September 19, 1984 / hoh
Technical assistance	V. Wuethrich
Start of experiments	August 17, 1984
Date of completion	September 14, 1984
Archiving	Raw data, protocol, report, duplicate of report: R C C Research and Consulting Company AG CH-4452 Itingen/Switzerland

1 INTRODUCTION

The objectives of the present test were to follow the biodegradability of FLUORAD FC-129 in a 28 days biodegradation test by following the DOC of the chemical in the incubation flasks by colorimetric methods.

2 MATERIALS AND METHODS

2.1 Chemicals

The following chemicals were tested:

Standard compound:	Aniline Purity: 99.5 %
Test compound:	Fluorad FC-129
Composition:	Potassium fluoro alkyl carboxylate 50 % Butyl ~~glycol~~ 18 % Carbitol Water 28 % Ethanol 4 %
	Batch: 2304
Water solubility:	>2 g/l at 25 degrees centigrade.

The test chemical was supplied by 3M Deutschland GmbH, on July 10, 1984.

2.2 Experimental design

The study was run according to the OECD method (1).

3 RESULTS

3.1 Degradation of test compound

The results are presented in Sample Form Sheet No 1 and Figure 1.

3.2 Degradation of standard compound (aniline)

The results are presented in Sample Form Sheet No 2 and Figure 2.

4 CONCLUSION

The test compound, Fluorad FC-129, was degraded under the test conditions within 28 days to 90 %.
The standard, aniline, was degraded within seven days to 97 %.
The compound is considered to be readily degradeable.

5 REFERENCE

OECD Guideline for testing of Chemicals:
301 E: "Ready Biodegradability: Modified OECD Screening Test".

SAMPLE FORM SHEET FOR THE MODIFIED OECD SCREENING TEST: NO 1

Date of start of test: August 17, 1984
Test/Standard material: Fluorad FC-129
Theoretical test conc.: 19.8 mg C/l
Inoculum: sec. effluent of a domestic sewage plant (ARA-Birsig, Therwil, CH)
Carbon analyser: Technicon Autoanalyser II, Method-No. 451-76 W — persulfate UV at ~~37°C~~ temp lamp brings it to

A: Organic carbon content of test material: 100 mg test material correspond to: 13.5 mg organic carbon *

Stock solution of the test material (734.5 mg/100 ml, dilution 20 ml/1000 ml of nutrient solution)

based on formulation I calculate product is ~22 % carbon if it contains 14% butyl Carbitol or ~25% carb if 18% butyl carbitol [illegible] 10/17/84

B: Carbon determinations:

Culture medium	Flask No.	Analyses	Theor. conc. mg/l	DOC ** mg/l 0 (CO)	7	14	21	27	28
Mineral nutrient solution with test material and with inoculum	1	a1	19.8	17.5	***	3.3	3.6	3.8	4.0
		a2	19.8	17.6	***	4.1	4.1	4.0	3.7
		m1=(a1+a2)/2	19.8	17.6	***	3.7	3.9	3.9	3.9
	2	b1	19.8	17.7	***	3.8	3.6	4.1	3.6
		b2	19.8	17.7	***	3.8	3.5	4.2	3.6
		m2=(b1+b2)/2	19.8	17.7	***	3.8	3.6	4.2	3.6
Mineral nutrient solution without test material but with inoculum	Blank	c1	-	1.3	1.8	1.2	1.4	1.6	1.9
		c2	-	0.7	2.7	1.8	1.1	2.4	2.1
		m3=(c1+c2)/2	-	1.0	2.3	1.5	1.3	2.0	2.0

* Value determined with carbon analyser.

** All DOC values determined after centrifugation.

*** Samples lost during analysis.

Sample form sheet No. 1 cont.

C: Evaluation of raw data:

	Flask No.		% DOC removal after x days 7	14	21	27	28
DOC-concentrations minus blanks	1	$D1 = 1 - \frac{m1t-m3t}{m1-m3} \cdot 100$	***	87	84	89	89
	2	$D2 = 1 - \frac{m2t-m3t}{m2-m3} \cdot 100$	***	86	86	87	90
	mean	$D = \frac{D1 + D2}{2}$ for day x	***	87	85	88	90

*** Samples lost during analysis.

SAMPLE FORM SHEET FOR THE MODIFIED OECD SCREENING TEST: NO 2

Date of start of test: August 17, 1984
Test/Standard material: Aniline
Theoretical test conc.: 19.4 mg C/l
Inoculum: sec. effluent of a domestic sewage plant (ARA-Birsig, Therwil, CH)
Carbon analyser: Technicon Autoanalyser II, Method-No. 451-76 W

A: Organic carbon content of test material: 100 mg test material correspond to: 77.4 mg organic carbon *

Stock solution of the test material (25 mg/l)

B: Carbon determinations:

Culture medium	Flask No.	Analyses	Theor. conc. mg/l	DOC ** after x days mg/l 0 (CO)	7	14	21	27	28
Mineral nutrient solution with test material and with inoculum	1	a1	19.4	12.4	0	2.1	1.2	1.8	1.9
		a2	19.4	11.9	2.4	1.8	1.1	1.6	2.3
		m1=(a1+a2)/2	19.4	12.2	1.2	2.0	1.2	1.7	2.1
	2	b1	19.4	12.0	3.1	1.8	1.9	1.9	2.1
		b2	19.4	10.9	2.7	1.6	1.4	1.8	2.1
		m2=(b1+b2)/2	19.4	11.5	2.9	1.7	1.7	1.7	2.1
Mineral nutrient solution without test material but with inoculum	Blank	c1	-	1.3	1.8	1.2	1.4	1.6	1.9
		c2	-	0.7	2.7	1.8	1.1	2.4	2.1
		m3=(c1+c2)/2	-	1.0	2.3	1.5	1.3	2.0	2.0

* Value calculated.

** All DOC values determined after centrifugation.

Sample form sheet No. 2 cont.

C: Evaluation of raw data:

	Flask No.		% DOC removal after x days 7	14	21	27	28
DOC-concentrations minus blanks	1	$D1 = 1 - \frac{m1t - m3t}{m1 - m3} \cdot 100$	100	96	100	100	99
	2	$D2 = 1 - \frac{m2t - m3t}{m2 - m3} \cdot 100$	94	98	96	100	99
	mean	$D = \frac{D1 + D2}{2}$ for day x	97	97	98	100	99

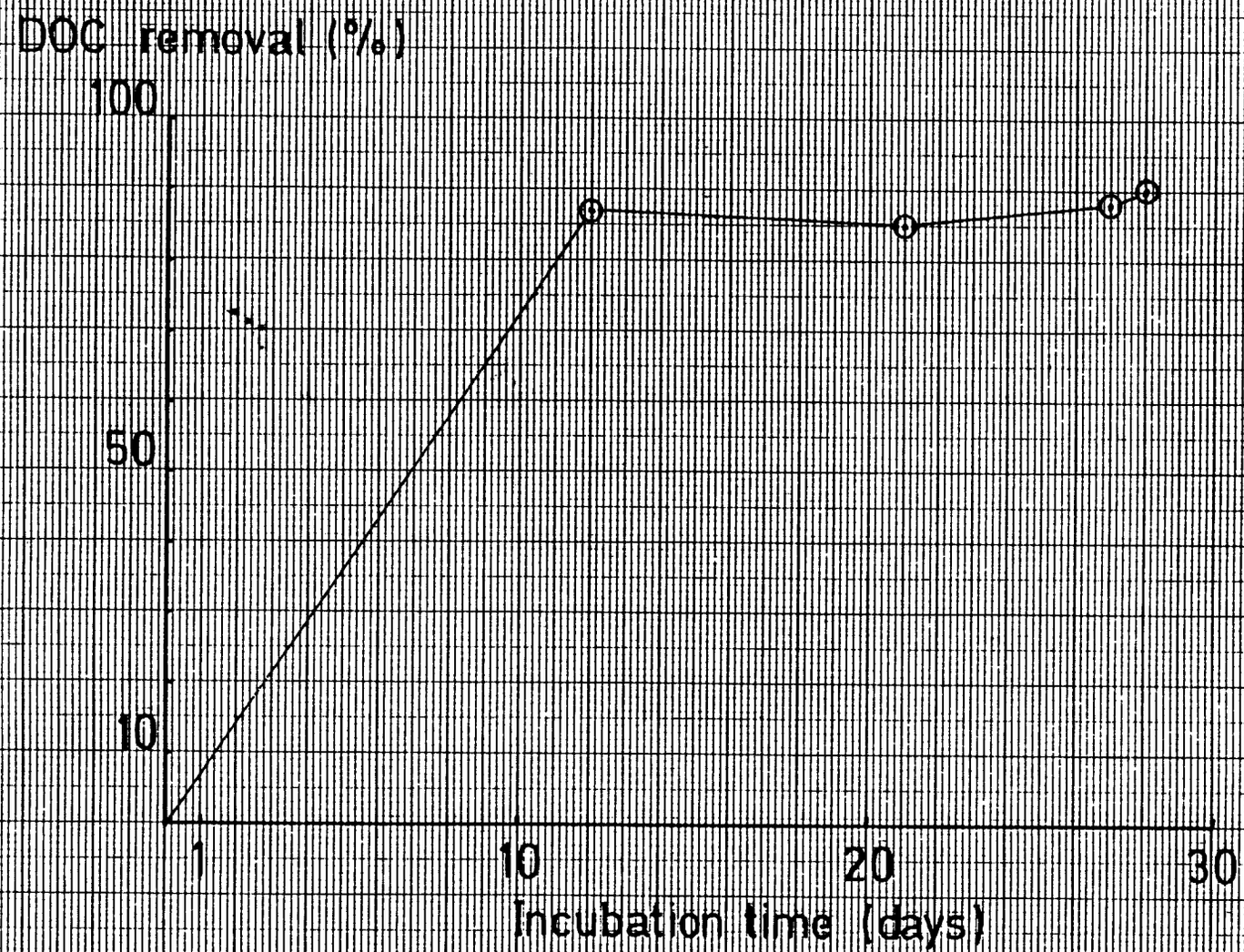

Figure 1: DOC-removal
Test material: Fluorad FC-129

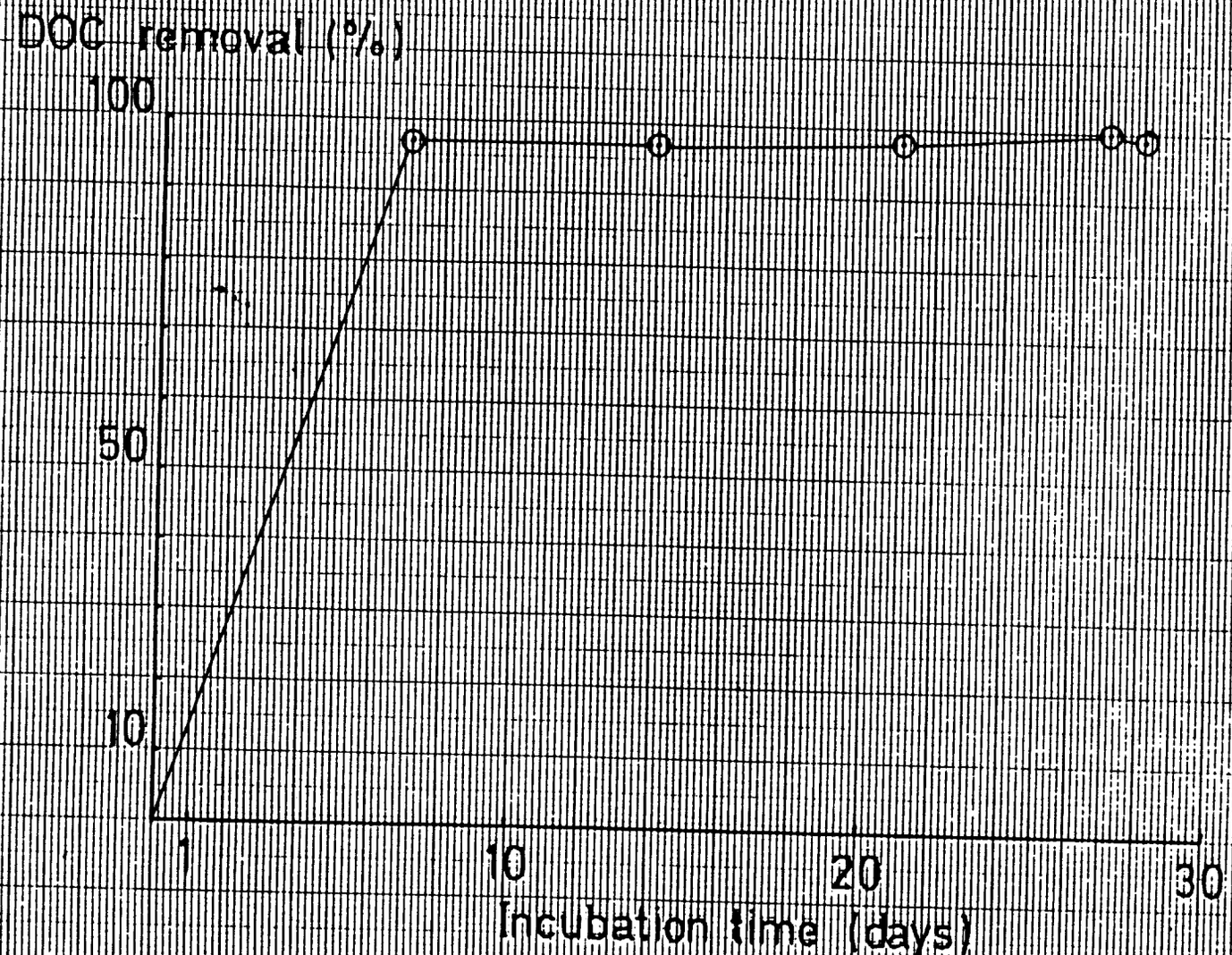

Figure 2: DOC-removal
Test material: Aniline

ThOD of FC-129						
1) 50% FC SOLIDS: C8F17SO2N(C2H5)CH2COO-K+						
11/91 NOTE: CARBON RATIOS ARE C4,C5,C6,C7,C8=2.5%,0.5%,3%,2%,42%						
2) 14% BUTYL CELLOSOLVE						
3) 32% WATER						
4) 4% ETHANOL						
FRACTION	COMPONENTS			MW	MW CARBON	% Carbon
42.00%	C8F17SO2N(C2H5)CH2COO-K+			623	144	9.71%
2.00%	C7F15SO2N(C2H5)CH2COO-K+			573	132	0.46%
3.00%	C6F13SO2N(C2H5)CH2COO-K+			523	120	0.69%
0.50%	C5F11SO2N(C2H5)CH2COO-K+			473	108	0.11%
2.50%	C4F9SO2N(C2H5)CH2COO-K+			423	96	0.57%
14%	C4H9OCH2CH2OCH2CH2OH			162	96	8.30%
4%	CH3CH2OH			46	24	2.09%
32%	WATER			18	0	0.00%
100.00%	TOTAL					21.92%

ACUTE TOXICITY TO FISH (FATHEAD MINNOW)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, FC-109-X or R1904. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-129 referred to by the test laboratory as R1904. Current information indicates it is a mixture of 42% test substance, 32% water, 14% 2-butoxyethanol, 4% ethanol, and 8% C_4-C_7 fluoroalkyl carboxylate compounds. Test material was from 3M production lot 930014, mfg. date 3/97.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD:

Methods: OECD 203
Test type: Static acute
GLP: No
Year Completed: 1997
Species: *Pimephales promelas*
Analytical monitoring: Temperature, pH, DO, and conductivity

RESULTS

Nominal concentrations: Bk control, 19, 32, 54, 90, 150, 250 mg/L (in duplicate)
Element values: 24-hour LC_{50} = >250 mg/L (C.I. not calculable)
48-hour LC_{50} = 211 mg/L (C.I. not calculable)
72-hour LC_{50} = 146 (128-167) mg/L
96-hour LC_{10} = 45 (22-80) mg/L
96-hour LC_{50} = 97 (81-116) mg/L
96-hour NOEC = 54 mg/L

All element values based on nominal concentrations.

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The value reported applies to that mixture and not the test substance. No attempt was made to

determine the impact of the presence of the organic solvents or what portion of the toxicity can be contributed to the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

The FC-129 96-hour LC_{50} for *Pimephales promelas* was determined to be 97 mg/L with a 95% confidence interval of 81-116 mg/L. The 96-hour no observed effect concentration was 54 mg/L.

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, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample.

REFERENCES

This study was conducted at AScI Corporation of Duluth, Minnesota at the request of the 3M Company.

OTHER

Last changed: 5/17/00

STUDY TITLE

ACUTE TOXICITY OF R1904 TO
FATHEAD MINNOW (*Pimephales promelas*)

DATA STANDARD

OECD GUIDELINE 203

STUDY COMPLETED

June 27, 1997

TESTING FACILITY

AScI Corporation/AScI-Duluth
Environmental Testing Division
4444 Airpark Boulevard
Duluth, MN 55811

Tel. No. (218) 722-4040
Fax No. (218) 722-2592

STUDY IDENTIFICATION NUMBERS

AScI Study ID# 5010-068

3M Company Lab Request# R1904

1.0 GENERAL INFORMATION

Study Title	Acute Toxicity of R1904 to Fathead Minnow (*Pimephales promelas*).	
Data Standard	OECD Guideline 203.	
Sponsor	3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133; Tel No. (612) 778-7452.	
Sponsor's Representative	Susan Beach, 3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133; Tel No. (612) 778-7452.	
Sponsor's Lab Request#	R1904.	
Testing Facility	AScI Corporation/AScI-Duluth Environmental Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.	
Principal Investigator	Joe Dierkes	
Project Director	Joe Amato	
Testing Facility Director	Donald Mount	
Definitive Test Dates	June 17-21, 1997.	
Data Validation and Report Review	Alan Mozol	Report Signature:
Retention of Raw Data and Final Report	Two years from the date study is completed.	
Location of Raw Data and Final Report	AScI Corporation/AScI-Duluth Environmental Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.	

2.0 OBJECTIVE(S)

To determine the 96-hour 10% and 50% effect concentrations (LC_{10} and LC_{50}), and the 96-hour no observable effect concentration (NOEC) of the test substance, R1904, for fathead minnow (*Pimephales promelas*) under static test conditions.

This study was conducted according to the AScI study plan retained by the Sponsor and OECD Guideline 203 (OECD 1993).

3.0 TEST METHODS

Test Substance	*Properties*: R1904, bright-yellow liquid, specific gravity = 1.3 (water=1.0), stable, and completely soluble in water. *Test concentrations*: 0 (dilution water control), 19, 32, 54, 90, 150, and 250 mg/L. Test solutions prepared by mass addition of test substance to dilution water followed by vigorous stirring.
Test Organisms	*Source*: Healthy, juvenile fathead minnows (*Pimephales promelas*) obtained from AScI-DETD in-house culture. *Holding/acclimation*: The fish used in the tests were obtained from AScI's in-house culture. The fish used in the study were hatched on May 24, 1997. For the 48 hours preceding test initiation mortality was 0%, and the fish were not fed for 24 hours before test initiation. The fish mean length was 17.0 ± 2.6 mm, and mean wet weight was 38 ± 23 mg. Loading rate was calculated to be 127 mg of fish per L test solution. *Use in test*: Used 10 organisms/replicate and 2 replicates/test concentration.
Dilution Water	*Source*: Dechlorinated City of Duluth, MN, tap water. The most recent chemical analysis is in Raw Data Package. *Characteristics*: At test time, the water had a hardness of 48 mg/L (as $CaCO_3$) and a pH of 8.03. Aerated for 24 h prior to use in the test.

Exposure Chambers	*Type*: 4-L glass jars. *Use in test*: 3.0 L of dilution water or test solution/chamber. During the test, kept covered, except when experimental observations were made. Solutions were not aerated during the exposures.
Incubation	*Duration*: 96 hours. *Daily photoperiod*: 16 h light and 8 h dark, maintained using cool white fluorescent lamps. *Temperature*: 21.0° ± 1°C.
Observations	*Biological*: Daily- Test organisms with abnormal behavior and signs of stress, and dead test organisms. *Water chemistry*: (1) At test initiation- total hardness and alkalinity, (2) daily- temperature, DO, conductivity, and pH. All determinations were made according to APHA methods (1995).
Endpoint Calculations	LC_{10}: Inhibition Concentration (ICp) Method. LC_{50}'s: Trimmed Spearman-Karber Method. *NOEC*: Fisher's Exact Test (TOXSTAT statistical software, Version 3.5, West Inc., and University of Wyoming).

3.0 RESULTS

Test Substance Toxicity (Pertinent data are in Table 1)	*24-h LC_{50}*: >250 mg/L (Not Calculable). *48-h LC_{50}*: 211 mg/L (Not Calculable). *72-h LC_{50}*: 146 mg/L (128-167). *96-h LC_{10}*: 45 mg/L (22-80). *96-h LC_{50}*: 97 mg/L (81-116). *96-h NOEC*: 54 mg/L.
Ranges of Water Chemistry Parameters	*Total hardness (as $CaCO_3$ mg/L)*: 46-48. *Alkalinity (as $CaCO_3$ mg/L)*: 44-46. *Temperature (°C)*: 20.8 (No Range). *DO (mg/L)*: 5.4-8.8. *pH*: 7.60-8.05. *Specific conductivity (µmhos/cm)*: 116-133.

4.0 TEST QA/QC

QA/QC Criteria	Data
Mortality in control exposure must not be more than 10%.	0% mortality.
The lowest DO concentration at test termination must be at least 60% of the air saturation value at test temperature.	The lowest DO concentration measured was 61% of the air saturation value at 21.0°C.
Test duration must be 96 h.	The test duration was 96 h.
Reference Substance Toxicity Test (this test is optional).	Control chart for sodium chloride reference toxicity testing is in Raw Data Package.

5.0 REFERENCES

American Public Health Association (APHA). 1995. Standard Methods for the Examination of Water and Wastewater. APHA, Washington, D.C.

Organization for Economic Cooperation and Development (OECD). 1981/1993. OECD Guidelines for Testing of Chemicals. OECD Publication Information Center, Washington, D.C.

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Karber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays. Environ. Sci. Technol. 11(7): 714-719; Correction 12 (4): 417 (1978).

USEPA. 1993. A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach. Version 2.0.

West Inc., and University of Wyoming. Toxstat Software Package. Version 3.5. Cheyenne, Wyoming.

TABLE 1. ACUTE TOXICITY OF R1904 TO FATHEAD MINNOWS (*P. promelas*)

Test Substance Concentration (mg/L)	Cumulative Number of Dead Organisms and Percent Mortality[a]			
	24 h	48 h	72 h	96 h
0 (control)	0 (0)	0 (0)	0 (0)	0 (0)
19	0 (0)	0 (0)	0 (0)	1 (5)
32	0 (0)	0 (0)	0 (0)	0 (0)
54	0 (0)	0 (0)	0 (0)	3 (15)
90	0 (0)	0 (0)	2 (10)	8 (40)
150	0 (0)	0 (0)	9 (45)	16 (80)
250	0 (0)	15 (75)	20 (100)	20 (100)

[a] Percentages are in parenthesis.

LETHAL AND NO-EFFECT CONCENTRATIONS WITH CORRESPONDING 95% CONFIDENCE INTERVALS

LC's and NOEC	Data (mg/L)[a]	95% Confidence Interval
24-h LC_{50}	>250	Not calculable
48-h LC_{50}	211	Not reliable
72-h LC_{50}	146	128-167
96-H LC_{10}	45	22-80
96-h LC_{50}	97	81-116
96-h NOEC	54	Not applicable

[a] Values based on nominal test concentrations.

ACUTE TOXICITY TO AQUATIC INVERTABRATES (DAPHNIA MAGNA)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, FC-109-X or R1904. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-129 referred to by the test laboratory as R1904. Current information indicates it is a mixture of 42% test substance, 32% water, 14% 2-butoxyethanol, 4% ethanol, and 8% C_4-C_7 fluoroalkyl carboxylate compounds. Test material was from 3M production lot 930014, mfg. Date 3/97.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD:

Methods: OECD 202
Test type: Static acute
GLP: No
Year Completed: 1997
Species: *Daphnia magna*
Analytical monitoring: Temperature, pH, DO, and conductivity

RESULTS

Nominal concentrations: Bk control, 1.4, 2.3, 3.9, 6.5, 10.8, 18, 30, 50 mg/L (in duplicate)
Element values: 24-hour EC_{50} = >50 mg/L (C.I. not calculable)
48-hour EC_{10} = 4.4 (4.3 – 4.6) mg/L
48-hour EC_{50} = 9.1 (7.4 - 11) mg/L
48-hour NOEC = 3.9 mg/L

All element values based on nominal concentrations.

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The value reported applies to that mixture and not the test substance. No attempt was made to determine the impact of the presence of the organic solvents or what

portion of the toxicity can be contributed to the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

The FC-129 48-hour EC_{50} for *Daphnia magna* was determined to be 9.1 mg/L with a 95% confidence interval of 7.4 - 11 mg/L. The 48-hour no observed effect concentration was 3.9 mg/L.

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, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample.

REFERENCES

This study was conducted at AScl Corporation of Duluth, Minnesota at the request of the 3M Company.

OTHER

Last changed: 5/17/00

STUDY TITLE

ACUTE TOXICITY OF R1904 TO *DAPHNIA MAGNA*

DATA STANDARD

OECD GUIDELINE 202

STUDY COMPLETED

June 17, 1997

TESTING FACILITY

AScI Corporation/AScI-Duluth
Environmental Testing Division
4444 Airpark Boulevard
Duluth, MN 55811

Tel. No. (218) 722-4040
Fax No. (218) 722-2592

STUDY IDENTIFICATION NUMBERS

AScI Study ID# 5010-068

3M Company Lab Request# R1904

1.0 GENERAL INFORMATION

Study Title	Acute Toxicity of R1904 to *Daphnia magna*.	
Data Standard	OECD Guideline 202.	
Sponsor	3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133.	
Sponsor's Representative	Susan A. Beach, 3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133. Tel No. (612) 778-7452.	
Sponsor's Lab Request#	R1904.	
Testing Facility	AScI Corporation/AScI-Duluth Environmental Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.	
Principal Investigator	Holly Kitterman.	
Project Director	Joe Amato.	
Testing Facility Director	Donald Mount.	
Definitive Test Dates	June 10-12, 1997.	
Data Validation and Report Review	Alan Mozol.	Report Signature:
Retention of Raw Data and Final Report	Two years from the date study is completed.	
Location of Raw Data and Final Report	AScI Corporation/AScI-Duluth Environmental Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.	

2.0 OBJECTIVE(S)

To determine the 48-h EC_{10} and EC_{50}, and 48-h NOEC (no observed effect concentration) of the test substance, R1904, for *Daphnia magna*.

This study was conducted according to the AScI study plan retained by the Sponsor and OECD Guideline 202 (OECD 1993).

3.0 TEST METHODS

Test Substance	*Properties*: R1904, yellow-colored liquid, specific gravity Ca. 1.3 (water = 1), stable, and completely soluble in water. *Test concentrations*: 0 (dilution water control), 1.4, 2.3, 3.9, 6.5, 10.8, 18, 30, and 50 mg/L. Stock solution prepared by diluting 200 mg of test substance in 1 L of dilution water. Test substance concentrations were prepared by direct addition of stock solution into dilution water. A 0.6 dilution series was utilized.
Test Organisms	*Source*: Healthy, less than 24-h old *Daphnia magna* neonates obtained from AScI stock cultures maintained in water of test dilution water quality. *Use in test*: Gravid adults isolated in dilution water 24 h before the test initiation. Used 5 organisms/replicate and 4 replicates/test concentration. Handled with a smooth bore pipet with an inside diameter of 6 mm. Did not feed during the test.
Dilution Water	*Source*: Dechlorinated City of Duluth, MN, tap water. The most recent chemical analysis is in Raw Data Package. *Characteristics*: At test time, the water had a hardness of 46 mg/L (as $CaCO_3$) and a pH of 7.93. Aerated for 24 h prior to using in the test.
Exposure Chambers	*Type*: 250-ml borosilicate glass beakers. *Use in test*: 200 ml of dilution water or test solution/chamber. During the test, kept covered, except when experimental observations were made.

Incubation	*Duration*: 48 h. *Daily photoperiod*: 16 h light and 8 h dark, maintained using cool-white fluorescent bulbs. *Temperature*: 21°C.
Observations	*Biological*: Daily- gently agitated the exposure chambers and then counted immobilized and dead test organisms (immobilized organisms were those that did not swim within 15 seconds after exposure chambers were gently agitated). Did not remove any daphnids from the exposure chambers at 24 h. *Water chemistry*: (1) At test initiation- total hardness and alkalinity, (2) daily- temperature, pH, DO, and conductivity. All determinations were made according to APHA methods (1995).
Endpoint Calculations	EC_{50}: Trimmed Spearman-Karber method. EC_{10}: Inhibition Concentration (10%) USEPA software package for Determining ICp concentrations. *NOEC*: Fisher's Test (TOXSTAT statistical software, Version 3.5, West, Inc., and University of Wyoming).

4.0 RESULTS

Test Substance Toxicity (Pertinent data are in Table 1)	*24-h* EC_{50}: >50 mg/L. *48-h* EC_{50}: 9.1 mg/L (7.4-11.0). *48-h* EC_{10}: 4.4 mg/L (4.3-4.6). *48-h NOEC*: 3.9 mg/L.

Ranges of Water Chemistry Parameters	*Total hardness (as $CaCO_3$ mg/L)*: 44-46. *Alkalinity (as $CaCO_3$ mg/L)*: 42-44. *Temperature (°C)*: 20.5-20.8°C. *DO (mg/L)*: 8.1-8.5. *pH*: 7.81-8.04. *Specific conductivity (µmhos/cm)*: 118-126.

5.0 TEST QA/QC

QA/QC Criteria	Data
Test organism dead, immobilized or trapped in control exposure must not be more than 10%.	0% dead, immobilized or trapped.
The lowest DO concentration at test termination must be at least 60% of the air saturation value at test temperature.	The lowest DO concentration measured was 91% of the air saturation value at 21.0°C.
Test duration must be 48 h.	The test duration was 48 h.
Reference substance toxicity test (this test is optional).	Current control chart for *D. magna* acute testing is included in the raw data package.

5.0 REFERENCES

American Public Health Association (APHA). 1995. Standard Methods for the Examination of Water and Wastewater. APHA, Washington, D.C.

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Karber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays. Environ. Sci. Technol. 11(7):714-719; Correction 12(4):417 (1978).

Organization for Economic Cooperation and Development (OECD). 1993. OECD Guidelines for Testing of Chemicals. OECD Publication Information Center, Washington, D.C.

West Inc., & University of Wyoming. TOXSTAT Software Package Version 3.5.

US EPA. 1993. A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach (Version 2.0). Environmental Research Laboratory, Duluth, Minnesota.

TABLE 1. ACUTE TOXICITY OF R1904 TO *DAPHNIA MAGNA*

Test Substance Concentration (mg/L)	Cumulative Number and Percent of Dead and Immobilized Organisms[a]	
	24 h	48 h
0	0 (0)	0 (0)
1.4	0 (0)	0 (0)
2.3	0 (0)	0 (0)
3.9	0 (0)	0 (0)
6.5	0 (0)	10 (50)
10.8	1 (5)	17 (85)
18	1 (5)	14 (70)
30	0 (0)	16 (80)
50	4 (20)	20 (100)

[a] Percentages are in parenthesis.

EFFECT AND NO-EFFECT CONCENTRATIONS WITH CORRESPONDING 95% CONFIDENCE INTERVALS

EC_{50} and NOEC	Data (mg/L)	95% Confidence Interval
24-h EC_{50}	>50	Not Calculable
48-h EC_{50}	9.1	7.4-11.0
48-h EC_{10}	4.4	4.3-4.6
48-h NOEC	3.9	Not Applicable

ACUTE TOXICITY TO AQUATIC PLANTS (ALGAE)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, FC-109-X or R1904. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-129 referred to by the test laboratory as R1904. Current information indicates it is a mixture of 42% test substance, 32% water, 14% 2-butoxyethanol, 4% ethanol, and 8% C_4-C_7 fluoroalkyl carboxylate compounds. Test material was from 3M production lot 930014, mfg. date 3/97.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD:

Methods: OECD 201
Test type: Static acute
GLP: No
Year Completed: 1997
Species: *Selenastrum capricornutum*
Analytical monitoring: Temperature, pH, and conductivity

RESULTS

Nominal concentrations: Bk control, 129.6, 216, 360, 600, 1000 mg/L (4 replicates per concentrations, 6 per blank controls)
Element values:

Endpoint	Data (based on nominal concentrations)		95% Confidence Interval (mg/L)
	Time	(mg/L)	
Cell Count	24-hour EC_{50}	431	385-484
	48-hour EC_{50}	395	356-439
	72-hour EC_{10}	113	94-130
	72-hour EC_{50}	248	225-272
	96-hour EC_{10}	163	54-228
	96-hour EC_{50}	298	278-319
	96-hour NOEC	216	Not Applicable

Endpoint	Data (based on nominal concentrations)		95% Confidence Interval (mg/L)
	Time	(mg/L)	
Average Specific Growth Rate (μ)	24-hour EC_{50}	586	524-656
	48-hour EC_{50}	859	777-950
	72-hour EC_{10}	234	197-268
	72-hour EC_{50}	610	551-676
	96-hour EC_{10}	287	248-322
	96-hour EC_{50}	604	550-664
	96-hour NOEC	216	Not Applicable

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The values reported apply to that mixture and not the test substance. No attempt was made to determine the impact of the presence of the organic solvents or what portion of the toxicity can be contributed to the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

The FC-129 96-hour EC_{50} for *Selenastrum capricornutum* was determined to be 298 mg/L (C.I.: 278-319 mg/L) by Cell Count and 604 mg/L (C.I.: 550-664 mg/L) by Average Specific Growth Rate. The 96-hour no observed effect concentration for both Cell Count and Average Specific Growth Rate was 216 mg/L. Effect determined to be algistatic based on recovery of cells previously exposed to the 1000 mg/L concentration.

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, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample.

REFERENCES

This study was conducted at AScI Corporation of Duluth, Minnesota at the request of the 3M Company.

OTHER

Last changed: 5/17/00

STUDY TITLE

GROWTH INHIBITION OF R1904 FOR
GREEN ALGA (*Selenastrum capricornutum*)

DATA STANDARD

OECD GUIDELINE 201

STUDY COMPLETED

June 18, 1997

TESTING FACILITY

AScI Corporation/AScI-Duluth
Environmental Testing Division
4444 Airpark Boulevard
Duluth, MN 55811

Tel. No. (218) 722-4040
Fax No. (218) 722-2592

STUDY IDENTIFICATION NUMBERS

AScI Study ID# 5010-068

3M Company Lab Request# R1904

1.0 GENERAL INFORMATION

Study Title	Growth Inhibition of R1904 for Green Alga (*Selenastrum capricornutum*).	
Data Standard	OECD Guideline 201.	
Sponsor	3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133; Tel No. (612) 778-7452.	
Sponsor's Representative	Susan Beach, 3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133; Tel No. (612) 778-7452.	
Sponsor's Lab Request#	R1904.	
Testing Facility	AScI Corporation/AScI-Duluth Environmental Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.	
Principal Investigator	Todd McGuire	
Project Director	Joe Amato	
Testing Facility Director	Donald Mount	
Definitive Test Dates	June 9-13, 1997.	
Data Validation and Report Review	Alan Mozol	Report Signature:
Retention of Raw Data and Final Report	Two years from the date study is completed.	
Location of Raw Data and Final Report	AScI Corporation/AScI-Duluth Environmental Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.	

2.0 OBJECTIVE(S)

To determine the 3-d and 4-d effect concentrations (EC_{10}'s and EC_{50}'s) and the 4-d no observable effect concentration (NOEC) of the test substance, R1904, for green alga (*Selenastrum capricornutum*) under static test conditions.

This study was conducted according to OECD Guideline 201 (OECD 1993).

3.0 TEST METHODS

Test Substance	*Properties*: R1904, amber liquid, specific gravity ca. 1.3 (water = 1), stable, and completely soluble in water. *Test concentrations*: 0 (algal medium control), 129.6, 216, 360, 600, and 1000 mg/L. A 2000 mg/L stock solution was prepared by mass addition to algal medium followed by mechanical stirring. Each test concentration was made by volume addition of the stock to algal medium.
Test Organisms	*Source*: ATCC 22662 *Inoculum preparation*: Algal stock was subcultured in OECD recommended medium for 4 days before test initiation. *Use in test*: Stock culture was diluted and spiked into test solutions to obtain an initial cell density of 1.0×10^4 cells per ml (1.0×10^4 actual concentration).
Algal Medium	*Preparation*: Deionized water was spiked with nutrient stocks (including Na_2EDTA) to OECD recommended concentrations. The most recent chemical analysis for deionized water is in Raw Data Package. *Characteristics*: At test time, the medium had a pH of 7.86. Sterilized via 0.22 μm filtration prior to use in the test.
Exposure Chambers	*Type*: Sterilized via autoclave 250-ml borosilicate-glass Erlenmeyer flasks sealed with foam stoppers. *Use in test*: 100 ml of algal medium or test solution/chamber. Six control replicates and four replicates per test substance concentration were employed. During the test, kept stoppered, except when experimental observations were made.

Incubation	*Duration*: 4 days. *Daily photoperiod*: Continuous at 740 ft-candles using cool-white fluorescent lamps. Test chambers held on shaker table set at 100 rpm. *Temperature*: 25.0 ± 2°C.
Observations	*Biological*: Daily cell counts using direct microscopic enumeration via Improved Neubauer hemacytometer. *Water chemistry*: (1) At test initiation and termination- pH, conductivity, and temperature, (2) daily- temperature. All determinations were made according to APHA methods (1995).
Endpoint Calculations	1-, 2-, 3-, and 4-d EC_{50}'s, 3- and 4-d EC_{10}'s, and 4-day NOEC's based on both cell counts and average specific growth rates (μ). Statistical analyses performed using Trimmed Spearman-Karber Method (EC_{50}'s), Toxstat (NOEC's) and EPA Probit software (EC_{10}'s).

4.0 RESULTS

Test Substance Toxicity (Pertinent data are in Tables 1, 2, and 3)	*Cell Count 1-d* EC_{50}: 431 mg/L (385-484). *μ 1-d* EC_{50}: 586 mg/L (524-656). *Cell Count 2-d* EC_{50}: 395 mg/L (356-439). *μ 2-d* EC_{50}: 859 mg/L (777-950). *Cell Count 3-d* EC_{10}: 113 mg/L (94-130). *μ 3-d* EC_{10}: 234 mg/L (197-268). *Cell Count 3-d* EC_{50}: 248 mg/L (225-272). *μ 3-d* EC_{50}: 610 mg/L (551-676). *Cell Count 4-d* EC_{10}: 163 mg/L (54-228). *μ 4-d* EC_{10}: 287 mg/L (248-322). *Cell Count 4-d* EC_{50}: 298 mg/L (278-319). *μ 4-d* EC_{50}: 604 mg/L (550-664). *Cell Count 4-d NOEC*: 216 mg/L. *μ 4-d NOEC*: 216 mg/L. Effect was determined to be algistatic based on recovery of cells previously exposed to the 1000 mg/L test substance concentration.
Test Conditions	*Temperature (°C)*: 24.8-26.0. *Light intensity ft-cdls*: 740. *pH*: 6.78-9.46. *Specific conductivity (μmhos/cm)*: 123-189.

5.0 TEST QA/QC

QA/QC Criteria	Data
Algal counts in control must increase by at least a factor of 16 during the first 3 d of the exposure.	During the first 3 d of the test control algal counts increased by a factor of 348.
Test duration must be 4 d.	The test duration was 4 d.

6.0 REFERENCES

American Public Health Association (APHA). 1995. Standard Methods for the Examination of Water and Wastewater. APHA, Washington, D.C.

Organization for Economic Cooperation and Development (OECD). 1993. OECD Guidelines for Testing of Chemicals. OECD Publication Information Center, Washington, D.C.

Hamilton, M.A., R.C. Russo, and R.V. Thurston, 1977. Trimmed Spearman-Karber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays. Environ. Sci. Technol. 11(7): 714-719; Correction 12 (4): 417 (1978).

U.S. Environmental Protection Agency (EPA). 1988. Computer Program Probit. Biological Methods Branch, EMSL, U.S. EPA, Cincinnati, OH 45268.

TABLE 1. EC_{10}'s EC_{50}'S, 95% Confidence Intervals, and 4-d NOEC'S from Algal Growth Inhibition Test with R1904

Endpoint	Data[a]		95% Confidence Interval (mg/L)
	Time	(mg/L)	
Cell Count	1-d EC_{50}	431	385-484
	2-d EC_{50}	395	356-439
	3-d EC_{10}	113	94-130
	3-d EC_{50}	248	225-272
	4-d EC_{10}	163	54-228
	4-d EC_{50}	298	278-319
	4-d NOEC	216	Not Applicable

Endpoint	Data[a]		95% Confidence Interval (mg/L)
	Time	(mg/L)	
Average Specific Growth Rate (μ)	1-d EC_{50}	586	524-656
	2-d EC_{50}	859	777-950
	3-d EC_{10}	234	197-268
	3-d EC_{50}	610	551-676
	4-d EC_{10}	287	248-322
	4-d EC_{50}	604	550-664
	4-d NOEC	216	Not Applicable

[a] Values Based on Nominal Test Concentrations

TABLE 2. Cells per ml and Percentage Difference from Mean Control Value for *Selenastrum capricornutum* Exposed to R1904

Test Conc. (mg/L)	Replicate	Cells/ml (10^4)			
		1 d	2 d	3 d	4 d
Control	A	11	52	342	605
	B	8	48	306	704
	C	8	60	348	616
	D	9	51	336	572
	E	7	64	390	627
	F	10	48	366	726
	Mean	8.8 (-)	53.8 (-)	348.0 (-)	641.7 (-)
129.6	A	9	50	264	506
	B	6	43	282	528
	C	7	50	318	627
	D	7	52	300	671
	Mean	7.3 (-17)	48.8 (-9)	291.0 (-16)	583.0 (-9)
216	A	10	37	240	517
	B	7	45	204	561
	C	6	44	198	583
	D	8	39	234	594
	Mean	7.8 (-11)	41.3 (-23)	219.0 (-37)	563.8 (-12)
360	A	3	24	72	143
	B	5	27	72	154
	C	6	27	96	165
	D	3	28	84	165
	Mean	4.3 (-51)	26.5 (-51)	81.0 (-77)	156.8 (-76)
600	A	3	24	33	37
	B	3	24	27	32
	C	4	17	30	32
	D	4	18	25	30
	Mean	3.5 (-60)	20.8 (-61)	28.8 (-92)	32.8 (-95)
1000	A	2	4	4	2
	B	1	6	2	3
	C	2	3	2	3
	D	1	6	3	4
	Mean	1.5 (-83)	4.8 (-91)	2.8 (-99)	3.0 (-100)

[a] Parenthetic Values = % Difference from Control Mean Value

* 0-d Mean Algal Count = 1.0 X 10^4

TABLE 3. Average Specific Growth Rates (μ) and Percentage Difference from Mean Control Value for *Selenastrum capricornutum* Exposed to R1904

Test Conc. (mg/L)	Average Specific Growth Rate (μ)[a]			
	1 d	2 d	3 d	4 d
Control	2.175 (-)	1.993 (-)	1.951 (-)	1.616 (-)
129.6	1.988 (-9)	1.944 (-2)	1.891 (-3)	1.592 (-1)
216	2.054 (-6)	1.860 (-7)	1.796 (-8)	1.584 (-2)
360	1.495 (-33)	1.639 (-18)	1.465 (-25)	1.264 (-22)
600	1.253 (-42)	1.517 (-24)	1.120 (-43)	0.873 (-46)
1000	0.405 (-81)	0.784 (-61)	0.343 (-82)	0.275 (-83)

[a] $\mu = \frac{\ln \text{Cells per ml } t_n - \ln \text{Cells/ml } t_0}{t_n - t_0}$

[b] Parenthetic Values = % Difference from Control Mean Value

* 0-d Mean Algal Count = 1.0×10^4

ACTIVATED SLUDGE RESPIRATION INHIBITION

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, FC-109-X or R1904. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-129 referred to by the test laboratory as R1904. Current information indicates it is a mixture of 42% test substance, 32% water, 14% 2-butoxyethanol, 4% ethanol, and 8% C_4-C_7 fluoroalkyl carboxylate compounds. Test material was from 3M production lot 930014, mfg. date 3/97.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD:

Methods: OECD 209
Test type: Static acute
GLP: No
Year Completed: 1997
Test organism source: Activated sludge collected from the City of Superior, Wisconsin, Wastewater Treatment Plant.

RESULTS

Nominal concentrations: Bk control, Reference substance, 3.75, 7.5, 15.0, 30 mg/L
Element values: 30-minute EC_{50} = >1000 mg/L
3-hour EC_{50} = >1000 mg/L (No inhibition exhibited)

All element values based on nominal concentrations.

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The value reported applies to that mixture and not the test substance. No attempt was made to determine the impact of the presence of the organic solvents or what portion of the toxicity can be contributed to the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

The FC-129 3-hour EC_{50} for activated sludge respiration inhibition was determined to be >1000 mg/L.

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, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample.

REFERENCES

This study was conducted at AScl Corporation of Duluth, Minnesota at the request of the 3M Company.

OTHER

Last changed: 5/17/00

STUDY TITLE

INHIBITION OF R1904 FOR ACTIVATED SLUDGE RESPIRATION

DATA STANDARD

OECD GUIDELINE 209

STUDY COMPLETED

JULY 10, 1997

TESTING FACILITY

AScI Corporation
AScI-Duluth Environmental Testing Division
4444 Airpark Boulevard
Duluth, MN 55811

Tel. No. (218) 722-4040

PROJECT IDENTIFICATION NUMBERS

AScI Study ID# 5010-068

3M Company Lab Request# R1904

1.0 GENERAL INFORMATION

Study Title	Inhibition of R1904 For Activated Sludge Respiration	
Data Standard	OECD Guideline 209.	
Sponsor	3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133; Tel No. (612) 778-7452.	
Sponsor's Representative	Susan Beach, 3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133; Tel No. (612) 778-7452.	
Sponsor's Lab Request#	R1904	
Testing Facility	AScI Corporation/AScI-Duluth Environmental Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.	
Principal Investigator	Joe Dierkes	
Project Director	Joe Amato	
Testing Facility Director	Donald Mount	
Definitive Test Date	July 1, 1997	
Data Validation and Report Review	Alan Mozol	Report Signature:
Retention of Raw Data and Final Report	Five years from the date study is completed.	
Location of Raw Data and Final Report	AScI Corporation/AScI-Duluth Environmental Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.	

2.0 OBJECTIVE

To determine if possible, the 30-minute and 3-h (contact times) EC_{50}'s of the test substance R1904 for activated sludge respiration.

This study was conducted according to the AScI study plan retained by the Sponsor and OECD Guideline 209 (OECD 1993).

3.0 TEST METHODS

Test Substance	*Properties*: R1904, yellow liquid, specific gravity ca. 1.3 (water = 1), stable, and completely soluble in water. *Test concentrations*: 0 and 1,000 mg/L prepared in duplicate by direct additions.
Reference Substance	*Name*: 3,5-dichlorophenol, purity 97%, Aldrich Chemicals, Lot# 02110JX. *Test concentrations*: 3.75, 7.5, 15.0, and 30 mg/L prepared by direct addition of 500 mg/L stock solution in Millipore® water.
Test Organisms	*Source*: Activated sludge from the City of Superior (Wisconsin) Wastewater Treatment Plant receiving approximately 95% domestic sewage. *Use in test*: (1) Centrifuged five minutes, three times (4°C, 7500 rpm), (2) diluted the sludge pellet with carbon-treated water to selected volume, final total solids concentration of 1.6 g/L was obtained by further dilution, and (3) added 50 ml of synthetic sewage feed per L of the diluted sludge, and aerated at 20°C until test initiation. The sludge was held for less than 24 hours between collection and test initiation.
Synthetic Sewage Feed	*Composition*: Chemical — g dissolved/L of deionized water Bacto. peptone — 16.0 Beef extract — 11.0 Urea — 3.0 NaCl — 0.7 $CaCl_2.2H_2O$ — 0.4 $MgSO_4.7H_2O$ — 0.2 K_2HPO_4 — 2.8
Deionized Water	Deionized water used in the study was obtained from a Millipore® Milli-Q System. The most recent chemical analysis is in Raw Data Package.

Test Setup	*Exposure vessels*: 8, 1-L borosilicate glass beakers; two inoculum controls, two test exposures, and four reference substance treatments. *Order of preparation*: At 10-minute time intervals: (1) first inoculum control, (2) test exposure concentration replicates, (3) from low to high reference substance exposure concentrations, and (4) second inoculum control. The final volume in each beaker was 800 ml. *Preparation*: (1) Added 284 ml of the sludge preparation in each beaker, (2) added 26 ml of synthetic sewage feed to each beaker, and (3) in test and reference exposures, added the required amounts of the test and reference substances, respectively, (4) and diluted each exposure to 800 ml with carbon-treated water. *Incubation and aeration*: 19.9° to 20.0°C and vigorous aeration.
Determination of Respiration	*Sequence*: Same as exposures were prepared. *Determination*: At 30-min and 3-h contact times: (1) filled one 300-ml BOD bottle with the exposure medium and continued to aerate the remaining medium, (2) using a YSI 54A oxygen meter with a self-stirring probe, determined the DO concentration in the exposure medium every 0.5 minutes for up to 5.5 minutes (the first determination was made after 0.5 minutes), and (3) the sub-sample was properly disposed. DO meter calibration checks were made between the sets of measurements for each control or treatment.
Calculation of Respiration Rate	For each exposure, correlated DO depletion and time interval, and calculated the slope of the regression line as the respiration rate.
Endpoint Calculation	*EC50*: Probit Analysis (EPA 1988).

3.0 RESULTS

Test Substance Toxicity (Pertinent data are in Table 1)	*30-minute* EC_{50}: >1,000 mg/L. *3-h* EC_{50}: >1,000 mg/L.
Reference Substance Toxicity (Pertinent data are in Table 1)	*30-minute EC50*: <3.75 mg/L. *3-h* EC_{50}: <3.75 mg/L.

4.0 TEST QA/QC

QA/QC Criteria	Data
Respiration rates of two inoculum controls must not vary by more than 15%.	Variations after 30-minute and 3-h contact times were less than 3%.
3-h EC_{50} of reference substance must be between 5-30 mg/L.	3-h EC_{50} was <3.75 mg/L indicating the sludge had greater than normal sensitivity. The lack of effect due to R1904 is a valid result.
Test duration must be 3 h.	The test duration was 3 h.

5.0 REFERENCES

Organization for Economic Cooperation and Development (OECD). 1993. OECD Guidelines for Testing of Chemicals. OECD Publication Information Center, Washington, D.C.

U.S. Environmental Protection Agency (EPA). 1988. Computer Program Probit. Biological Methods Branch, EMSL, U.S. EPA, Cincinnati, OH 45268.

TABLE 1.0
OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
CALCULATION OF RESULTS

Stdy N 5010-068
Date: 7\1\97

ASci Corporation/ASci-Duluth
Environmental Testing Division
ASci Study ID# 5010-068

Test Substance: R1904

Analyst: DIERKES

30-Minute Exposure Dissolved Oxygen Concentrations, mg/L

mg/L	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	Temp, °C	R (1)	Ra (2)	% Inh. (3)
BK1	6.8	6.5	6.0	5.6	5.1	4.6	4.2	3.7	3.2	2.7	2.2	19.9	0.9503	0.9569	
1000A	6.9	6.5	6.2	5.8	5.4	5.0	4.6	4.2	3.8	3.3	3.0	19.9	0.7976	0.8031	16
1000B	6.8	6.4	6.0	5.6	5.2	4.8	4.4	4.0	3.6	3.2	2.8	20.0	0.8000	0.8000	16
3.75ref	7.6	7.3	7.1	6.9	6.6	6.4	6.2	5.9	5.7	5.5	5.2	19.9	0.4655	0.4687	51
7.5ref	7.6	7.4	7.3	7.1	7.0	6.8	6.6	6.4	6.2	6.1	5.9	20.0	0.3442	0.3442	64
15ref	8.1	8.0	7.9	7.8	7.8	7.7	7.6	7.5	7.4	7.3	7.3	19.9	0.1636	0.1648	83
30ref	8.7	8.6	8.6	8.5	8.5	8.4	8.4	8.3	8.2	8.2	8.1	20.0	0.1139	0.1139	88.0
BK2	6.9	6.4	6.0	5.5	5.1	4.5	4.1	3.7	3.1	2.6	2.2	20.0	0.9479	0.9479	

% diff in controls(4) 1.0 **EC50 (95% CI): >1000 mg/L (NC)** RaCm (5): 0.9524

3-Hour Exposure Dissolved Oxygen Concentrations, mg/L

mg/L	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	Temp, °C	R (1)	Ra (2)	% Inh. (3)
BK1	7.5	7.0	6.7	6.2	5.8	5.4	5.0	4.6	4.1	3.7	3.2	20.0	0.8448	0.8448	
1000A	7.3	6.8	6.4	5.9	5.5	5.1	4.6	4.2	3.7	3.2	2.8	20.0	0.8945	0.8945	-5
1000B	7.0	6.6	6.2	5.7	5.3	4.8	4.3	3.9	3.4	2.9	2.5	19.9	0.9236	0.9301	-9
3.75ref	8.3	8.1	7.9	7.7	7.5	7.3	7.1	6.9	6.8	6.5	6.1	20.0	0.4158	0.4158	51
7.5ref	8.5	8.3	8.2	8.0	7.9	7.7	7.6	7.4	7.3	7.2	7.0	19.9	0.2885	0.2905	66
15ref	8.9	8.8	8.7	8.6	8.6	8.5	8.4	8.4	8.3	8.3	8.2	20.0	0.1261	0.1261	85
30ref	9.0	9.0	8.9	8.9	8.9	8.8	8.8	8.8	8.7	8.7	8.7	20.0	0.0655	0.0655	92.3
BK2	7.9	7.4	7.0	6.6	6.2	5.7	5.3	4.9	4.4	4.0	3.5	20.0	0.8655	0.8655	

% diff in controls(4) 2.4 **EC 50 (95% CI): >1000 mg/L (NC)** RaCm (5): 0.8552

(1) R= Oxygen uptake rate (mg O2/L-min)
(2) Ra= R adjusted to 20°C= [R/ø.Ex]
(3) % Inhibition= [(RaCm)-RaS/(RaCm)]100
(4) % difference in controls: [IRaBk1-RaBk2I/RaCm]100 (Two controls must be within 15% of each other.)
(5) Mean adjusted R, controls= [RaBk1+RaBk2]/2

ø= 1.072
RaCm= mean adjusted R, controls
Bk= blank control, S= sample
Tobs= sample temp
x= Tobs-20°C

Sponsor: 3M Company
Sponsor Study ID# R1904

MICROBICS MICROTOX® TOXICITY TEST

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, FC-109-X or R1904. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-129 referred to by the test laboratory as R1904. Current information indicates it is a mixture of 42% test substance, 32% water, 14% 2-butoxyethanol, 4% ethanol, and 8% C_4-C_7 fluoroalkyl carboxylate compounds. Test material was from 3M production lot 930014, mfg date 3/97.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD:

Method: Microbics Microtox® "BASIC" Procedure
Test type: Static acute
GLP: No
Year Completed: 1997
Species: *Vibrio fischeri* formerly *Photobacterium phosphoreum*
Analytical monitoring: pH and osmotic condition
Replicates: Two
Statistical methods: EC10 and EC50 values calculated by statistical linear regression program provided for Microtox®
Test organism source: Microbics Microtox® Reagent.

RESULTS

Nominal concentrations: Blank control, 125, 250, 500, 1000 mg/L
Element value: 5-minute EC_{50} = 354 (316-396) mg/L
15-minute EC_{50} = 290 (261-323) mg/L
30-minute EC_{50} = 274 (248-302) mg/L

All element values based on nominal concentrations.

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The value reported applies to that mixture and not the test substance. No attempt was made to

determine the impact of the presence of the organic solvents or what portion of the toxicity can be contributed to the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

The FC-129 30-minute EC_{50} for *Vibrio fischeri* was determined to be 274 mg/L with a 95% Confidence Interval of 248-302 mg/L.

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, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample.

REFERENCES

This study was conducted at AScl Corporation of Duluth, Minnesota at the request of the 3M Company.

OTHER

Last changed: 5/17/00

1 of 9
JT 7/30

AScI Corporation

TEST 3M R1904 Microtox ©

DATE 7/9/97

TECHNICIAN HHK, TG

2 of 9
JT 7/80

INFORMATION ABOUT THE STUDY

Sponsor:	3M
Sponsor Study ID#:	R1904
AScI Study ID#:	5010-068
Study Director:	HHK, TG
Test Substance:	R1904
Reference Substance:	
Test Organism:	Vibrio fischeri
Type of Test:	Micro tox toxicity test
Test Guideline:	
In-Life Phase Dates:	

Provide comments below if any additional observations are made or data provided:

Stock of 2000 mg/L ~~made by~~ RCHHK 7/3/97

200 ml volume	Amount wanted	Amount weighed
(2000 mg/L)(0.2 L) = 400 mg	400 mg	402

Provide an explanation below if the test is unsuccessful:

Chemical is a dark yellow color and the consistency of cough syrup

369
#7/30

MICROTOX DATA REPORT
Basic Test

FILE: 97070901.K5
R1904 dark yellow in color;consistency of cough syrup. 2000mg/l stock
Test Time: 5 minutes Osmotic Adjustment:NaCl

NUMBER	IO/IT	CONC.	CR/GAMMA	% EFFECT	AV % q DIFF.
Control	96.99/ 78.33	0.0	0.8076 #		
Control	110.50/ 89.45	0.0	0.8095 #		
1	116.03/ 67.32	125.0000	0.394 #	28.2	
2	110.55/ 60.09	125.0000	0.488 #	32.8	30.5 q 2.3
3	122.19/ 53.45	250.0000	0.848 #	45.9	
4	108.70/ 53.42	250.0000	0.645 #	39.2	42.6 q 3.3
5	103.95/ 35.95	500.0000	1.338 #	57.2	
6	110.81/ 37.84	500.0000	1.368 #	57.8	57.5 q 0.3
7	111.12/ 26.72	1000.0000	2.363 #	70.3	
8	109.79/ 27.90	1000.0000	2.182 #	68.6	69.4 q 0.8

CR = CoCORRECTION FACTOR = 0.8086
# Used for calculations

EC50 354.0 ~~%~~ mg/l (95% CONFIDENCE RANGE:316.3 TO 396.3)

Signature Hollie Hutterman TEST DATE: 7/9/97
TIME: 1530

CONTINUED TO NEXT PAGE...

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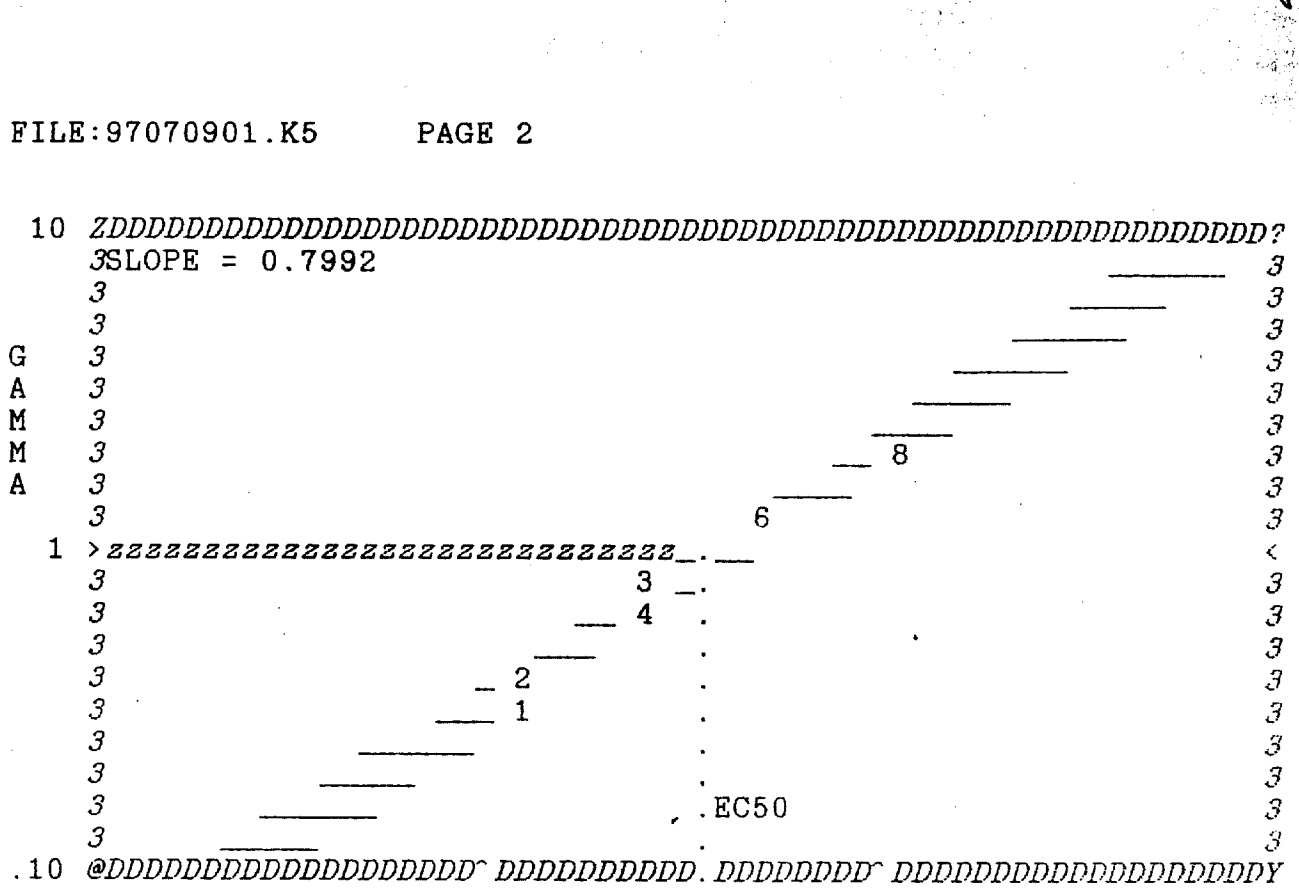
#7/30

FILE:97070901.K5 PAGE 2



ESTIMATING EQUATION: LOG C = 1.2247 x LOG *b* +2.5491
95% CONFIDENCE FACTOR: 1.11940 FOR EC50
COEFFICIENT OF DETERMINATION: R^2 = 0.97877

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JH 7/30

MICROTOX DATA REPORT
Basic Test

FILE: 97070901.K15
R1904 dark yellow in color;consistency of cough syrup. 2000mg/l stock
Test Time: 15 minutes Osmotic Adjustment:NaCl

NUMBER	IO/IT	CONC.	CR/GAMMA	% EFFECT	AV % q DIFF.
Control	96.99/ 72.50	0.0	0.7475 #		
Control	110.50/ 81.76	0.0	0.7399 #		
1	116.03/ 58.65	125.0000	0.471 #	32.0	
2	110.55/ 52.30	125.0000	0.572 #	36.4	34.2 q 2.2
3	122.19/ 45.05	250.0000	1.017 #	50.4	
4	108.70/ 44.87	250.0000	0.802 #	44.5	47.5 q 3.0
5	103.95/ 30.48	500.0000	1.536 #	60.6	
6	110.81/ 32.57	500.0000	1.530 #	60.5	60.5 q 0.0
7	111.12/ 22.36	1000.0000	2.696 #	72.9	
8	109.79/ 23.43	1000.0000	2.485 #	71.3	72.1 q 0.8

CR = CoCORRECTION FACTOR = 0.7437
# Used for calculations

EC50 290.1 ~~%~~ mg/l (95% CONFIDENCE RANGE:261.0 TO 322.5)

Signature [signature] Hollie Kitterman TEST DATE: 7/9/97
TIME: 1530

CONTINUED TO NEXT PAGE...

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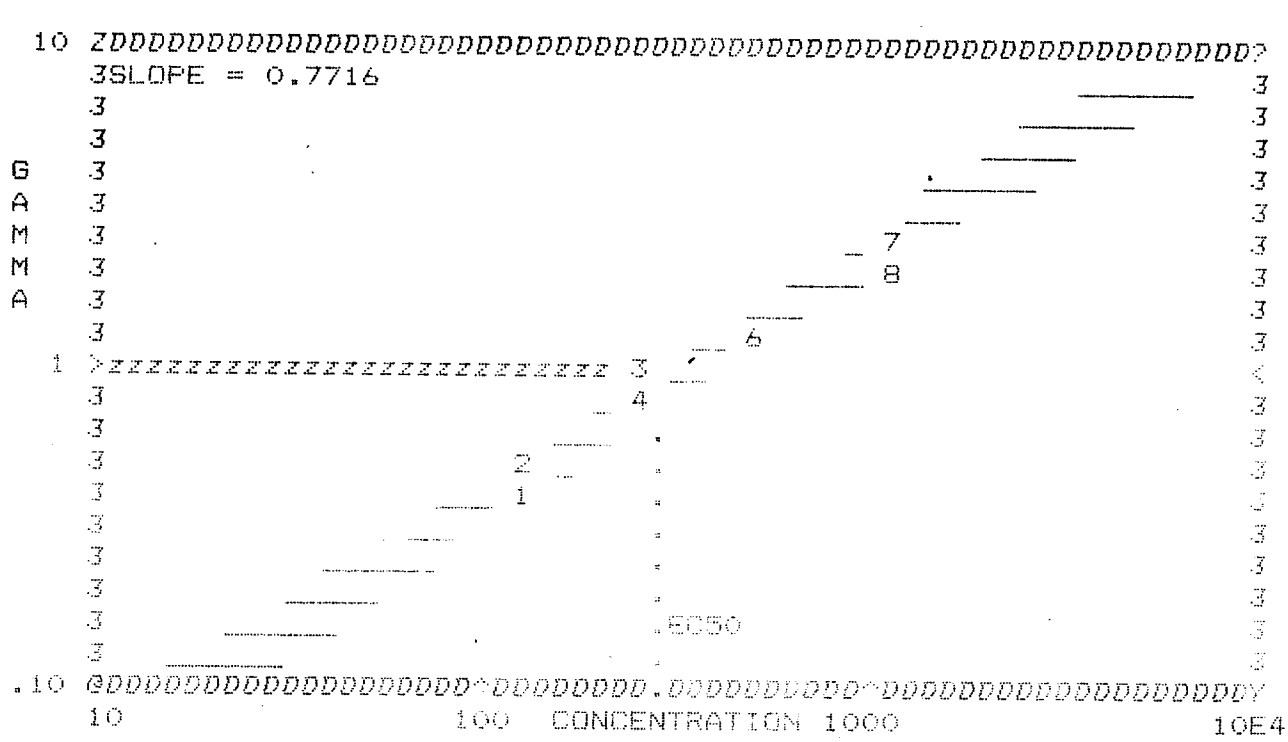
H7130

FILE:97070901.K15 PAGE 2



ESTIMATING EQUATION: LOG C = 1.2733 x LOG b +2.4626
95% CONFIDENCE FACTOR: 1.11152 FOR EC50
COEFFICIENT OF DETERMINATION: R^2 = 0.98251

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JT 7/30

MICROTOX DATA REPORT
Basic Test

FILE: 97070901.K30
R1904 dark yellow in color;consistency of cough syrup. 2000mg/l stock
Test Time: 30 minutes Osmotic Adjustment:NaCl

NUMBER	IO/IT	CONC.	CR/GAMMA	% EFFECT	AV % q DIFF.
Control	96.99/ 67.40	0.0	0.6949 #		
Control	110.50/ 76.81	0.0	0.6951 #		
1	116.03/ 54.30	125.0000	0.485 #	32.7	
2	110.55/ 48.04	125.0000	0.599 #	37.5	35.1 q 2.4
3	122.19/ 42.35	250.0000	1.005 #	50.1	
4	108.70/ 41.06	250.0000	0.840 #	45.7	47.9 q 2.2
5	103.95/ 27.41	500.0000	1.636 #	62.1	
6	110.81/ 29.19	500.0000	1.638 #	62.1	62.1 q 0.0
7	111.12/ 19.34	1000.0000	2.993 #	75.0	
8	109.79/ 20.62	1000.0000	2.701 #	73.0	74.0 q 1.0

CR = CoCORRECTION FACTOR = 0.6950
# Used for calculations

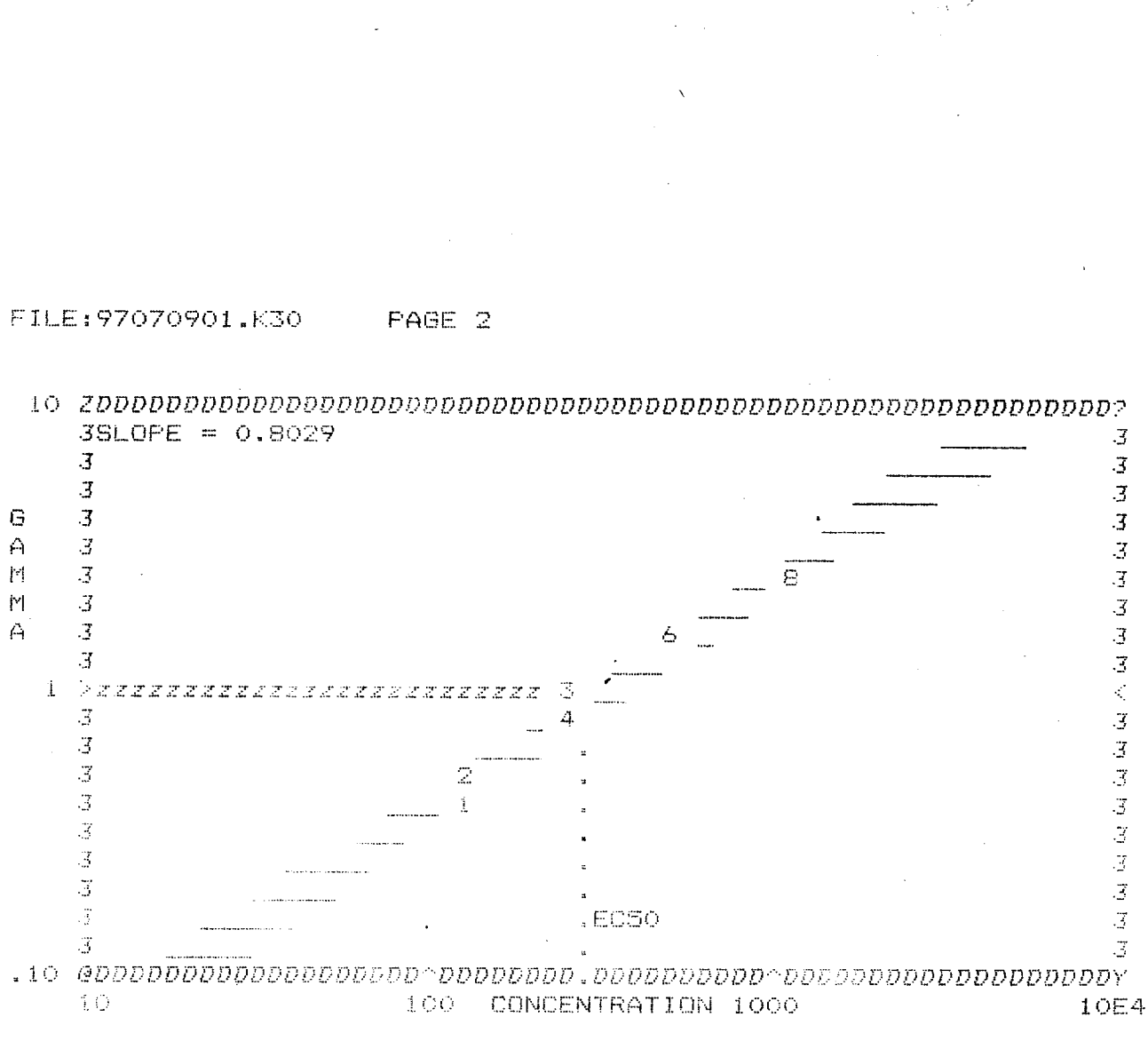
EC50 273.6 ~~%~~ mg/l (95% CONFIDENCE RANGE:248.2 TO 301.5)

Signature [signature] TEST DATE: 7/9/97
TIME: 1530

CONTINUED TO NEXT PAGE...

8 of 9
H 7/30

FILE:97070901.K30 PAGE 2



ESTIMATING EQUATION: LOG C = 1.2279 x LOG b +2.4370
95% CONFIDENCE FACTOR: 1.10200 FOR EC50
COEFFICIENT OF DETERMINATION: R^2 = 0.98583

9 of 9
#7130

Attachment A
Environmental Laboratory

LR/Study Number: R1904
Date/Time: 7/9/97, 1530
Analyst: HAK, TG

Microtox® Test Solution Preparation

TEST SUBSTANCE: R1904

1. Preparation of Stock Solution or 100% Solution

(If testing a 100% solution, record final concentration as 100% and skip to section 2)

Mass/Volume or volume/volume of Test Substance: 402 mg/200 ml
Diluent Used: milipore water

Final Concentration of Stock Solution (mg/L or % v/v): 2000 mg/l

Balance Used: MEHLER AT250 Analytical

2. pH Adjustment of Stock Solution or 100% Solution

Initial pH: 8.46

pH Adjusted to: 7.64

pH Adjusted with: HCl

3. Osmotic Adjustment of Stock Solution or 100% Solution

Osmotic Adjuster: NACl 400 *mg added to* 20 *mL solution*

Comments: Sodium Chloride Crystals J.T. Baker Lot # K02636

4. Appearance of pH and Osmotically Adjusted Stock Solution

CLEAR or OPAQUE *(Circle one)*

Color: Light yellow

Comments: R1904 dark yellow color, consistency of cough syrup

5. Comments

Reagent lot # ACV005-3 Exp. 1/98

EEF-M-3.0

MICROTOX® PHENOL REFERENCE TOXICITY CONTROL CHART

Test Date	5-Min EC_{50} (mg/L)	Running Mean (mg/L)	Standard Deviation	Upper Limit	Lower Limit	CV (%)
July 1997	23.59	--	--	--	--	--
July 1997	18.26	20.93	3.769	28.47	12.99	18.0
July 1997	18.23	20.03	3.086	26.20	13.86	15.4

MICROTOX® ZINC REFERENCE TOXICITY CONTROL CHART

Test Date	15-Min EC_{50} (mg/L)	Running Mean (mg/L)	Standard Deviation	Upper Limit	Lower Limit	CV (%)
June 1997	3.898	--	--	--	--	--
June 1997	4.481	4.190	0.412	5.014	3.366	9.8
July 1997	4.125	4.168	0.294	4.756	3.580	7.1

PHYSICAL/CHEMICAL PROPERTIES

TEST SUBSTANCE

Identity: [2-(N-Ethylperfluorooctanesulfonamido) ethyl acrylate; may also be referred to as B1228, D-1, EtFOSEA, or FX-13. (2-Propenoic acid, 2-[ethyl[heptadecafluorooctyl)sulfonyl]amino]ethyl ester, CAS # 423-82-5)

Remarks: Material is an amber solid.

Remarks: Report states that sample purity is 99% or more based on information supplied by Sponsor. The purity/identity of the test substance cannot be substantiated. As presented in the report, the structural formula indicates that "purity" cannot be assigned as "99% or more". The lot number was 101.

METHOD

Water Solubility: OECD 105, Shake Flask Method
Hydrolysis as a Function of pH: OECD 111
Dissociation Constants in Water: OECD 112, Spectrophotometric Method
Partition Coefficient (1-octanol/water) by HPLC: OECD 117
Vapor Pressure: OECD 104, Gas Saturation Method

GLP (Y/N): Yes
Year completed: 1994
Remarks:
Details from these studies can be found in one report entitled: "Determination of Physico-chemical Properties of Sample D-1".

RESULTS

Water Solubility: 0.89 mg/L at 25°C
Hydrolysis as a Function of pH: At 25°C the reaction rate constants of the test substance at pH 4, 7, and 9 were 0.017, 0.020, and 0.046 day^{-1} with half life times of 42, 35, and 15 days, respectively.
Dissociation Constants in Water: No dissociation constants of the test substance were determined because the spectra of the test substance at different pH values did not significantly differ from one another.
Partition Coefficient: $K_{ow} > 6$
Vapor Pressure: 6.0×10^{-3} Pa at 25°C

Remarks: The study results indicated that the test substance was composed of different components which, in some cases, yielded results different from each other.

Water solubility: It is suggested that the water solubility of each component of the test substance is different from the others.
Hydrolysis as a function of pH: The chemical structure of the test substance suggests that the reaction rate of each component is similar to each other.
Dissociation constants in water: The water solubility of the test substance is too low to find ionized and unionized forms of the test substance. The laboratory did not investigate its constants further by either the titration method or the conductometric method in the OECD Guideline because both methods are considered not suitable for substances with such a low solubility. However, the chemical structure of the test substance suggests that its dissociation potential is very low.
Parition coefficient: The test substance was detected as ten peaks or more by HPLC.
Vapor Pressure: The chemical structure of the test substance suggests that the vapor pressure of each component is different from the others.

DATA QUALITY

Water Solubility
Reliability: Klimisch ranking 2.
The report states a purity of the substance of "99% or more". Since the purity/identity of the test substance cannot be substantiated, the stated average solubility of the test substance is not defensible. As presented, the structural formula indicates that "purity" cannot be assigned as "99 % or more". Since the method of detection is not compound specific, and was not validated, it cannot be determined if the peaks detected in the sample chromatograms do indeed arise from the test substance.

Hydrolysis
Reliability: Klimisch ranking 2.
Without the purity/identity information on the test substance and compound specific analytical techniques, the experimentally determined rate constant cannot be substantiated.

Dissociation Constants in Water
Reliability: Klimisch ranking 1.
Potential for a compound of the given structure to ionize at any pH is low.

Octanol/Water Partition Coefficient

Reliability: Klimisch ranking 3.
Since the purity/identity of the test substance cannot be substantiated, the stated partition coefficient is not defensible. It was not established in this study what the retention time(s) of the test substance(s) is. The UV detector is not compound specific and it has not been definitively established that the >10 peaks assigned as belonging to the test compound are actually related to the test material. Furthermore, it has not been established that this method for determining the octanol/water partition coefficient applies to fluorochemical compounds similar in structure to the test material.

Vapor Pressure
Reliability: Klimisch ranking 3.
The purity/identity of the test substance cannot be substantiated. The chromatogram of the test substance has a distinctly different profile that that of the standard. It has not been established that under the conditions of the experiment the nitrogen passed over the substance has become saturated with gas phase test substance. The reference compound (benzoic acid) has a measured vapor pressure ~ 16 times that determined for the test substance. It would be appropriate to choose a reference compound with a vapor pressure closer to that determined for the test compound The lower molecular weight constituents will have a higher vapor pressure than the n=8 component.

REFERENCES
Study conducted at the request of Sumitomo 3M LTD by Mitsubishi Chemical Safety Institute Ltd., Yokohama, Japan

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

Last changed: 5/22/00

M.S.I. Report No. 4B223-4B227

Determination of Physico-chemical Properties of Sample D-1

Submitted to:

SUMITOMO 3M LTD.

Prepared by:

Mitsubishi Chemical Safety Institute Ltd.

February 14, 1996
(The original report was submitted on August 8, 1994)

000435

RECEIVED
25 1996

Determination of Physico-chemical Properties of Sample D-1

(English version)

Study No.: 4B223-4B227
Study Title: Determination of Physico-chemical Properties of Sample D-1
Sponsor: SUMITOMO 3M LTD.

This test was conducted in Yokohama Laboratory of Mitsubishi Chemical Safety Institute Ltd., 1000 Kamoshida-cho, Aoba-ku, Yokohama 227, Japan.

This report is the English version of the original, which was written in Japanese. The undersigned hereby declare that this version faithfully reflects the original report to the best of our knowledge.

Translated by

Shiro Iwami Date: Feb. 14, 1996

Shiro IWAMI, M.Sc.
Environmental Science Division of the Yokohama Laboratory

Approved by

Jun Toriya Date: Feb. 14, 1996

Jun TORIYA, B.Sc.
Head of the Yokohama Laboratory,

Testing Facility

Study No.: 4B223-4B227
Study Title: Determination of Physico-chemical Properties of Sample D-1
Sponsor: SUMITOMO 3M LTD.

Testing Facility:

Yokohama Laboratory, Mitsubishi Chemical Safety Institute Ltd.† (M.S.I.),
1000 Kamoshida-cho, Aoba-ku, Yokohama 227, Japan (telephone: 045-963-3541)

† *The company's name was changed on October 1, 1994.*
(The former name: Mitsubishi-kasei Institute of Toxicological and Environmental Sciences)

Facility Management:

Jun TORIYA, B.Sc. Sealed date: August 8, 1994
Head of the Yokohama Laboratory

Study Management:

Tadayoshi SHIGEOKA, Ph.D. Sealed date: August 8, 1994
Chief Research Scientist,
Environmental Science Division of the Yokohama Laboratory

Study Director:

Takemi NAKANOME, B.Sc. Sealed date: August 8, 1994
Senior Research Scientist,
Environmental Science Division of the Yokohama Laboratory

Experimental Scientist:

Akiko TAKEDA, M.Sc. Sealed date: August 8, 1994
Environmental Science Division of the Yokohama Laboratory

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  1.2 Source 6

2 Water Solubility [Study No. 4B223] 7
  2.1 Materials and Methods 7
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3 Hydrolysis as a function of pH [Study No. 4B224] 10
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  5.1 Materials and Methods 19
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(59 pages in all)

Abstract

Study No.: 4B223-4B227
Study Title: Determination of Physico-chemical Properties of Sample D-1
Sponsor: SUMITOMO 3M LTD.

1 Test Substance

Name: Sample D-1
Chemical name: 2-[N-ethyl-N-perfluoroalkyl (C=1-8) sulfonylamino] ethyl acrylate
Structural formula: $C_nF_{2n+1}SO_2N(C_2H_5)CH_2CH_2OC(=O)CH=CH_2$
n=1-8 (n=8: approx. 78 %; n=1-7: approx. 21 %)

2 Water Solubility [Study No. 4B223]

2.1 Methods: No.105† "Water Solubility" (Flask Method)
2.2 Results: Water solubility of the test substance was 0.89 mg/L at 25°C.

3 Hydrolysis as a function of pH [Study No. 4B224]

3.1 Methods: No.111† "Hydrolysis as a function of pH", (testing at pH 4, 7, and 9 at 25°C)
3.2 Results: At 25°C reaction rate constants of the test substance at pH 4, 7, and 9 were 0.017, 0.020, and 0.046 day^{-1} with half life time of 42, 35, and 15 days, respectively.

4 Dissociation Constants in Water [Study No. 4B225]

4.1 Methods: No.112† "Dissociation Constants in Water" (Spectrophotometric Method)
3.2 Results: No dissociation constants of the test substance were determined because the spectra of the test substance at different pH did not significantly differ from one another. (But the chemical structure of the test substance suggests that its dissociation potential is very low.)

5 Partition Coefficient (1-octanol/water) by HPLC Method [Study No. 4B226]

5.1 Methods: No.117† "Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method"
5.2 Results: The test substance was detected as ten peaks or more by HPLC. The log Pow value of the main component was more than 6.

6 Vapor Pressure [Study No. 4B227]

6.1 Methods: No.104† "Vapor Pressure Curve" (Gas Saturation Method)
6.2 Results: Vapor pressure of the test substance was 6.0×10^{-3} Pa at 25°C.

† *Number in Organization for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals*

1 Test Substance

1.1 Identification

1)Name†: Sample D-1

Chemical name†: 2-[N-ethyl-N-perfluoroalkyl(C=1-8) sulfonylamino] ethyl acrylate

2)Structural formula†: $C_nF_{2n+1}SO_2N(C_2H_5)CH_2CH_2OC(=O)CH=CH_2$
n=1-8
(n=8: approx. 78 %; n=1-7: approx. 21 %)

3)Physico-chemical properties:

Solubility†:	water:	insoluble
	acetone:	50 % or more
	DMSO:	insoluble
Melting point†:	27-42°C	
Boiling point†:	approx. 150°C (1 mm Hg)	

† *provided by the sponsor*

1.2 Source

1)Batch‡:	Lot No. 101
2)Supplier:	Misao SHIBA, SUMITOMO 3M LTD.
3)Supplied quantity‡:	approx. 100 g
4)Purity‡:	99 % or more
5)Appearance:	amber like wax

‡ *provided by the sponsor*

2 Water Solubility [Study No. 4B223]

A solution is a homogeneous mixture of different substances in a solvent. The particle sizes of the dispersed substances are of the same magnitude as molecules and ions. Therefore, the smallest volumes which can be obtained from a solution are always of uniform composition.

Solubility in water is a significant parameter because

- the spatial and temporal movement (mobility) of a substance is largely determined by its solubility in water;
- water soluble substances gain ready access to humans and other living organisms;
- the knowledge of the solubility in water is a prerequisite for testing biological degradation and bio-accumulation in water and for other tests.

2.1 Materials and Methods

This study was conducted in accordance with the standard procedure "Water Solubility" (Flask Method) in the OECD† Guidelines for Testing of Chemicals No.105 (1981). This method is summarized as follows:

The test substance (Solids must be pulverized.) is dissolved in water at a temperature somewhat above the test temperature. When saturation is achieved the mixture is cooled and kept at the test temperature, stirring as long as necessary to reach equilibrium. Subsequently, mass concentration of the test substance in the aqueous solution, which must not contain any undissolved particles, is determined by a suitable analytical method.

† *Organization for Economic Cooperation and Development*

2.1.1 Reagents

n-hexane:	Wako Pure Chemical Industries, Ltd.,	guaranteed reagent
acetone:	Wako Pure Chemical Industries, Ltd.,	guaranteed reagent

2.1.2 Apparatus

incubator:	Taitec Corporation,	model M-100
centrifugal separator:	Hitachi Ltd.,	model SCT 15B
gas chromatograph(GC):	Shimadzu Corporation,	model GC-14A
integrator:	Shimadzu Corporation,	model C-R3A

2.1.3 Test procedure

Two hundred milligrams of the test substance was dissolved in 10 mL of acetone in an Erlenmeyer glass flask. The solvent was evaporated with stirring to deposit a thin layer of the test substance onto inner surface of the flask. The residual solvent was thoroughly removed by stream of nitrogen gas . Four hundred milliliters of distilled water was added to the flask, which was then shaken at 40±0.2°C over a 3-day period. A 50-mL aliquot of the water phase was sampled after 1, 2, and 3 days. The aliquot was subsequently shaken at 25±0.2°C for 1 day or more to reach equilibrium of dissolution of the test substance.

The operation by the above procedure was repeated once more.

2.1.4 Analytical methods

The concentration of the test substance was measured by gas chromatography (GC). Prior to GC analysis, each equilibrated aliquot was treated as follows:

The aliquot was centrifuged at 4000 rpm (3000 × g) at 25°C. Sodium chloride, 1.2 g, was dissolved in the supernatant, to which 4 mL of *n*-hexane was then added. They were shaken for 1 minutes and then centrifuged at 4000 rpm (3000 × g) at 25°C. The *n*-hexane phase (supernatant) was analyzed by GC under the following conditions:

GC conditions

column:	Shimadzu Corporation, wide bore column CBP20-W25-100, 0.53 mm i.d., 25 m in length
temperature:	column: 120°C; injector: 200°C; detector: 240°C
carrier:	nitrogen gas (flow rate: 20 mL/min)
detector:	electron capture detector (ECD)
injection volume:	3 μL

As informed by the sponsor, the components of the test substance differ in length of the perfluoroalkyl chain (see §1.1). In fact, the test substance was detected as four major peaks with retention time of 3.8, 4.5, 5.1, and 6.0 minutes by GC under the conditions described above. Therefore, the concentration of the test substance was based on total area of the four peaks.

2.1.5 Calibration curve

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Standard solutions of the test substance were prepared to make concentrations of 0, 0.2, 1.0, and 5.0 mg/L in *n*-hexane. These standard solutions were analyzed by GC under the conditions described in §2.1.4. The total peak area of the four peaks

was calculated and plotted against the concentration of the test substance. The calibration curve yielded a straight line passing through the origin and its correlation coefficient was calculated to be 0.999 by the least square method described in Japanese Industrial Standards (JIS) Z 9041-1968.

[Figure 2.1 and Figure 2.2]

At measurement of the test substance concentration, the standard solution with 1.0 mg/L was analyzed. Concentration in each test sample was calculated from ratio of total peak area for the sample to that for the standard solution.

2.1.6 Recovery

An acetone solution of the test substance was prepared to make a concentration of 483 mg/L. Two milliliters of the solution was dissolved in 1000 mL of distilled water (final concentration of the test substance: 0.97 mg/L).

Sodium chloride, 1.2 g, was dissolved in 4 mL of the water solution, to which 4 mL of *n*-hexane was then added. They were shaken for 1 minutes and then centrifuged at 4000 rpm (3000 × g) at 25 °C. The concentration of the test substance in the *n*-hexane phase (supernatant) was measured by GC under the conditions described in §2.1.4 to evaluate recovery.

[Table 2.2 and Figure 2.3]

The recovery of the test substance was 94 %. The concentrations of the test substance reported below were corrected by this factor.

2.2 Results and Discussion

Average water solubility of the test substance was 0.89 mg/L at 25 °C.

[Table 2.1 and Figure 2.4.1 to Figure 2.4.3]

The component with GC retention time of 4.5 minutes was rather soluble in water compared with the others. Dissolution of this component at 40 °C reached equilibrium within 1 day.

It is suggested that water solubility of each component of the test substance differs from one another.

3 Hydrolysis as a Function of pH [Study No. 4B224]

The testing of substance for hydrolysis is relevant to their persistence. Hydrolysis is one of the most common reactions controlling abiotic degradation and is therefore one of the main degradation paths of substances in the environment.

A procedure to determine hydrolysis is important also in indicating whether other testing should be carried out on a parent compound or its hydrolysis product. It is the degradation products that are crucial.

Hydrolysis behavior needs to be examined at pH values normally found in the environment (pH 4-9) and under more acidic conditions (pH 1-2) for physiological purpose.

Surface-controlled reactions can sometimes predominate over bulk solution hydrolysis, especially in the soil environment. This may result in different degradation rates than would be predicted from this methods based upon rates in homogeneous solutions.

3.1 Materials and Methods

This study was conducted in accordance with the standard procedure "Hydrolysis as a function of pH" in the OECD Guidelines for Testing of Chemicals No.111 (1981).

In the environment, chemicals usually occur in dilute solution, which means that water is present in large excess, and therefore, the kinetics of hydrolysis are generally pseudo-first order at fixed pH and temperature.

The hydrolysis reaction may be influenced by acidic or basic species H_3O^+ (H^+) and OH^-, in which case it is referred to as specific acid or specific base catalysis.

The concentration of the test substance is determined as a function of time. The logarithms of the concentrations are plotted against time and the slope of the resulting straight line (assuming first-order or pseudo-first order behavior) gives the rate constant.

3.1.1 Reagents and water

acetone:	Wako Pure Chemical Industries, Ltd.,	guaranteed reagent
n-hexane:	Kishida Chemical Co., Ltd.	guaranteed reagent
sodium chloride:	Junsei Chemical Co., Ltd.,	guaranteed reagent
sodium hydroxide:	Junsei Chemical Co., Ltd.,	guaranteed reagent
monopotassium phosphate:	Wako Pure Chemical Industries, Ltd.,	guaranteed reagent
monopotassium citrate:	Nacalai Tesque Inc.,	extra pure reagent
boric acid:	Wako Pure Chemical Industries, Ltd.,	guaranteed reagent
potassium chloride:	Junsei Chemical Co., Ltd.	guaranteed reagent
water:	deionized water purified by Milli-Q®	

3.1.2 Apparatus

incubator:	Taitec Corporation,	model M-100
pH meter:	Toa Electronics Ltd.,	model HM-50S
gas chromatograph(GC):	Shimadzu Corporation,	model GC-14A
	[equipped with an electron capture detector (ECD)]	
integrator:	Shimadzu Corporation,	model C-R3A

3.1.3 Test procedure

3.1.3.1 Preparation of buffer solutions

Buffer solutions were prepared by the following two methods described in the annex of the Guideline No. 111:

- Buffer mixtures of Clark and Lubs
- Citrate buffer of Kolthoff and Vleeschhouwer

Each buffer solution of pH values 4, 7, and 9 was prepared to be 1000 mL using the following reagents:

1) pH 4: 0.1 M monopotassium citrate and 0.1 *N* sodium hydroxide
2) pH 7: 0.1 M monopotassium phosphate and 0.1 *N* sodium hydroxide
3) pH 9: 0.1 M potassium chloride, 0.1 M boric acid and 0.1 *N* sodium hydroxide

The buffer solutions obtained were passed through Millipore® filter of pore size 0.25 μm. The pH values of the filtrates were determined to be 4.05, 7.02, and 9.05 for nominal values of 4, 7, and 9, respectively.

3.1.3.2 Preparation of stock solution of the test substance

The test substance, 80 mg, was dissolved and diluted with acetone to prepare a stock solution with 40 mg/L.

3.1.3.3 Preliminary test (at 50 °C)

Four milliliters of the stock solution (prepared in §3.1.3.2) was added to 400 mL each of the three buffer solutions to prepare test solutions of pH values 4, 7, and 9. The solutions thus prepared were tested under the following conditions:

I) Conditions

pH:	4, 7, and 9
concentration:	0.40 mg/L
temperature:	50.0±0.1 °C
testing period:	5 days with continuous shaking
test volume:	400 mL (containing 1 % acetone)
test vessel:	Erlenmeyer glass flask

II) Analytical methods

The concentration of the test substance was measured by GC. Prior to GC analysis, the test solutions were treated by the following procedure:

Sodium chloride, 128 g, was dissolved in each of the three 400-mL solutions, from which the test substance was then extracted with 100 mL of *n*-hexane. The extraction was repeated twice more. The three extracts were combined and concentrated to 50 mL, which was then transferred into a 200-mL glass volumetric flask and filled to 200 mL with *n*-hexane. The hexane solution was analyzed by GC under the following conditions:

GC conditions

column:	Shimadzu Corporation, wide bore column CBP20-W25-100, 0.53 mm i.d., 25 m in length
temperature:	column: 120 °C; injector: 200 °C; detector: 240 °C
carrier:	nitrogen gas (flow rate: 20 mL/min)
detector:	ECD
injection volume:	3 μL

As described in §2.1.4, the concentration of the test substance was based on total area of the four peaks detected by GC (retention time: 3.6, 4.3, 4.9, and 5.8 minutes).

III) Recovery

Four milliliters of the stock solution (prepared in §3.1.3.2) was added to 400 mL of water in an Erlenmeyer glass flask (final concentration: 0.4 mg/L). Sodium chloride, 128 g, was dissolved in this solution, from which the test substance was then extracted with 100 mL of *n*-hexane. The extraction was repeated twice more.

The three extracts were combined and concentrated to 50 mL, which was then transferred into a 200-mL glass volumetric flask and filled to 200 mL with *n*-hexane. The concentration of the test substance in the final solution was measured by GC under the conditions described in §3.1.3.3.II.

Recovery was determined to be 101 %. The concentrations of the test substance reported for this preliminary test were corrected by this factor.

[Table 3.6 and Figure 3.1]

3.1.3.4 Further investigation (at 25 °C)

Four milliliters of the stock solution (prepared in §3.1.3.2) was added to 400 mL each of the three buffer solutions to prepare test solutions of pH values 4, 7, and 9. Six 5-mL aliquots from each of the test solutions were transferred into glass tubes. These solutions were examined as a function of time under the following conditions:

I) Conditions

pH:	4, 7, and 9
concentration:	0.40 mg/L
temperature:	25.0±0.2 °C
testing period:	33 days with no shaking
test volume:	5 mL (containing 1 % acetone)
test vessel:	glass tube

II) Analytical methods

The concentration of the test substance was measured by GC. Prior to GC analysis, the test solutions were treated by the following procedure:

Sodium chloride, 1.6 g, was dissolved in the 5-mL aliquot treated in the tube. Two milliliters of *n*-hexane was added to this solution. They were shaken for 1 minutes and then centrifuged at 3000 rpm. The *n*-hexane phase (supernatant) was analyzed by GC as described in §3.1.3.3.

As described in §2.1.4, the concentration of the test substance was based on total area of the four peaks detected by GC (retention time: 3.6, 4.3, 4.9, and 5.8 minutes).

III) Recovery

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Four milliliters of the stock solution (prepared in §3.1.3.2) was added to 400 mL of water in an Erlenmeyer glass flask (the final concentration: 0.4 mg/L). Sodium

chloride, 1.6 g, was dissolved in a 5-mL sample of this solution, to which two milliliters of *n*-hexane was then added. They were shaken for 1 minutes and then centrifuged at 3000 rpm. The *n*-hexane phase (supernatant) was analyzed by GC under the conditions described in §3.1.3.3.

Recovery was determined to be 95 %. The concentrations of the test substance reported for this further investigation were corrected by this factor.

[Table 3.7 and Figure 3.3]

3.1.3.5 Calibration curve

The calibration curve prepared in §2.1.5 (Determination of Water Solubility) was used.

[Figure 2.1 and Figure 2.2]

At measurement of the test substance concentration, the standard solution of the test substance with 1.0 mg/L was analyzed. Concentration in each test sample was calculated from ratio of total peak area for the sample to that for the standard solution.

3.1.3.6 Calculations

Residual percent of the test substance was calculated as follows:

$$r_t = 100\,(C_t / C_{t_0})$$

where

r_t:	residual percent of the test substance after "t" days
C_t:	concentration of the test substance after "t" days (mg/L)
C_{t_0}:	initial concentration of the test substance (0.4 mg/L)

Assuming that the logarithms of the residual concentrations are first order as a function of time, their reaction rate constants were calculated as follows:

$$k = t^{-1}\ ln\,(100/r_t)$$

where

k:	reaction rate constant of the test substance (day^{-1})

Using the average of these rate constants, half life time of the test substance was calculated as follows:

$$t_{1/2} = k^{-1}\ ln\ 2$$

where

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$t_{1/2}$: half life time of the test substance (days)

3.2 Results and Discussion

1) Preliminary test

After 5 days at 50°C the residual percents of the test substance at pH 4, 7, and 9 were 76, 76, and 52, respectively.

[Table 3.5 and Figure 3.2]

On the basis of the results, it is suggested that the test substance is transformed at least by 24 %.

Relating to the preliminary test result, there is the following description in the OECD Guideline:

If less than 10 per cent of the reaction is observed after 5 days ($t_{1/2}$ > 1 year), the chemical is considered hydrolytically stable and no additional testing is required.

Therefore, we decided to perform the further investigation described below.

2) Further investigation

At 25°C the reaction rate constants of the test substance at pH 4, 7, and 9 were 0.017, 0.020, and 0.046 day^{-1} with half life time of 42, 35, and 15 days, respectively.

[Table 3.1 to Table 3.4 and Figure 3.4]

The chemical structure of the test substance (shown in §2.2) suggests that the reaction rate of each component is similar to each other.

4 Dissociation Constants in Water [Study No. 4B225]

The dissociation of a chemical in water is of importance in assessing its impact upon the environment. It governs the form of the substance which in turn determines its behavior and transport. It may affect the adsorption of the chemical on soils and sediments and adsorption into biological cells.

4.1 Materials and Methods

This study was conducted in accordance with the standard procedure "Dissociation Constants in Water" (Spectrophotometric Method) in the OECD Guidelines for Testing of Chemicals No.112 (1981). General summary of this method is as follows:

A wavelength is found where the ionized and unionized forms of the compound have appreciably different extinction coefficients. The UV-visible absorption spectrum is obtained from solutions of constant concentration under a pH condition where the substance is essentially unionized and fully ionized and at several intermediate pH's. This may be done, either by adding increments of concentrated acid (base) to a relatively large volume of a solution of the compound in a multicomponent buffer, initially at high (low) pH, or by adding equal volumes of a stock solution of the compound in e.g. water, methanol, to constant volumes of various buffer solutions covering the desired pH range. From the pH and absorbance values at the chosen wavelength, a sufficient number of values for the pKa is calculated using data from at least 5 pH's where the compound is at least 10 per cent and less than 90 per cent ionized.

4.1.1 Reagents and water

methanol:	Junsei Chemical Co., Ltd., guaranteed reagent
0.1 *N* hydrochloric acid:	Kishida Chemical Co., Ltd., reagent for titration (diluted to 0.01 *N* with water prior to use)
0.1 *N* sodium hydroxide:	Kishida Chemical Co., Ltd., reagent for titration (diluted to 0.01 *N* with water prior to use)
water:	Deionized water was distilled.

4.1.2 Apparatus

UV-visible spectrophotometer: Shimadzu Corporation, Model UV-260

4.1.3 Test procedure

4.1.3.1 Preparation of stock solution of the test substance

A stock solution of the test substance with 80 mg/L in methanol was prepared using 20.0 mg of the substance.

4.1.3.2 Preparation of test solutions

For measurement of UV-visible spectra of the test substance, three solutions of the test substance were prepared as follows:

1) Acid solution of the test substance

Two milliliters of the stock solution (prepared in §4.1.3.1.) and 2.4 mL of 0.01 *N* hydrochloric acid were transferred into a 200-mL glass volumetric flask, which was then filled to 200 mL with water. (final concentration of the test substance: 0.8 mg/L)

2) Alkaline solution of the test substance

Two milliliters of the stock solution and 2.4 mL of 0.01 *N* sodium hydroxide were transferred into a 200-mL glass volumetric flask, which was then filled to 200 mL with water. (final concentration of the test substance: 0.8 mg/L)

3) Neutral solution of the test substance

Two milliliters of the stock solution was transferred into a 200-mL glass volumetric flask, which was then filled to 200 mL with water. (final concentration of the test substance: 0.8 mg/L)

4.1.3.3 Measurement of UV-visible spectrum

The UV-visible spectra of the test substance in the solutions of three different pH values were measured with the apparatus shown in §4.1.2.

4.2 Results and Discussion

No dissociation constants of the test substance were determined because the spectra of the test substance at different pH did not significantly differ from one another.

[Figure 4.1]

The water solubility of the test substance is too low to find an ionized and unionized forms of the test substance. We did not investigate its constants further by either "Titration Method" or "Conductometric Method" in the OECD Guideline because the both methods are considered not suitable for the substance having such a low solubility (0.89 mg/L, reported in §2.2).

But the chemical structure of the test substance (shown in §1.1.2) suggests that its dissociation potential is very low.

5 Partition Coefficient (1-octanol/water) by HPLC Method [Study No. 4B226]

The partition coefficient (P) is defined as the ratio of the equilibrium concentrations of a dissolved substance in a two-phase system consisting of two largely immiscible solvents. In case of 1-octanol and water,

$$P_{ow} = C_o / C_w$$

where

P_{ow}: 1-octanol/water partition coefficient
C_o: concentration in 1-octanol phase
C_w: concentration in water phase

The partition coefficient being the quotient of two concentrations is usually given in the form of its logarithm to base ten (log P_{ow}).

P_{ow} is a key parameter in studies of the environmental fate of chemical substances. A highly-significant relationship between the P_{ow} of substances and their bioaccumulation in fish has been shown. It has also been shown that P_{ow} is a useful parameter in the prediction of adsorption on soil and sediments and for establishing quantitative structure-activity relationships for a wide range of biological effects.

5.1 Materials and Methods

This study was conducted in accordance with the standard procedure "Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method" in the OECD Guidelines for Testing of Chemicals No.117(1989). Principle of this method is as follows:

HPLC is performed on analytical columns packed with a commercially available solid phase containing long hydrocarbon chains (e.g. C_8, C_{18}) chemically bound onto silica.

Chemicals injected onto such a column move along it by partitioning between the mobile solvent phase and the hydrocarbon stationary phase. The chemicals are retained in proportion to their hydrocarbon-water partition coefficient, with water soluble chemicals eluted first and oil-soluble chemicals last. This enables the relationship between the retention time on a reverse-phase column and the 1-octanol/water partition coefficient to be established.

5.1.1 Reference compounds

As reference compounds whose Pow values are well known, we selected five substances: methyl benzoate, bromobenzene, diphenyl, dibenzyl, and DDT. In addition, thiourea was used for determination of the dead time. A mixture of the six substances was prepared in acetonitrile.

5.1.2 Correlation between retention time and Pow

The mixture of the reference compounds (shown in §5.1.1) was analyzed by high performance liquid chromatography (HPLC) under the conditions described below:

HPLC conditions

column:	GL Sciences Inc., Inertsil ODS-2 4.6 mm i.d., 250 mm in length (C_{18} chemically bound onto silica)
mobile phase:	acetonitrile/water = 75/25 (v/v)
flow rate:	1.0 mL/min.
wavelength:	210 nm
temperature:	25 °C

The HPLC retention time of the reference compounds were corrected as follows:

$$Rt = Rt' - Rt_0$$

where

Rt:	corrected retention time of the compound (minutes)
Rt':	retention time of the compound (minutes)
Rt_0:	retention time of thiourea (minutes)

Correlation equation between the corrected retention time of the five compounds and their corresponding Pow was computed by the least square method, resulting in the following equation:

$$\log P_{ow} = 5.274 \log Rt - 0.0219 \quad (r = 0.973)$$

[Figure 5.1 and Figure 5.2]

5.1.3 Retention time of the test substance

A mixture of the test substance and thiourea was prepared in acetonitrile. The mixture was analyzed by HPLC under the conditions described in §5.1.2. Based on the chromatogram, retention time (Rt', minutes) were determined for components of the test substance.

5.2 Results and Discussion

The test substance was detected as ten peaks or more by HPLC.

The corrected retention time (Rt) of the main component was determined to be 29.245 minutes, while that of DDT (Log Pow is reported to be 6.20.) was 13.263 minutes.

Based on the correlation equation shown in §5.1.2, the log Pow value of the main component was more than 6.

[Figure 5.2]

6 Vapor pressure [Study No. 4B227]

The environmental relevance of vapor pressure is accounted for by the following reasons:

- The vapor pressure gives an indication of the probability of the phase transitions, liquid/gas and solid/gas.
- The vapor pressure, together with the solubility in water, is the major auxiliary variable for calculating the volatility of a substance from an aqueous solution.
- Vapor pressure is thus a significant factor for predicting atmospheric concentrations.
- The vapor pressure of a substance can furthermore be useful as a basis for deciding whether or not a photochemically induced degradation study (in the homogeneous gas phase or in an absorbed phase) is necessary.

6.1 Materials and Methods

This study was conducted in accordance with the standard procedure "Vapor Pressure Curve" (Gas Saturation Method) in the OECD Guidelines for Testing of Chemicals No.104 (1981). This method is summarized as follows:

A stream of inert carrier gas (nitrogen gas) is passed over the substance in such a way that it becomes saturated with vapor of the substance and the vapor is then collected in a trap adsorbent. Measurement of the amount of material transported by a known amount of carrier gas is used to calculate the vapor pressure at a given temperature.

6.1.1 Reagents

acetonitrile:	Wako Pure Chemical Industries, Ltd.,	reagent for HPLC
adsorbent:	GL Sciences Inc.,	Tenax GC®, 60-80 mesh

6.1.2 Apparatus

saturator column:	Sibata Scientific Technology Ltd., 12 mm i.d., 150 mm in length	
adsorbent column:	Sibata Scientific Technology Ltd., 12 mm i.d., 150 mm in length	
glass bead:	Iuchi Seieido Co., Ltd.,	1 mm in diameter
flow meter:	Shinagawa Corporation,	model NWK-IC
gas chromatograph:	Shimadzu Corporation,	model GC-14A
data processor:	Shimadzu Corporation,	model C-R3A

6.1.3 Vapor pressure measuring apparatus

For measurement of vapor pressure of the test substance, an apparatus shown in Figure 6.5 was assembled and set in a room air-conditioned at 25±1 °C.

[Figure 6.5]

The glass beads coated with the test substance were packed into the saturator column up to 7 cm in length. Nitrogen gas, kept at a constant flow rate by the pressure controller and the flow gauge, was introduced to this column.

The nitrogen gas saturated with vapor of the test substance was delivered to the Tenax GC®-packed column to trap the test substance. Total amount of the nitrogen gas which passed through this trap column was measured by the flow meter.

Preparation of saturator column

1) Ten milligrams of the test substance was dissolved in 10 mL of acetonitrile in a 100-mL round bottom glass flask.

2) Eight milliliters of glass beads was added into the flask. The solvent was removed by rotary evaporation. The residual solvent was thoroughly eliminated with a vacuum pump at room temperature. (The test substance was thus coated onto the glass beads.)

3) The glass beads coated with the test substance was packed into the saturator column up to 7 cm in length. Both open sides of the column were held by quartz glass wool, which was then incorporated in the vapor pressure measuring apparatus.

nitrogen gas

1) Nitrogen gas was delivered into the vapor pressure measuring apparatus for 70 hours. Total volume of the carrier gas was measured with the flow meter and determined to be 1.017 m^3. (average flow rate: 242 mL/min)

2) The total volume of the gas was corrected as follows:

$$V = Vr\,[(273/T)(760-P)\,/\,760]^{1/2}$$

where

V: total gas volume corrected (m^3)
Vr: amount of the gas measured by the flow meter (1.017 m^3)
T: temperature of the nitrogen gas (298 °K)
P: pressure difference between at the flow meter and at the absorber-packed column (0.4 mm Hg)

The total volume of the carrier was thus corrected by factors of the temperature and pressure difference, resulting in 0.973 m^3.

Adsorbent column

1) Eight milliliters of the adsorbent (Tenax GC®) was packed into the absorbent column. Both open sides of the column were retained with quartz glass wool. The column thus prepared was connected to the saturator column.

2) The test substance trapped by the Tenax GC® was desorbed by the procedure described in §6.1.4.

6.1.4 Analytical methods

The test substance trapped by the adsorbent was desorbed as follows:

1) The adsorbent packed in the column was transferred into a glass filter. The inside of the vacant column was rinsed with 20 mL of acetonitrile, which was then transferred in the glass filter. The rinse of the Tenax GC® was repeated twice more with 20 mL and 10 mL each of acetonitrile.

2) The three filtrates were combined and transferred into a 50-mL volumetric glass flask, which was then filled to 50 mL with acetonitrile. An aliquot of the solution was diluted 50 fold in volume.

3) The concentration of the test substance in the final solution was measured by GC under the following conditions:

GC conditions

column:	Shimadzu Corporation, wide bore column CBP20-W25-100, 0.53 mm i.d., 25 m in length
temperature:	column 120 °C, injector 200 °C, detector 240 °C
carrier:	nitrogen gas (flow rate: 20 mL/min)
detector:	ECD
injection volume:	3 μL

Calibration curve

Standard solutions of the test substance were prepared to make concentrations of 0, 0.25, 0.5, and 1.0 mg/L in acetonitrile. They were analyzed by GC under the above conditions. A calibration curve prepared by the method described in §2.1.5 generated a straight line which crosses the origin and its correlation coefficient was calculated to be 0.999.

[Figure 6.1 and Figure 6.2]

At measurement of the test substance concentration, the standard solution with 1.0 mg/L was analyzed. Concentration in each test sample was calculated from ratio of total peak area for the sample to that for the standard solution.

Desorption efficiency (Recovery)

Desorption efficiency of the test substance from Tenax GC® was determined according to the following procedure:

1) Twenty five milliliters of the solution of the test substance with 1 mg/L in acetonitrile (mass of the test substance: 25 μg) was added to 4 mL of the adsorbent in a 50-mL round bottom glass flask. The solvent was removed by rotary evaporation to adsorb the test substance onto the Tenax GC®.

2) The test substance adsorbed on the Tenax GC® was recovered by the desorption procedure described above (except that the substance was extracted with 10, 10, and 5 mL of acetonitrile and the filtrates were transferred into 25-mL volumetric glass flask, which was then filled to 25 mL). The concentration of the test substance in the final solution was measured by GC to evaluate desorption efficiency.

The desorption efficiency was determined to be 97 %. For measurement of vapor pressure, the concentration of the test substance was corrected by this factor.

[Table 6.3 and Figure 6.3]

6.1.5 Calculation of vapor pressure

Vapor pressure of the test substance was calculated as follows:

$$P = (W/M)(RT/V)$$

where

- P: vapor pressure (Pa)
- W: amount of the test substance trapped (g)
- M: molecular weight of the test substance (g $mole^{-1}$)
- R: gas constant (8.31 Pa m^3 $mole^{-1}$ K^{-1})
- V: total volume of the carrier gas corrected (m^3)
- T: temperature (°K)

6.2 Results and Discussion

Vapor pressure of the test substance was 6.0×10^{-3} Pa.

[Table 6.1 to Table 6.2, and Figure 6.4]

But the chemical structure of the test substance (shown in §1.1.2) suggests that vapor pressure of each component is different from one another.

Vapor pressure of benzoic acid (a reference substance in the OECD Guideline) was 0.10 Pa (24 °C) under the conditions employed in this study.

Table 2.1 Calculation of concentration of the test substance dissolved in water

Measurement No. 1

time at 40 °C, days		1	2	3
conc. in Std., mg/L	A	1.01	1.01	1.01
peak area, μV·sec				
Std.	B	2589644	2717449	2732276
test solution	C	2030738	2414771	2230296
concentration factor	D	1	1	1
conc. of the substance, mg/L	E	0.84	0.95	0.88

Calculation equation

$$E = A(C/B)(1/D)(1/0.94)$$

Measurement No. 2

time at 40 °C, days		1	2	3
conc. in Std., mg/L	A	1.01	1.01	1.01
peak area, μV·sec				
Std.	B	2589644	2717449	2732276
test solution	C	2115067	2319263	2293546
concentration factor	D	1	1	1
conc. of the substance, mg/L	E	0.88	0.92	0.90

Calculation equation

$$E = A(C/B)(1/D)(1/0.94)$$

average water solubility: 0.89mg/l

Table 2.2 Calculation of recovery

conc. of the substance added, mg/L	A	0.97
conc. in Std., mg/L	B	1.01
peak area, μV·sec		
Std.	C	2723221
test solution	D	2456907
concentration factor	E	1
conc. of the substance recovered, mg/L		0.91
recovery (%)	F	94

Calculation equation

$$F=B(D/C)(1/E)(100/A)$$

Figure 2.1 Calibration curve of the test substance

Curve Fitting [Least Square Method]

Input Data

No.	Concentration X(mg/l)	Peak Area Y(μV·sec)
1	0	0
2	0.2016	550784
3	1.008	2758732
4	5.04	13119948

$Y = 5.0483 \times 10^4 + 2.5966 \times 10^6 \times X^1$

$r = 0.99995$

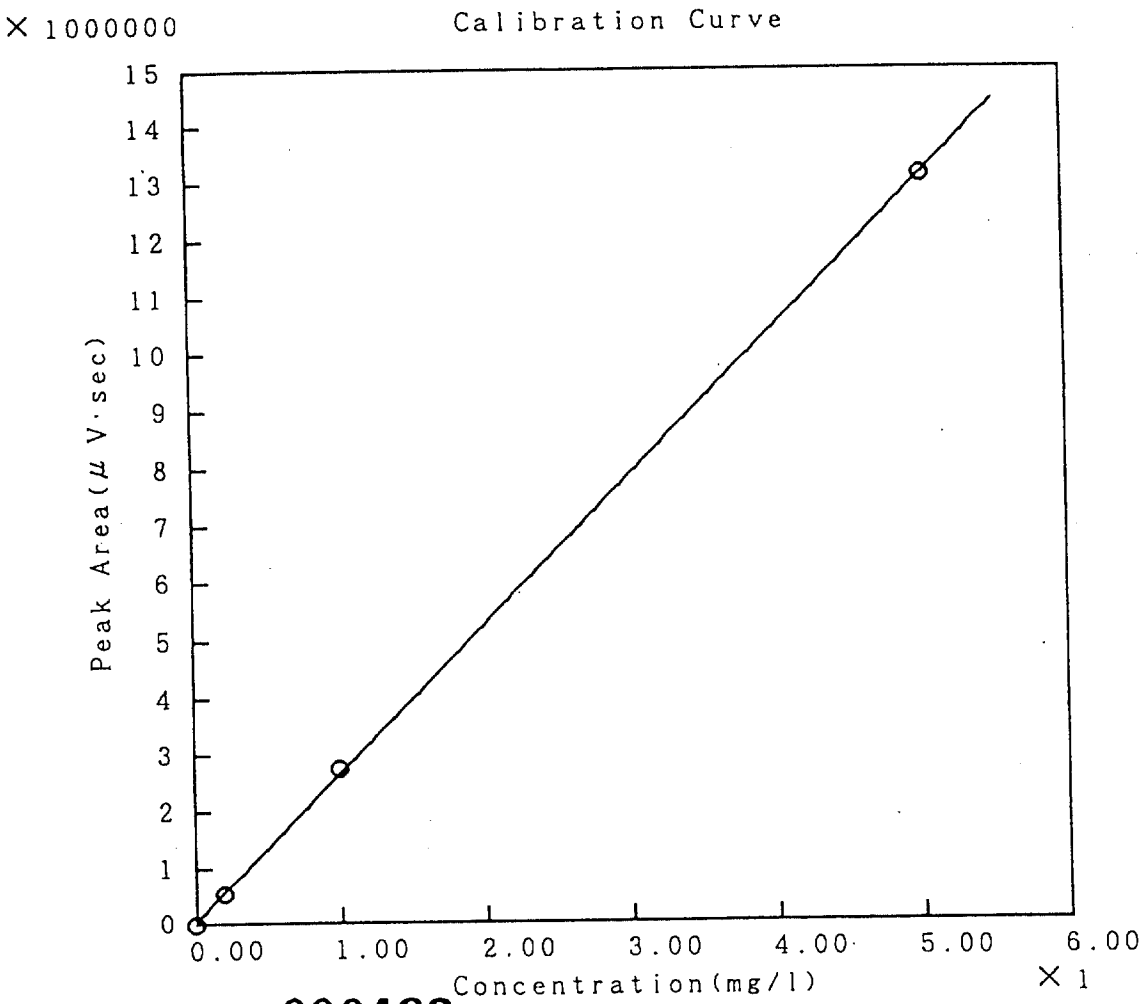
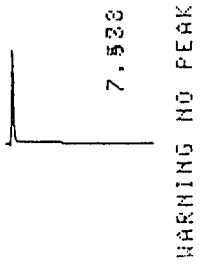
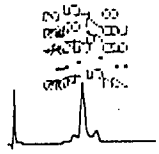


Figure 2.2 GC chromatograms of the test substance
--- for calibration curve ---

Std. 0mg/l

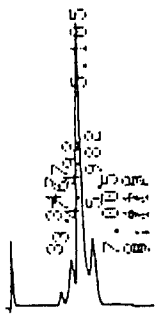


Std. 0.202mg/l



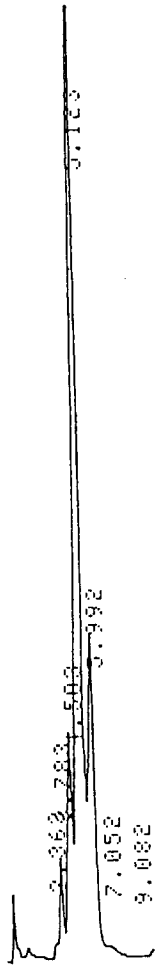
PKNO	TIME	AREA	MK
1	3.76	15993	V
2	4.485	64568	V
3	5.1	373677	V
4	5.97	96547	V
	TOTAL	550784	

Std. 1.008mg/l



PKNO	TIME	AREA	MK
1	3.347	11070	
2	3.767	80396	V
3	4.492	317962	V
4	5.105	1821019	V
5	5.982	539354	SV
	TOTAL	2769805	

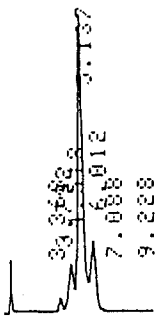
Std. 5.04mg/l



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.363	50334			0.4073
2	3.783	467507	V		3.5436
3	4.503	1545826	V		11.7168
4	5.123	8586993	V		65.0866
5	5.992	2519622	SV		19.0979
6	7.052	19504	T		0.1478
	TOTAL	13193186			100

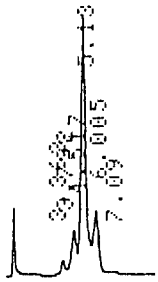
Figure 2.3 GC chromatograms of the test substance
--- for recovery---

Std. 1.008mg/l



PKNO	TIME	AREA	MK
1	3.368	11920	
2	3.792	74532	V
3	4.518	308316	V
4	5.137	1812582	V
5	6.012	527793	SV
TOTAL		2735141	

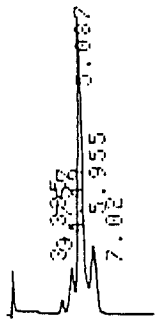
test solution



PKNO	TIME	AREA	MK
1	3.368	11050	
2	3.792	69258	V
3	4.517	280909	V
4	5.13	1628500	V
5	6.005	478240	SV
TOTAL		2467957	

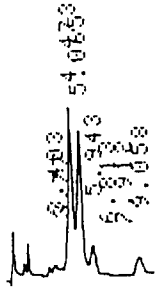
Figure 2.4.1 GC chromatograms of the test substance
--- for measurement of concentration in water
(after 1 day at 40°C)---

Std. 1.008mg/l



PKNO	TIME	AREA	MK
1	3.225	11030	
2	3.757	67922	V
3	4.49	293103	V
4	5.087	1732292	V
5	5.955	496327	SV
	TOTAL	2600681	

test solution
n=1



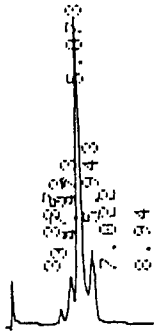
PKNO	TIME	AREA	MK
1	3.403	47759	
2	3.77	24910	V
3	4.473	927788	V
4	5.065	879701	V
5	5.943	198339	V
6	7.012	21500	
7	9.058	153840	
	TOTAL	2253882	

test solution
n=2

PKNO	TIME	AREA	MK
1	3.262	39773	
2	3.763	16505	V
3	4.477	1039350	V
4	5.072	859751	V
5	5.953	199461	V
6	7.047	17090	
7	9.05	131063	
	TOTAL	2303814	

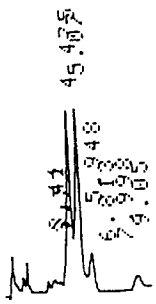
Figure 2.4.2 GC chromatograms of the test substance
--- for measurement of concentration in water
(after 2 days at 40°C)---

Std. 1.008mg/l



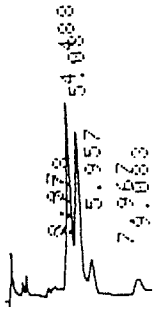
PKNO	TIME	AREA	MK
1	3.037	11413	
2	3.753	74675	V
3	4.473	310527	V
4	5.073	1802535	V
5	5.943	529712	SV
	TOTAL	2728862	

test solution
n=1



PKNO	TIME	AREA	MK
1	3.41	51425	
2	3.77	35983	V
3	4.475	1004015	V
4	5.07	1119855	V
5	5.948	254918	V
6	7.998	10137	
7	9.05	13067.4	
	TOTAL	2613016	

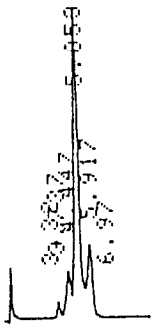
test solution
n=2



PKNO	TIME	AREA	MK
1	3.37	40012	
2	3.778	23320	V
3	4.488	1053734	V
4	5.08	1002276	V
5	5.957	239933	V
6	7.967	20401	
7	9.083	143424	
	TOTAL	2523247	

Figure 2.4.3 GC chromatograms of the test substance
--- for measurement of concentration in water
(after 3 days at 40°C)---

Std. 1.008mg/l

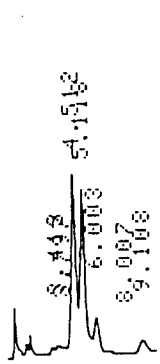


PKNO	TIME	AREA	MK
1	3.22	11934	
2	3.797	76145	V
3	4.447	311144	V
4	5.053	1819770	V
5	5.917	525217	SV
	TOTAL	2744109	

test solution
n=1

PKNO	TIME	AREA	MK
1	3.407	52984	
2	3.777	29460	V
3	4.478	1015002	V
4	5.073	965788	V
5	5.947	220046	V
6	6.07	17482	
7	7.907	22211	V
8	9.053	166957	
	TOTAL	2497030	

test solution
n=2



PKNO	TIME	AREA	MK
1	3.413	20661	
2	3.797	24657	V
3	4.512	1008133	V
4	5.118	1020090	V
5	6.003	240666	V
6	6.007	11329	
7	9.108	116910	
	TOTAL	2463445	

Table 3 Calculations of reaction rate constant and half life time of the test substance (Table 3.1-Table 3.3)

Table 3.1 (pH 4 at 25°C)

time, days (t)	residual percent of the substance, mg/L (C)	rate constant, day^{-1} (k)
5	89.7	0.022
8	87.3	0.017
15	77.0	0.017
19	76.8	0.014
26	68.3	0.015
33	62.1	0.014

average rate constant $0.017 day^{-1}$
half life time ($t_{1/2}$) 42 days

calculation equations

$$k = 1/t \times \ln 100/c$$

$$t_{1/2} = 1/k \times \ln 2$$

Table 3.2 (pH 7 at 25°C)

time, days (t)	residual percent of the substance, mg/L (C)	rate constant, day^{-1} (k)
5	88.6	0.024
8	86.9	0.018
15	74.1	0.020
19	69.5	0.019
26	59.9	0.020
33	53.8	0.019

average rate constant $0.020 day^{-1}$
$t_{1/2}$ 35 days

Table 3. (pH 9 at 25°C)

time, days (t)	residual percent of the substance, mg/L (C)	rate constant, day^{-1} (k)
2	92.4	0.040
5	78.4	0.049
8	69.0	0.046
12	57.1	0.047
15	50.2	0.046
19	37.7	0.051

average rate constant $0.046 day^{-1}$
$t_{1/2}$ 15 days

Table 3.4 Calculations of residual concentration of the test substance
--- for *Further investigation* (at 25.0 ± 0.2°C) ---

time, days	pH	peak area, μV*sec Std B	peak area, μV*sec test solution C	conc. in test soln.	residual percent D
2	9	2889193	2523765	0.371	92.4
5	4	2951466	2502799	0.361	89.7
	7	2951466	2473261	0.356	88.6
	9	2951466	2186759	0.315	78.4
8	4	2968192	2449526	0.351	87.3
	7	2968192	2439536	0.350	86.9
	9	2968192	1936885	0.278	69.0
12	9	2939623	1585436	0.229	57.1
15	4	2871668	2089210	0.309	77.0
	7	2871668	2011015	0.298	74.1
	9	2871668	1361482	0.202	50.2
19	4	2871088	2084603	0.309	76.8
	7	2871088	1886031	0.279	69.5
	9	2871088	1022942	0.152	37.7
26	4	2962788	1912723	0.275	68.3
	7	2962788	1676378	0.241	59.9
33	4	2928913	1718699	0.250	62.1
	7	2928913	1490039	0.216	53.8

concentration of the standard solution (A): 1.01 mg/L
concentration factor (E): 2.5
initial concentration of the test substance(F): 0.402 mg/L
recovery(G): 95 %

calculation equation: $D = A(C/D) * E^{-1} * F^{-1} (100/G) * 100$

Table 3.5 Calculations of residual concentration of the test substance
--- for *Preliminary test* (after 5 days at 50.0±0.1°C) ---

		pH 4	pH 7	pH 9
initial concentration of the test substance, mg/L	A	0.402	0.402	0.402
after 5 days				
conc. in Std., mg/L	B	1.00	1.00	1.00
peak area, μV*sec				
Std.	C	3022269	3022269	3022269
test solution	D	1867539	1876183	1264465
concentration factor	E	2	2	2
conc. in the test solution		0.306	0.307	0.207
residual percent of the test substance	F	76	76	52

calculation equation

$$F = B(D/C)(1/E)(1/A)(1/1.01)100$$

Table 3.6 Calculation of recovery
--- for *Preliminary test* ---

conc. of the test substance added, mg/L	A	0.402
conc. in Std., mg/L	B	1.00
peak area, μV*sec		
Std.	C	2977220
test solution	D	2420272
concentration factor	E	2
conc. of the test substance recovered, mg/L		0.406
recovery, %	F	101

calculation equation

$$F = B(D/C)(1/E)(1/A)100$$

Table 3.7 Calculation of recovery
--- for *Further investigation* ---

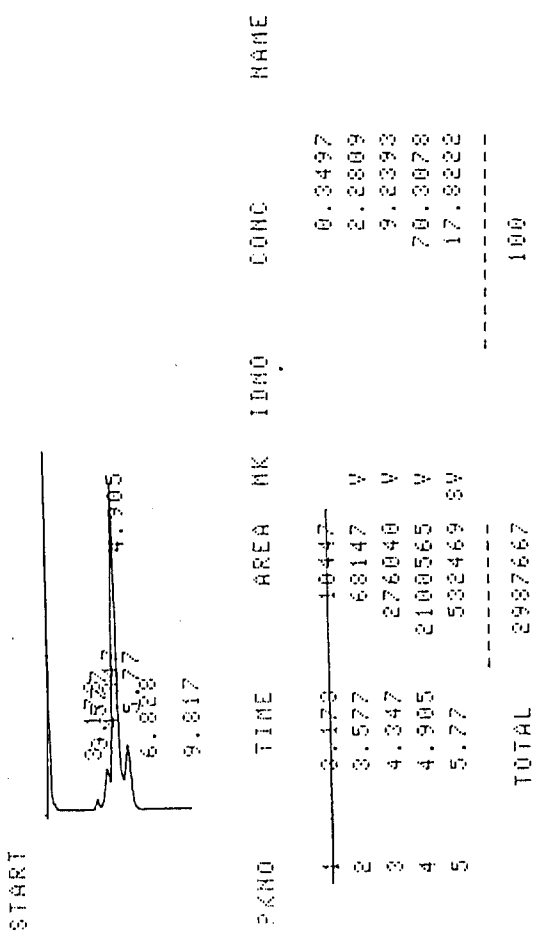
conc. of the test substance added, mg/L	A	0.402
conc. in Std., mg/L	B	1.01
peak area, μV*sec		
Std.	C	2782217
test solution	D	2639636
concentration factor	E	2.5
conc. of the test substance recovered, mg/L		0.383
recovery, %	F	95

calculation equation

$$F = B(D/C)(1/E)(1/A)100$$

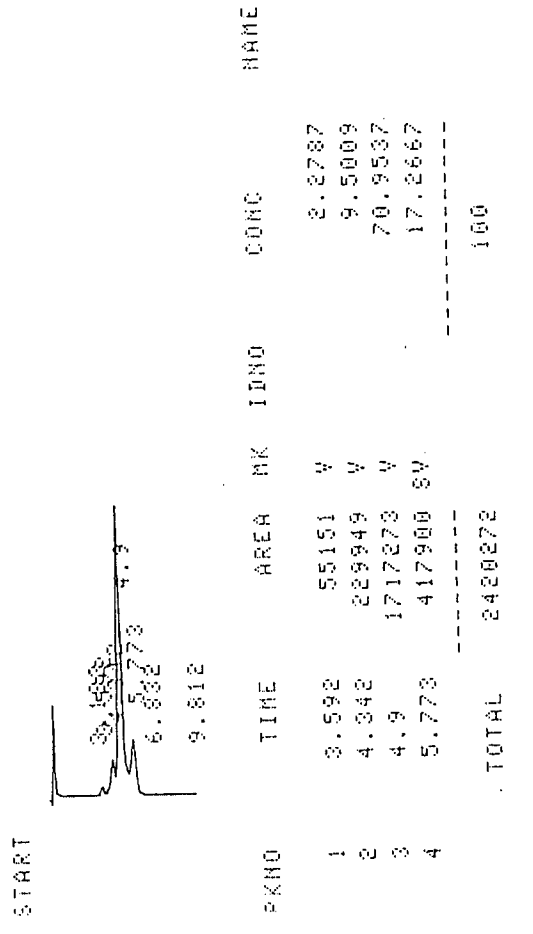
Figure 3.1 GC chromatograms of the test substance
--- for recovery in *Preliminary test* ---

Std. (1.0mg/ℓ)



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.172	10447			0.3497	
2	3.577	68147	V		2.2809	
3	4.347	276040	V		9.2393	
4	4.905	2100565	V		70.3078	
5	5.77	532469	SV		17.8222	
TOTAL		2987667			100	

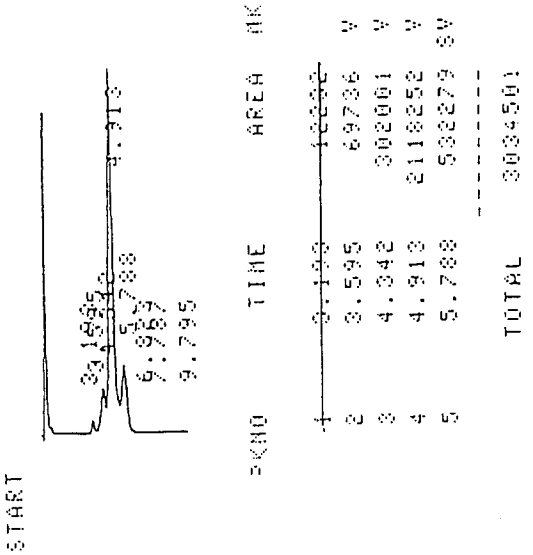
test solution



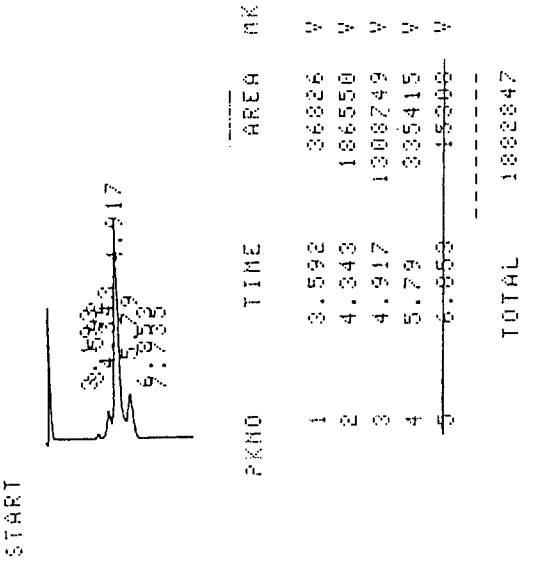
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.592	55151	V		2.2787	
2	4.342	229949	V		9.5009	
3	4.9	1717273	V		70.9537	
4	5.773	417900	SV		17.2667	
TOTAL		2420272			100	

Figure 3.2 GC chromatograms of the test substance
--- for measurement of concentration in water in *Preliminary test* ---

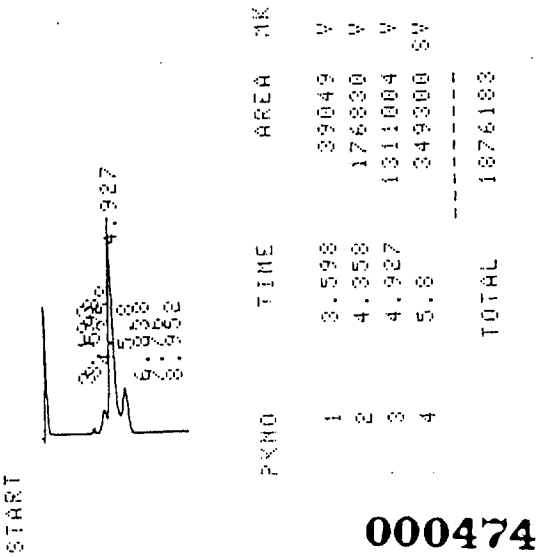
Std. (1.0mg/ℓ)



PH 4



PH 7



PH 9

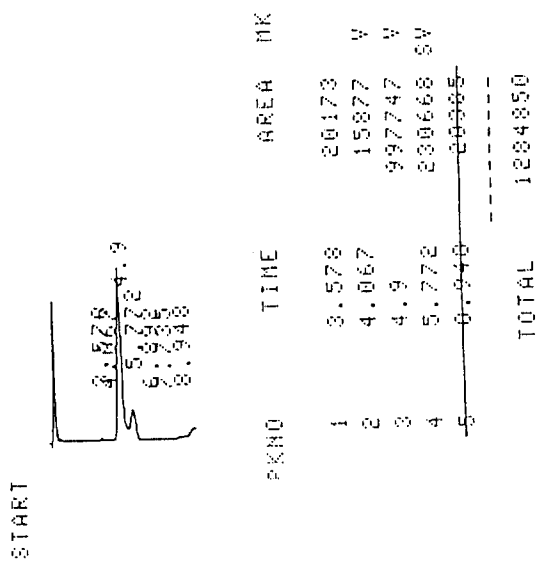
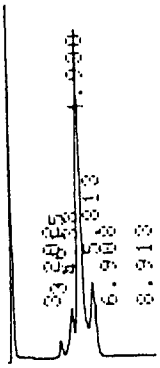


Figure 3.3 GC chromatograms of the test substance
--- for recovery in *Further investigation* ---

Std. 1.01mg/l



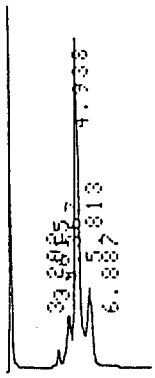
PKNO	TIME	AREA	MK
1	3.202	11582	
2	3.615	78688	V
3	4.38	307633	V
4	4.938	1888329	V
5	5.813	507568	V
6	6.908	22732	V
	TOTAL	2816536	

test solution

PKNO	TIME	AREA	MK
1	3.162	10263	
2	3.572	71301	V
3	4.322	270242	V
4	4.882	1790986	V
5	5.757	507107	V
6	6.81	27406	V
	TOTAL	2677385	

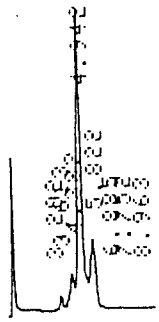
Figure 3.4 GC chromatograms of the test substance
--- for measurement of concentration in water
in *Further investigation* --- [1] *after 2 days*

Std. 1.01mg/l



PKNO	TIME	AREA	MK
1	3.203	12991	
2	3.615	81198	V
3	4.367	331785	V
4	4.938	1928231	V
5	5.813	547979	V
6	6.887	26503	V
	TOTAL	2928667	

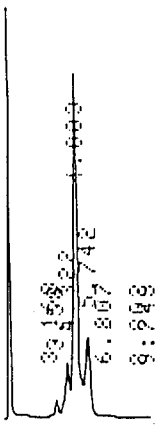
pH 9



PKNO	TIME	AREA	MK
1	3.613	69455	V
2	4.382	241338	V
3	4.942	1738036	V
4	5.822	474936	V
5	6.885	19064	V
	TOTAL	2542849	

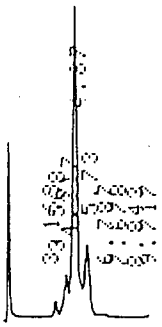
Figure 3.4 (continued) [2] *after 5 days*

Std. 1.01mg/l



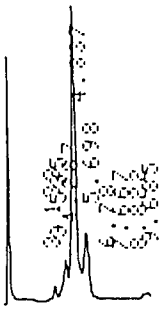
PKNO	TIME	AREA	MK	IDNO
1	3.168	11917		
2	3.58	84103	W	
3	4.322	332077	W	
4	4.883	1973856	W	
5	5.742	561432	SW	
	TOTAL	2963383		

pH 4



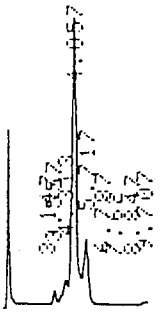
PKNO	TIME	AREA	MK	IDNO
1	3.168	10310		
2	3.568	72548	W	
3	4.317	264864	W	
4	4.87	1763114	W	
5	5.73	402272	SW	
	TOTAL	2513109		

pH 7



PKNO	TIME	AREA	MK	IDNO
1	3.535	69921	W	
2	4.287	265288	W	
3	4.837	1672584	W	
4	5.698	465468	SW	
5	9.685	18761		
	TOTAL	2492022		

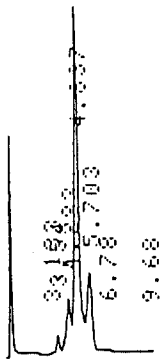
pH 9



PKNO	TIME	AREA	MK	IDNO
1	3.557	61419	W	
2	4.313	171877	W	
3	4.857	1626920	W	
4	5.717	326543	SW	
5	9.707	10937		
	TOTAL	2197696		

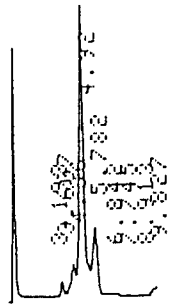
Figure 3.4 (continued) [3] *after 8 days*

Std. 1.01mg/l



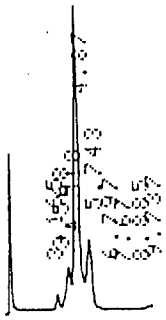
PKNO	TIME	AREA	MK	IDNO
1	3.188	12095		
2	3.54	84865	V	
3	4.283	342423	V	
4	4.837	1976176	V	
5	5.703	564727	V	
6	6.78	27643	V	
	TOTAL	3008870		

pH 4



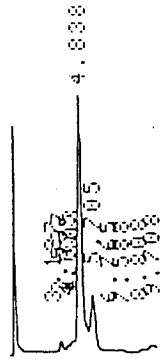
PKNO	TIME	AREA	MK	IDNO
1	3.199	11099		
2	3.607	70742	V	
3	4.36	225643	V	
4	4.92	1689464	V	
5	5.782	463678	SV	
	TOTAL	2460565		

pH 7



PKNO	TIME	AREA	MK	IDNO
1	3.568	72575	V	
2	4.318	276869	V	
3	4.87	1764394	V	
4	5.743	325699	SV	
	TOTAL	2439536		

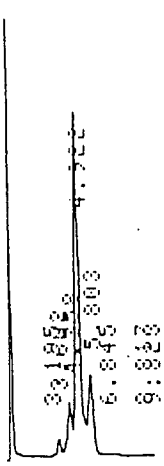
pH 9



PKNO	TIME	AREA	MK	IDNO
1	3.537	47846	V	
2	4.035	34361	V	
3	4.303	56378	V	
4	4.838	1442243	V	
5	5.705	356058	SV	
6	6.808	13215	T	
7	9.708	17944		
	TOTAL	1968049		

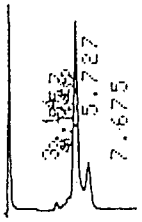
Figure 3.4 (continued) [4] *after 12 days*

Std. 1.01mg/l



PKNO	TIME	AREA	MK	IDNO
1	3.125	12673		
2	3.602	85290	V	
3	4.358	341391	V	
4	4.922	2006286	V	
5	5.803	506656	SV	
	TOTAL	2952296		

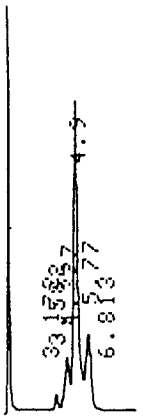
pH 9



PKNO	TIME-	AREA	MK	IDNO
1	3.557	41384	V	
2	4.048	27159	V	
3	4.332	70038	V	
4	4.86	1115007	V	
5	5.727	331848	V	
	TOTAL	1585436		

Figure 3.4 (continued) [5] *after 15 days*

Std. 1.01mg/l



PKNO	TIME	AREA	MK	IDNO
1	3.172	13294		
2	3.582	78196	V	
3	4.327	329297	V	
4	4.9	1883392	V	
5	5.77	580784	SV	
TOTAL		2884962		

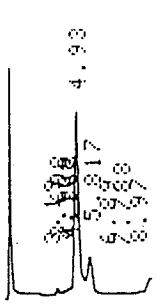
pH 4

PKNO	TIME	AREA	MK	IDNO
1	3.117	10243		
2	3.525	59779	V	
3	4.267	169135	V	
4	4.812	1444165	V	
5	5.65	416131	SV	
TOTAL		2099453		

pH 7

PKNO	TIME	AREA	MK	IDNO
1	3.54	54941	V	
2	4.275	176806	V	
3	4.833	1362655	V	
4	5.687	416614	SV	
TOTAL		2011015		

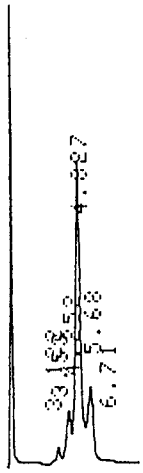
pH 9



PKNO	TIME	AREA	MK	IDNO
1	3.608	32900	V	
2	4.108	22387	V	
3	4.368	35866	V	
4	4.93	1010484	V	
5	5.817	259845	SV	
6	0.97	16005		
TOTAL		1378387		

Figure 3.4 (continued) [6] *after 19 days*

Std. 1.01mg/l

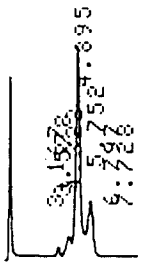


PKNO	TIME	AREA	MK	IDNO
1	3.122	13139		
2	3.53	80003	V	
3	4.252	328533	V	
4	4.827	1879027	V	
5	5.68	583525	SV	
TOTAL		2884227		

pH 4

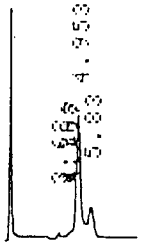
PKNO	TIME	AREA	MK	IDNO
1	3.597	55724	V	
2	4.347	149067	V	
3	4.917	1414172	V	
4	5.783	465641	SV	
TOTAL		2084603		

pH 7



PKNO	TIME	AREA	MK	IDNO
1	3.578	52284	V	
2	4.322	155916	V	
3	4.895	1248817	V	
4	5.752	429014	SV	
TOTAL		1886031		

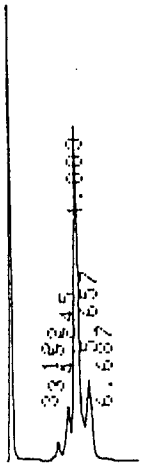
pH 9



PKNO	TIME	AREA	MK	IDNO
1	3.62	22481		
2	4.108	14349	V	
3	4.417	40911	V	
4	4.953	723651	V	
5	5.83	221549	V	
TOTAL		1022942		

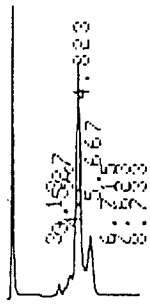
Figure 3.4 (continued) [7] *after 26 days*

Std. 1.01mg/l



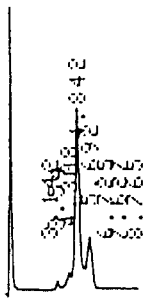
PKNO	TIME	AREA	MK	IDNO
1	3.12	11819		
2	3.52	86807	V	
3	4.245	338231	V	
4	4.803	1970817	V	
5	5.657	566933	SV	
	TOTAL	2974607		

pH 4



PKNO	TIME	AREA	MK	IDNO
1	3.527	58084	V	
2	4.28	152256	V	
3	4.823	1349836	V	
4	5.667	352547	SV	
	TOTAL	1912723		

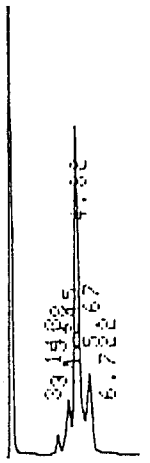
pH 7



PKNO	TIME	AREA	MK	IDNO
1	3.54	54270	V	
2	4.308	130219	V	
3	4.842	1100240	V	
4	5.692	391650	V	
	TOTAL	1676378		

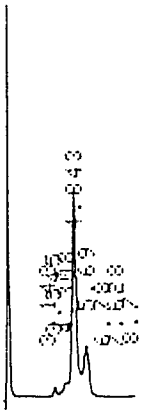
Figure 3.4 (continued) [8] *after 33 days*

Std. 1.01mg/l



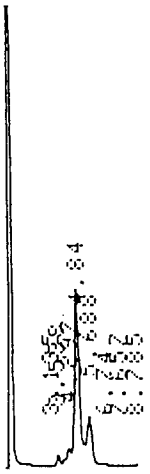
PKNO	TIME	AREA	MK	IDNO
1	3.113	10906		
2	3.518	86430	V	
3	4.245	341379	V	
4	4.82	1937852	V	
5	5.67	563253	V	
6	6.722	27141	V	
TOTAL		2966930		

pH 4



PKNO	TIME	AREA	MK	IDNO
1	3.545	48236	V	
2	4.308	114485	V	
3	4.843	1193570	V	
4	5.69	362408	SV	
TOTAL		1718699		

pH 7



PKNO	TIME	AREA	MK	IDNO
1	3.538	46752	V	
2	4.297	116978	V	
3	4.84	1032199	V	
4	5.688	294110	SV	
TOTAL		1490039		

Figure 4.1 UV spectrum of the test substance
in acidic, neutral, and alkaline solutions

concentration of the test substance 0.8mg/ℓ

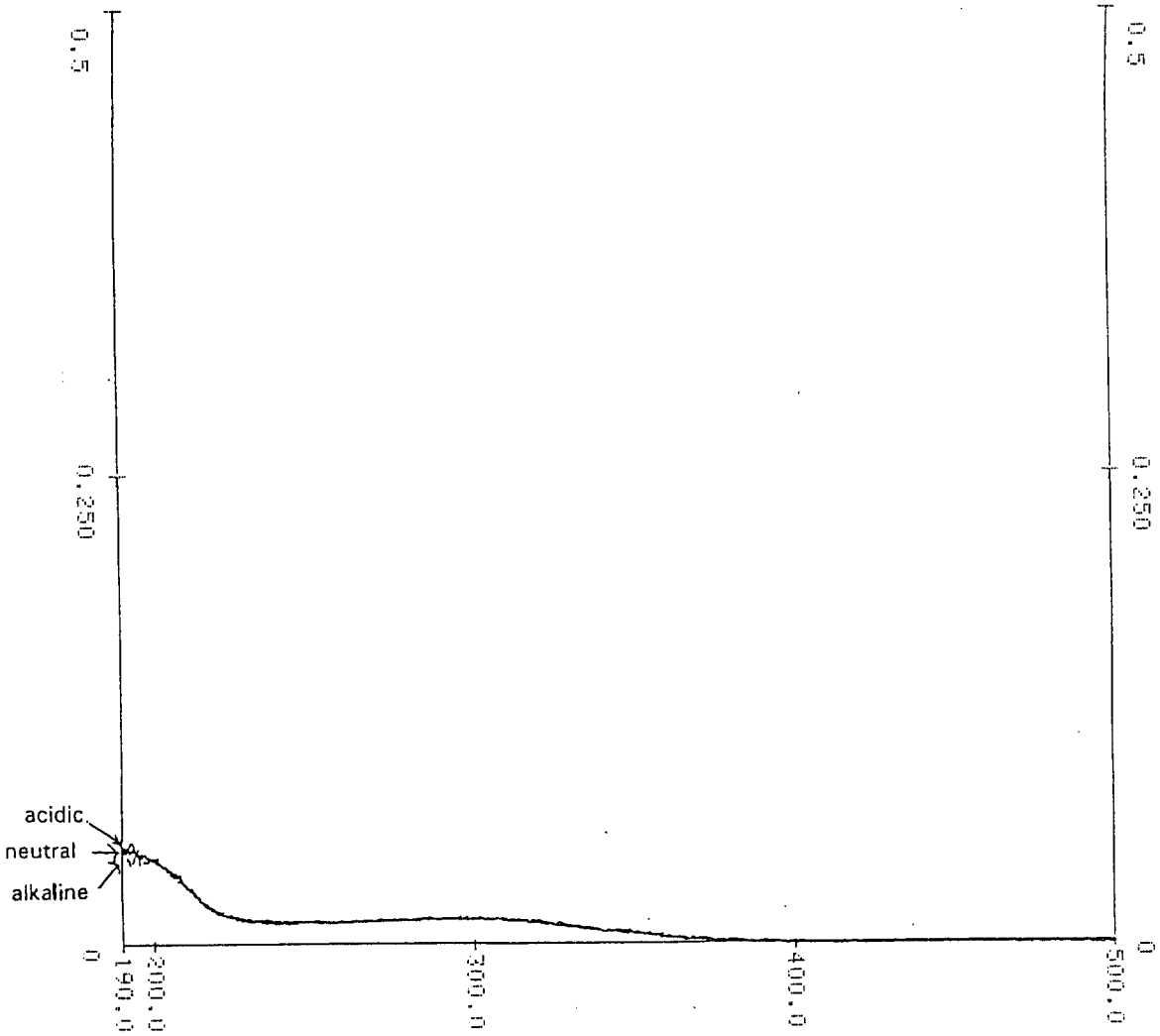


Figure 5.1 Correlation between HPLC retention time and Pow of the reference compounds

Curve Fitting [Least Square Method]

Input Data

No.	log Rt	log Pow
1	0.355	2.12
2	0.626	2.99
3	0.813	3.76
4	0.981	4.81
5	1.123	6.20

$Y = -2.1919 \times 10^{-2} + 5.1282 \times X^{1}$

$r = 0.97258$

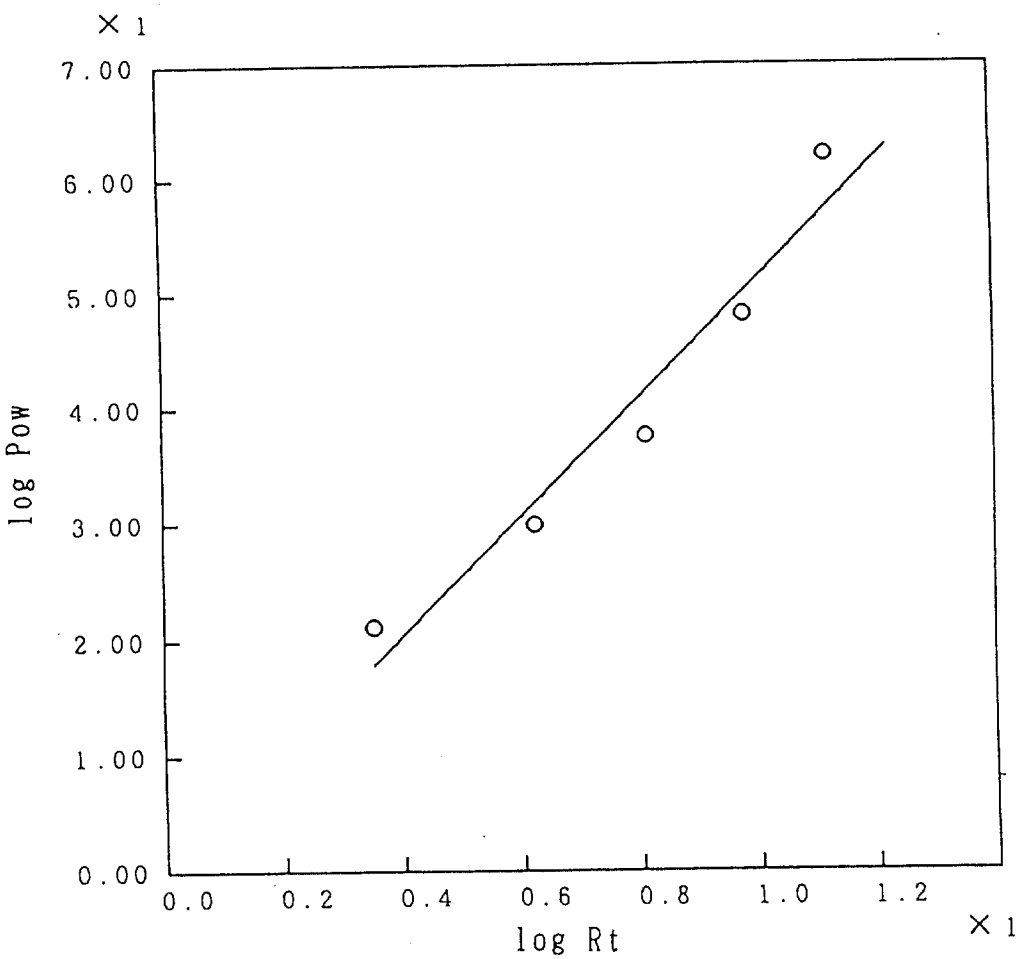
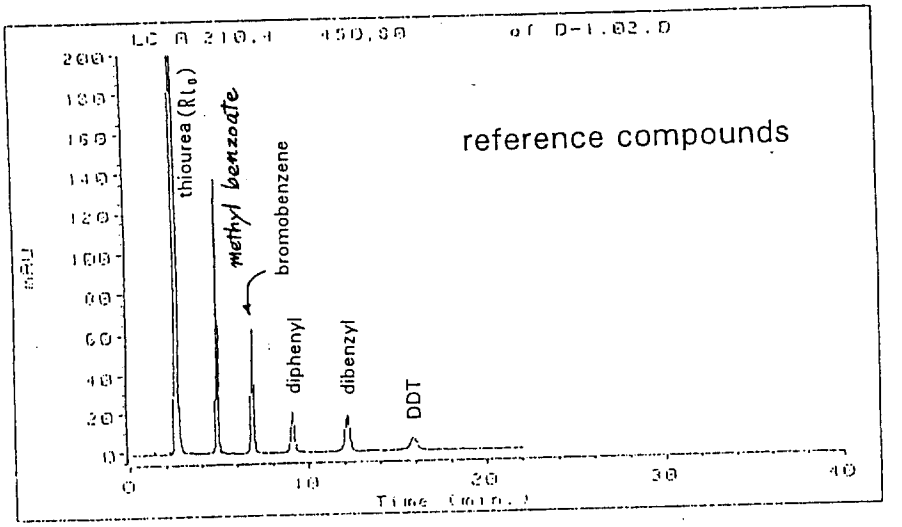
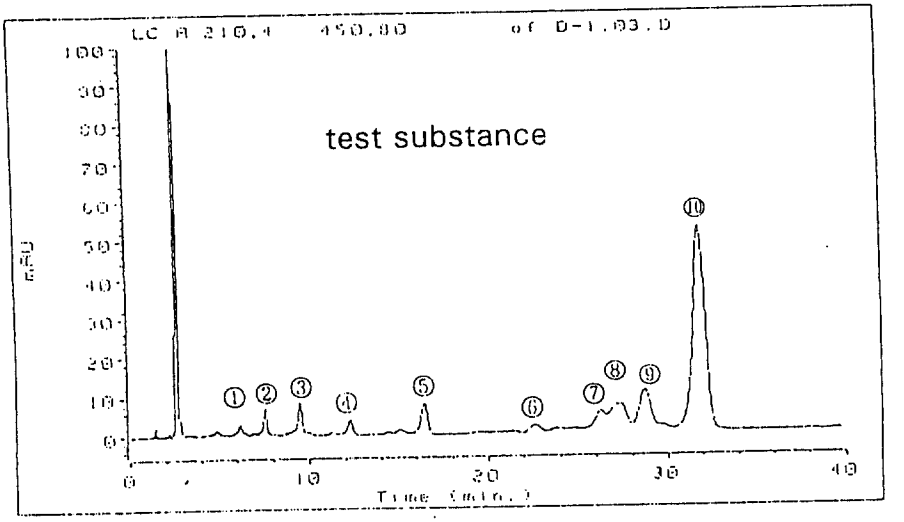


Figure 5.2 HPLC chromatograms
of the reference compounds and the test substance



Peak#	Ret Time	Type	Width	Area		Rt-Rt₀	log Rt	log Pow
1	2.637	PV	0.078	11214	thiourea			
2	4.903	BV	0.116	1042	methyl benzoate	2.266	0.355	2.12
3	6.867	BV	0.157	633.02	bromobenzene	4.230	0.626	2.99
4	9.139	BV	0.208	278.54	diphenyl	6.502	0.813	3.76
5	12.204	BV	0.274	353.74	dibenzyl	9.567	0.981	4.81
6	15.900	VV	0.325	169.83	DDT	18.263	1.123	6.20



Peak#	Ret Time	Type	Width	Area	thiourea	Rt-Rt₀	log Rt	log Pow
1	2.633	PV	0.069	2559				
2	6.144	VV	0.236	53.99	①	3.511	0.545	2.77
3	7.555	VV	0.199	90.09	②	4.922	0.692	3.75
4	9.502	BV	0.261	138.64	③	6.869	0.837	4.27
5	12.304	BV	0.255	79.43	④	9.671	0.985	5.03
6	16.445	VV	0.377	214.84	⑤	13.812	1.140	5.82
7	22.562	BV	0.416	67.83	⑥	19.929	1.299	6.64
8	26.308	BV	0.462	168.18	⑦	23.675	1.374	7.02
9	27.359	VV	0.751	359.48	⑧	24.726	1.393	7.12
10	28.722	VV	0.482	371.23	⑨	26.089	1.416	7.24
11	31.878	BV	0.669	2576	⑩	29.245	1.466	7.50

000486

Table 6.1 Calculation of vapor pressure

trapped test substance (*corrected* mass), g	W	1.47×10^{-3}
molecular weight of the test substance	M	625
gas constant, $Pa*m^3*mole^{-1}*K^{-1}$	R	8.31
corrected total volume of the carrier gas, m^3	V	0.973
temperature, °K	T	298
vapor pressure, Pa	P	6.0×10^{-3}

calculation equations

$$P = (W/M)(RT/V)$$

Table 6.2 Calculation of mass of the trapped test substance

conc. in Std., mg/L	A	1.006
peak area, $\mu V*sec$		
Std.	B	2489067
test solution	C	1412087
conc. in the test solution, mg/L		0.571
final volume, mL	D	50
dilution factor	E	50
trapped test substance as *measured* mass, g		1.43×10^{-3}
trapped test substance as *corrected* mass, g	W	1.47×10^{-3}

calculation equation

$$W = A(C/B)E(D/1000)(1/0.97)(1/1000)$$

Table 6.3 Calculation of desorption efficiency

conc. of the test substance added, mg/L	A	0.02515
conc. in Std., mg/L	B	1.006
peak area, $\mu V*sec$		
Std.	C	2451409
test solution	D	2372240
final volume, mL		25
conc. of the test substance recovered, mg/L	E	0.02434
desorption efficiency, %	F	97

calculation equations

$E = B(D/C)(25/1000)$ $F = (E/A)100$

Figure 6.1 Calibration curve of the test substance

Curve Fitting [Least Square Method]

Input Data

No.	Concentration X(mg/l)	Peak Area Y(μV·sec)
1	0	0
2	0.252	602790
3	0.503	1234186
4	1.003	2475687

$Y = -9.1506 \times 10^3 + 2.4740 \times 10^6 \times X^1$

$r = 0.99997$

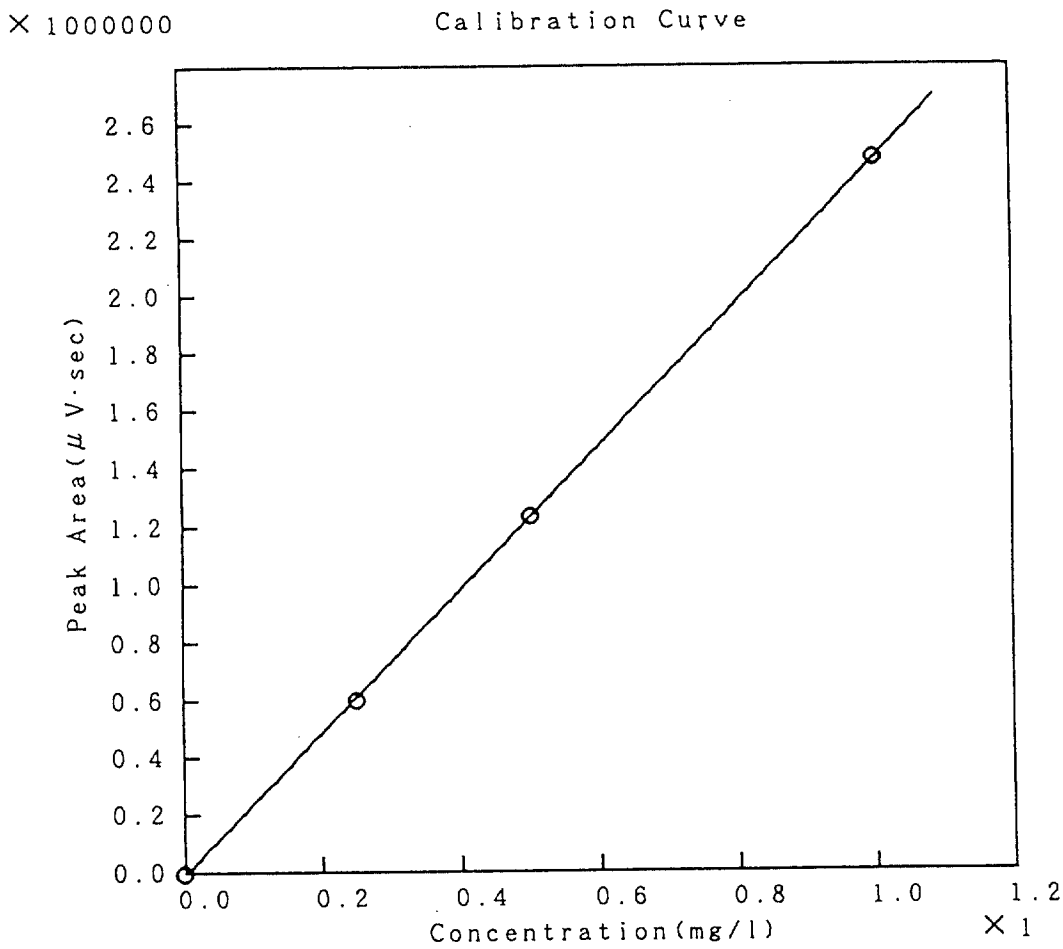
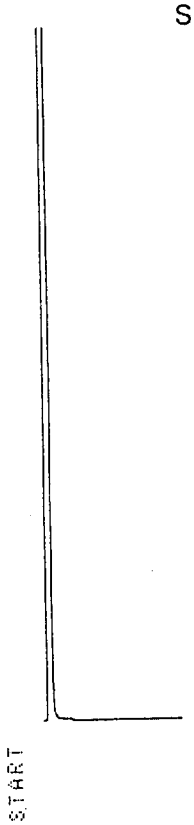
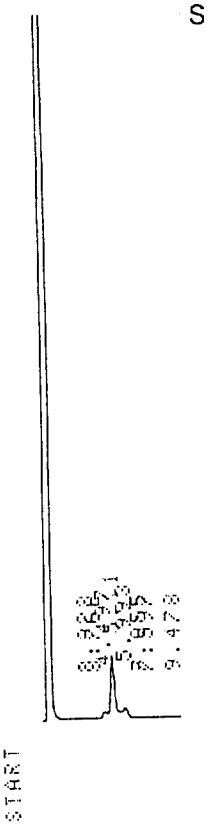


Figure 6.2 GC chromatograms of the test substance
--- for calibration curve ---

Std. 0mg/ℓ

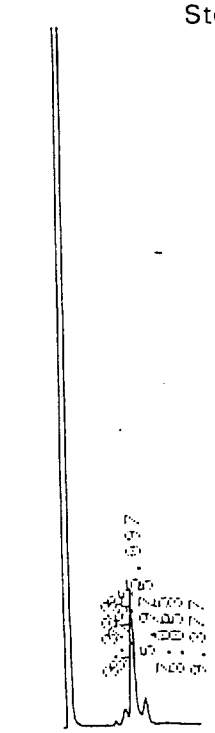


Std. 0.25mg/ℓ



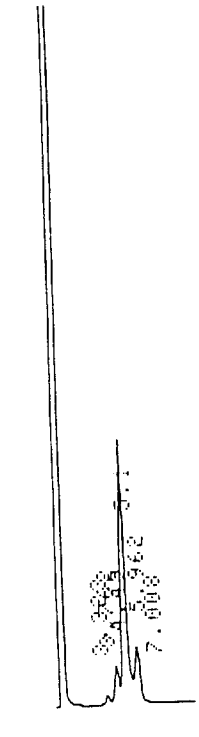
PKNO	TIME	AREA	MK	IDNO	CONC
1	3.762	13433	V		2.2284
2	4.497	63505	V		10.5351
3	5.11	417290	V		69.2264
4	5.998	108563	SV		18.0101
TOTAL		602790			100

Std. 0.50mg/ℓ



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.762	28988	V		2.3488
2	4.495	131742	V		10.6744
3	5.097	843020	V		68.306
4	5.975	230432	SV		18.6708
TOTAL		1234186			100

Std. 1.0 mg/ℓ

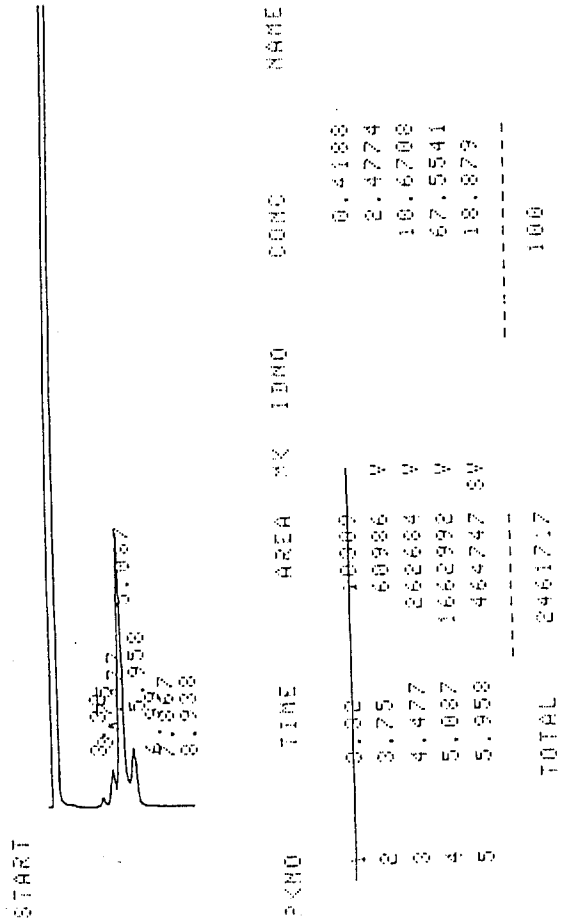


PKNO	TIME	AREA	MK	IDNO	CONC
1	3.326	10240			0.4111
2	3.762	62275	V		2.5051
3	4.49	266548	V		10.7224
4	5.1	1674132	V		67.3449
5	5.962	472732	SV		19.0165
TOTAL		2485905			100

000490

Figure 6.3 GC chromatograms of the test substance
--- for desorption efficiency ---

Std. (1.0mg/ℓ)



test solution

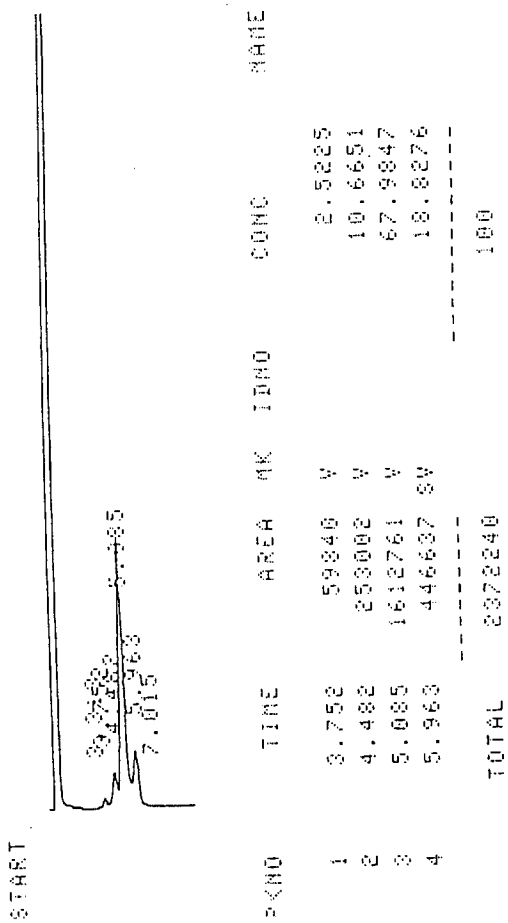
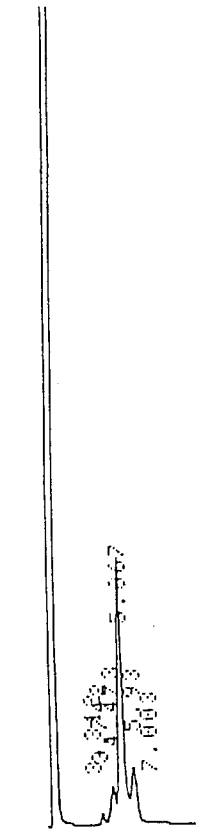


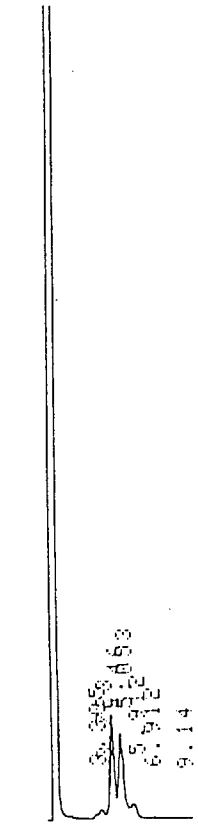
Figure 6.4 GC chromatograms of the test substance
.--- for measurement of mass of the trapped test substance ---

Std. (1.0mg/ℓ)



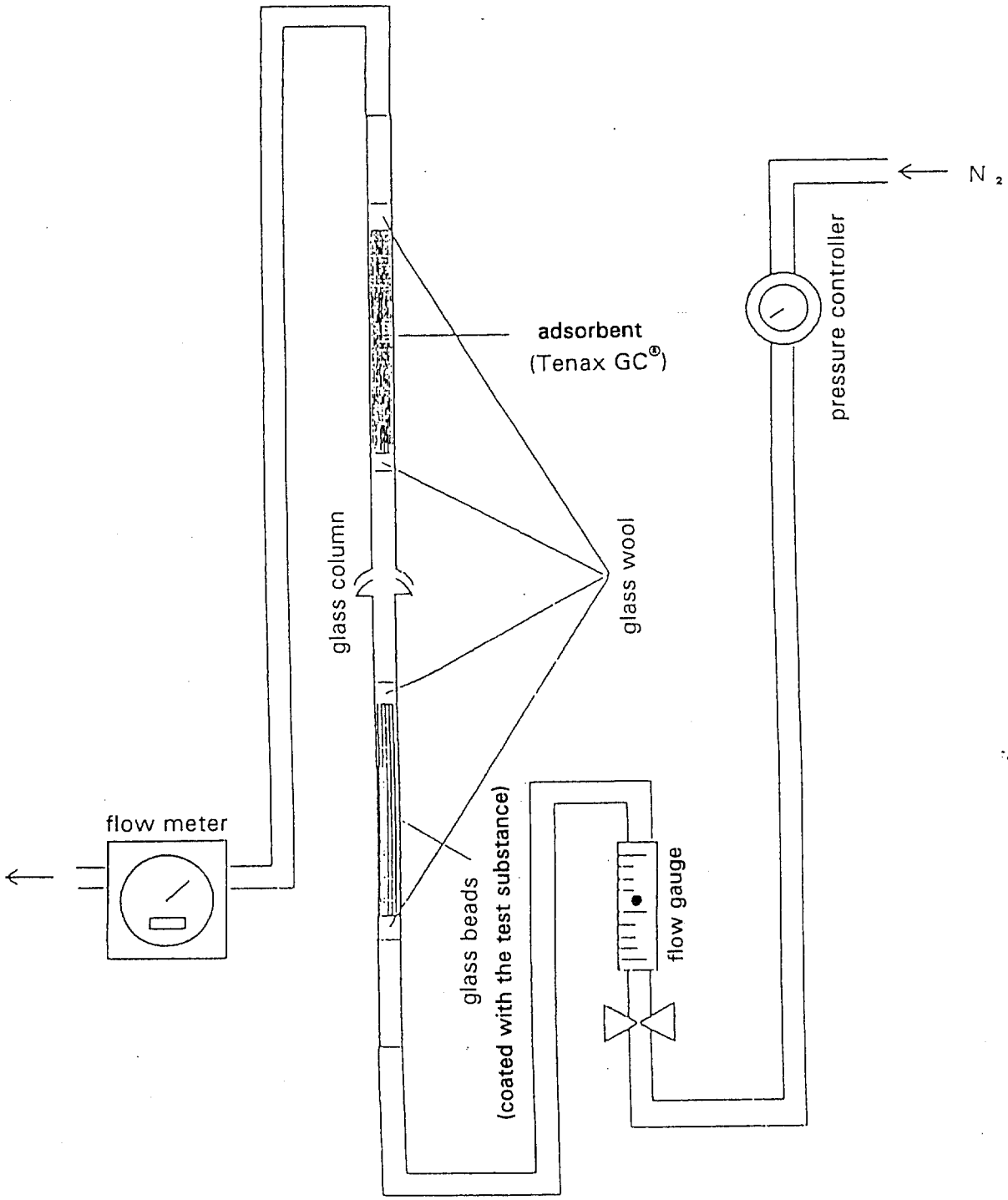
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.742	58593	V		2.354	
2	4.473	262690	V		10.5538	
3	5.067	1683711	V		67.6442	
4	5.93	484073	SV		19.448	
TOTAL		2489067			100	

test solution



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.305	21006			1.5067	
2	3.723	66097	V		4.6103	
3	4.46	623477	V		43.4876	
4	5.053	588125	V		41.0218	
5	5.912	134388	V		9.3736	
TOTAL		1433689			100	

Figure 6.5 Vapor pressure measuring apparatus



BIODEGRADATION

TEST SUBSTANCE

Identity: [2-(N-Ethylperfluorooctanesulfonamido) ethyl acrylate; may also be referred to as B1228, D-1, EtFOSEA, or FX-13. (2-Propenoic acid, 2-[ethyl[heptadecafluorooctyl)sulfonyl]amino]ethyl ester, CAS # 423-82-5)

Remarks: Material is an amber solid. Sample purity not recorded. 3M lot number 13.

METHOD

Method: Not noted.
Type: Aerobic
GLP: No
Year completed: 1984
Test solution preparation: Sample weighed onto a cover glass, dissolved in acetone and washed into a bottle with acetone. Bottle coated with acetone solution. Acetone was then purged from the bottle with nitrogen.
Contact time: 20 days
Inoculum: Not noted

RESULTS

Test substance:

5-day BOD:	Not measurable
10-day BOD:	18,990 mg/kg
20-day BOD:	23,000mg/kg
COD:	240,000 mg/kg

Reference substance: The glucose-glutamic acid standard achieved a BOD of 201 mg/L in 5-days, which is within the recommended range for the Standard Methods BOD test method.

CONCLUSIONS

This testing indicated that the test substance is not capable of readily biodegrading in aerobic environments.

DATA QUALITY

Reliability: Klimisch ranking 3. This study, while well conducted using the methodology acceptable at the time, lacks analytical data for determination of the sample purity. Testing was also done well above the solubility limit of the test substance with the use of acetone as a solubilizing agent.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, Lab Request number B1228, 4/26/84

OTHER

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

Last changed: 5/22/00

Environmental Laboratory
BOD Work Sheet - Blanks, Controls, and Standards

2ND C-BOD RUN

3M

Lab Request No. ▶ B1334, B1439 B1228, B1230, B1237, B1292		BOD Run No. ▶ 84-1314	
Analyst [illegible]	Date Started 4-6-84	5 c-Day BOD Readings	
Seed Type & Source State Sewage/Chemolite	Seed Concentration 6ml/L	Analyst [illegible]	Date 4-11-84

Nonseeded Dilution - Water Blank Incub. temp 4-6-84 20.5 °C

Bottle No.	Initial D.O. (A_1)	Present D.O. (A_2)	D.O. Depletion ($A_1 - A_2$)	Average D.O. Depletion ($\bar{A}_1 - \bar{A}_2$)
79	8.40	8.10	0.30	0.27
70	8.40	8.10	0.30	
140	8.40	8.20	0.20	

Seeded Dilution - Water Blank (optional)

Bottle No.	Initial D.O. (C_1)	Present D.O. (C_2)	D.O. Depletion ($C_1 - C_2$)	Average D.O. Depletion ($\bar{C}_1 - \bar{C}_2$)
72	8.40	7.85	0.55	0.42
4	8.40	8.00	0.40	
34	8.40	8.10	0.30	

Seed Controls

Bottle No.	Sample Size	Initial D.O. (B_1)	Present D.O. (B_2)	D.O. Depletion ($B_1 - B_2$)
97	20	8.40	7.50	0.90
85	40	8.20	6.80	1.40
84	80	8.15	5.20	2.95

Glucose - Glutamic Acid Standard

Bottle No.	Sample Size	Initial D.O. (D_1)	Present D.O. (D_2)	Total D.O. Depletion ($D_1 - D_2$)	Depletion Due To Standard	5 / C-BOD · Day	Average 5 / C-BOD · Day
50	3	8.40	6.00	2.40	1.99	199	201 mg/L
83	5	8.40	4.60	3.80	3.39	203	
62	7	8.40	3.30	5.10	4.69	201	

Acetone ~~water~~ coated bottle blanks

~~Potassium Hydrogen Phthalate Standard (optional)~~ - Non seeded dilution

Bottle No.	Sample Size	Initial D.O. (D_1)	Present D.O. (D_2)	Total D.O. Depletion ($D_1 - D_2$)	~~Depletion Due To Standard~~	~~/ BOD~~ Day	~~Average~~ / ~~BOD~~ · Day
74	-	8.40	8.10	0.30	average DO depletion 0.28		
53	-	8.40	8.10	0.30			
8	-	8.40	8.15	0.25			

Form 19755 PWO

...al Laboratory
...k Sheet - Blanks,
...s, and Standards

3M

Lab Request No. ▶ B1334, B1434 B1228, B1230, B1237, B1292		BOD Run No. ▶ 84-~~13~~ 14	
Analyst Karen Spit	Date Started 4-6-84	10 c - Day BOD Readings	
Seed Type & Source	Seed Concentration	Analyst Kapit	Date 4-16-84

Nonseeded Dilution - Water Blank Incub. temp 4-6-84 20.5 °C 4-16 20.7

Bottle No.	Initial D.O. (A_1)	Present D.O. (A_2)	D.O. Depletion ($A_1 - A_2$)	Average D.O. Depletion ($\bar{A}_1 - \bar{A}_2$)
79	8.40	7.90	0.50	
70	8.40	7.90	0.50	0.50
140	8.40	7.90	0.50	

Seeded Dilution - Water Blank (optional)

Bottle No.	Initial D.O. (C_1)	Present D.O. (C_2)	D.O. Depletion ($C_1 - C_2$)	Average D.O. Depletion ($\bar{C}_1 - \bar{C}_2$)
72	8.40	7.50	0.90	
4	8.40	7.85	0.55	0.575
34	8.40	7.80	0.60	

Seed Controls

Bottle No.	Sample Size	Initial D.O. (B_1)	Present D.O. (B_2)	D.O. Depletion ($B_1 - B_2$)
97	20	8.40	7.00	1.40
85	40	8.20	6.20	2.00
84	80	8.15	4.25	3.90

Glucose - Glutamic Acid Standard

Bottle No.	Sample Size	Initial D.O. (D_1)	Present D.O. (D_2)	Total D.O. Depletion ($D_1 - D_2$)	Depletion Due To Standard	10 / c - BOD - Day	Average 10 / c - BOD - Day
50	3	8.40	5.70	2.7	2.13	213	
83	5	8.40	4.30	4.10	3.53	212	215 mg/l
62	7	8.40	2.70	5.70	2.20	220	220 mg/l

Potassium Hydrogen Phthalate Standard (optional)

Bottle No.	Sample Size	Initial D.O. (D_1)	Present D.O. (D_2)	Total D.O. Depletion ($D_1 - D_2$)	Depletion Due To Standard	___ / BOD - Day	Average ___ / BOD - Day

Form 19755 PWO

...mental Laboratory
...Work Sheet - Blanks,
...rols, and Standards

3M

Lab Request No. ▶ B1334, B1434 B1228, B1230, B1237, B1292		BOD Run No. ▶ 84-1314	
Analyst [illegible]	Date Started 4-6-84	20 c - Day BOD Readings	
Seed Type & Source	Seed Concentration	Analyst [illegible]	Date 4-26-84

Nonseeded Dilution - Water Blank Incub. temp 4-6-84 20.5 °C 21.0 °C

Bottle No.	Initial D.O. (A_1)	Present D.O. (A_2)	D.O. Depletion ($A_1 - A_2$)	Average D.O. Depletion ($\bar{A}_1 - \bar{A}_2$)
79	8.40	7.55	0.85	
70	8.40	7.60	0.80	0.85
140	8.40	7.50	0.90	

Seeded Dilution - Water Blank (optional)

Bottle No.	Initial D.O. (C_1)	Present D.O. (C_2)	D.O. Depletion ($C_1 - C_2$)	Average D.O. Depletion ($\bar{C}_1 - \bar{C}_2$)
72	8.40	7.30	1.10	
4	8.40	7.55	0.85	0.93
34	8.40	7.55	0.85	

Seed Controls

Bottle No.	Sample Size	Initial D.O. (B_1)	Present D.O. (B_2)	D.O. Depletion ($B_1 - B_2$)
97	20	8.40	6.10	2.30
85	40	8.20	5.20	3.00
84	80	8.15	<0.50	> 7.65

Glucose - Glutamic Acid Standard

Bottle No.	Sample Size	Initial D.O. (D_1)	Present D.O. (D_2)	Total D.O. Depletion ($D_1 - D_2$)	Depletion Due To Standard	$\frac{20}{c\text{-BOD}}$ - Day	Average $\frac{20}{c\text{-BOD}}$ - Day
50	3	8.40	5.10	3.30	2.38	237.6	
83	5	8.40	3.60	4.80	3.88	232.9	237 mg/l
62	7	8.40	1.90	6.50	5.59	239.5	240 g/l

Potassium Hydrogen Phthalate Standard (optional)

Bottle No.	Sample Size	Initial D.O. (D_1)	Present D.O. (D_2)	Total D.O. Depletion ($D_1 - D_2$)	Depletion Due To Standard	$\frac{\quad}{\text{BOD}}$ - Day	Average $\frac{\quad}{\text{BOD}}$ - Day

Form 19755 PWO

ironmental Laboratory
JD Work Sheet - Samples

3M

Lab Req. No. ♦ B1228		BOD Run No. ♦ 84-~~13~~ 14	
Analyst	Date Started	5 - Day BOD Readings	
		Analyst Kabet	Date 4-11-84

Sample Description: B1228-1 Fx-13 (L-2514) FM-4519 lot 13

Initial Dilution	_____ of Sample diluted to _____ ml with DI water
Initial Dilution Factor	1 (Dilution factor = 1 if undiluted)

Bottle No.	Conc. of Sample[1]	Sample Size[2]	Initial D.O. (D_1)	Present D.O. (D_2)	Total D.O. Depletion ($D_1 - D_2$)	Depletion Due To Sample	7 - Day BOD	Average 5 - Day BOD
20	19.0	0.0057g	8.45	8.00	0.45	0.03	(1755)	
17	34.7	0.0104g	8.40	6.55	1.85	1.43	(41350)	
8	74.0	0.0222g	8.40	7.50	0.90	0.48	(6531)	
7	133	0.0399g	8.40	6.10	2.30	1.88	(14160)	
23	272	0.0817g	8.60	7.80	0.80	0.38	(1408)	
73	502	0.1505g	8.55	1.90	6.65	6.23	12425	

Note: 1. Conc. = Concentration of sample (ml/l or mg/l) in BOD bottles.

2. Sample Size = ml or g of sample or diluted sample added to BOD Bottle.

Comments

COD = 240000 mg/kg

sample weighed into ~~aluminum boat~~ cover glass - dissolved in acetone & washed into bottle with acetone. Bottle coated with acetone solution. Acetone purged from bottle with ~~filtered air~~ nitrogen

go to 20 days

4-11-84 sample flaking from bottle #'s 7, 23 + 73

Form 19756 PWO

mental Laboratory
Work Sheet - Samples

3M

Lab Req. No. ▶ B1228		BOD Run No. ▶ 84-~~13~~ 14	
Analyst Kalt	Date Started 4-6-84	10 C - Day BOD Readings	
		Analyst Kalt	Date 4-16-84

Sample Description: B1228-1 Fx-13 (L-25-14) FM-4519 lot 13

Initial Dilution	_____ of Sample diluted to _____ ml with DI water
Initial Dilution Factor	_____ (Dilution factor = 1 if undiluted)

Bottle No.	Conc. of Sample[1]	Sample Size[2]	Initial D.O. (D_1)	Present D.O. (D_2)	Total D.O. Depletion ($D_1 - D_2$)	Depletion Due To Sample	$\frac{10}{C\text{-BOD}}$ · Day	Average $\frac{10}{C\text{-BOD}}$ · Day
20		0.0057g	8.45	7.50	0.95	0.38	(19740)	
17		0.0104g	8.40	5.90	2.50	1.93	(55530)	
8		0.0222g	8.40	6.80	1.60	1.03	(13850)	
7		0.0399g	8.40	5.30	3.10	2.53	18990	mg/kg
23		0.0817g	8.60	7.60	1.00	0.43	(1561)	
73		0.1505g	8.55	<0.50	>7.55	>6.98	≥13900	

Note: 1. Conc. = Concentration of sample (ml/l or mg/l) in BOD bottles.
2. Sample Size = ml or g of sample or diluted sample added to BOD Bottle.

Comments

COD = 240000 mg/kg

sample weighed onto ~~aluminum boat~~ cover glass - dissolved in acetone & washed into bottle with acetone. Bottle coated with acetone solution. Acetone purged from bottle with ~~filtered air~~ nitrogen

4-16-84 bottle #'s 7, 23 & 73 flaking

Form 19756 PWO

ntal Laboratory
rk Sheet - Samples

3M

Lab Req. No. ♦ B1228		BOD Run No. ♦ 84-~~13~~ 14	
Analyst	Date Started 4-6-84	20 · Day BOD Readings	
		Analyst [illegible]	Date 4-26-84

Sample Description B1228-1 Fx-13 (L-25-14) FM-4519 lot 13

Initial Dilution	_____ of Sample diluted to _____ ml with DI water
Initial Dilution Factor	1 (Dilution factor = 1 if undiluted)

Bottle No.	Conc. of Sample[1]	Sample Size[2]	Initial D.O. (D_1)	Present D.O. (D_2)	Total D.O. Depletion ($D_1 - D_2$)	Depletion Due To Sample	$\frac{20}{c-BOD}$ · Day	Average $\frac{20}{c-BOD}$ · Day
20		0.0057g	8.45	7.10	1.35	0.42	(21900)	
17		0.0104g	8.40	6.15	2.25	1.32	(38000)	
8		0.0222g	8.40	5.90	2.50	1.57	(21200)	
7		0.0399g	8.40	4.40	4.0	3.07	23100	23000 mg/kg
23		0.0817g	8.60	6.70	1.7	0.77	(2820) *	
73		0.1505g	8.55	<0.50	>7.55	>6.50	>12900	

Note: 1. Conc. = Concentration of sample (ml/l or mg/l) in BOD bottles.

2. Sample Size = ml or g of sample or diluted sample added to BOD Bottle.

Comments

COD = 240000 mg/kg

sample weighed into ~~aluminum boat~~ cover glass - dissolved in acetone & washed into bottle with acetone. Bottle coated with acetone solution. Acetone purged from bottle with ~~filtered~~ nitrogen

* possible bottle contamination

Form 19756 PWO

BIODEGRADATION (Modified MITI Test)

TEST SUBSTANCE

Identity: [2-(N-Ethylperfluorooctanesulfonamido) ethyl acrylate; may also be referred to as B1228, D-1, EtFOSEA, or FX-13. (2-Propenoic acid, 2-[ethyl[heptadecafluorooctyl)sulfonyl]amino]ethyl ester, CAS # 423-82-5)

Remarks: Material is an amber solid. Report lists sample as > 99%, as provided by sponsor. The purity/identity of the test substance cannot be substantiated. As presented in the report, the structural formula indicates that "purity" cannot be assigned as "99% or more". Lot number 4.

METHOD

Method: OECD 301C
Type: Aerobic
GLP: Yes
Year completed: 1995
Inoculum source: Chemical Biotesting Center, Chemical Inspection and Testing Institute, Japan

Remarks: It appears that the inoculum was obtained from only one source, rather than the ten sources required in the method.

RESULTS

Test substance: After 28-days, the percent biodegradability of the test substance was determined by three methods: Biochemical Oxygen Demand (BOD), Dissolved Organic Carbon (DOC), and Residual Test Substance Concentration. The results are as follows:

Percent Biodegradability (of 3 replicates):
BOD: 4, -1, and 6%
DOC: Not calculated; test substance not dissolved in solution
Test Substance Concentration: 5 and 1% (only two determined)

Reference substance: The aniline achieved 68% biodegradability in 28-days.

Measurement of test substance: The test substance concentration was only measured at the end of the study. Although this laboratory followed the

procedures as outlined in OECD 301C, the starting concentration should also be determined rather than relying on nominal concentrations. In addition, subsamples were removed from the bioreactors for pH and DOC determinations at the end of the study. The sample used for pH determinations and the remaining sample after centrifugation for DOC were returned to the bioreactor prior to sampling for the measurement of the test substance concentration. These samples should have been removed after the bioreactors were sampled for test substance concentration determination.

Calculations of theoretical oxygen demand should have made which account for possible oxygen consumption due to nitrification.

CONCLUSIONS

This testing indicated that the test substance is not capable of readily biodegrading in aerobic environments.

DATA QUALITY

Reliability: Klimisch ranking 3. There is no reference to the solubility information provided in the study. The quoted purity/identity of the test substance is not substantiated. Measurement of test substance concentration was only performed at the end of the study. The bioreactors were potentially contaminated by the practice of returning subsamples after pH and DOC determinations. The calculation of theoretical oxygen demand should have been performed using the correction for nitrification.

REFERENCES

Study conducted at the request of Sumitomo 3M LTD by Mitsubishi Chemical Safety Institute Ltd., Yokohama, Japan

OTHER

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

Last changed: 5/22/00

M.S.I. REPORT No.1B363(2)G

Ready Biodegradability Test of D-1

Submitted to :

SUMITOMO 3M LIMITED

Prepared by :

Mitsubishi Chemical Safety Institute Ltd.

October 31, 1995

000504

RECEIVED
NOV 2 8 1995

Ready Biodegradability Test of D-1
(English version)

Study No. : 1B363(2)G

Study Title : Ready Biodegradability Test of D-1

Sponsor : SUMITOMO 3M LIMITED

This report was conducted in the Yokohama Laboratory of the Mitsubishi Chemical Safety Institute Ltd., 1000 Kamoshida-cho, Aoba-ku, Yokohama 227, Japan.

This report is an English version of the orginal, which was written in Japanese. The underesigned hereby declare that this version faithfully reflects the original report to the best of our knowledge.

Mitsubishi Chemical Safety Institute Ltd.

Date : October 31, 1995

Kikuo Yoshida, Ph.D.

Environmental Science Division of Yokohama Laboratory

Approved by

Date : October 31, 1995

Jun Toriya, B.Sc.

Head of Yokohama Laboratory

COMPLIANCE WITH THE GLP STANDARDS

Study No. : 1B363(2)G

Study Title : Ready Biodegradability Test of D-1

Sponsor : SUMITOMO 3M LIMITED

To the best of our knowledge and belief, the study described in this report was conducted in compliance with the Good Laboratory Practice (GLP) standards applied to industrial chemicals under the Chemical Substances Control Law.

Facility management

Sakae Koike, B.Sc. Sealed date :April 28, 1992

Facility management

Head of Yokohama Laboratory

Study director

Kikuo Yoshida, Ph.D. Sealed date :April 28, 1992

Study Director

Environmental Science Division

QUALITY ASSURANCE STATEMENT

Study No. : 1B363(2)G

Study Title : Ready Biodegradability Test of D-1

Sponsor : SUMITOMO 3M LIMITED

We hereby certify that the above study has been performed in accordance with the Good Laboratory Practice (GLP) standards applied to industrial chemicals under the Chemical Substances Control Law.

Dates of inspections and reportings to the Study Director and the Facility Management are as follows :

	Date of Inspection	Date of Reporting
In-progress study :	March 18, 1992	March 30, 1992
	April 15, 1992	April 15, 1992
	April 16, 1992	April 16, 1992
Final report :	April 28, 1992	April 28, 1992

Quality Assurance Staff

Junko Katoh, Ph.D. Sealed date :April 28, 1992

Yumiko Miura, B.Sc. Sealed date :April 28, 1992

We, the undersigned declare that the work was performed according to the procedures herein described, and that this report provides a true and correct record of the results obtained.

Experiment Staff

Ayumi Okuda, M.Sc. Sealed date :April 28, 1992
Environmental Science Division

Junko Koyasu, B.Sc. Sealed date :April 28, 1992
Environmental Science Division

Yukiko Mizoguchi Sealed date :April 28, 1992
Environmental Science Division

Staff for cultivation of activated sludge

Yasuo Sato Sealed date :April 28, 1992
Environmental Science Division

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EXPLANATORY REMARKS

Study No. : 1B363(2)G

Study Title : Ready Biodegradability Test of D-1

The ready biodegradability test of D-1 was originally started on January 29, 1992, and the BOD measurement of the test substance was initiated on February 13, 1992(Study No.1B363G). However, the degradability value of aniline based on the BOD measurements did not exceed 40%.

Therefore, the test was terminated on February 20, 1992 and the new test (Study No. 1B363(2)G) was started with the same test substance and the same test method after approval of the sponsor.

Some of data used in this report (IR chart, calibration curve and recovery) are those obtained in the former study (Study No. 1B363G).

SUMMARY

STUDY TITLE

Ready Biodegradability Test of D-1

METHOD

The method described in the Order, which prescribes the procedure of testing new chemical substances as required by the Chemical Substances Control Law(Japanese Law 117, 1973). ("Ready biodegradability, Modified MITI test" in OECD Guidelines for Testing of Chemicals).

Test bottle	:	Bottle 1	: aniline + sludge + basal medium
		Bottle 2	: sludge + basal medium
		Bottles 3~5	: test substance + sludge + basal medium
		Bottle 6	: test substance + water

Measurements	:	Biochemical oxygen demand (BOD)	(for 28 days)
		Dissolved organic carbon (DOC)	(at day 28)
		Residual test substance concentration	(at day 28)

RESULTS

Measured values (day 28)

	Bottle No.				Theoretical
	3	4	5	6	value
BOD, mg	1.0*	-0.3*	1.6*	0.0	27.7
DOC, mg/l	0.7*	0.3*	1.6*	2.0	28.8
Test substance, mg/l	101.3	105.2	91.5	106.7	100.0

* value corrected with BOD or DOC value of Bottle 2.

Degradabilities (%)

Bottle No.	3	4	5
BOD	4	-1	6
DOC	NA*	NA*	NA*
Test substance	5	1	NA**

* not calculated because the test substance was not dissolved in the test solution.

** not calculated because the test substance might be lost accidentally during condensation of the solution under vacuum.

CONCLUSION

From the degradability results based on the BOD and the residual test substance concentration , it can be concluded that the test substance is not readily biodegradable and is not transformed under the conditions prescribed by the Modified MITI test.

1 STUDY TITLE

Ready Biodegradability Test of D-1

2 PURPOSE

This study was conducted to evaluate the ready biodegradability of the test substance for notification under the Chemical Substance Control Law of Japan.

3 TEST GUIDELINES

The test was conducted according to the method described in the Order, which prescribes the procedure of testing new chemical substances as required by the Chemical Substances Control Law (Japanese Law 117, 1973). This method is also known as the "Ready biodegradability, Modified MITI test" in the OECD Guidelines for the Testing of Chemicals No. 301C.

4 GLP COMPLIANCE

The test was conducted according to the Good Laboratory Practice (GLP) standards applied to industrial chemicals under the Chemical Substances Control Law.

5 STUDY PERIOD

March 17, 1992 to April 28, 1992

(BOD measurement : March 18, 1992 to April 15, 1992)

6 SPONSOR

SUMITOMO 3M LIMITED

8-8 Minamihashimoto 3-chome, Sagamihara 229, Japan

7 TESTING FACILITY

Yokohama Laboratory

Mitsubishi Chemical Safety Institute Ltd. (M.S.I.)

1000 Kamoshida-cho, Aoba-ku, Yokohama 227, Japan

Head Office:

Mitsubishi Chemical Safety Institute Ltd. (M.S.I.)

1-30 Shiba 2-chome, Minato-ku, Tokyo 105, Japan

8 STORAGE AND RETENTION OF TEST SUBSTANCE AND RECORDS

The following records, the raw data, and a small sample of the test substance will be stored in the archives of M.S.I. for 10 years after the submission of the final report. After this period, the sponsor will be contacted for the approval of the disposal of data. Data can be stored in the archives for a further specified period at the sponsor's request with an additional fee.

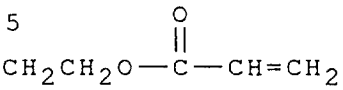
1) Protocol.
2) Final report.
3) Raw data.
4) Quality assurance reports.
5) Test substance (approx. 2 g).
6) Other documents required under the GLP standards.

9 TEST SUBSTANCE

1) Name* : D-1
(M.S.I. identification No. 1B363(2)G)

2) Chemical name* : 2-[N-Ethyl-N-perfuloroalkyl(C=1~8)sulfonyl-amino]ethylacrylate

3) Structural formula* :



$$C_nF_{2n+1}SO_2-\overset{\overset{C_2H_5}{|}}{N}-CH_2CH_2O-\overset{\overset{O}{||}}{C}-CH=CH_2$$

(n=1~8, main component; n=8)

4) Elemental composition : C;28.8%, H; 1.9%, N; 2.3%, O; 9.8%, F;52.0% S; 5.2% (Measured by M.S.I.)

5) Physico-chemical properties

Solubility* : insoluble in water
insoluble in DMSO
>50% in acetone

Melting point* : 27~42℃

Boiling point* : ca. 150℃/1mmHg

8) Batch* : Lot No. 4

9) Purity* : >99% (n=1~7;ca.21 %(total), n=8;ca.78%)

10) Appearance : amber solid

11) Date of receipt : September 25, 1991

12) Supplied quantity* : 100 g

* provided by the sponsor.

10 CONFIRMATION OF IDENTITY AND STABILITY

10.1 IDENTITY

For the identification of the test substance, the infrared absorption spectrum of the test substance was measured. The measured spectrum was consistent with that supplied by the sponsor.

1)Apparatus : Perkin-Elmer model 1640 infrared spectrophotometer.

[Figure 1 (P-23)]

10.2 STABILITY

After the exposure period, the infrared absorption spectrum of the test substance that had been stored in a refrigerator during the course of the study was measured. The spectrum was consistent with that shown in Fig. 1, indicating that the test substance was stable under the storage condition.

1)Apparatus : Perkin-Elmer model 1640 infrared spectrophotometer.

[Figure 2 (P-24)]

11 TEST METHODS

The test was performed according to the method described in the Order, which prescribes the procedure of testing new chemical substances as required by the Chemical Substances Control Law (Japanese Law 117, 1973). This method is also known as the "Ready biodegradability, Modified MITI test" in OECD Guidelines for Testing of Chemicals No.301C (1981).

The test substance was exposed to the activated sludge in a closed-system oxygen consumption measuring apparatus. The biochemical oxygen demand (BOD) was measured over a 28 day period. After this period, the concentrations of the dissolved organic carbon (DOC) and the residual test substance in the test bottles were measured.

The biodegradability of the test substance was evaluated from these results.

11.1 ACTIVATED SLUDGE

1) Mixed liquor suspended solid (MLSS) : 3300 mg/l

2) Source : Chemical Biotesting Center, Chemical Inspection and Testing Institute, Japan

3) Date of receipt : January 23, 1992

11.2 EXPOSURE CONDITIONS

1) Temperature : 25 ± 1 ℃

2) Exposure period : 28 days

3) Test volume : 300 ml

4) Concentration :

test substance (bottles 3~6)	: 100 mg/l
aniline*1 (bottle 1)	: 100 mg/l
activated sludge (bottles 1~5)	: 30 mg/l

*1 Reference substance : Syowa Chemicals, Lot No.SC-2726

5) Test bottle contents :

Bottle 1 : Aniline + activated sludge + basal medium
29μl (30 mg) of aniline was added to the basal medium*2, then activated sludge was added.

Bottle 2 : Activated sludge + basal medium
Activated sludge was added to the basal medium*2.

Bottles 3~5 : Test substance + activated sludge + basal medium
30 mg of the test substance was added to the basal medium*2, then activated sludge was added.

Bottle 6 : Test substance + deionized water
30 mg of the test substance was added to 300 ml of deionized water (purified by Milli-Q).

*2 The total volume of the basal medium and the sludge was held fixed at 300 ml.

11.3 BOD MEASUREMENT

The BOD was measured for 28 days.

1)Apparatus : closed system oxygen consumption measuring apparatus Ohkura Electric Co., Model OM-2001 (M.S.I. ID No.B).

11.4 pH MEASUREMENT

After the exposure period, 20 ml of the test solution in each test bottle was transferred into a 20 ml glass beaker for pH measurement.

After pH measurement, the test solution was returned to each bottle for measurement of residual test substance concentration.

1)Apparatus : pH meter, Denki Kagaku Keiki, Model COM-10

11.5 DOC MEASUREMENT

The concentration of the DOC was measured as follows.

1)Apparatus : TOC analyzer, Shimadzu Co., model TOC-5000

2)Conditions :

Furnace temperature	: 680°C(TC)
Air flow rate	: 150 ml/min.
Sensitivity	: × 5
Injection volume	: 50 μl

3)Calibration curve

The following standard solutions were injected into the TOC analyzer. The calibration curve was prepared by the data processor of the analyzer.

standard solutions

TC(total carbon)	20 and 50 mg C/l aqueous solutions of potassium biphthalate.
IC(inorganic carbon)	0 mg C/l deionized water (purified by Milli-Q) and 10 mg C/l aqueous solution of sodium hydrogen carbonate.

4)Measurement of DOC in the bottles

Ten ml of the test solution in each bottle was transferred into a 10 ml centrifuge tube and centrifuged at 3000 rpm for 10 minutes. Five ml of supernatant was used for the measurement of DOC.

The remaining supernatant and the precipitate were returned to each bottle for the measurement of the residual test substance concentration.

11.6 MEASUREMENT OF RESIDUAL TEST SUBSTANCE CONCENTRATION

The concentration of the residual test substance was measured by the gas chromatography (GC) as follows:

1) Apparatus

GC	:Hewlett-Packard. Model 5890A (No.2) with FID detector
Integrator	:Hewlett-Packard. Model 3392A

2) Conditions

Column	:J & W. DB-17(50%Phenyl 50%methyl polysiloxane), 30 m × 0.25 mm i.d., 0.25μm (film thickness)
Temperature	:[Column oven]100℃(1min.)→10℃/min.→200℃(1min.) [Injector]250℃, [Detector]280℃
Carrier gas	:He 30 ml/min.
Detector flow	:H_2 40ml/min., air 400 ml/min.
Attenuation	:[GC] 1 V
Injection volume	:1 μl

3) Calibration curve

The standard solutions of the test substance with 0, 250, 500, and 1000 mg/l in ethyl acetate were analyzed. The total peak area of test substance was calculated by adding the area of the peaks with retention time of about 5.6, 6.2 and 6.5 min. The total area was plotted against the concentration. The correlation coefficient was calculated to be 1.00 by the method described in Japanese Industrial Standards Z9041-1968.

[Figure 3 (P-25) and Figure 4 (P-26)]

4) Recovery test

Duplicate test bottles (identical to the test bottles 3~5 described in § 11.2) were kept in a closed-system oxygen consumption measuring apparatus at 25±1℃ for 30 minutes.

Each test solution was treated by the procedures described in § 11.6.6. The concentration of the test substance was measured by GC. The average recovery of the test substance was 97%.

[Table 3 (P-21) and Figure 5 (P-27)]

The concentration of the residual test substance measured for bottles 3 ~6 were corrected with the average recovery value.

5) Detection limit

The detection limit of the test substance was calculated to be 3 mg/l based on the minimum detectable peak area of 100000 μV・sec for the largest peak (R.T. 6.2 min.) in the GC chromatogram.

[Table 3 (P-21) and Figure 5 (P-27)]

6) Measurement of test substance in test bottles

The contents in each bottle was separately transferred into a 500 ml glass separatory funnel, which was then extracted twice with 100 ml of

ethyl acetate. The extract was filtered through a grass funnel containing 50 g sodium sulfate, anhydrous to remove water. Then filtrate was condensed to ca. 20 ml under vacuum (<50℃). The solution was brought up to 50 ml with ethyl acetate.

The ethyl acetate solution was then analyzed by GC. The concentration of the residual test substance was determined as described in §11.6.2.

11.7 EQUATIONS FOR CALCULATION OF DEGRADABILITY

Equations for calculation of degradability based on BOD, DOC, and residual test substance concentration are as follows:

1) Degradability based on BOD

Degradability (%) = (BODs- BODb)/ThOD × 100

where BODs : Oxygen consumption (mg) in bottles 1, 3, 4, and 5.
BODb : Oxygen consumption (mg) in bottle 2.
ThOD : Theoretical oxygen demand (mg) of aniline and the test substance.

ThOD

aniline : $C_6H_7N + 35/4O_2 \rightarrow 6CO_2 + 7/2H_2O + NO_2$
ThOD : 90.2 mg O_2/30 mg-aniline

test substance : $C+O_2 \rightarrow CO_2$, $H+1/4O_2 \rightarrow 1/2H_2O$, $N+O_2 \rightarrow NO_2$, $S+O_2 \rightarrow SO_2$, $F \rightarrow F$
ThOD : 27.7 mg O_2/30 mg-test substance

2) Degradability based on DOC

Degradability (%) = {1 - (DOCs-DOCb)/DOCc}×100

where DOCs : DOC (mg/l) in bottles 3, 4, and 5.
DOCb : DOC (mg/l) in bottle 2.
DOCc : DOC (mg/l) in bottle 6.

3) Degradability based on residual test substance concentration

Degradability (%) = (1 - Cs/Cc) × 100

where Cs : concentration (mg/l) in bottles 3, 4, and 5.
Cc : concentration (mg/l) in bottle 6.

12 RESULTS

There was no specific circumstances which might have affected the reliability of the test results.

12.1 OBSERVATION OF TEST BOTTLES AFTER EXPOSURE PERIOD

The solutions in the bottles were colorless except the bottle 1, in which the solution was cloudy.

Growth of the sludge was observed in bottle 1 in contrast with the bottle 2. No growth was observed in the bottles 3, 4 and 5.

12.2 pH MEASUREMENT

After 28 days of exposure, the pH was determined to be 7.4, 7.4, 7.5 and 7.4 for bottles 3, 4, 5 and 6, respectively.

[Table 1 (P-19)]

12.3 ACTIVITY OF SLUDGE

The degradability value of aniline based on the BOD measurements was 59% after 7 days. The activity of the sludge was thus shown to be satisfactory.

[Table 1 (P-19) and Figure 6 (P-28~31)]

12.4 DEGRADABILITY BASED ON BOD

BOD*[1] in bottles 3, 4, and 5 (as corrected with the value in bottle 2) were 1.0, -0.3 and 1.6 mg, respectively. BOD in bottle 6 was 0.0 mg.

(*[1] Maximum theoretical value = 27.7 mg)

The degree of degradability based on the BOD measurements were 4, -1 and 6% for bottles 3, 4, and 5, respectively.

[Table 1 (P-19) and Figure 6 (P-28~31)]

12.5 DEGRADABILITY BASED ON DOC

DOC*[2] in bottles 3, 4 and 5 (as corrected with the value in bottle 2) were 0.7, 0.3, and 1.6 mg/l, respectively. DOC in bottle 6 was 2.0 mg/l.

(*[2] Maximum theoretical value = 59.8 mg/l)

Degradability based on the DOC measurements was not calculated because the test substance was not dissolved in the test solution.

[Table 1 (P-19) and Table 2 (P-20)]

12.6 DEGRADABILITY BASED ON THE RESIDUAL TEST SUBSTANCE CONCENTRATION

The test substance*3 was detected at concentrations of 101.3, 105.2, 91.5 and 106.7 mg/l in bottles 3, 4, 5 and 6, respectively.

(*3 Initial concentration = 100 mg/l)

The degree of degradability based on the residual test substance concentration were 5 and 1 % for the bottle 3 and 4, respectively.

[Table 1 (P-19), Table 4 (P-22), and Figure 7 (P-32~33)]

During condensation of the filtrate of the bottle 5 under vacuum, a part of the filtrate spouted from an evaporating flask. The spouted filtrate was recovered and then condensed with the remaining filtrate. However, the residual test substance concentration in the bottle 5 was obviously lower than those in the bottle 3 and 4 and it would impair reliability of biodegradability of the test substance in the bottle 5. Therefore, the degree of degradability based on the residual test substance concentration was not calculated for the bottle 5.

13 CONCLUSIONS

From the degradability results based on the BOD and the residual test substance concentration, it can be concluded that the test substance is not readily biodegradable and is not transformed under the conditions prescribed by the Modified MITI test.

Table 1 Summary of the test results

Degradabilities were calculated by the equations shown in Sec. 11.7.

a) Degradability based on BOD

Bottle No.	Test substance	ThOD mg	day 7		day 14		day 21		day 28	
			BOD mg	% Degra-dability	BOD mg	% Degra-dability	BOD mg	% Degra-dability	BOD mg	% Degra-dability
1	aniline	90.2	56.2	59	63.7	65	66.3	67	67.4	68
2	------	---	3.1	---	5.0	---	5.5	---	5.8	---
3	D-1	27.7	3.0	0	5.0	0	6.1	2	6.8	4
4	D-1	27.7	2.4	-3	4.1	-3	4.8	-3	5.5	-1
5	D-1	27.7	3.5	1	5.7	3	6.7	4	7.4	6
6	D-1	27.7	0.0	---	0.0	---	0.0	---	0.0	---

Where % degradability was calculated to be negative, this value is shown in brackets.

b) pH measurement

Bottle No.	pH	
	day 0	day 28
1	---	8.3
2	6.9	7.4
3	6.9	7.4
4	6.9	7.4
5	6.9	7.5
6	6.8	7.4

c) Degradability based on DOC

Bottle No.	Organic carbon added, mg/l	TC mg/l	IC mg/l	DOC mg/l
2	------	1.7	0.0	1.7
3	28.8	2.5	0.1	2.4
4	28.8	2.1	0.1	2.0
5	28.8	3.3	0.0	3.3
6	28.8	2.0	0.0	2.0

d) Degradability based on residual test substance

Bottle No.	Concentration mg/l	Degradability %
2	<3	---
3	101.3	5
4	105.2	1
5	91.5	NA*
6	106.7	---

* not calculated because the test substance might be lost accidentally during condensation of the solution under vacuum.

Table 2 Results of DOC measurements

Standard solution

TC 50.0 mg/L
vial :s1

#	arer
1	34637
2	34795
3	34640
MN	34690

TC 20.0 mg/L
vial :s2

#	arer
1	14107
2	13981
3	14048
MN	14045

IC 10.0 mg/L
vial :s3

#	arer
1	7185
2	7107
3	7187
MN	7159

IC 0.0 mg/L
vial :s4

#	arer
1	0
2	0
3	0
MN	0

Bottle 2

TC

#	arer	mg/L	RMK
1	1178	1.71	
2	1157	1.68	
3	1135	1.65	
MN	1156	1.68	

IC

#	arer	mg/L	RMK
1	0	0.00	
2	0	0.00	
3	0	0.00	
MN	0	0.00	

Bottle 3

TC

#	arer	mg/L	RMK
1	1743	2.53	
2	1743	2.53	
3	1713	2.49	
MN	1733	2.52	

IC

#	arer	mg/L	RMK
1	0	0.00	
2	0	0.00	
3	166	0.23	
MN	55	0.08	

Bottle 4

TC

#	arer	mg/L	RMK
1	1470	2.14	
2	1418	2.06	
3	1374	2.00	
MN	1420	2.06	

IC

#	arer	mg/L	RMK
1	157	0.22	
2	0	0.00	
3	0	0.00	
MN	52	0.07	

Bottle 5

TC

#	arer	mg/L	RMK
1	2340	3.40	
2	2302	3.35	
3	2215	3.22	
MN	2285	3.32	

IC

#	arer	mg/L	RMK
1	0	0.00	
2	0	0.00	
3	0	0.00	
MN	0	0.00	

Bottle 6

TC

#	arer	mg/L	RMK
1	1442	2.10	
2	1326	1.93	
3	1354	1.97	
MN	1374	2.00	

IC

#	arer	mg/L	RMK
1	0	0.00	
2	0	0.00	
3	0	0.00	
MN	0	0.00	

SPL #	TC,mg/L	RMK	IC,mg/L	RMK	TOC,mg/L	
2	1.68		0.00		1.68	<---- Bottle 2
3	2.52		0.08		2.44	<---- Bottle 3
4	2.06		0.07		1.99	<---- Bottle 4
5	3.32		0.00		3.32	<---- Bottle 5
6	2.00		0.00		2.00	<---- Bottle 6

Table 3 Calculation of recovery and detection limit

	Peak area μV·sec (A)	Concentration in solution, mg/l for GC analysis (B)	Concentration in solution, mg/l in bottle (C)	Recovered mass mg (D)	Added mass mg (E)	Recovery % (F)	Detection limit mg/l (G)
Recovery test 1	4805210	578.8	96.5	29.0	30.0	97	----
Recovery test 2	4815940	580.1	96.7	29.0	30.0	97	----
Blank	<132800	<16.0	<2.7	----	0.0	----	3

Average recovery: 97 %

Standard solution	Concentration	(H):	500 mg/l
	Peak area	(I):	4150910 μV·sec
Volume of solution for GC analysis		(J):	0.050 l
Volume of test solution for GC analysis		(K):	0.300 l

Equation :B=H×A÷I C=B×J÷K D=C×K F=D÷E×100 G=C raise decimal fractions to unit.

Calculation of detection limit

Retention time, min	500 mg/l std. Peak area μV·sec	500 mg/l std. Rate of peak area, %	Blank Peak area μV·sec
5.6	298720	7.2	----
6.2	3127000	75.3	<100000
6.5	725190	17.5	----
Total	4150910	100.0	<132800

The minimum detectable peak area of the test substance was calculated by the following equation based on the minimum detectable peak area of 100000 μV·sec for the largest peak (retention time 6.2 min.) in GC chromatogram.

$$\text{Minimum detectable peak area} = 4150910\ (\mu V\cdot sec) \times \frac{100000\ (\mu V\cdot sec)}{3127000\ (\mu V\cdot sec)} = 132745\ (\mu V\cdot sec)$$

$$< 132800\ (\mu V\cdot sec)$$

Table 4 Residual test substance concentration in test bottles

Bottle No.	Peak area μV·sec (A)	Concentration in solution, mg/l	
		for GC analysis (B)	in bottle (C)
2	<132800	<15.2	<3
3	5149880	589.3	101.3
4	5349150	612.1	105.2
5	4654900	532.7	91.5
6	5428120	621.2	106.7

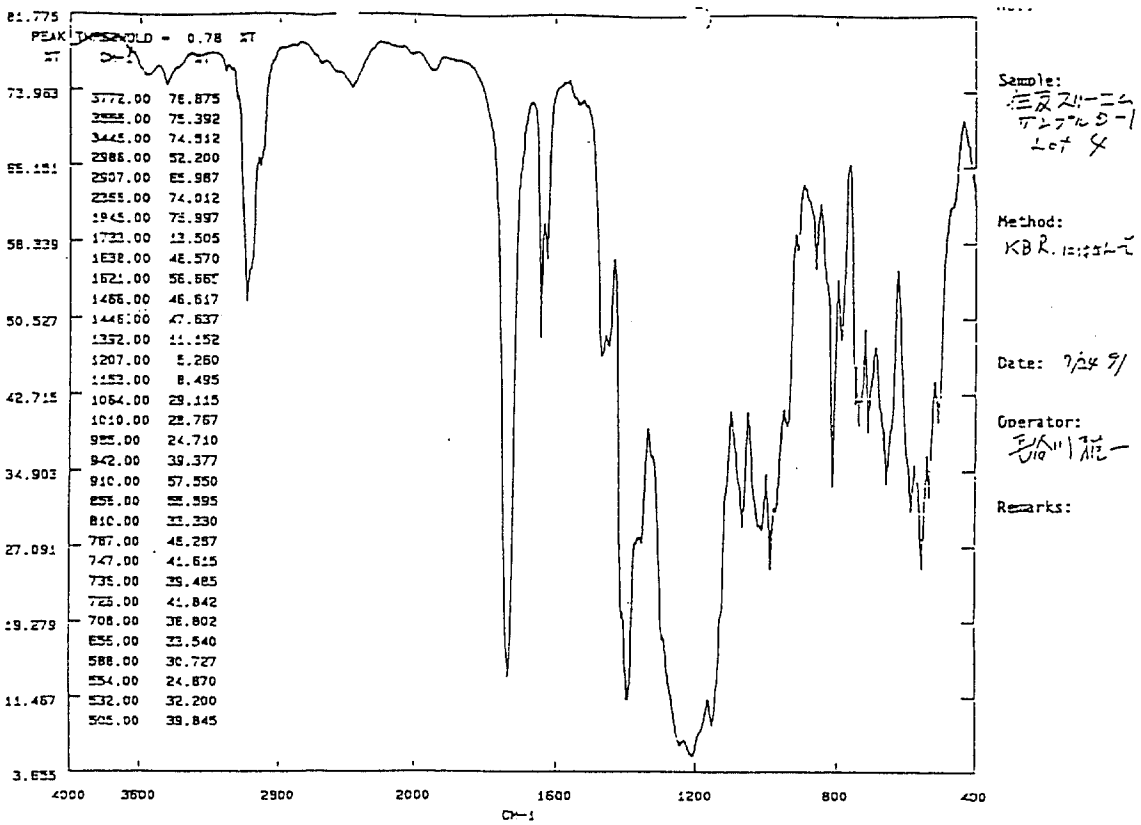
Standard solution	Concentration	(D):	500 mg/l
	Peak area	(E):	4369340 μV·sec
Volume of solution for GC analysis		(F):	0.050 l
Volume of test solution for GC analysis		(G):	0.300 l
Average recovery		(H):	97 %

Equation :B=A×D÷E C=A×D÷E×F÷G÷H×100

000525

Figure 1 Infrared absorption spectra of the test substance

Measure by the sponsor



Measure in M.S.I.

X: 4000.0, 400.00 cm-1; 0.07, 60.53 %T 92/01/13 09.31
4 scans; mode ratio; resol 4.00 cm-1; apod strong
1B3630 D-1 Lot No.4 A. Okuda
threshold 6.00%; band

cm-1	%	cm-1	%	cm-1	%	cm-1	%
2988.3	24.17	2364.1	50.05	1729.3	4.34	1639.4	19.44
1619.8	28.11	1468.8	18.77	1382.0	5.27	1202.0	2.18
1062.1	7.99	1017.3	7.50	904.3	7.19	858.1	36.16
808.1	9.87	784.1	22.25	747.4	18.34	653.7	11.30

16 peaks found

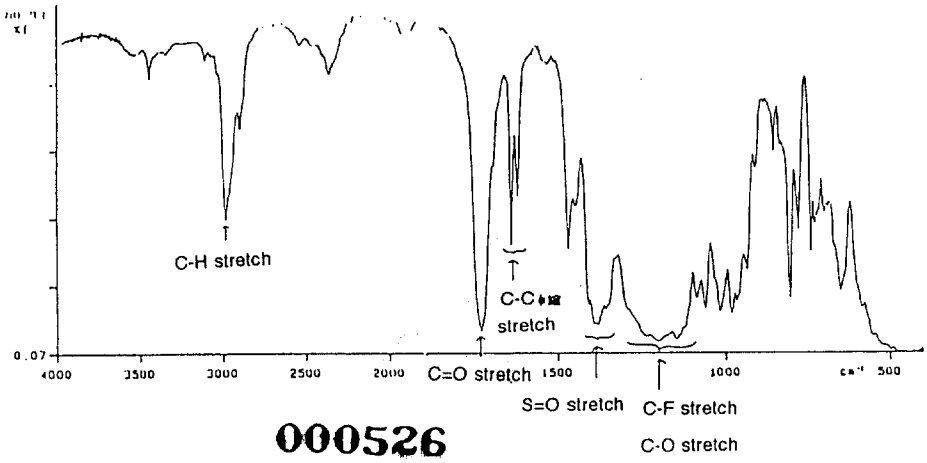


Figure 2 Infrared absorption spectrum of the test substance stored in a refrigerator

X: 4000.0, 400.00 cm-1; 0.14, 77.86 %T 92/04/16 16.39
4 scans; mode ratio; resol 4.00 cm-1; apod strong
1B363(2)G O-1 Lot No.4 A. Okuda
threshold 5.00%; band

cm-1	%	cm-1	%	cm-1	%	cm-1	%
2987.2	51.57	2363.0	68.77	1731.1	3.85	1638.8	42.99
1619.8	54.81	1468.8	40.04	1392.9	5.38	1205.5	1.32
1062.3	20.58	1018.0	17.95	985.1	16.75	808.1	25.32
784.2	46.59	747.2	40.04	735.8	43.83	653.6	25.40

16 peaks found

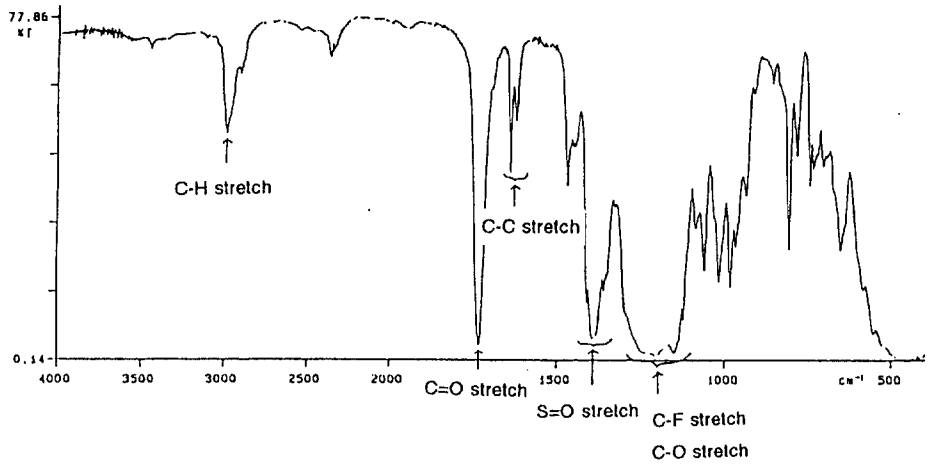


Figure 3 Calibration Curve of the Test Substance

Calibration Curve

Concentration X(mg/l)	Peak Area Y(μV•sec)
0	0
250	1.92785E+06
500	3.89009E+06
1000	8.12341E+06

$Y = -7.552 \times 10^4 + 8.139 \times 10^3 X$

r= 0.999707

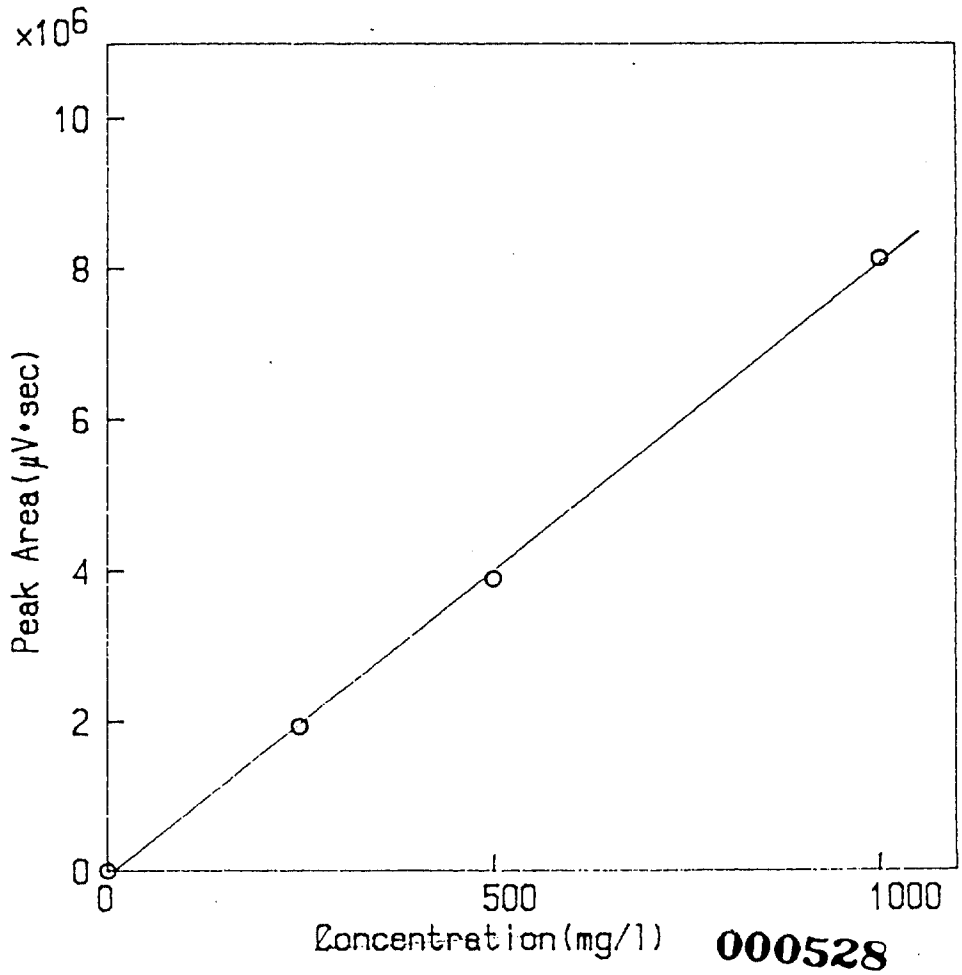
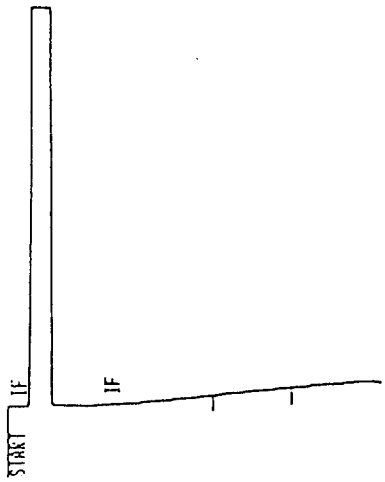


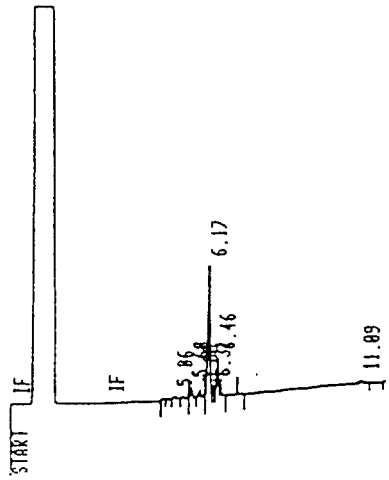
Figure 4 GC chromatograms of the test substance -- Calibration curve

0 mg/l in ethylacetate



NO RUN PEAKS STORED

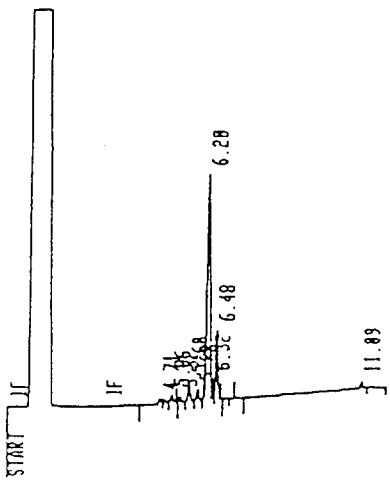
250 mg/l in ethylacetate



AREA% RT	AREA TYPE	AR/HT	AREA%
5.60	145420 D BP	0.096	2.952
6.17	1432000 D BV	0.078	29.070
6.46	350430 D VB	0.069	7.114
11.09	2998400 BV	1.670	60.865

total peak area:1927850µv·sec

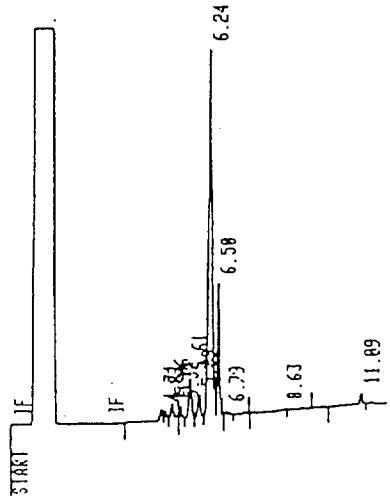
500 mg/l in ethylacetate 夜



AREA% RT	AREA TYPE	AR/HT	AREA%
5.60	291280 D PP	0.098	4.136
5.88	156380 D PV	0.090	2.217
6.28	2885100 D VV	0.090	40.899
6.32	188170 D VV	0.064	2.668
6.48	713210 D VB	0.071	10.110
11.09	2819600 PV	1.335	39.970

total peak area:3890090µv·sec

1000 mg/l in ethylacetate



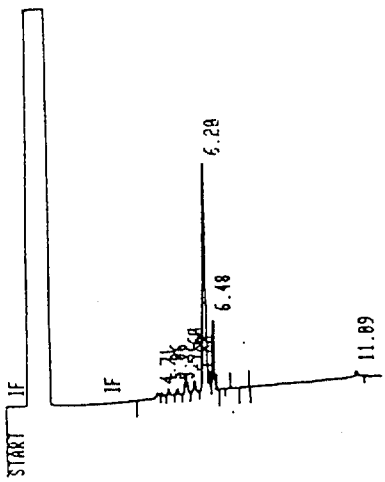
AREA% RT	AREA TYPE	AR/HT	AREA%
4.03	100220 D VV	0.090	1.154
5.06	177070 D VB	0.086	1.988
5.32	117440 D PP	0.076	1.252
5.61	580310 PP	0.103	6.188
5.89	318480 D PV	0.097	3.396
6.24	6123600 VV	0.118	65.293
6.59	1419500 D VB	0.076	15.135
11.09	534090 D BV	0.26.	5.695

total peak area:8123410µv·sec

Figure 5 GC chromatograms of the test substance -- Rocovery and detection limit

Recovery and detection limit are shown in Table 3.

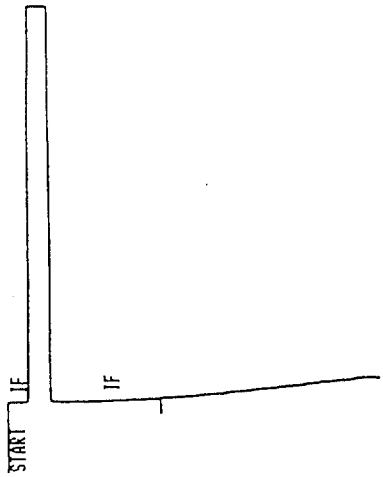
500 mg/l in ethylacetate



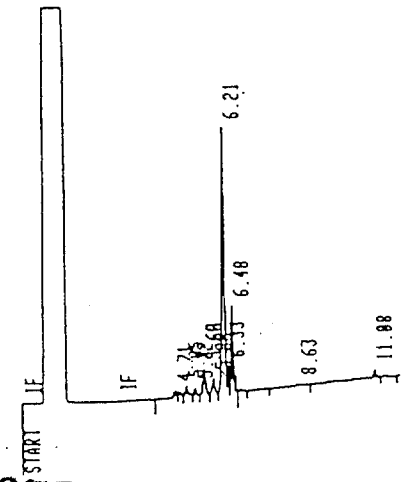
AREA% RT	AREA	TYPE	AR/HT	AREA%
5.06	105990	D VV	0.096	1.664
5.60	298720	D VP	0.099	4.689
5.88	161710	D PV	0.091	2.538
6.20	3122000	D VV	0.097	49.029
6.48	725190	D VB	0.071	11.382
11.89	1952700	PV	1.091	30.648

total peak area:4150910μv·sec

Blank



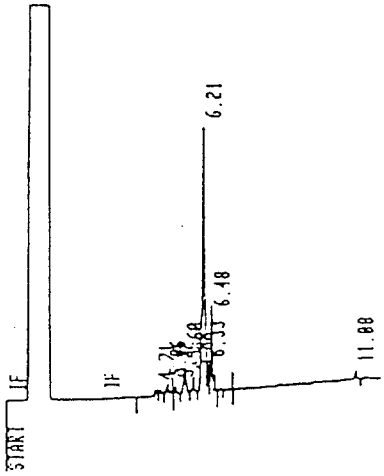
Recovery test-1



AREA% RT	AREA	TYPE	AR/HT	AREA%
5.05	144480	D VV	0.105	1.606
5.60	362910	D VP	0.100	4.035
5.88	194960	D PV	0.092	2.167
6.21	3565700	D VV	0.095	39.641
6.33	232790	D VV	0.064	2.588
6.48	876600	D VB	0.072	9.745
8.63	618280	VV	0.756	6.874
11.08	2999300	VV	1.182	33.344

total peak area:4805210μv·sec

Recovery test-2 2

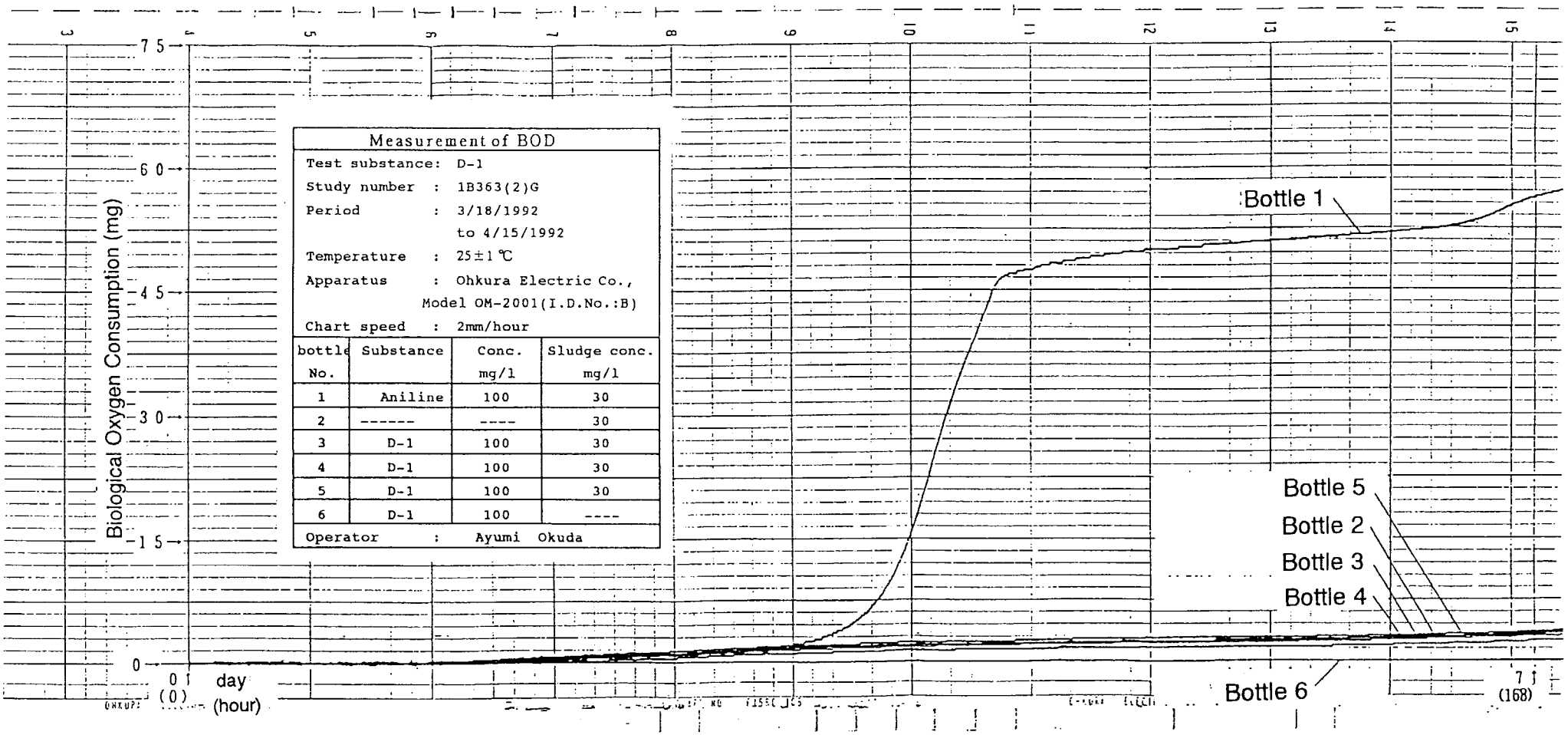


AREA% RT	AREA	TYPE	AR/HT	AREA%
5.06	104890	D PB	0.084	1.269
5.60	360210	D PP	0.099	4.359
5.88	195258	D PV	0.092	2.363
6.21	3529700	D VV	0.096	43.315
6.33	232240	D VV	0.064	2.810
6.48	876030	D VB	0.073	10.600
11.08	2915900	BV	1.250	35.284

total peak area:4815940μv·sec

000530

Figure 6 BOD chart



Measurement of BOD

Test substance: D-1
Study number : 1B363(2)G
Period : 3/18/1992 to 4/15/1992
Temperature : 25±1 ℃
Apparatus : Ohkura Electric Co., Model OM-2001(I.D.No.:B)
Chart speed : 2mm/hour

bottle No.	Substance	Conc. mg/l	Sludge conc. mg/l
1	Aniline	100	30
2	------	----	30
3	D-1	100	30
4	D-1	100	30
5	D-1	100	30
6	D-1	100	----

Operator : Ayumi Okuda

Figure 6 (Continued)

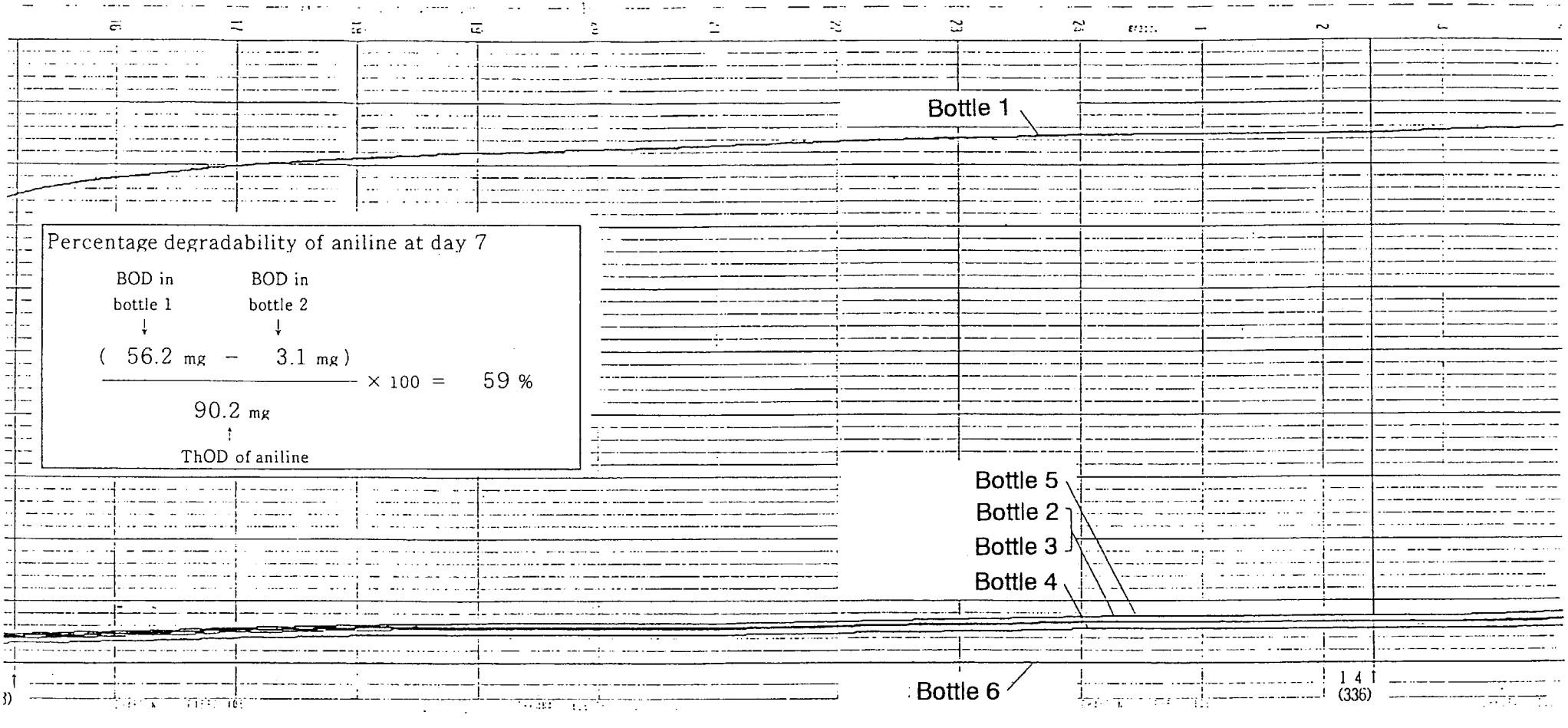


Figure 6 (Continued)

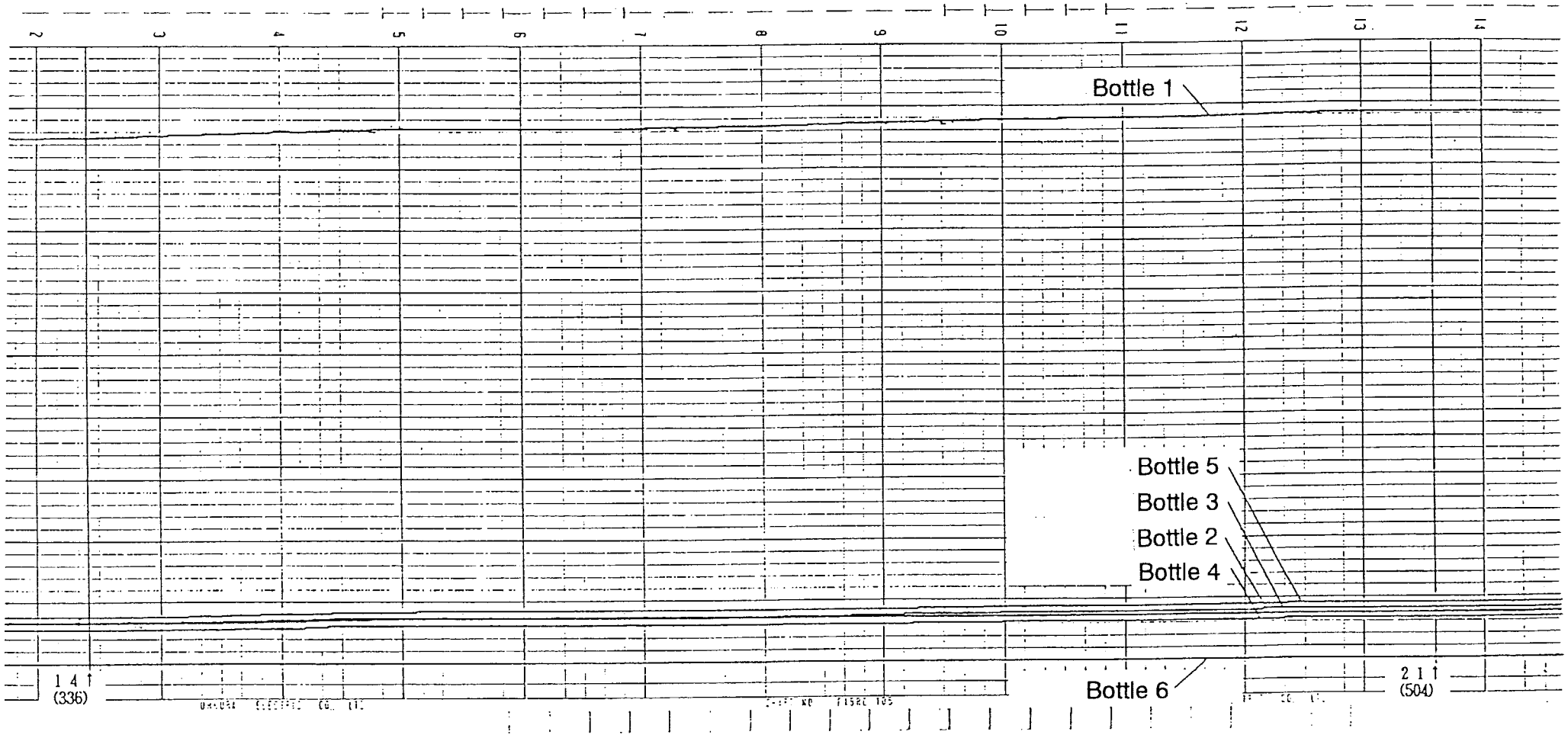


Figure 6 (Continued)

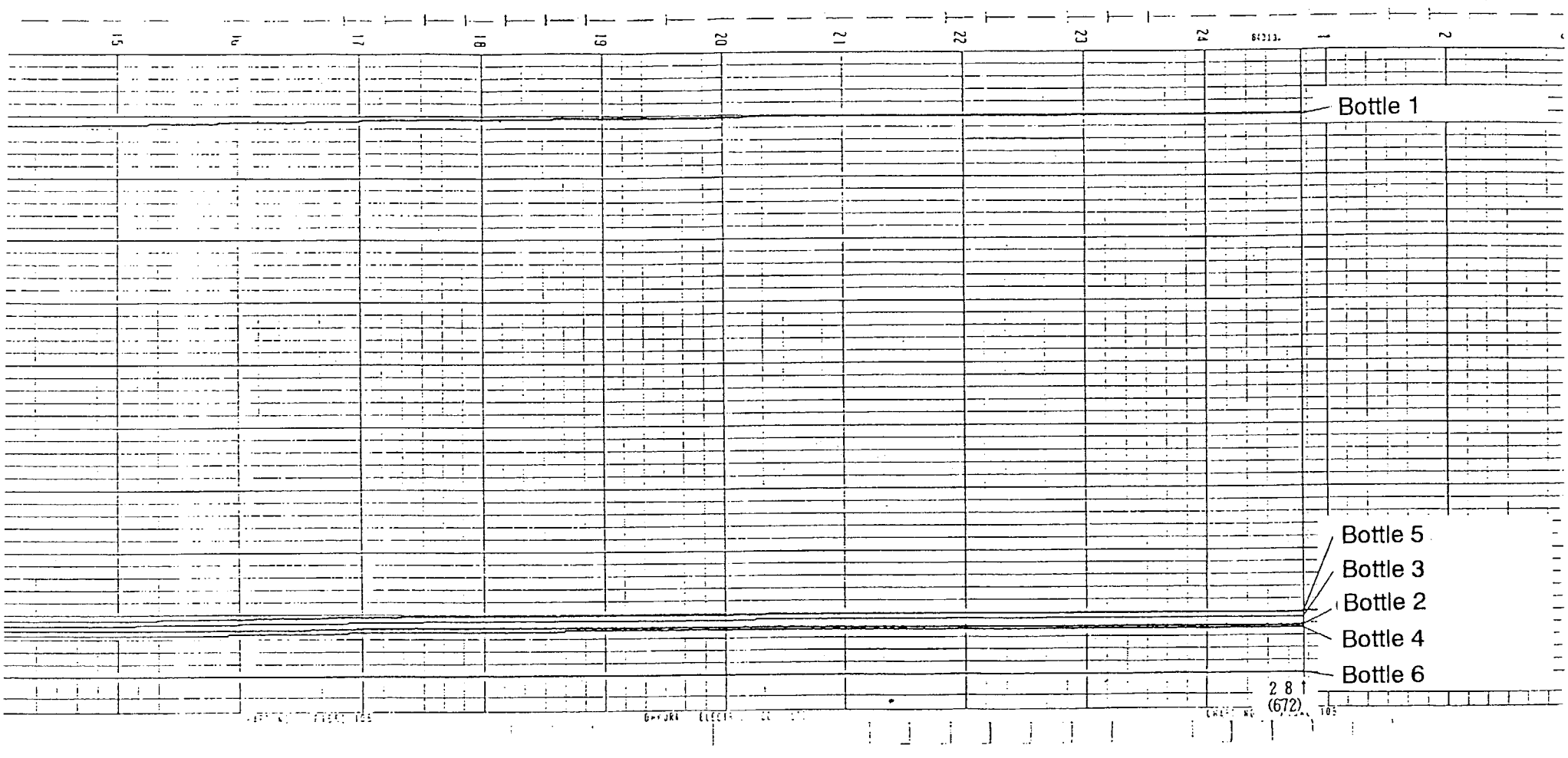
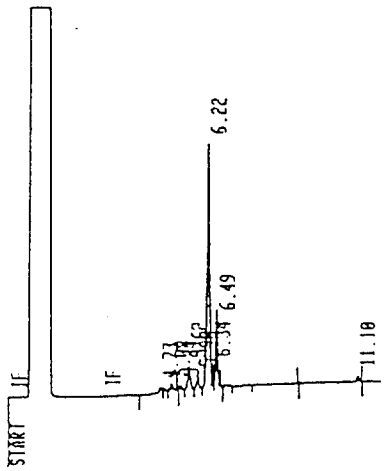


Figure 7 GC chromatograms of the test substance -- Measurement of residual test substance concentration

The concentrations of the residual test substance in the bottles are shown in Table 4.

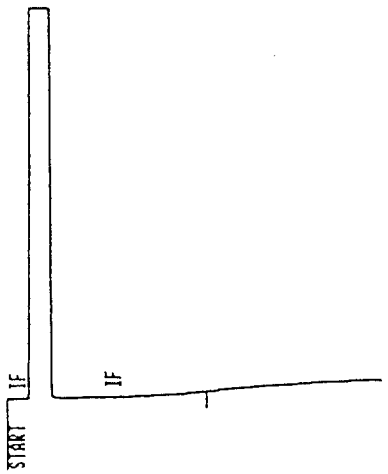
500 mg/l in ethylacetate



AREA% RT	AREA	TYPE	AR/HT	AREA%
5.62	325870	D PP	0.098	6.948
5.90	178020	D PV	0.092	3.741
6.22	3241400	D VV	0.092	68.114
6.34	211410	D VV	0.064	4.443
6.49	802070	D VB	0.072	16.855

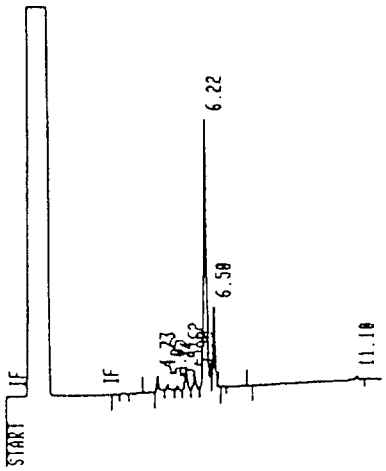
total peak area:4369340μv·sec

Bottle 2



NO RUN PEAKS STORED

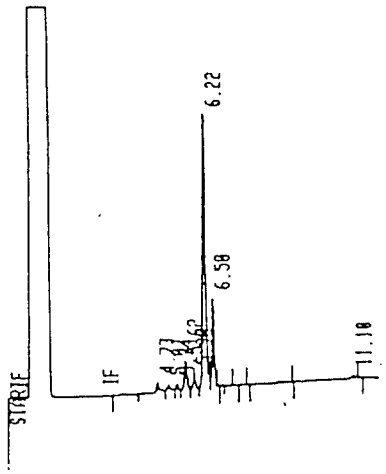
Bottle 3



AREA% RT	AREA	TYPE	AR/HT	AREA%
4.73	215680	D PV	0.096	3.106
5.05	121510	VV	0.115	1.750
5.62	334190	D VP	0.100	4.913
5.90	185690	D PV	0.092	2.674
6.22	3979000	VV	0.102	57.300
6.50	836690	D VB	0.072	12.049
11.10	1271400	BV	1.199	18.309

total peak area:5149880μv·sec

Bottle 4

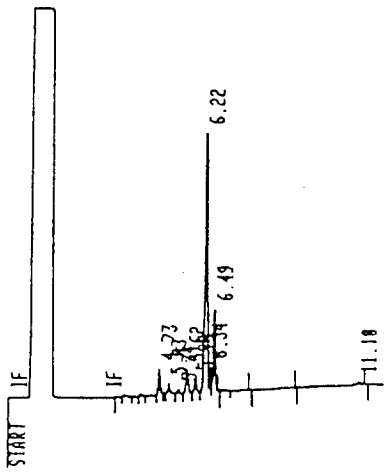


AREA% RT	AREA	TYPE	AR/HT	AREA%
4.73	140480	D PV	0.110	2.421
5.07	108370	D VV	0.108	1.867
5.62	379960	D VP	0.101	6.547
5.90	205250	D PV	0.093	3.537
6.22	4053900	VV	0.102	69.856
6.50	915290	D VB	0.072	15.772

total peak area:5349150μv·sec

Figure 7 (Continued)

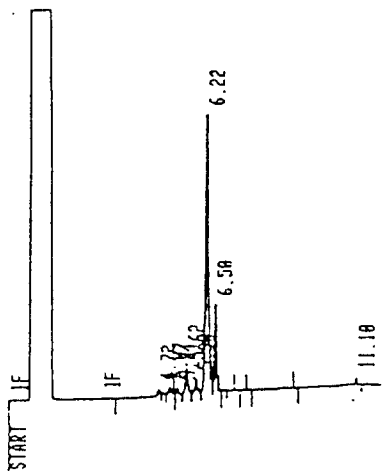
Bottle 5



AREA% RT	AREA	TYPE	AR/HT	AREA%
4.73	322600	D PV	0.085	5.810
5.03	164900	D VV	0.103	2.971
5.62	335320	D VP	0.101	6.039
5.90	183900	D PV	0.092	3.312
6.22	3472200	D VV	0.093	62.540
6.34	226410	D VV	0.064	4.077
6.49	846800	D VB	0.072	15.251

total peak area:4654900µv·sec

Bottle 6



AREA% RT	AREA	TYPE	AR/HT	AREA%
5.62	361480	D PP	0.100	5.850
5.90	200840	D PV	0.093	3.250
6.22	4159200	VV	0.103	67.306
6.50	907440	D VB	0.072	14.685
11.10	550590	D BV	0.418	8.910

total peak area:5428120µv·sec

ACUTE TOXICITY TO FISH

TEST SUBSTANCE

Identity: [2-(N-Ethylperfluorooctanesulfonamido) ethyl acrylate; may also be referred to as B1228, D-1, EtFOSEA, or FX-13. (2-Propenoic acid, 2-[ethyl[heptadecafluorooctyl)sulfonyl]amino]ethyl ester, CAS # 423-82-5)

Remarks: Material is an amber solid. Sample purity and not recorded. 3M lot number 13.

METHOD

Method: Not given
Type: Static acute range-finder
GLP: No
Year completed: 1984
Species: *Pimphales promelas*
Supplier: Brady, Neb.
Analytical monitoring: Nominal concentrations only. Temperature, pH and dissolved oxygen recorded.
Exposure period: 96-hours
Statistical methods: LC_{50} could not be calculated as there was insufficient toxicity at concentrations above the water solubility.
Test fish age: Not given
Length and weight::
Length: 3.79 cm
Weight: 0.359 g
Loading: 0.6 g/L

Test conditions:
Dilution water: Carbon-filtered well water
Dilution water chemistry during the study:

DO:	6.5 – 9.3 mg/L
pH:	7.9-8.2
Test temp. range:	21-22°C

Stock and test solution preparation: Test solutions prepared by mass addition of test substance to 3L of dilution water.
Concentrations dosing rate: Once
Stability of the test chemical solutions: Not recorded
Exposure vessels: Wide mouth one gallon glass jars
Exposure volume: 3 liters
Number of replicates: one
Number of fish per replicate: five

Number of concentrations: Four plus a negative control

RESULTS

Nominal loading concentrations: Bk control, 10, 100, 500, 1000 mg/L
Element value: 96-hour LC_{50} > 1000 mg/L

Percent Mortality

Nominal Test Concentration, mg/L	24-Hours	48-Hours	72-Hours	96-Hours
Negative Control	0	0	0	0
10	0	0	0	0
100	0	0	0	0
500	0	0	0	0
1000	0	20	20	20

Remarks: All values reported are for nominal concentrations. No measurement of test concentrations was conducted.

CONCLUSIONS

The Et FOSEA 96-hour LC_{50} for fathead minnow was determined to be > 1000 mg/L

DATA QUALITY

Reliability: Klimisch ranking 2. This study, while conducted using the methodology acceptable at the time, lacks analytical data for determination of the test substance concentration in the test solutions and determination of the sample purity. Testing was also done well above the solubility limit of the test substance.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, Lab Request number B1228, 4/1/84

OTHER

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

Last changed: 5/22/00

ENVIRONMENTAL LABORATORY
CHEMICAL ENVIRONMENTAL EFFECTS EVALUATION
TOXICITY TEST: SUMMARY REPORT

Chemical Tested EX13 (CC8511-26) LOT 13 FM4519 Test Type: RANGE FINDER (EXPLORATORY)

LC50/EC50/IC50/GR50 (95% C.L.): 96HR LC50 GREATER THAN 1000 mg/l

Test Species: FATHEAD MINNOW (PIMEPHALES PROMELAS)

Test Date: 3-28-84 / 4-1-84 L.R. #: R1228-1

Analyst(s): [illegible]

(Exposure/Test Conditions)

Exposure Type:

Static V ACUTE Static/Continuous Orbital Shaking _____

Static Recirculatory _____ Static Renewal _____ Flow-Through _____

Diluent:

Aerated Well Water (Initial D.O. 9.3 mg/l; pH 8.2; Temp. 21 °C (70°F))

Water Hardness: 240 ppm Hardness Classification: HARD

Dissolved Oxygen % Saturation:

	48-hr	96-hr	Extended Exposure
Control(s)	80	79	
Lowest Experimental Dose 10 mg/l	75	76	
Highest Experimental Dose 1000 mg/l	73	75	

pH Levels:

	48-hr	96-hr	Extended Exposure
Control(s)	8.1	8.0	
Lowest Experimental Dose 10 mg/l	8.1	7.9	
Highest Experimental Dose 1000 mg/l	8.2	8.1	

Test Temperature °C (°F):

48-hr	96-hr	Extended Exposure
21°C (70)	22°C (72)	°C ( )

Photoperiod: Sixteen hours light with a 15-30 minute transition period.

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ENVIRONMENTAL LABORATORY
AQUATIC TOXICITY DATA SHEET
EXPLORATORY (RANGE-FINDING TEST)

LR B1228

Type Test Acute Static Material Tested EX13 (CC8311-26) Lot 13, EM4519

Test Organism Fathead Minnow Source Brady, Neb Date Rec'd. 3/6/84 Batch No. 3-84

Avg. Wt. 0.359 grams Avg. Size 3.79 CM No. Organisms per dose 5

Test Solution Volume 3 Liters/All-Glass Tank Tank Dimensions: WIDE-MOUTH 1 GALLON GLASS JAR

Time Started 12:15pm Date Started 3/28/84 Date Completed 4/1/84

Dilution Water Carbon-filtered well water (Temp. 21 °C., D.O. 9.3 ppm, pH 8.2)

Investigator (Full Signature) [signature] LC_{50} (95% Confidence Limits) >1000 mg/l

Tank No.	Init. Conc. mg/l	Total Amount Added	24 Hours Total No. Dead	24 Hours pH	24 Hours D.O. ppm	48 Hours Total No. Dead	48 Hours pH	48 Hours D.O. ppm	72 Hours Total No. Dead	72 Hours pH	72 Hours D.O. ppm	96 Hours Total No. Dead	96 Hours pH	96 Hours D.O. ppm
-	Control	* mg	0	8.2	7.1	0	8.1	7.0	0	8.1	6.9	0	8.0	6.8
-	10	30	0	8.0	6.5	0	8.0	6.6	0	7.9	6.6	0	7.9	6.6
2	100	300	0	8.1	6.5	0	8.1	6.5	0	8.0	6.4	0	8.0	6.1
3	500	1500	0	8.1	6.7	0	8.1	6.5	0	8.0	6.5	0	8.0	6.3
4	1000	3000	0	8.1	6.7	1	8.1	6.4	1	8.0	6.5	1	8.0	6.4
Test Temp. °C.			21°C			21°C			21°C			22°C		
Initials & Date			3/29/84			3/30/84			3/31/84			4/1/84		

Comments: * INDIVIDUAL WEIGHTS. LIGHT AMBER, SOLID MATERIAL.

Effects other than Mortality EC_{50}

Tank No.	Symptoms or Reactions	Total No. Affected 24-Hr.	48 Hr.	72-Hr.	96-Hr.
Control		None	None	None	None
1					
2					
3					
4					

Comments:

000540

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST

TEST SUBSTANCE

Identity: [2-(N-Ethylperfluorooctanesulfonamido) ethyl acrylate; may also be referred to as B1228, D-1, EtFOSEA, or FX-13. (2-Propenoic acid, 2-[ethyl[heptadecafluorooctyl)sulfonyl]amino]ethyl ester, CAS # 423-82-5)

Remarks: Material is an amber solid. Sample purity and not recorded. 3M lot number 13.

METHOD

Method: Protocol Reference EAR/WAS 5/9/84
Test type: Static acute
GLP: No
Year Completed: 1984
Analytical monitoring: Dissolved oxygen concentrations.
Test organism source: Activated sludge collected from the St Paul/Minneapolis, MN Metro Wastewater Plant on 5/10/84

Test conditions:

Dilution water: Deionized water

Test solution preparation: Placed 0.30 g test substance in a 300 mL BOD bottle with 50 mL deionized water. The mixture was mixed with a magnetic stirring bar and box for ~ 30-minutes in order to disperse sample. After mixing the sample was finely suspended in the water.
Test vessels: 300 mL BOD bottles
Number of concentrations: One
Temperature: Not recorded
Total Suspended Solids of sludge on day of testing: Not recorded. Sludge was used the day of collection.
Exposure period: 6.65 minutes
Element Basis: Respiration inhibition as determined by dissolved oxygen concentration.

RESULTS

Nominal concentrations: Two blank (deionized water) controls, one 1000mg/L test material solution.

Statistical Analyses: EC_{50} values was not determined as the oxygen depletion rate for the controls was judged to be equal to that of the deionized water. controls

Analytical Methodology: Method for the analysis of dissolved oxygen concentrations was not recorded.

Control response: Satisfactory; difference between controls was 6.9%.

CONCLUSIONS

The test substance exhibited a no inhibitory effect on respiration at a nominal test substance concentration of 1000 mg/L after 6.65 minutes of exposure. The EC_{50} (respiration inhibition) is therefore greater than 1000 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking 3. This study was not conducted using an established method. Testing was also done well above the solubility limit of the test substance. This study lacks sufficient documentation and characterization of the test substance purity, and analytical measurement of test concentrations.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, Lab Request number B1228, 4/1/84

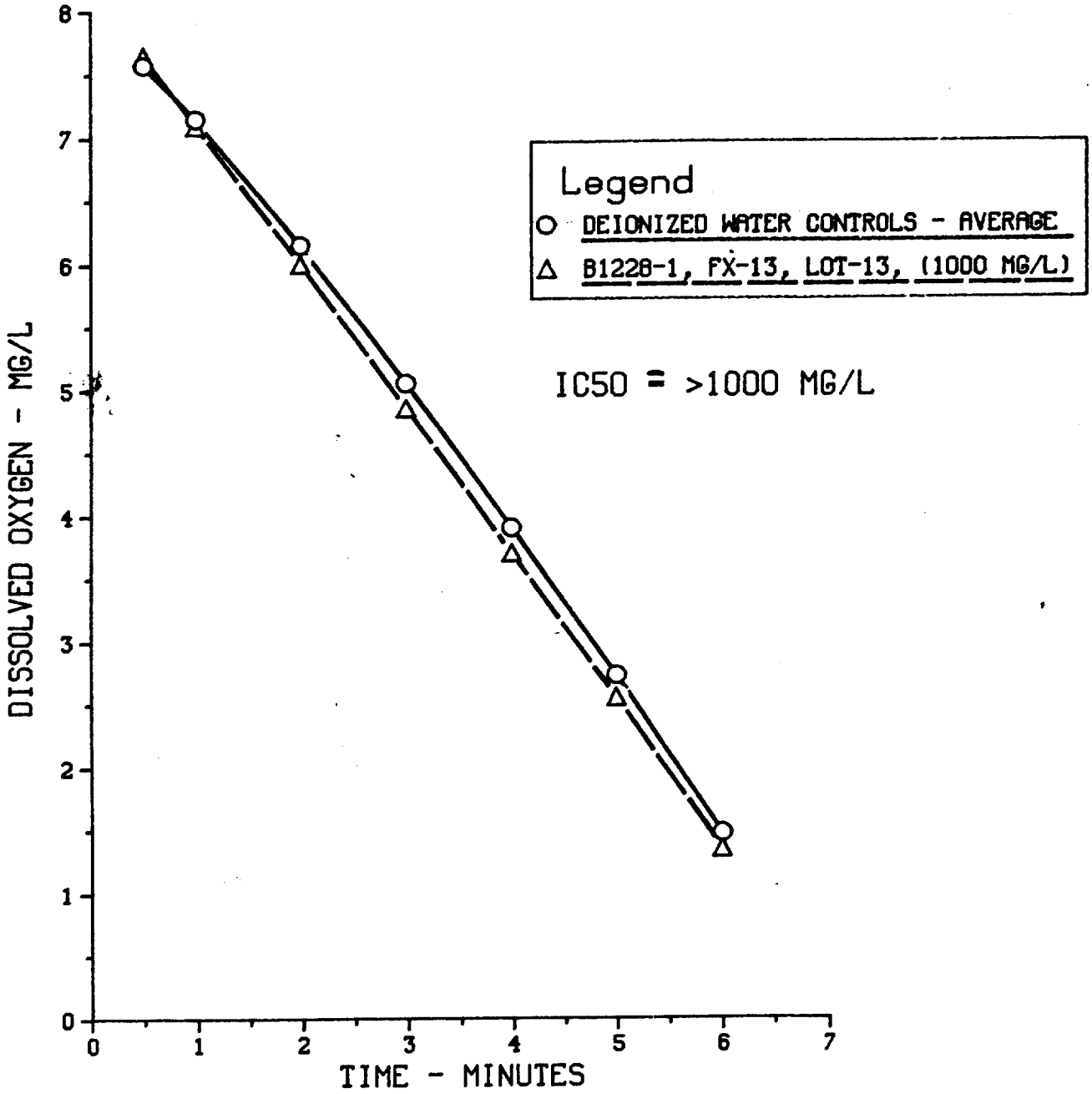
OTHER

Last changed: 5/22/00

5/10/84

3M Environmental Laboratory

Acute Toxicity of a Substance to
Activated Sludge Mixed Liquor (SLUDGE-TOX)
Protocol Reference: EAR/WAS 5/09/84, Lab Request No. B1228



W.A. Schiel
5-17-84

STOX.DAT

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3M Environmental Laboratory
Acute Toxicity of a Compound (Substance) to Activated Sludge Mixed Liquor
Protocol Reference: EAR/WAS 5-09-84

Lab request no.: B1228 Date: 5/10/84 Analyst: W. A. Schei[illegible]

The activated sludge mixed liquor was collected from the St. Paul/Minneapolis Metro Wastewater Plant on 5/10/84.

Sample description: (B1228-1) FX-13, L-2514, FM-4519, LOT-13

Preparation of stock solution: _____ grams of sample were diluted to _____ ml with deionized reagent water. Date of preparation: _____.

See comments below.

TIME min.	TIME sec.	CONCENTRATION OF TEST MATERIAL IN ACTIVATED SLUDGE MIXTURE: DI H2O CONTROL	1000 mg/L			DI H2O Control		CONTROL AVERAGE
	30	7.60	7.65			7.55		7.575
1	-	7.15	7.10			7.15		7.15
2	-	6.15	6.00			6.15		6.15
3	-	5.00	4.85			5.10		5.05
4	-	3.80	3.70			4.00		3.90
5	-	2.60	2.55			2.85		2.725
6		1.30 ~~6.50~~	1.35			1.65		1.475
7		0.50 ~~6.65 minutes~~	0.30			0.55		
			6.65 minutes					

WAS 5/10/84

% difference between controls = 6.9%

COMMENTS:

Test solution preparation: 0.30 g sample was placed in a 300 ml BOD bottle with 50 ml deionized water. The mixture was ~~placed on WAS mixture~~ mixed with a magnetic stirring bar and box for ≈30 minutes in order to disperse sample. After mixing the sample was finely suspended in the water.

BIOACCUMULATION

TEST SUBSTANCE

Identity: [2-(N-Ethylperfluorooctanesulfonamido) ethyl acrylate; may also be referred to as B1228, D-1, EtFOSEA, or FX-13. (2-Propenoic acid, 2-[ethyl[heptadecafluorooctyl)sulfonyl]amino]ethyl ester, CAS # 423-82-5)

Remarks: Material is an amber solid. Report states that sample purity is 99% or more based on information supplied by Sponsor. The purity/identity of the test substance cannot be substantiated. As presented in the report, the structural formula indicates that "purity" cannot be assigned as "99% or more. Lot number 101.

METHOD

Method/guideline followed: MITI described in "Chemical Substances Control Law" (Japanese Law No. 117, 1974). OECD 305C.
GLP (Y/N): Yes
Year: 1995
Exposure period: 8-weeks
Exposure concentrations: 0.01 and 0.1 mg/L
Test species: *Cyprinus carpio*

RESULTS

Bioconcentration Factors:

	Week 2	Week 4	Week 6	Week 8
0.01 mg/L exposure:	0.4	0.6	0.6	0.5
duplicate	0.4	0.7	0.4	0.5
0.1 mg/L exposure	3	4	2	2
duplicate	3	3	2	2

CONCLUSIONS

Neither mortality nor abnormalities in appearance or behavior were observed in the treated and control fish during the 8-week test period.

The test substance is not bioaccumulative in carp under the prescribed test conditions.

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

DATA QUALITY

Reliability: Klimisch ranking 3.
The purity/identity of the test substance cannot be substantiated. The extraction/analytical methodology has not been validated therefore it is not possible to know if the test compound did not bioconcentrate in the fish or simply was not extractable by the methodology of the experiment. The report states that the chromatographic peaks eluting from "1 to 3 minutes" are considered to be metabolites. While this statement does not change the conclusions of the report, this assumption is cannot be justified.

OTHER

Last changed: 5/22/00

M. S. I. REPORT No. 2B121G

Bioaccumulation Study of Sample D-1

with Carp (Cyprinus carpio)

Submitted to :

SUMITOMO 3M LTD.

Prepared by :

Mitsubishi Chemical Safety Institute Ltd.

Nov. 30, 1995

000547

RECEIVED
NOV 25 1995

TRANSLATION OF THE TEST REPORT

Study No. 2B121G

Study Title Bioaccumulation Study of Sample D-1 with Carp (*Cyprinus carpio*)

Study Period From May 18, 1992 to Sept. 24, 1992

Sponsor SUMITOMO 3M LTD.

This test was conducted in Yokohama Laboratory, Mitsubishi Chemical SafetyInstitute Ltd.(M.S.I.), 1000 Kamoshida-cho, Aoba-ku, Yokohama 227, JAPAN.

The original test report was written in Japanese and this report was translated into English. The persons, the undersigned, hereby declare that this report reflects faithfully the original report as much as possible to our knowledge.

Translator from original Japanese version

Tadayoshi Shigeoka Nov. 30, 1995.

Tadayoshi SHIGEOKA, Ph.D. Date
Chief Research Scientist of Environmental Sciences Division

Shoko Tanimoto Nov. 30. 1995

Shoko TANIMOTO, B.Sc. Date
Scientist of Environmental Sciences Division

Approved by

Jun Toriya Nov. 30. 1995

Jun TORIYA, B.Sc. Date
Head of Yokohama Laboratory

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARD

Study No. 2B121G

Study Title Bioaccumulation Study of Sample D-1 with Carp (*Cyprinus carpio*)

Sponsor SUMITOMO 3M LTD.

To the best of our knowledge and belief, the study described in this report was conducted in compliance with the Good Laboratory Practice (GLP) regulations recognized by Basic Industries Bureau, Japanese Ministry of International Trade and Industry (MITI).

Yokohama Laboratory,

Mitsubishi Chemical Safety Institute Ltd. * (M.S.I.)

* The company's name was changed on October 1, 1994.
(Former name : Mitsubishi-kasei Institute of Toxicological and Environmental Sciences)

Test Facility Management

Sakae KOIKE, B.Sc. Sealed Date : Sept. 24, 1992

Head of Yokohama Laboratory

Study Management

Tadayoshi SHIGEOKA, Ph.D. Sealed Date : Sept. 24, 1992

Chief Research Scientist of Environmental Sciences Division

Study Director

Ayao NISHIKAWA Sealed Date : Sept. 24, 1992

Scientist of Environmental Sciences Division

We, the undersigned, declare that the work was performed according to the procedures herein described, and this report provides a correct and faithful record of the results obtained.

Experimental Scientist

Yasuo SATO Sealed Date : Sept. 24, 1992

Scientist of Environmental Sciences Division

Naomi SAEKI, B.Sc. Sealed Date : Sept. 24, 1992

Scientist of Environmental Sciences Division

QUALITY ASSURANCE STATEMENT

Yokohama Laboratory,

Mitsubishi Chemical Safety Institute Ltd.

Study Title Bioaccumulation Study of Sample D-1 with Carp (*Cyprinus carpio*)

Study No. 2B121G

We hereby certify that the above study was performed in compliance with the Good Laboratory Practice (GLP) regulations recognized by the Basic Industries Bureau, Japanese Ministry of International Trade and Industry (MITI).

Dates of inspections and findings reported to the Study Director and the Facility Management are as follows:

	Dates of Inspection	Dates of Reporting
In-progress study	May 26, 1992 June 23, 1992 July 21, 1992	May 26, 1992 June 23, 1992 July 22, 1992
Final report	Sept. 24, 1992	Sept. 24, 1992

Quality Assurance Staffs

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Summary

Sponsor : SUMITOMO 3M LTD.

Study Title : Bioaccumulation study of Sample D-1 with carp (Cyprinus carpio)

Study No. : 2B121G

Test Substance : Sample D-1

Test Period : From May 18, 1992 to Sept. 24, 1992

Testing Method : Testing method of Ministry of International Trade and Industry (MITI) described in "Chemical Substances Control Law" (Japanese Law No.117, 1974).
(OECD testing guideline 305C)
Supply of test water : Continuous flow-through diluter system
flow rate : 1600 L/ day
Nominal concentration of Sample D-1 in test water :
High exposure level ; 0.1 μg/mL
Low exposure level ; 0.01 μg/mL
Exposure period : 8 weeks
Analytical method : GC analysis after pre-treatment

Results : Bioconcentration factors (BCFs) obtained in this study were as follows:

Bioconcentration factors (BCFs)				
level \ exposure period (weeks)	2	4	6	8
High exposure level, sample fish No.1	0.4	0.6	0.6	0.5
No.2	0.4	0.7	0.4	0.5
Low exposure level, sample fish No.1	3	4	2	2
No.2	3	3	2	2

Conclusion : On the basis of the above results, it can be concluded that the test substance is not bioaccumulative under the prescribed test conditions.

1. Introduction

1.1 Study Title

Bioaccumulation Study of Sample D-1 with Carp (Cyprinus carpio)

1.2 Objective

The objective of this study was to evaluate bioaccumulation potential of D-1 for application of a new chemical substance in Japan.

1.3 Testing Method

Testing method of Ministry of International Trade and Industry (MITI) described in "Chemical Substances Control Law" (Japanese Law No.117, 1974). (OECD testing guideline 305C)

1.4 GLP Compliance

The study was conducted in accordance with the Good Laboratory Practice (GLP) regulations recognized by Basic Industries Bureau, Japanese Ministry of International Trade and Industry (MITI).

1.5 Study Period

From May 18, 1992 to Sept. 24, 1992

(exposure period : From May 26, 1992 to July 21, 1992)

1.6 Storage and retention of records and sample

Following records, raw data, and sample will be retained in the archives of MITES for 10 years after submission of the final report. After this period, the sponsor will be contacted for approval of the disposal of any data. Data will be retained in the archives for a further specified period at the sponsor's request (additional fee).

1) The protocol and its ammendment record.
2) The final report.
3) Raw data.
4) Quality assurance reports.
5) Test substance (ca. 2g)
6) Other documents required by MITI GLP regulations.

2. Test substance

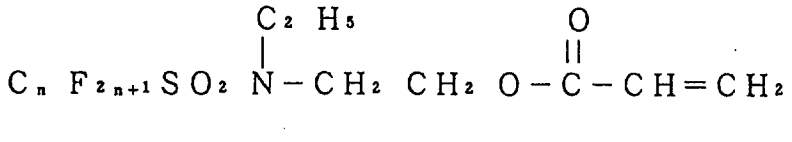
2.1 Chemical name, Structural formula, Molecular formula and weight.

Chemical name *:

2-[N-Ethyl-N-perfluoroalkyl(C= 1～8)sulfonylamino]ethylacrylate

[Abbreviated name: Samle D-1, identification No.2B121G]

Structural formula *:



$$C_nF_{2n+1}SO_2N(C_2H_5)-CH_2CH_2O-C(=O)-CH=CH_2$$

n = 1～8, n = 8 : ca. 78%, n = 1～7 : ca. 21%

Molecular formula : $C_{15}H_{12}O_4NSF_{17}$ (n = 8)

Molecular weight * : 625 (n = 8)

(* Data from the sponsor)

2.2 Physico-chemical properties

Appearance : amber like wax

Melting point * : 27 ～42℃

Boiling point * : ca.150 ℃ (1mmHg)

Solubility :

water	: <	0.1% (25 ℃)
methanol	: >	5 % (25 ℃)
acetonitrile	: >	5 % (25 ℃)
acetone	: >	50%
n-hexane	: >	5 % (25 ℃)
THF	: >	5 % (25 ℃)
DMSO	:	insoluble

Infrared spectrum* : shown in Appendix-1

(* Data from the sponsor)

2.3 Source

(1) Supplier	:	SUMITOMO 3M LTD.
(2) Supplied quantity*	:	50 g
(3) Date of receipt	:	April 1, 1992.
(4) Lot. No. *	:	101
(5) Purity *	:	> 99 %
Impurity *	:	Phenothiazine 60 ±20ppm
		Hydroquinone monomethylether 170 ± 35ppm

(* provided by the sponsor)

2.4 Confirmation of test substance

Before starting the study, infrared (IR) spectrum of Sample D-1 was measured. The spectrum (Fig.1(p.28)) was compared with the IR data provided by the sponsor (Appendix-1) and was confirmed the identity. And it also corresponded to the structural formula shown by the sponsor.

2.5 Stability of test substance under storage conditions

At the termination of the study, IR spectrum of Sample D-1 was measured and the spectrum (Fig.2(p.28)) was compared with the IR spectrum obtained at the start of this study (Fig.1). Test substance was confirmed to be stable under storage conditions stored in the refrigerator during the test period of ca.2.5 months.

3. Materials

3.1 Apparatus for bioaccumulation test

The flow-through test system is shown in Fig.3 (p.29).

[For test aqruarium]

Dilution water supply pump	: Yamazen (FMI) Co., QD-2CSC
Feed solution supply pump	: Yamazen (FMI) Co., QG6-RHOOSTY

[For control aqruarium]

Dilution water supply pump	: Yamazen (FMI) Co., RP-D-2CKC
Feed solution supply pump	: Tokyo Rika Kikai Co., GMW-8

3.2 Apparatus for analysis

Infrared spectrophotometer	: Parkin Elmer Co., Model 1640
Homogenizer	: Nihon Seiki Kaisha Ltd., AM-8
Shaker	: Taiyo Kagaku Kogyo Co., SR-II
Rotary evaporator	: Buchi Ltd., RE-111
Aspirator	: Yamato Kagaku Co., BP-51
Electronic balance	: Mettler Ltd., PL1200
Gas chromatograph	: Shimadzu Ltd., GC-14A

3.3 Reagents

Acetonitrile	: Kokusan Chemical Co., Guaranteed Reagent
Acetone	: Junsei Chemical Co., Guaranteed Reagent
Chloroform	: Junsei Chemical Co., Guaranteed Reagent
Ethyl Acetate	: Kokusan Chemical Co., Guaranteed Reagent
Tetrahydrofuran (THF)	: Kishida Chemical Co., Guaranteed Reagent
n-Hexane	: Kishida Chemical Co., Guaranteed Reagent
Methanol	: Junsei Chemical Co., Guaranteed Reagent
Sodium chloride	: Kishida Chemical Co., Guaranteed Reagent
Sodium sulfate, anhydrous	: Kokusan Chemical Co., Guaranteed Reagent

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4. Analytical methods

4.1 Gas chromatographic(GC) conditions

Column : Shimadzu Ltd., Wide Bore column, CBP20-W25-100 ϕ0.53mm×25m

Temperature : Column ; 130℃

Injector ; 200℃

Detector ; 240℃

Flow rate of N_2 : 20mL/min

Range : 10^1

Attenuation : 7

Chart speed : 5mm/min

Injection volume : 3μL

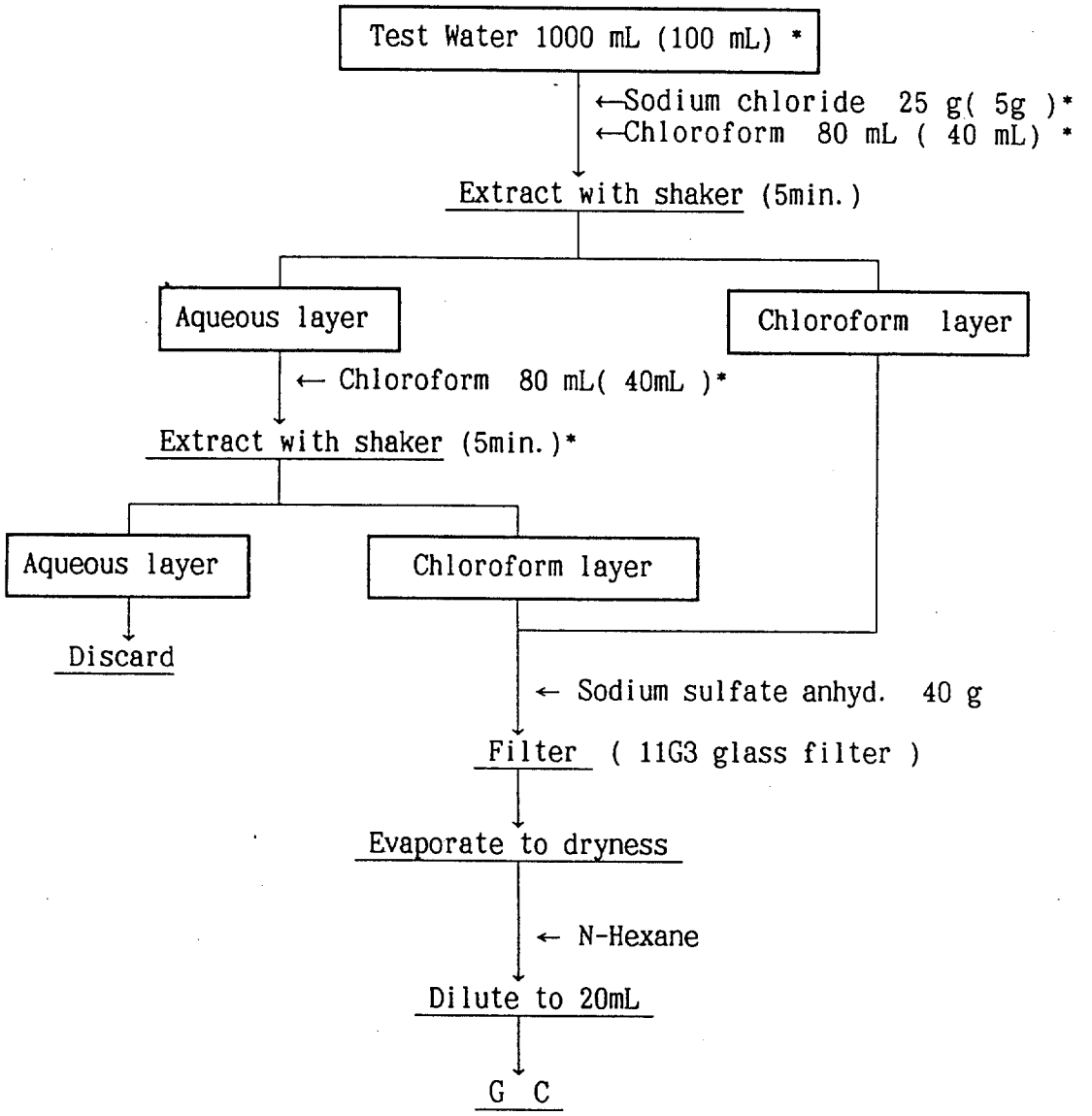
4.2 Calibration curve

Standard solution of 1000 μg/mL was prepared by dissolving 100 mg of D-1 in 100 mL of n-hexane and this standard stock solution was diluted with n-hexane to prepare 0.25, 0.50, 1.00μg/mL n-hexane solutions of D-1. These solutions were injected into GC described above. The GC peak areas(μV・sec) were plotted against the concentrations of D-1. The GC chromatograms and the calibration curve thus obtained are shown in Fig.4(p.30), and Fig.5(p.31), respectively. Quantity of D-1 was calculated from the total area of peaks at 3.9 and 4.5 min. on the chromatogram. The calibration curve is straight line through the origin and its correlation coefficient is 1.000.

D-1 was determined with the ratio of area on GC chromatograms against the standard solution (0.5 μg/mL) per measurement.

4.3 Analytical method of test substance in test water

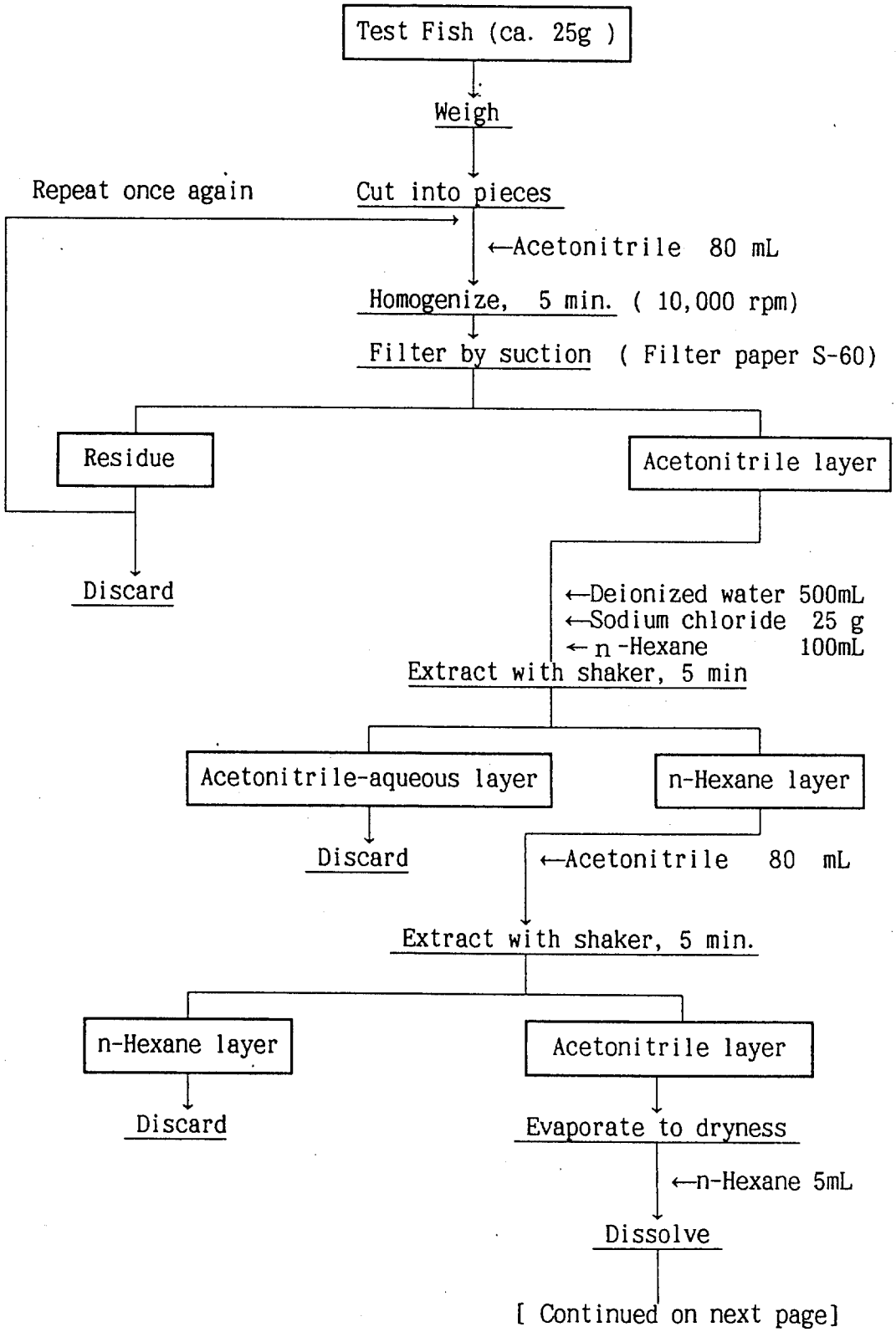
Analysis was performed according to the following procedure.

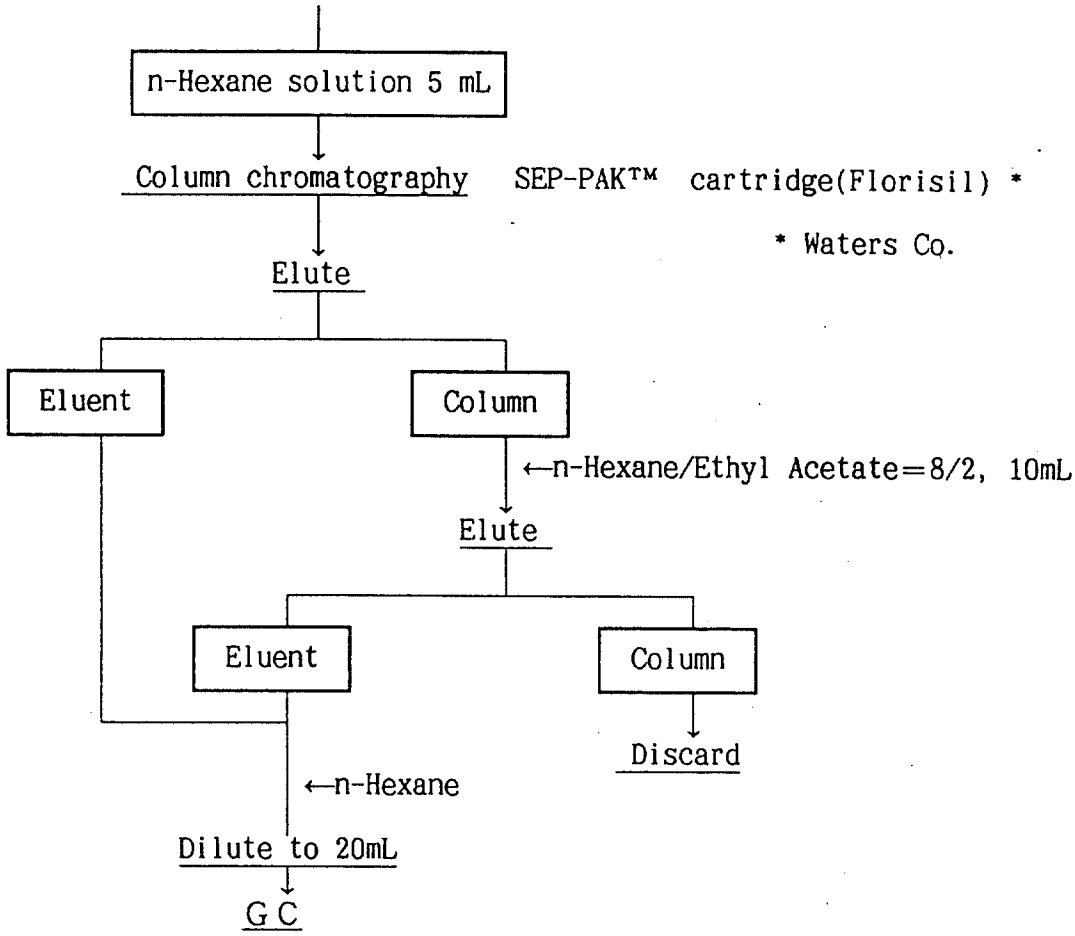


( ) * High exposure level

4.4 Analytical method of test substance in test fish

Analysis was performed according to the following procedure.




n-Hexane solution 5 mL
Column chromatography
SEP-PAK™ cartridge(Florisil) *
* Waters Co.
Elute
Eluent
Column
←n-Hexane/Ethyl Acetate=8/2, 10mL
Elute
Eluent
Column
Discard
←n-Hexane
Dilute to 20mL
GC

4.5 Blank test

(1) Water

The blank tests were performed twice according to the procedure shown in 4.3, using 1000 mL of the test water in the absence of D-1(Control aquarium). There was no peak which disturbed the analysis of D-1 in GC chromatograms from water(Fig.6 (p.32)).

(2) Fish

The blank tests were performed according to the procedure shown in 4.4, using the each two untreated test fish with D-1 (Control aquarium) ,at the biginning and the termination of the exposure period. There was no peak which disturbed the analysis of D-1 in GC chromatograms from fish(Fig.7(p.33, 34)).

4.6 Recovery test

(1) Water

Two mL of acetone solution of D-1 (5 μg/mL) was added to 1000 mL of the test water in the absence of D-1. D-1 disperse solution of 0.01 μg/mL (the same as Low exposure level concentration in the test water) in the test water was thus prepared and the recovery tests were performed twice according to the procedure shown in 4.3. As shown in Table 1(p.35)and Fig.8(p.36), the mean value of the recovery ratios was 94.6%. Analytical values of the test water were corrected by this value.

(2) Fish

Two mL of acetone solution of D-1 (5 μg/mL) were added to the untreated test fish with D-1 and the recovery test was peformed twice according to the procedure shown in 4.4. In case that a fish body weight is 25g, the concentration of D-1 in fish would be 0.3 μg/g and correspond to 30 times of that in the test water. As shown in Table 2 (p.35) and Fig.9 (p.37), the mean value of the recovery ratios was 99.2%. Analytical values of the test fish were corrected by this value.

4.7 Detection limit

There was no peak which disturbed the analysis of D-1 in GC chromatograms from blank water and blank fish. Therefore, the area of the minimum detectable peaks, which can be recognized visually, was set to be 30,000 μV・Sec.. This value corresponds to ca. 0.02 μg/mL(D-1) on the calibration curve. Detection limit is calculated according to following calculation formula and conditions. The results are as follows;

Fish	:	0.02 μg/g	
Water	:	High exposure level	0.004 μg/mL
		Low exposure level	0.0004 μg/mL

It would be possible to calculate bioconcentration factors (BCFs) of following values in the case of fish weight 20g.

High exposure level (0.1 μg/mL)	:	> 0.2 times
Low exposure level (0.01 g/mL)	:	> 2 times

.....

[Calculation formula]

Detection limit = Concentration in the final solution × Volume of the final solution / Amount of sample

[Conditions]

Fish	:	Volume of the final solution	20 mL
		Amount of sample (fish weight)	20 g
Water	:		
High exposure level	;	Volume of the final solution	20 mL
	;	Amount of sample	100 mL
Low exposure level	;	Volume of the final solution	20 mL
		Amount of sample	1000 mL

5. Acute toxicity to fish

The static acute toxicity test of the test substance to fish was conducted according to the Japanese Industrial Standard Method "JIS K 0102-1986, Industrial Waste Water Testing Method, 71. Acute toxicity study using fish".

The result obtained in this test were used as a reference for determining the nominal concentrations to be used in the bioaccumulation test because the nominal concentrations of high and low exposure levels should be lower than 1/100 and 1/1000 of 48hr-LC50, respectively.

5.1 Preparation of test solutions

Four mL of 400mg/mL THF solution of HCO-20 was mixed with 4mL of 50mg/mL THF solution of D-1. After evaporation of THF in the resultant solution by blowing N_2 gas, it was mixed with HCO-20 solution(6.4g in 100mL water) and dispersed. This dispersed solution was diluted to 2L with declorinated water (Yokohama municipal tap water was treated with activated charcoal)to prepare 100 μg/mL solution of D-1.

〔control level 〕

Eight g of HCO-20 was dissolved with 100 mL of deionized water and diluted to 2L with dechlorinated water.

5.2 Test conditions

(1) Test fish : Orange killifish (Oryzias latipes)

Source	;	Miyazawa Fish Farm(1323, Suenaga, Takatsu-ku, Kawasaki)
Date of receipt	;	March 3, 1992.
Lot No.	;	92-H-0303

Body length ; approx. 2 cm

Body weight ; approx. 0.2 g

(2) Acclimation :

Aquarium No. ; C-5

Water temperature ; 25±2 ℃

Period ; Over one week

Feed ; Tetramin ®(Tetra Werke, Germany)

Fish were fed once a day except holidays and not fed 48 hours prior to the test.

Percentage of mortal fish during one week befor test ;less than 5%

(3) Test conditions :

The number of fish ; 10 per one aquarium

Volume of water ; 2 L

Temperature ; 24.4～25.4℃

Duration ; 48 hours

Dissolved oxygen ; 4.2～7.4 ppm

Aeration ; Test water was aerated during the test period.

Renewal of test water ; non

5.3 Results

The 48hr-LC_{50} value of test substance was more than 100 μg/mL as shown in Table-3 (p 35).

6. Stability of test substance under the test conditions

6.1 Stability in water

(1) Preparation of test solution

The feed solution of high exposure level (concentration of D-1: 4.0mg/mL) was prepared by the procedure in 7.2(1). Then, 2.5mL of this solution was diluted to 1000mL with deionized water. It(concentration of D-1: 10 μg/mL) was used for the stability test of D-1 in water.

(2) Method of measurement

The solution prepared by the procedure shown in 6.1 was left under room conditions, and 5mL of this solution was withdrawn and diluted with 100 mL of water on days 0, 1, and 2. Each of them was pre-treated by the procedure in Sec.4.3 and diluted to 100 mL with n-hexane. The resultant solution was analyzed by GC and variation of the test substance concentration with time was evaluated.

(3) Results

Typical GC chromatograms are shown in Fig.10(p.38) and the variation of concentration is shown in Fig.11(p.39). Based on the result that test substance remained 104% (9.5 μg/mL → 9.9μg/mL) of initial concentrations after 2 days, it is concluded that D-1 is stable in water.

6.2 Stability in feed solution

(1) Preparation of test solution

The feed solution of low exposure level (concentration of D-1: 400μg/mL) was prepared by the procedure in 7.2(1).

000567 (2) Method of measurement

The test solution prepared by (1) was left under the room conditions.

At days 0, 3, 7, and 14, 2.5mL of the test solution was withdrawn and was diluted to 50 mL with acetone. Then each of 2.5mL of these solution was withdrawn and was diluted to 100mL with acetone, and analyzed by GC. Variation of the test substance concentration with time was evaluated.

(3) Results

Typical GC chromatograms are shown in Fig.12(p.40) and the variation of concentration is shown in Fig.13(p.41). Based on the result that test substance remained 99% (394 μg/mL → 390 μg/mL) of initial concentrations after 14 days, it is concluded that D-1 is stable in feed stock solution.

7. Bioaccumulation test method

The study was performed in accordance with the testing method of Ministry of International Trade and Industry (MITI) described in "Chemical Substances Control Law" (Japanese Law No.117, 1974).

7.1 Test fish

Carp(Cyprinus carpio) were purchased from Sankyo Suisan Co. (1-1 Ichigayatamachi, Shinjuku-ku, Tokyo) on December 6, 1991. Prior to the initiation of the test, these fish were acclimatized over a week under the test condition (water temperature 25 ±2 ℃, fed with commercial pellets once a day) and the mean body length of them is ca.11 cm. Mortal fish were less than 5 % during the acclimation of a week prior to the introduction of fish into the test system.

7.2 Methods and conditions

(1) Preparation of feed solution

・High exposure level

The stock solution was prepared by dissolving 2.0 g of D-1 and 80 g of HCO-20 in 500mL of THF. (Conc. of D-1 : 4.0 mg/mL, Conc. of HCO-20: 160 mg /mL)

・Low exposure level

Fifty mL of the feed solution of high exposure level was diluted to 500 mL with THF (Conc. of D-1 : 0.40 mg /mL, conc. of HCO-20 : 16 mg/mL).

・Control aquarium

The 160g of HCO-20 was dissolved in 1L of THF(stock solution). The 370mL of this solution was diluted to 11 L with deionized water. (Conc. of HCO-20 : 5.4 mg /mL).

The feed solutions were renewed every 7 -10 days.

(2) Preparation of test solution

The feed solution prepared by the procedure shown in 7.2(1) was supplied to mixing tube by feed solution supply pump and dilution water (dechlorinated tap water) was also supplied to the same tube. Then these were mixed and the concentration of test substance reached nominal value. The test solution in mixing tube was supplied to an aquarium. Dilution water is the tap water of Yokohama city which was dechlorinated with activated charcoal. (Fig.3 (p.29))

(3) Conditions

Flow rate of feed solution : 40m L/day for high and low exposure levels and 1.2 L/day for control level

Flow rate of dilution water : 1600 L/day (The turnover rate for each aquarium : 20 times/ day)

Test fish : Carp (Cyprinus carpio, Lot.No. 91-K-1206, aquarium No. B5)

- Body weight : 31～40 g
- Body length : 11～ 12 cm
- Fat content of fish : 3.7 % (n=4, 3.3～ 4.3%)
- Water temperature : 24.2～24.7℃
- Dissolved oxygen : 4.9 ～ 8.2 mg/L
- Feeding : Fish were fed with commercial pellets (Minipet®(Kyorin Co. Ltd.)) once a day except holidays.
- Nominal concentration of D-1 in water :

  High exposure level ; 0.1 μg/mL (conc. of HCO-20, 4mg/mL)

  Low exposure level ; 0.01 μg/mL (conc. of HCO-20, 0.4mg/mL)

  Control ; 0 μg/mL (conc. of HCO-20, 4mg/mL)
- Population density (at the biginning of the exposure period) :

  High exposure level ; 15 fish / 80L test water

  Low exposure leve ; 15 fish / 80L test water

  Control ; 12 fish / 80L test water

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· Exposure period : 8 weeks

(4) Introduction of fish, biological observation and environmental control

The test system was operated for 4 days prior to the introduction of fish to confirm the stability of the concentration of D-1 in water. After that, fish were introduced into the test system. Fish were observed for mortality, behavior and some abnormalities twice a week. Dissolved oxygen and temperature of test water were monitored twice a week. These results were recorded.

(5) Analysis of D-1 in test water

Aliquot of test water was withdrawn twice a week and was analyzed by the procedure shown in 4.3.

(6) Analysis of D-1 in test fish

Three fish were collected in 2, 4, 6 and 8 week after exposure. Two of them were analyzed by the procedure shown in 4.4.

* Flow rate of dilution water was 800 L/day in first 3 days , but because of concentration decrease of D-1 due to uptake by fish, it was changed to 1600 L/day after the day for each aquaria.

8. Results and Discussion

8.1 Circumstances that may have affected the reliability of the test results.

There was no significant matter that may have affected the reliability of the test results during the test period of 8 weeks.

8.2 Biological observations of test fish

Neither mortality nor abnormality in appearance or behavior was observed in treated and control fish during the test period of 8 weeks.

8.3 Concentrations of test substance in test water

The results are shown in Table 4, 5(p.42, 43) and Fig.14, 15(p.44). (Appendix-2) The mean values during the exposure period were 0.092 μg/mL (high exposure level) and 0.0082 μg/mL (low exposure level).

The decreases of D-1 concentration in both levels were observed at 3 days from the initiation of test. This phenomena will be attributable to uptake of D-1 by fish. Therefore, flow rates of the feed solution for both aquaria were increased by a factor of two, and as a result, each concentration is approached to each nominal value. The coefficients of variation are 8.4 % (high exposure level) and 13.5 % (low exposure level), respectively.

8.4 Concentrations of test substance in test fish and bioconcentration factors(BCFs)

The results are shown in Table 6, 7 (p.45, 46) and Fig.16 ~18 (p.47~55). Concentrations of D-1 in fish are 0.032~0.062 μg/g (high exposure level) and 0.013 ~0.034 μg/g (low exposure level). The BCFs are 0.4~0.7 (high exposure level) and 2~4 (low exposure level).

Peaks observed at the retention time from 1 min. to 3min. will be considered as metabolites of D-1.

On the basis of the above results, it can be concluded that sample D-1 is not bioaccumulative in carp under the prescribed test conditions.

Fig. 1 Infrared spectrum of D-1 (before starting the study)

X: 4000.0, 400.00 cm-1: 0.06, 65.92 %T 92/05/20 11:00
4 scans; mode ratio; resol 4.00 cm-1; apod strong
28121G D-1 Lot.No.101 N.Saeki
threshold 3.00%; band

cm-1	%	cm-1	%	cm-1	%	cm-1	%
2986.8	46.13	1730.3	13.67	1638.4	46.05	1619.8	53.28
1468.6	46.61	1392.0	16.90	1202.5	6.36	1148.9	7.71
1062.0	29.33	1017.7	28.37	984.6	27.24	857.9	57.01
808.0	35.17	784.1	48.20	746.7	44.54	735.7	45.63
706.4	44.98	653.5	33.43				

18 peaks found

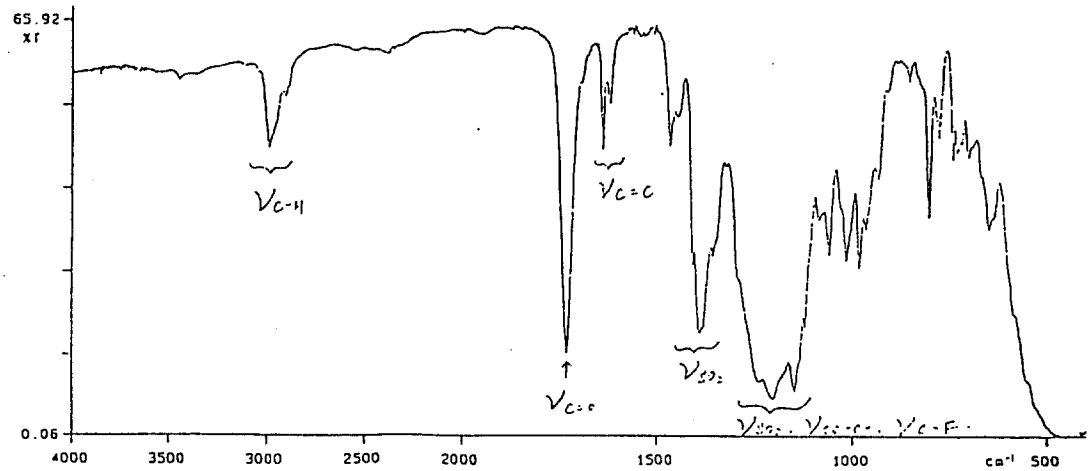

Fig. 2 Infrared spectrum of D-1 (at the termination of the study)

X: 4000.0, 400.00 cm-1: 0.23, 76.30 %T 92/08/07 09:57
4 scans; mode ratio; resol 4.00 cm-1; apod strong
28121G D-1 Lot.No.101 N . Saeki
threshold 3.00%; band

cm-1	%	cm-1	%	cm-1	%	cm-1	%
3854.2	70.59	3751.9	71.78	3676.2	71.50	3650.1	71.29
3630.0	71.45	3587.7	71.30	2987.1	64.35	1731.7	29.76
1638.4	60.89	1467.8	60.04	1392.9	31.11	1202.3	23.91
1062.4	46.04	1018.3	44.44	985.2	42.65	808.2	49.13
784.3	59.95	746.9	56.19	735.7	56.80		

19 peaks found

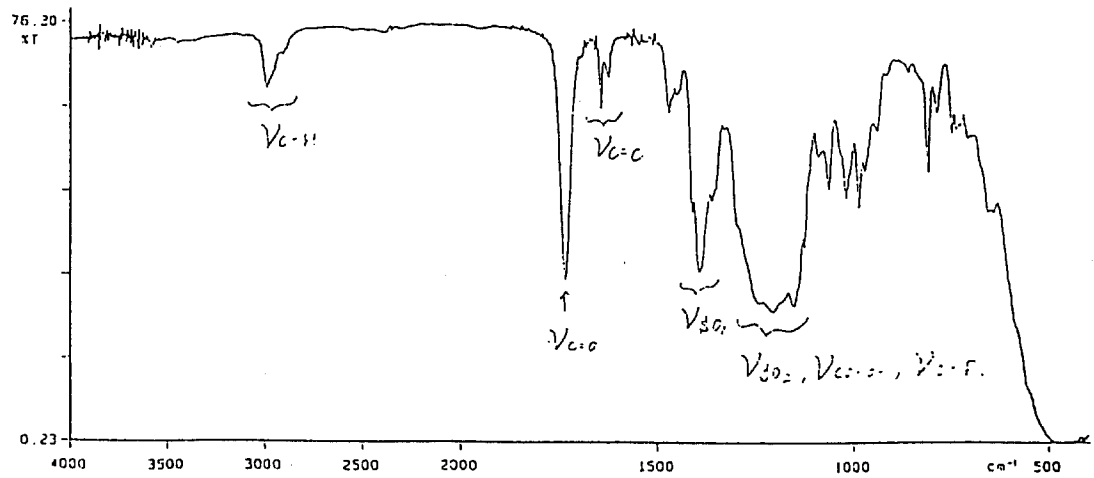

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Fig. 3 Bioconcentration test system

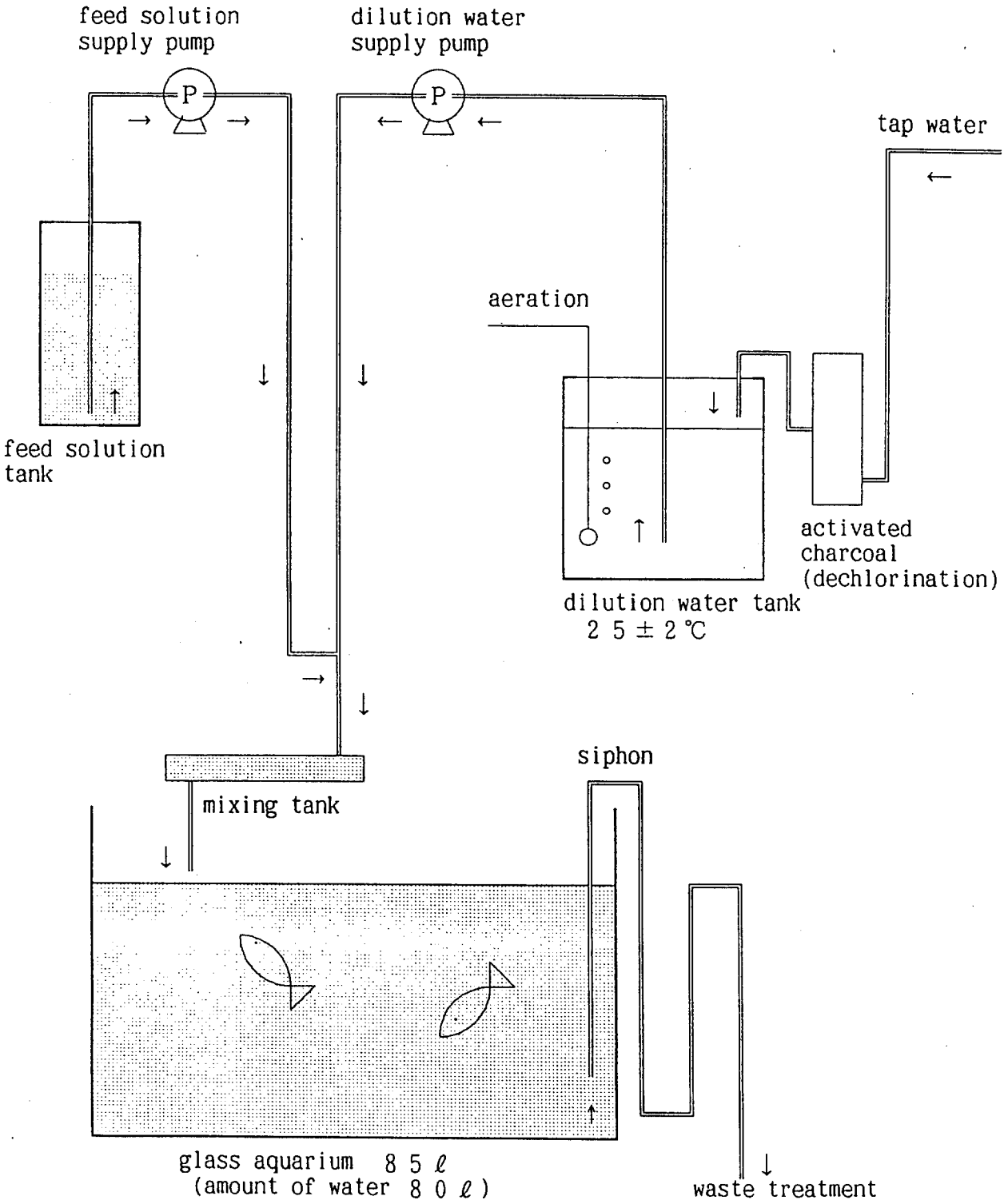


Fig.4 Gas chromatograms of D-1(Standard solution) for calibration curve

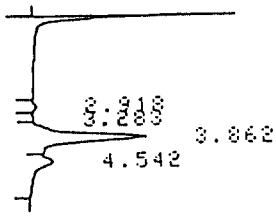
0 μg/ml (n-hexane only)

START

WARNING NO PEAK

0.25 μg/ml

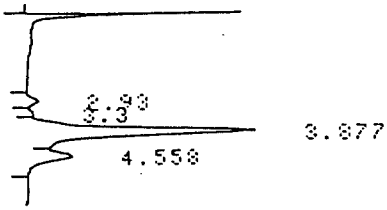
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.918	8026			2.0726
2	3.283	5253			1.3564
3	3.862	299049	V		77.2208
4	4.542	74936	V		19.3502
	TOTAL	387265			100

0.50 μg/ml

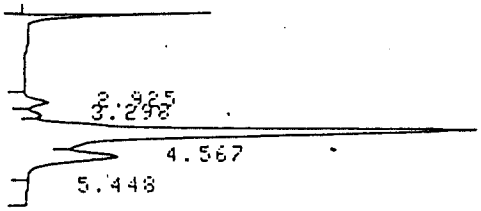
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.93	18629			2.4738
2	3.3	11731	V		1.5579
3	3.877	594555	V		78.9546
4	4.558	128119	V		17.0137
	TOTAL	753034			100

1.00 μg/ml

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.925	40165			2.6298
2	3.298	26487	V		1.7342
3	3.877	1179476	V		77.2246
4	4.567	271999	V		17.8087
5	5.448	9205	V		0.6027
	TOTAL	1527332			100

Fig.5 Calibration curve of D-1

$$Y = 3.8338 \times 10^3 + 1.4473 \times 10^6 \times X^1$$
$$r = 0.99995$$

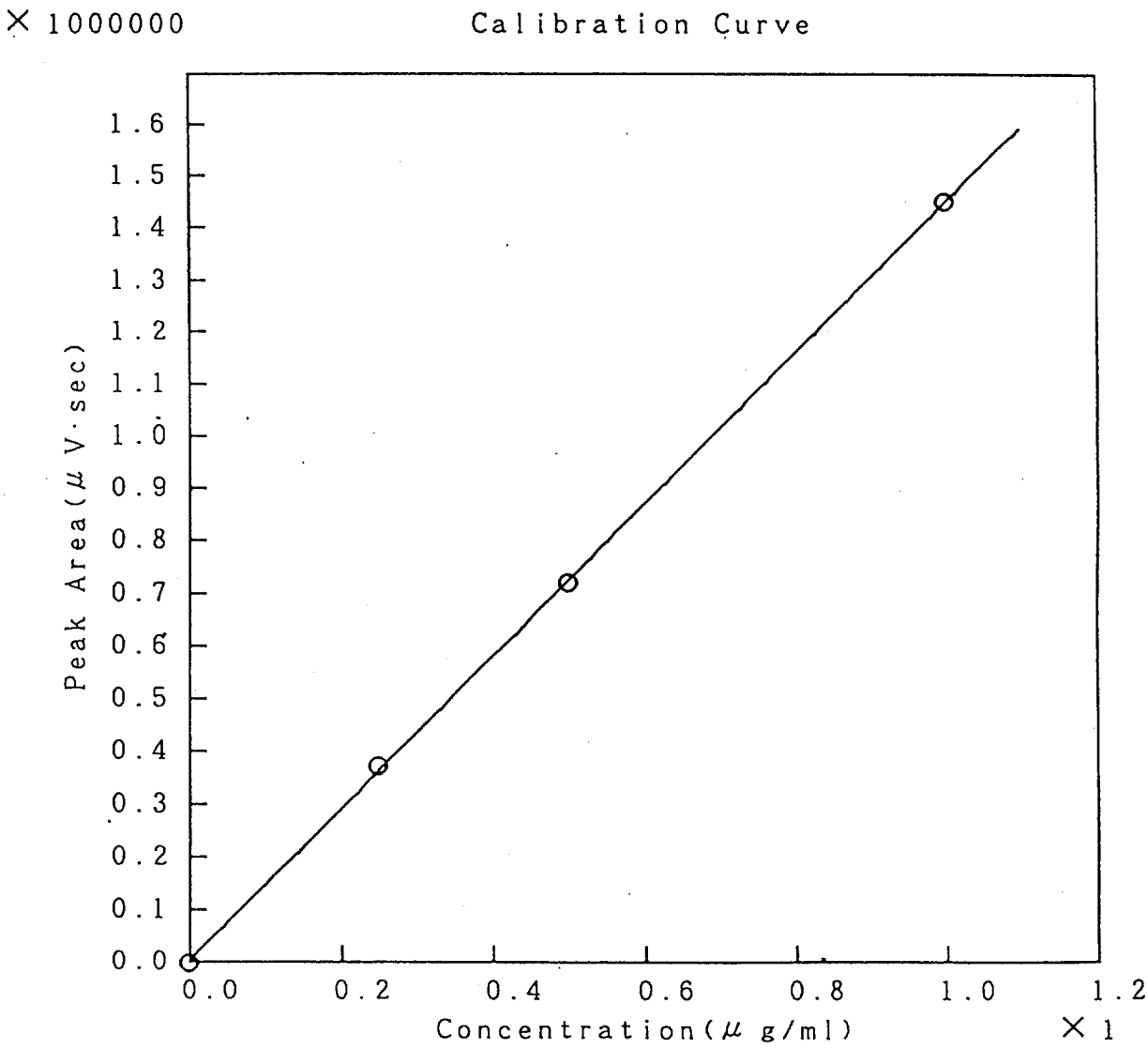
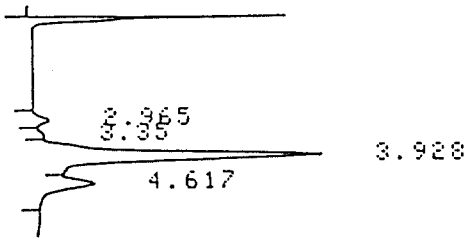


Fig.6 Gas chromatograms (Blank test from water)

Std.0.50 μg/ml

1

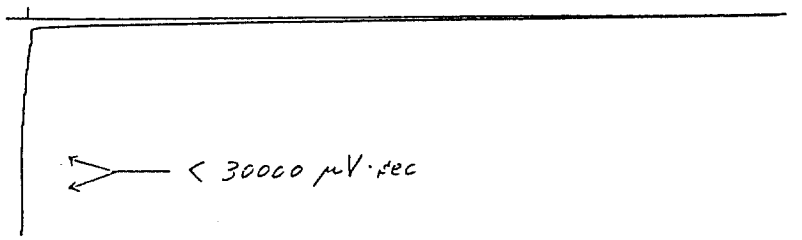
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.965	20297			2.5362
2	3.35	13721	V		1.7146
3	3.928	628854	V		78.5797
4	4.617	137403	V		17.1695
	TOTAL	800275			100

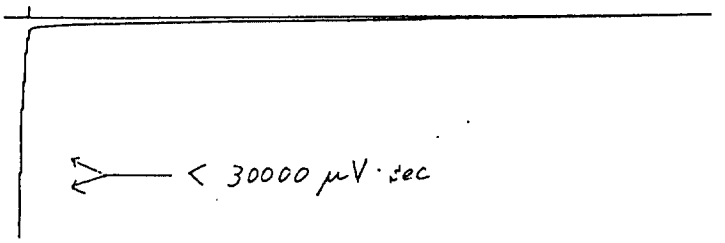
2

START



WARNING NO PEAK

START



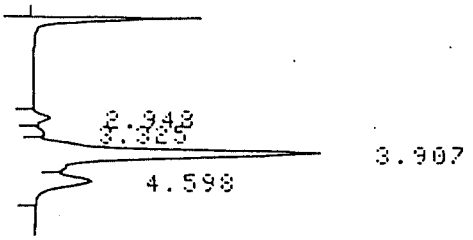
WARNING NO PEAK

Fig.7-1 Gas chromatograms (Blank test from fish)

(before starting the study)

Std.0.50 μg/ml

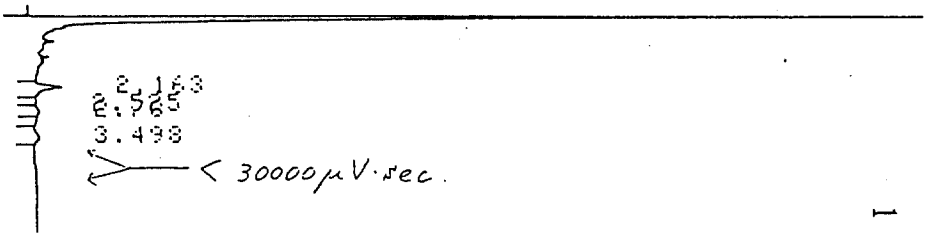
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.948	21215			2.6632
2	3.325	14409	V		1.8088
3	3.907	625133	V		78.4757
4	4.598	135839	V		17.0524
	TOTAL	796595			100

1

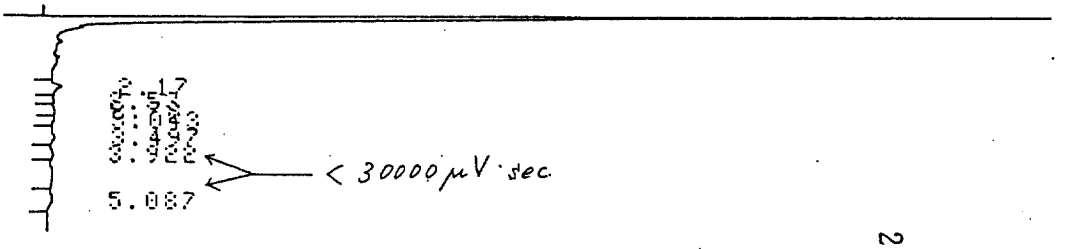
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.163	26237			62.7341
2	2.78	5053	V		12.0813
3	3.498	10533			25.1846
	TOTAL	41823			100

2

START



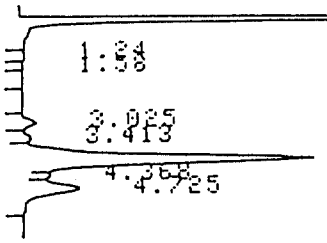
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.17	11941			27.8025
2	2.78	8120	V		18.905
3	3.497	8755			20.3835
4	3.922	5657			13.1711
5	5.087	8477			19.7379
	TOTAL	42949			100

Fig.7-2 Gas chromatograms (Blank test from fish)

(at the termination of the study)

Std.0.50 μg/ml

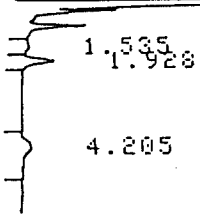
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.025	19079			2.3534
2	3.413	12804	V		1.5793
3	4.01	639675	SV		78.9034
4	4.725	139148	V		17.1639
	TOTAL	810706			100

3

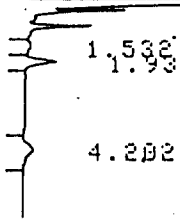
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	1.535	8144			10.3422
2	1.928	35586			45.1906
3	4.205	35016			44.4672
	TOTAL	78746			100

4

START



< 30000 μV·sec

PKNO	TIME	AREA	MK	IDNO	CONC
1	1.532	7659			11.3524
2	1.93	33256			49.2902
3	4.202	26554			39.3574
	TOTAL	67470			100

Table 1 Recovery test from water

W		A	B	C	D	E	R	
amount of water		added amount	peak area μV・sec		conc. in final volume	measured amount	recovery	Fig. No.
mL		μg	Sample	Std.	μg/mL	μg	%	
1	1000	10	723856	762903	0.474	9.48	94.8	8
2	1000	10	720337	762903	0.472	9.44	94.4	8

Average : 94.6 %

Concentration of standard solution(Cstd.) : 0.56 mg/mL
Final volume (V) :20 mL

Calculation

$$D = Cstd \times B / C \quad ; \quad E = D \times V \quad ; \quad R = E / A \times 100$$

Table 2 Recovery test from fish

W		A	B	C	D	E	R	
Fish weight		added amount	peak area μV・sec		conc. in final volume	measured amount	recovery	Fig. No.
g		μg	sample	Std.	μg/mℓ	μg	%	
1	36.92	10	803266	813996	0.493	9.86	98.6	9
2	34.25	10	813149	813996	0.499	9.98	99.8	9

Average : 99.2 %

Concentration of standard solution(CStd.) : 0.5 μg/mL
Final volume (V) :20.0mL

Calculation

$$D = Cstd \times B / C \quad ; \quad E = D \times V \quad ; \quad R = E / A \times 100$$

Table 3 Acute toxicity test (48 hrs)

D - 1 μg/mL	number of test fish	dead fish	mortality %
0 (control)	10	0	0
100	10	0	0

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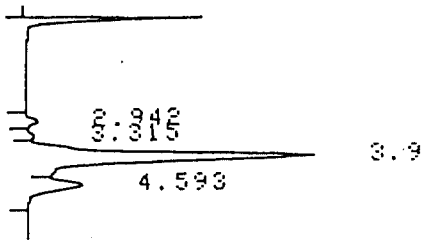
48hr-LC_{50} = >100 μg/mL

Gas chromatograms (Recovery test from water)

Std.0.50μg/ml

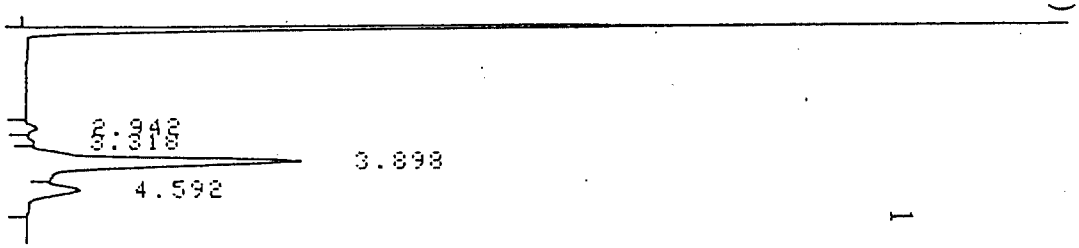
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.942	19610			2.4623
2	3.315	13901	V		1.7455
3	3.9	625788	V		78.5757
4	4.593	137115	V		17.2165
	TOTAL	796415			100

1

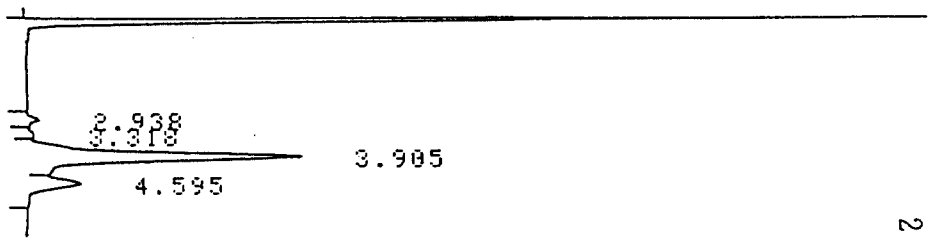
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.942	16717			2.2177
2	3.318	13213	V		1.7529
3	3.898	593258	V		78.7037
4	4.592	130598	V		17.3256
	TOTAL	753786			100

2

START

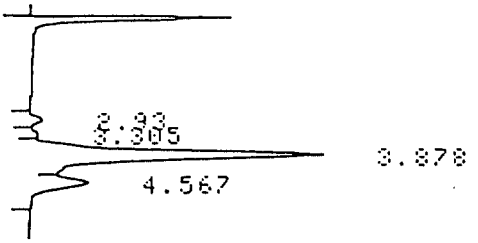


PKNO	TIME	AREA	MK	IDNO	CONC
1	2.938	16595			2.215
2	3.318	12282	V		1.6393
3	3.905	592950	V		79.1429
4	4.595	127387	V		17.0027
	TOTAL	749214			100

Gas chromatograms (Recovery test from fish)

Std.0.50μg/ml

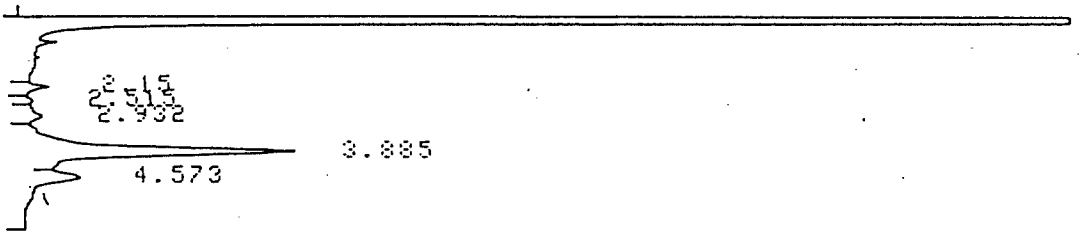
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.93	20325			2.3961
2	3.305	13939	V		1.6432
3	3.878	667862	V		78.7332
4	4.567	146134	V		17.2275
	TOTAL	848260			100

1

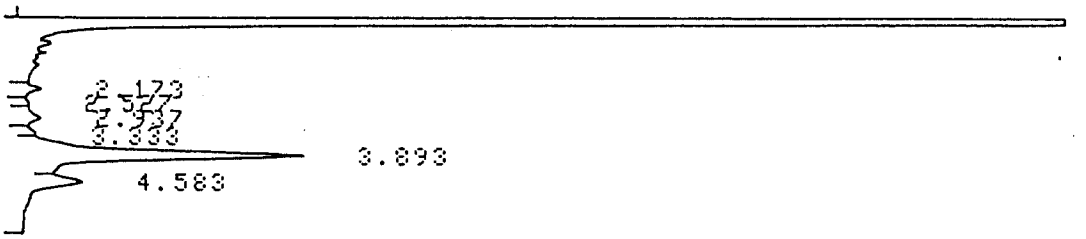
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.15	20166			2.3366
2	2.515	6354			0.7362
3	2.932	33246	V		3.8523
4	3.885	650164	V		75.3348
5	4.573	153102	V		17.74
	TOTAL	863032			100

2

START

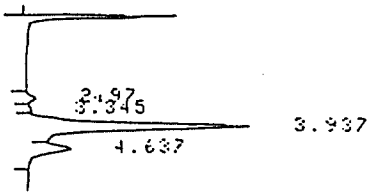


PKNO	TIME	AREA	MK	IDNO	CONC
1	2.173	15969			1.8185
2	2.937	32435	V		3.6937
3	3.333	16576	V		1.8876
4	3.893	657675	V		74.8951
5	4.583	155474	V		17.7051
	TOTAL	878128			100

Fig. 10 Gas chromatograms (Stability in water)

Std. 0.50 μg/ml

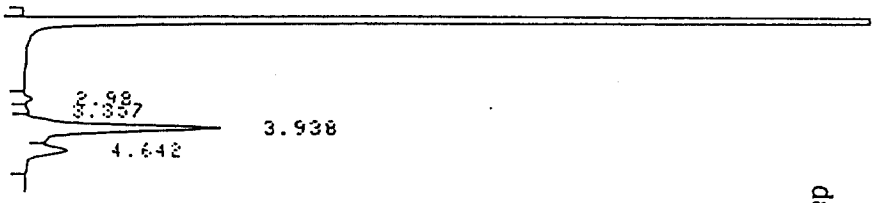
START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.97	18144			2.2954	
2	3.345	11585	V		1.4656	
3	3.937	624824	V		79.0451	
4	4.637	135911	V		17.1938	
	TOTAL	790465			100	

day 0

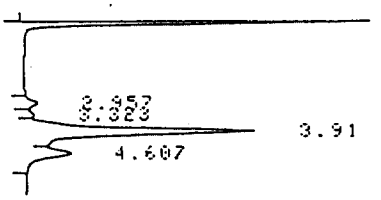
START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.98	17187			2.405	
2	3.357	11017	V		1.5416	
3	3.938	551147	V		77.1226	
4	4.642	135287	V		18.9309	
	TOTAL	714637			100	

Std. 0.50 μg/ml

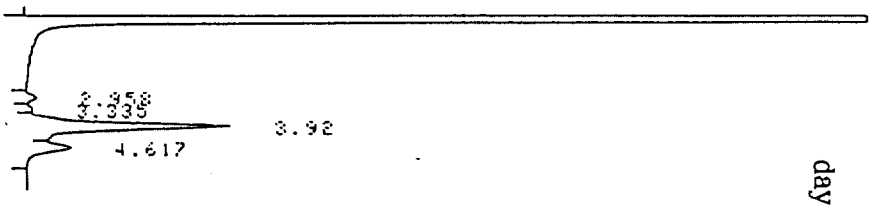
START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.957	20717			2.5946	
2	3.323	13763	V		1.7237	
3	3.91	627773	V		78.6225	
4	4.607	136211	V		17.0592	
	TOTAL	798464			100	

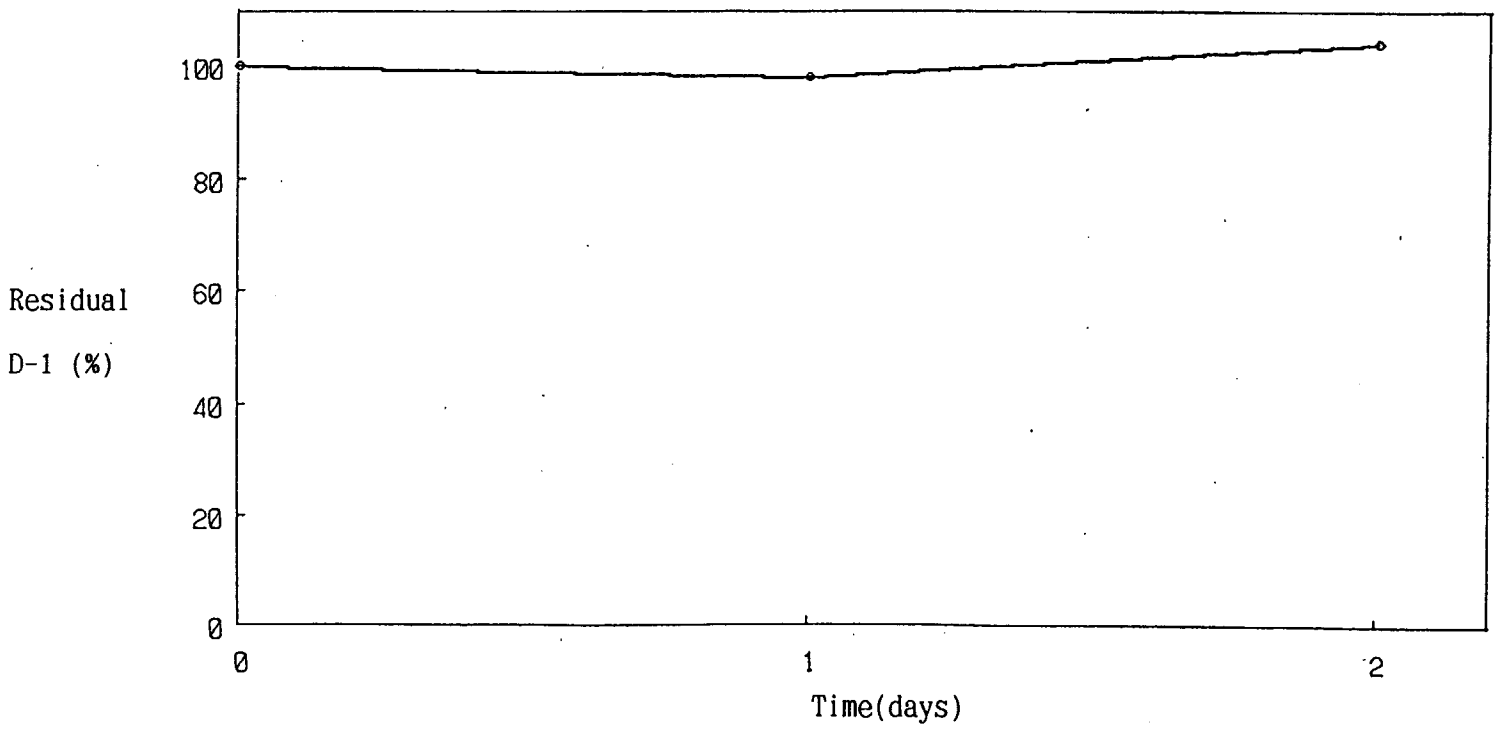
day 2

START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.958	17479			2.3471	
2	3.335	11633	V		1.5621	
3	3.92	576722	V		77.4419	
4	4.617	138882	V		18.6489	
	TOTAL	744716			100	

Fig.11 Stability in water

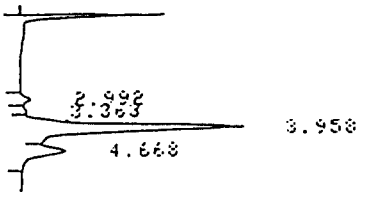


000585

Fig. 12 Stabirity in feed solution in bioaccumulation test

Std.0.50 μg/ml

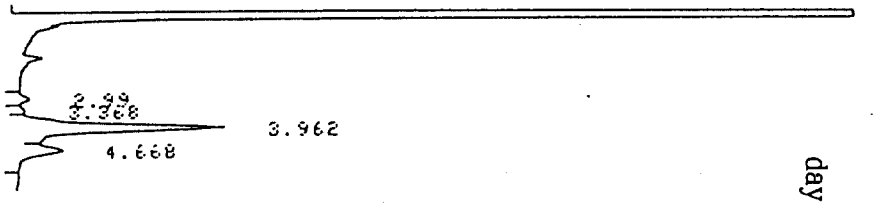
START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.992	18283			2.3097	
2	3.363	11934	V		1.5077	
3	3.958	625846	V		79.0634	
4	4.668	135512	V		17.1192	
	TOTAL	791574			100	

day 0

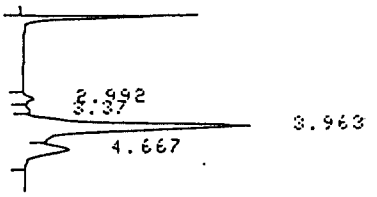
START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.99	18476			2.3703	
2	3.368	11096			1.4235	
3	3.962	605198	V		77.6418	
4	4.668	144705	V		18.5644	
	TOTAL	779474			100	

Std.0.50 μg/ml

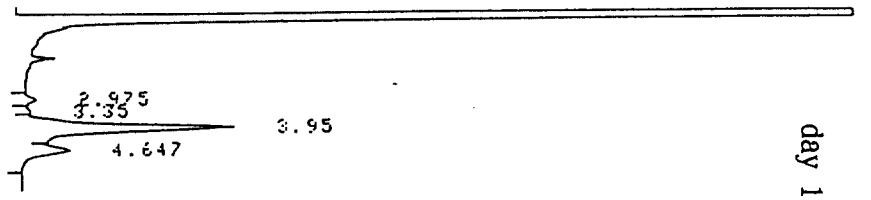
START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.992	19238			2.2871	
2	3.37	13961	V		1.6598	
3	3.963	662222	V		78.7271	
4	4.667	145740	V		17.326	
	TOTAL	841161			100	

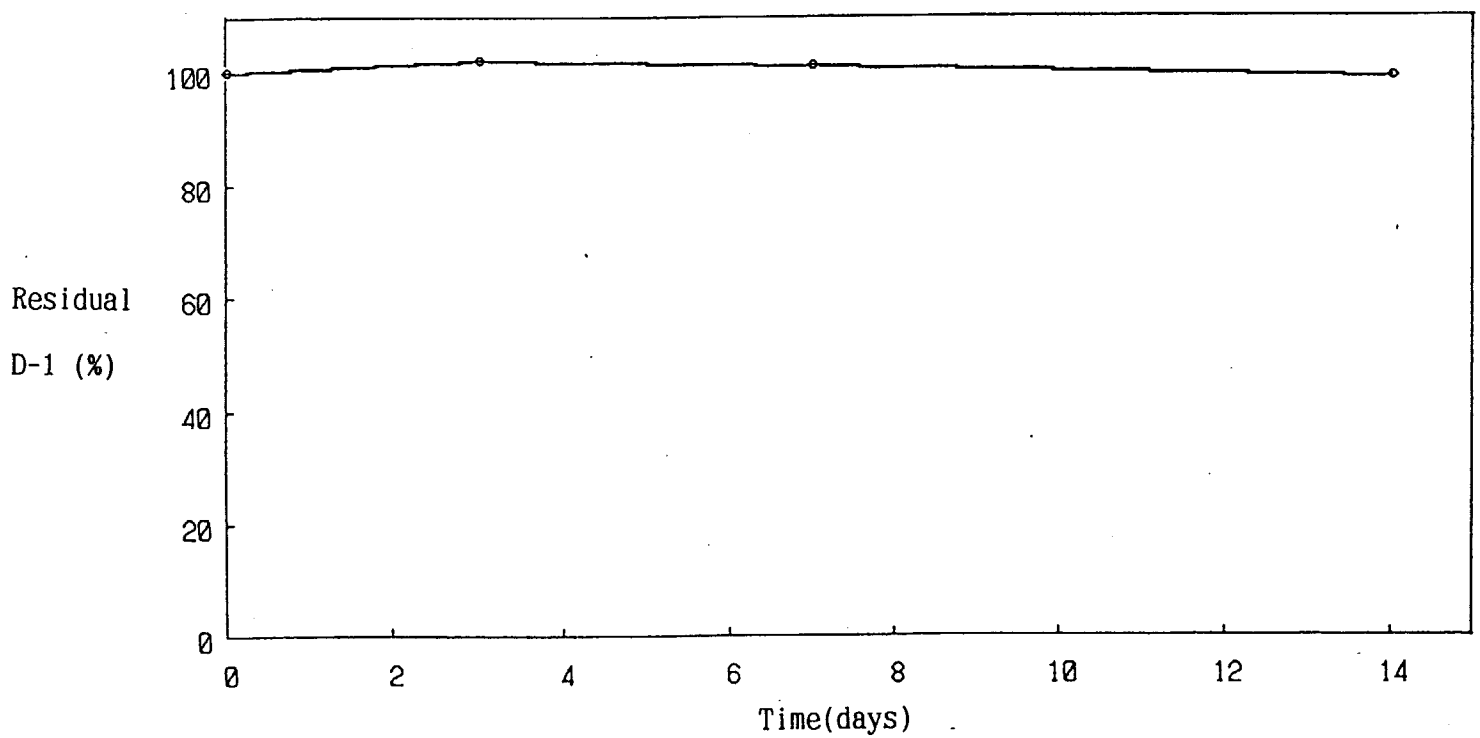
day 14

START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.975	20201			2.4675	
2	3.35	12086	V		1.4762	
3	3.95	635744	V		77.6537	
4	4.647	150660	V		18.4025	
	TOTAL	818691			100	

Fig. 13 Stability in feed solution



000587

Table 4 Analysis of D-1 in test water (High exposure level)

			i	A	B	C	E	
sampling date	exposure period			peak area μV・sec		conc. in water	mean conc.	Fig. No. *
	day	week		Sample	Std.	μg/mL	μg/mL	
5.26, '92	0	0	1	691668	761885	0.096	0.096	1-1
29	3		2	551911	768251	0.076	0.086	2
6. 2	7	1	3	665450	759199	0.093	0.088	3
5	10		4	684891	764544	0.095	0.090	4
9	14	2	5	655152	752597	0.092	0.090	5
12	17		6	742762	794882	0.099	0.092	6
16	21	3	7	600994	794596	0.080	0.090	7
19	24		8	705686	824875	0.090	0.090	8
23	28	4	9	716171	792816	0.095	0.091	9
26	31		10	687002	809639	0.090	0.091	10
30	35	5	11	776203	831148	0.099	0.091	11
7. 3	38		12	709193	787890	0.095	0.092	12
7	42	6	13	674771	771065	0.093	0.092	13
10	45		14	742351	808504	0.097	0.092	14
14	49	7	15	701150	791505	0.094	0.092	15
17	52		16	544487	775383	0.074	0.091	16
21	56	8	17	733512	779453	0.099	0.092	17

(* Appendix-2)

Concentration of standard solution Cstd : 0.5 μg/mL
Final volume V : 20 mL
Sample volume W : 100 mL
Recovery R : 94.6 %

Calculation

$C = Cstd \times A / B \times F$; $(F = V / W / (R / 100))$

000588 $E = \Sigma Ci / i$ (i: number of analysis)

Mean concentration in water ± standard deviation : 0.092 ± 0.0077 μg/mL

Table 5 Analysis of D-1 in test water (Low exposure level)

			i	A	B	C	E	
sampling date	exposure period			peak area μV・sec		conc. in water	mean conc.	Fig. No. *
	day	week		Sample	Std.	μg/mL	μg/mL	
5.26, '92	0	0	1	738542	761885	0.0102	0.0102	1-1
29	3		2	359497	768251	0.0049	0.0076	2
6. 2	7	1	3	515797	759199	0.0072	0.0047	3
5	10		4	599070	764544	0.0083	0.0077	4
9	14	2	5	570779	752597	0.0080	0.0077	5
12	17		6	572050	794882	0.0076	0.0077	6
16	21	3	7	659682	794596	0.0088	0.0079	7
19	24		8	572309	824875	0.0073	0.0078	8
23	28	4	9	676137	792816	0.0090	0.0079	9
26	31		10	689946	809639	0.0090	0.0080	10
30	35	5	11	701085	831148	0.0089	0.0081	11
7. 3	38		12	636904	787890	0.0085	0.0081	12
7	42	6	13	615794	771065	0.0084	0.0082	13
10	45		14	662294	808504	0.0087	0.0082	14
14	49	7	15	606422	791505	0.0081	0.0082	15
17	52		16	591058	775383	0.0081	0.0082	16
21	56	8	17	600028	779453	0.0081	0.0082	17

(* Appendix-2)

Concentration of standard solution Cstd : 0.5 μg/mL
Final volume V : 20 mL
Sample volume W : 1000 mL
Recovery R : 94.6 %

Calculation

$C = Cstd \times A / B \times F \quad (F = V / W / (R / 100))$

000589 $E = \Sigma \; C_i / i$ (i: number of analysis)

Mean concentration in water ± standard deviation : 0.0082 ± 0.00111ng /mL

Fig. 14 Concentrations of test substance in water High exposure level

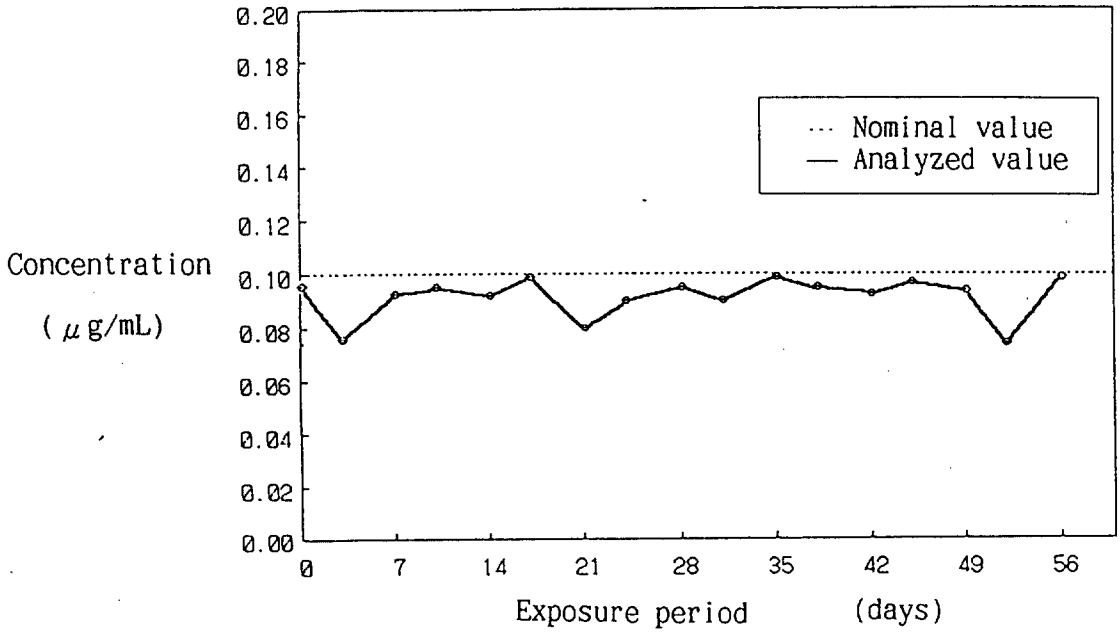


Fig. 15 Concentrations of test substance in water Low exsposure level

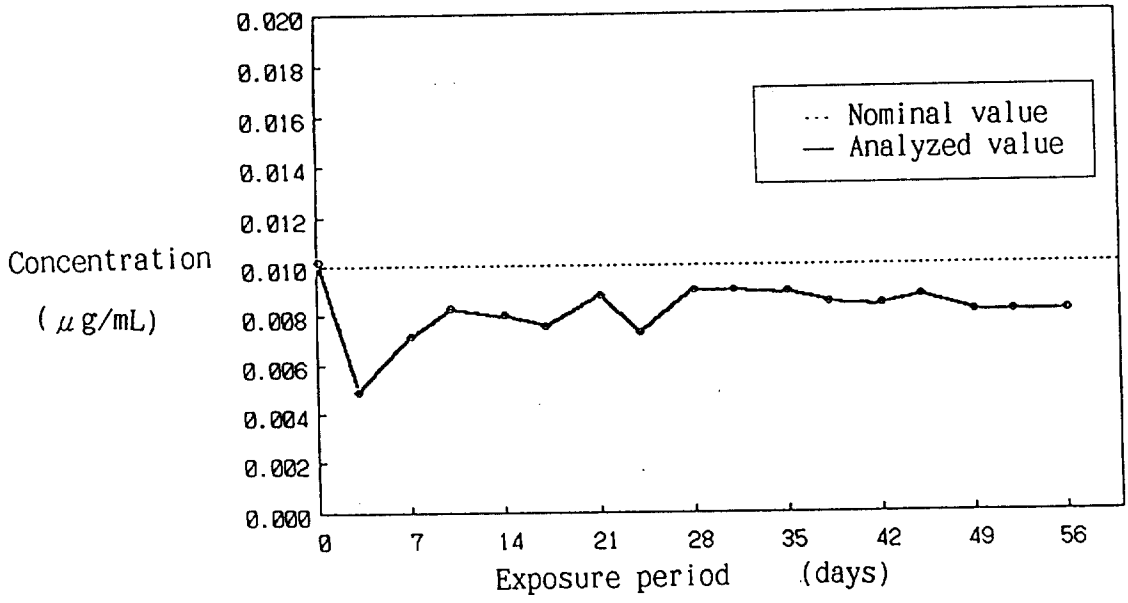


Table 6 Analysis of D-1 in test fish (High exposure level)

		W	A	B	C	D	E	F	
exposure period		fish weight	peak area μV・sec		conc. in final volume	conc. in fish measured	mean conc. in water	BCF	Fig. No.
week		g	Sample	Std.	μg/mL	μg/g	μg/mL		
2	1	34.14	90324	839568	0.054	0.032	0.090	0.4	18-1
	2	34.80	94243	796908	0.059	0.034	0.090	0.4	18-1
4	1	37.18	168720	807176	0.105	0.057	0.091	0.6	18-2
	2	39.96	192918	790270	0.122	0.062	0.091	0.7	18-2
6	1	36.95	161768	839289	0.096	0.052	0.092	0.6	18-3
	2	37.47	113668	804605	0.071	0.038	0.092	0.4	18-3
8	1	35.54	132230	879847	0.075	0.043	0.092	0.5	18-4
	2	36.77	145406	885954	0.082	0.045	0.092	0.5	18-4

Concentration of standard solution(Std.) Cstd : 0.5 μg/mL
Final volume V : 20 mL
Recovery R : 99.2 %

Calculation

$C = Cstd \times A / B$; $D = C \times V / W \times 100 / R$

$F = D / E$

Table 7 Analysis of D-1 in test fish (Low exposure level)

		W	A	B	C	D	E	F	
exposure period		fish weight	peak area μV・sec		conc. in final volume	conc. in fish measured	mean conc. in water	BCF	Fig. No.
week		g	Sample	Std.	μg/mL	μg/g	μg/mL		
2	1	34.73	72837	839568	0.043	0.025	0.0077	3	18-5
	2	35.61	61435	796908	0.039	0.022	0.0077	3	18-5
4	1	34.23	93655	807176	0.058	0.034	0.0079	4	18-6
	2	35.70	63936	790270	0.040	0.023	0.0079	3	18-6
6	1	37.23	38016	804605	0.024	0.013	0.0082	2	18-7
	2	40.00	53795	798557	0.034	0.017	0.0082	2	18-7
8	1	35.27	45555	851984	0.027	0.015	0.0082	2	18-8
	2	35.73	46433	864383	0.027	0.015	0.0082	2	18-8

Concentration of standard solution(Std.) Cstd : 0.5 μg/mL
Final volume V : 20 mL
Recovery R : 99.2 %

Calculation

$C = Cstd \times A / B$; $D = C \times V / W \times 100 / R$

$F = D / E$

Fig. 16 Bioconcentration factors (BCFs) of D-1 High exposure level

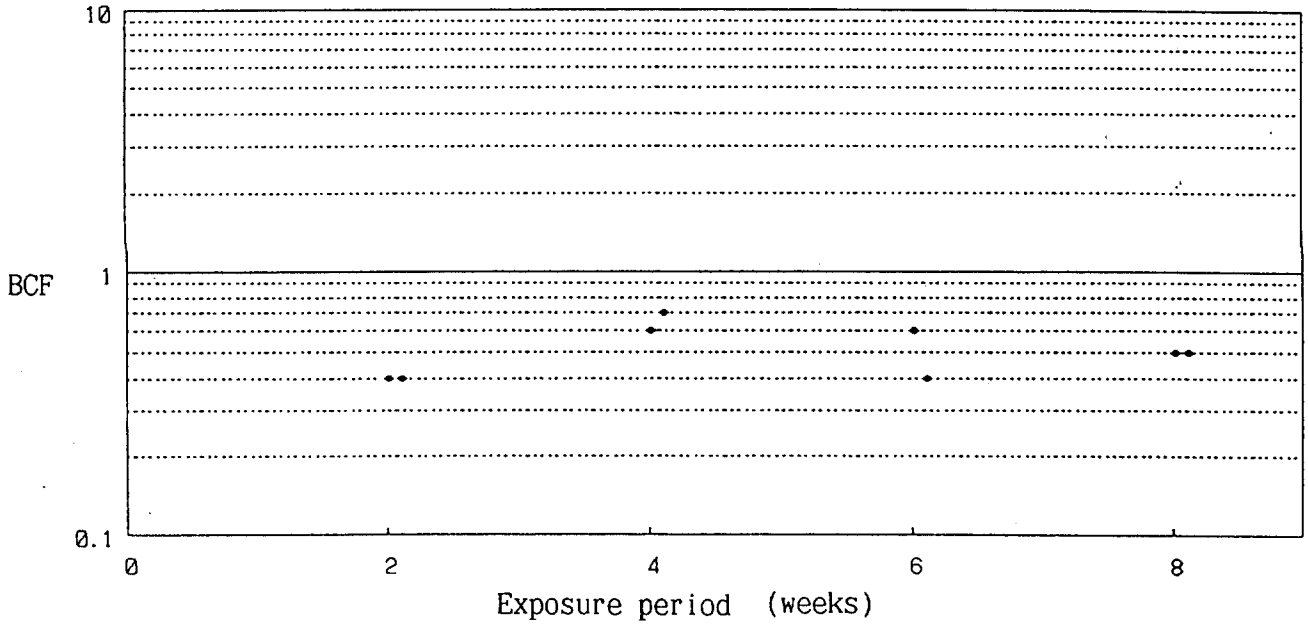


Fig. 17 Bioconcentration factors (BCFs) of D-1 Low exsposure level

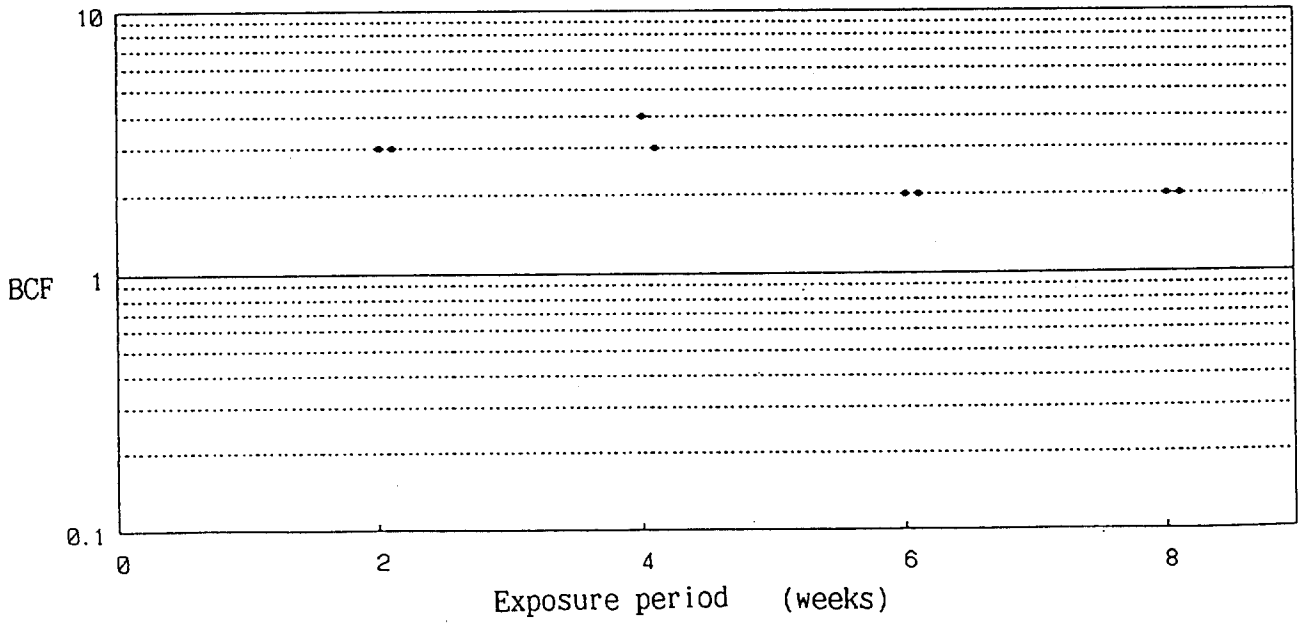


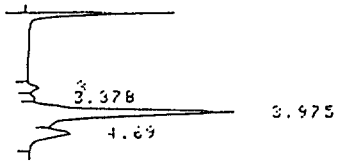
Fig.18-1 Gas chromatograms (Analysis of test fish)

— High exposure level —

Std.0.50 μg/ml

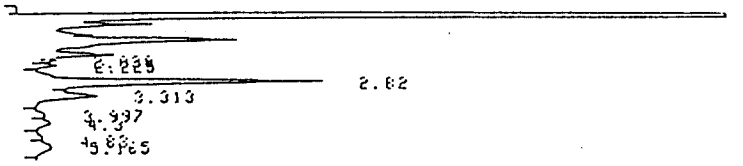
week 2
No. 1

START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3	21899			2.4988	
2	3.378	14921	V		1.7026	
3	3.975	688849	V		78.6009	
4	4.69	150719	V		17.1977	
	TOTAL	876388			100	

START

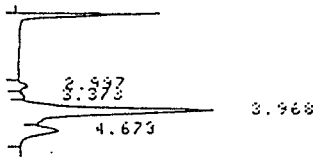


PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.225	16851			1.746	
2	2.82	617839			64.0153	
3	3.313	177252	V		18.3654	
4	3.997	27456			2.8443	
5	4.3	43991	V		4.558	
6	4.83	18377	V		1.9559	
7	5.165	62874	V		6.5145	
	TOTAL	965141			100	

Std.0.50 μg/ml

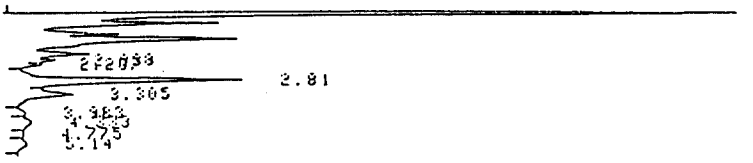
week 2
No. 2

START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.997	18595			2.2492	
2	3.373	11236	V		1.3591	
3	3.968	655877	V		79.3329	
4	4.673	141031	V		17.0587	
	TOTAL	826740			100	

START



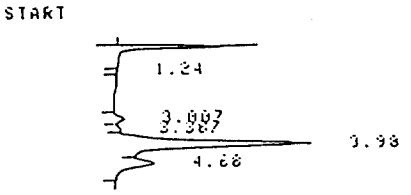
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.038	6617			0.8577	
2	2.207	9363			1.2136	
3	2.81	469854			60.9007	
4	3.305	156703	V		20.3113	
5	3.983	20682			2.6807	
6	4.333	55854	V		7.2396	
7	4.775	17707	V		2.2951	
8	5.14	34727	V		4.5012	
	TOTAL	771509			100	

000594

Fig. 18-2 Gas chromatograms (Analysis of test fish)

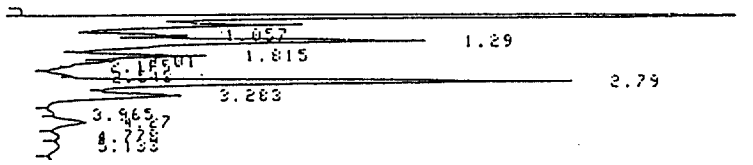
— High exposure level —

Std. 0.50 μg/ml



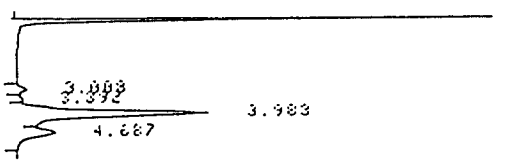
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.007	20342			2.4131	
2	3.387	13694	V		1.6279	
3	3.98	662318	V		78.7338	
4	4.68	144858	V		17.2201	
	TOTAL	841211			100	

week 4
No. 1



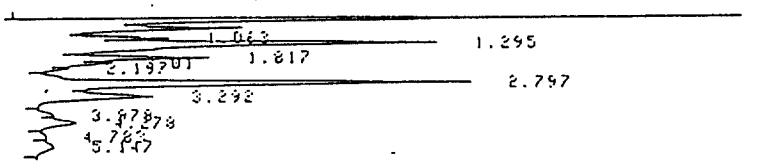
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.057	30556			1.1685	
2	1.29	626677			23.9654	
3	1.815	194391			7.4339	
4	2.01	49740	V		1.9021	
5	2.175	5320			0.2034	
6	2.548	36284			1.3876	
7	2.79	1099665	V		42.0534	
8	3.283	359586	V		13.7513	
9	3.965	18810			0.7193	
10	4.27	114698	V		4.3863	
11	4.778	35212	V		1.3466	
12	5.133	43987	V		1.6822	
	TOTAL	2614927			100	

Std. 0.50 μg/ml



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.008	18556			2.257	
2	3.392	13323	V		1.6205	
3	3.983	647973	V		78.8145	
4	4.687	142297	V		17.308	
	TOTAL	822150			100	

week 4
No. 2

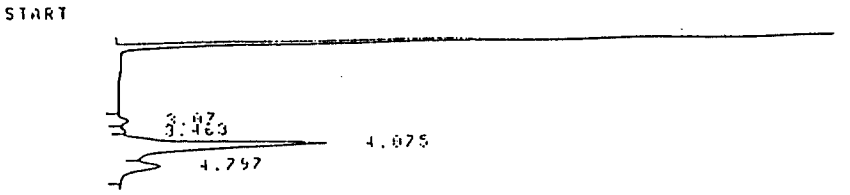


PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.063	33016			1.3371	
2	1.295	676331			27.3909	
3	1.817	197896			8.0147	
4	2.01	50849	V		2.0593	
5	2.197	12586			0.5097	
6	2.797	930669			37.6914	
7	3.292	306954	V		12.4314	
8	3.978	42276			1.7122	
9	4.278	119995	V		4.8597	
10	4.783	30647	V		1.2412	
11	5.147	67961	V		2.7524	
	TOTAL	2469181			100	

Fig. 18-3 Gas chromatograms (Analysis of test fish)

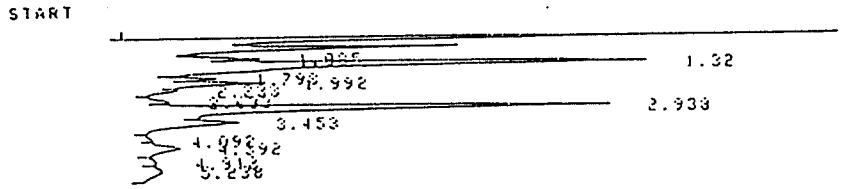
— High exposure level —

Std. 0.50 μg/ml



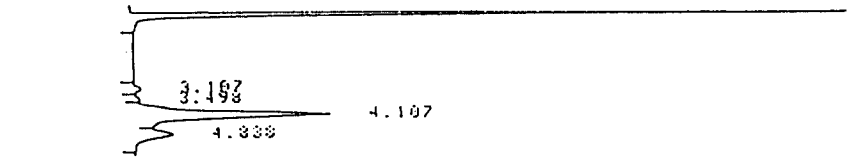
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.07	20996			2.4017	
2	3.463	13919	V		1.5921	
3	4.075	689392	V		78.8595	
4	4.797	149897	V		17.1467	
	TOTAL	874203			100	

week 6 No. 1



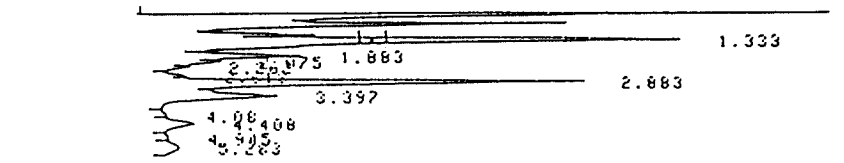
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.085	51438			1.8378	
2	1.32	960403	V		34.3138	
3	1.798	58126			2.0767	
4	1.992	164295	V		5.87	
5	2.233	34433	V		1.2302	
6	2.633	34348			1.2451	
7	2.938	1027735	V		36.7195	
8	3.453	236231	V		8.4402	
9	4.092	15458			0.5523	
10	4.392	111682	V		3.9902	
11	4.912	34628	V		1.2372	
12	5.238	69606	V		2.4869	
	TOTAL	2798831			100	

Std. 0.50 μg/ml



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.107	17820			2.1328	
2	3.498	13081	V		1.5656	
3	4.107	659094	V		78.8857	
4	4.838	145511	V		17.4159	
	TOTAL	835505			100	

week 6 No. 2

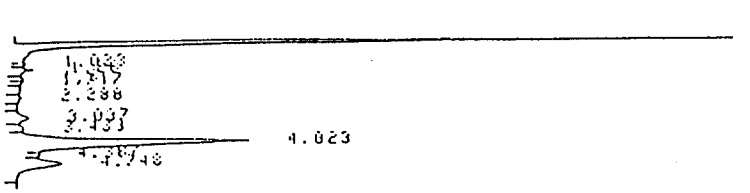


PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.1	103145			3.7683	
2	1.333	1029289	V		37.604	
3	1.883	171012			6.2478	
4	2.075	39547	V		1.4448	
5	2.262	9979			0.3646	
6	2.618	31831			1.1647	
7	2.883	862603	V		31.5143	
8	3.397	323153	V		11.8061	
9	4.08	10321			0.3771	
10	4.408	89255	V		3.2608	
11	4.945	14092	V		0.5148	
12	5.283	52900	V		1.9327	
	TOTAL	2737177			100	

Fig. 18-4 Gas chromatograms (Analysis of test fish)

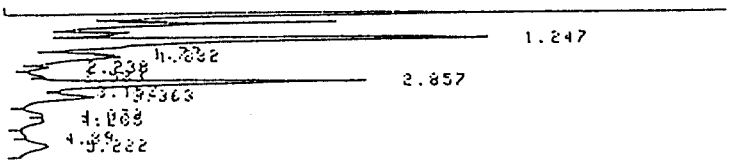
— High exposure level —

Std. 0.50 μg/ml



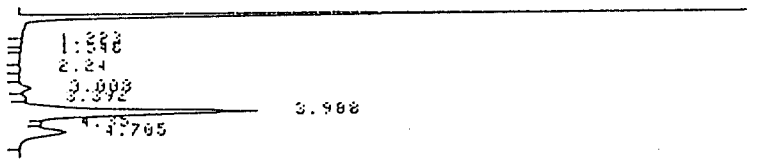
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.26	10192			1.0889	
2	1.6	5476			0.5851	
3	3.037	25349			2.7083	
4	3.433	15119	V		1.6154	
5	4.023	724795	SV		77.4367	
6	4.748	155052	V		16.5656	
	TOTAL	935983			100	

week 8 No. 1



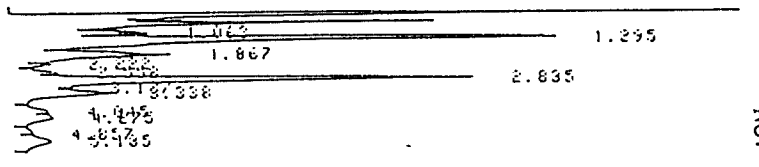
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.247	876064			39.2078	
2	1.77	73667			3.2969	
3	1.882	127216	V		5.6935	
4	2.238	18456			0.826	
5	2.583	29488			1.3197	
6	2.857	708399	SV		31.704	
7	3.363	171384	V		7.6702	
8	4.073	55055			2.464	
9	4.285	62817	V		2.8113	
10	4.89	14358			0.6426	
11	5.222	97511	V		4.3641	
	TOTAL	2234414			100	

Std. 0.50 μg/ml



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.008	23440			2.5347	
2	3.392	15362	V		1.6612	
3	3.988	728895	SV		78.8203	
4	4.705	157059	V		16.9839	
	TOTAL	924756			100	

week 8 No. 2



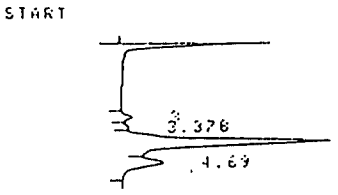
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.063	18429			0.7008	
2	1.295	962267			36.5932	
3	1.867	253568			9.6427	
4	2.222	12999			0.4943	
5	2.558	43157			1.6412	
6	2.835	911925	SV		34.6788	
7	3.338	193849	V		7.3717	
8	4.045	51798			1.9698	
9	4.275	78581	V		2.9883	
10	4.857	15027			0.5714	
11	5.185	88035	V		3.3478	
	TOTAL	2629632			100	

000597

Fig.18-5 Gas chromatograms (Analysis of test fish)

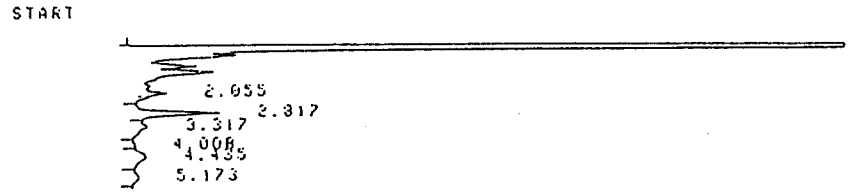
— Low exposure level—

Std.0.50 μg/ml



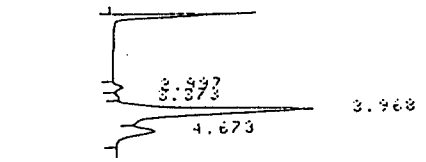
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3	21899			2.4988	
2	3.378	14921	V		1.7026	
3	3.975	688849	V		78.6009	
4	4.69	150719	V		17.1977	
	TOTAL	876388			100	

week 2
No. 1



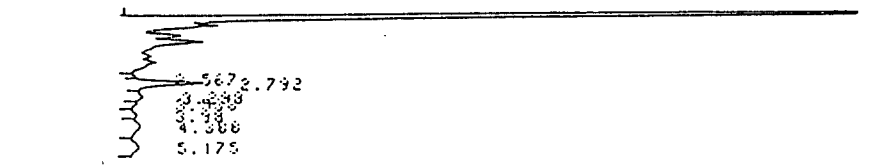
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.055	9129			2.7017	
2	2.317	174936			51.7735	
3	3.317	53912	V		15.9557	
4	4.008	8412			2.4896	
5	4.435	64425	V		19.0671	
6	5.173	27073	V		8.0125	
	TOTAL	337888			100	

Std.0.50 μg/ml



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.997	18595			2.2492	
2	3.373	11236	V		1.3591	
3	3.968	655877	V		79.3329	
4	4.673	141031	V		17.0587	
	TOTAL	826740			100	

week 2
No. 2



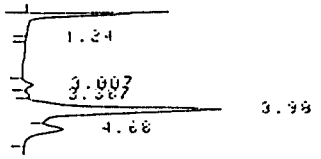
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.567	7704			2.5412	
2	2.792	150318	V		49.5867	
3	3.298	37928	V		12.5117	
4	3.533	9787	V		3.2285	
5	3.98	15856			5.2305	
6	4.388	45579	V		15.0355	
7	5.175	35970	V		11.8659	
	TOTAL	303141			100	

Fig.18-6 Gas chromatograms (Analysis of test fish)

— Low exposure level—

Std.0.50μg/ml

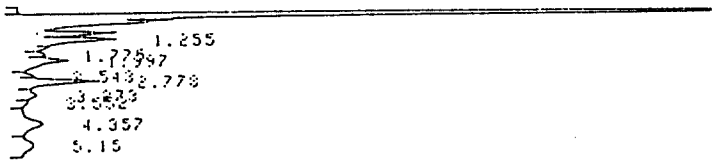
START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.007	20342			2.4181	
2	3.387	13694	V		1.6279	
3	3.98	662318	V		78.7338	
4	4.68	144858	V		17.2201	
TOTAL		841211			100	

week 4
No. 1

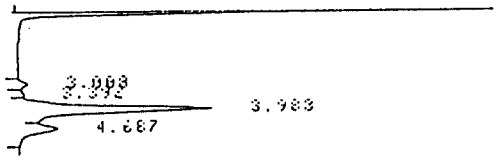
START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.255	95976			16.8791	
2	1.775	18292			3.2169	
3	1.997	84906	V		14.9322	
4	2.543	14179			2.4936	
5	2.778	154964	V		27.2532	
6	3.273	44587	V		7.8413	
7	3.552	9302	V		1.6359	
8	4.357	93655			16.471	
9	5.15	52749	V		9.2768	
TOTAL		568608			100	

Std.0.50μg/ml

START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.008	18556			2.257	
2	3.392	13323	V		1.6205	
3	3.983	647973	V		78.8145	
4	4.687	142297	V		17.308	
TOTAL		822150			100	

week 4
No. 2

START

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.265	192021			32.3603	
2	1.788	26276			4.4282	
3	2.002	10004	V		1.686	
4	2.222	9218			1.5535	
5	2.567	6386			1.0761	
6	2.795	179136	V		30.1889	
7	3.292	52169	V		8.7918	
8	3.562	8808	V		1.4843	
9	3.99	37967			6.3983	
10	4.333	25969	V		4.3764	
11	5.163	45430			7.6561	
TOTAL		593382			100	

000599

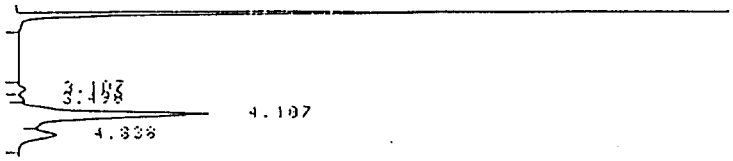
Fig. 18-7 Gas chromatograms (Analysis of test fish)

— Low exposure level—

Std. 0.50 μg/ml

week 6
No. 1

START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.107	17820			2.1328	
2	3.498	13081	V		1.5656	
3	4.107	653034	V		78.8857	
4	4.838	145511	V		17.4159	
	TOTAL	835505			100	

Std. 0.50 μg/ml

week 6
No. 2

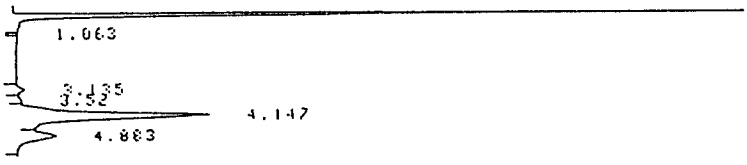
START

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.087	17334			2.3713	
2	1.322	286125	V		39.1416	
3	1.85	33241			4.5473	
4	2.075	15858	V		2.1694	
5	2.633	6451			0.8825	
6	2.09	225211	V		30.8086	
7	3.4	68115	V		9.3181	
8	4.1	6959			0.952	
9	4.457	31057	V		4.2486	
10	5.315	40648			5.5606	
	TOTAL	731000			100	

Std. 0.50 μg/ml

week 6
No. 2

START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.135	16317			1.9734	
2	3.52	11983	V		1.4492	
3	4.147	654673	V		79.2003	
4	4.883	143684	V		17.3771	
	TOTAL	826656			100	

START

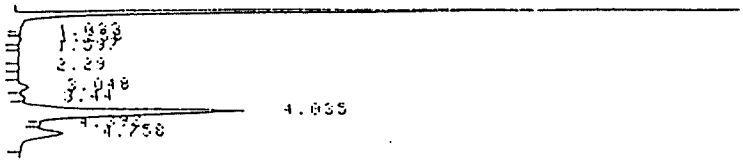
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.073	40008			4.346	
2	1.323	296921			32.2542	
3	1.867	42823			4.6518	
4	2.092	55448	V		6.0233	
5	2.658	13616			1.4791	
6	2.917	288337	V		31.3272	
7	3.428	93474	V		10.154	
8	4.167	6861			0.7453	
9	4.51	46934	V		5.0983	
10	5.363	36034			3.9209	
	TOTAL	920567			100	

Fig. 18-8 Gas chromatograms (Analysis of test fish)

— Low exposure level—

Std. 0.50 μg/ml

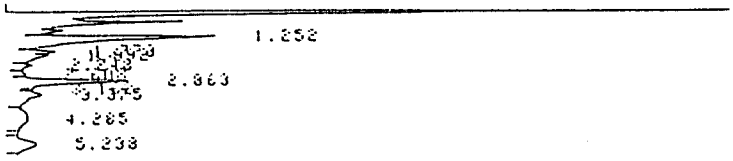
START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.048	23924			2.6823	
2	3.44	15990	V		1.7928	
3	4.035	698118	SV		78.2733	
4	4.758	153866	V		17.2515	
	TOTAL	891898			100	

week 8
No. 1

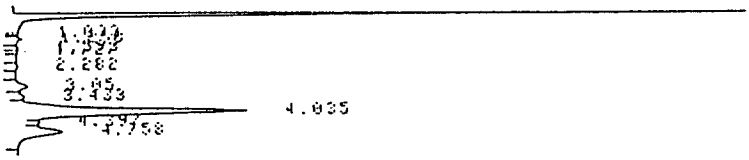
START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.252	357169			42.5117	
2	1.773	32220			3.835	
3	1.892	34497	V		4.1059	
4	2.248	10112			1.2036	
5	2.608	7523			0.8955	
6	2.863	225477	SV		26.8371	
7	3.375	62919	V		7.4889	
8	4.285	45555			5.4221	
9	5.238	64694			7.7002	
	TOTAL	840166			100	

Std. 0.50 μg/ml

START



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.258	5224			0.5736	
2	3.05	24542			2.6946	
3	3.433	16652	V		1.8283	
4	4.035	708883	SV		77.8307	
5	4.758	155500	V		17.0728	
	TOTAL	910802			100	

week 8
No. 2

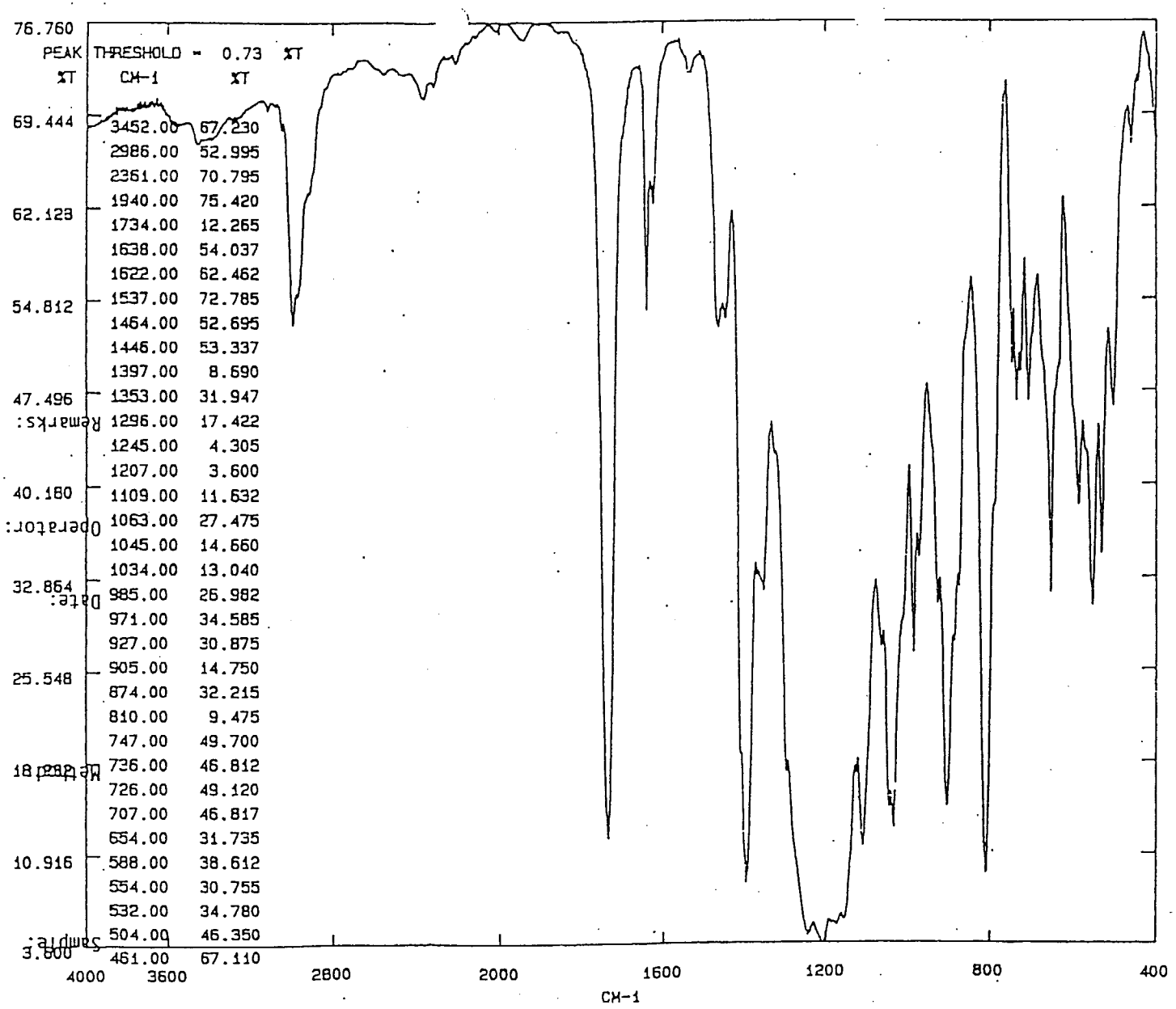
START

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.275	292876			38.5866	
2	1.795	23210			3.0579	
3	1.908	37124	V		4.8911	
4	2.273	7754			1.0216	
5	2.88	238992	V		31.4874	
6	3.392	57528	V		7.5794	
7	4.213	46433			6.1175	
8	5.258	55093			7.2585	
	TOTAL	759009			100	

A p p e n d i x — 1

Infrared spectrum of D-1 (Data provided by the sponsor)

(1 page in all)



CM-1	%T
3452.00	67.230
2986.00	52.995
2361.00	70.795
1940.00	75.420
1734.00	12.265
1638.00	54.037
1622.00	62.462
1537.00	72.785
1464.00	52.695
1446.00	53.337
1397.00	8.690
1353.00	31.947
1296.00	17.422
1245.00	4.305
1207.00	3.600
1109.00	11.632
1063.00	27.475
1045.00	14.660
1034.00	13.040
985.00	26.982
971.00	34.585
927.00	30.875
905.00	14.750
874.00	32.215
810.00	9.475
747.00	49.700
736.00	46.812
726.00	49.120
707.00	46.817
654.00	31.735
588.00	38.612
554.00	30.755
532.00	34.780
504.00	46.350
461.00	67.110

サンプル 往反スリーエム
サンプル "D-1"
lot 101

測定担当者
瀬川晴貴

測定年月日
H4.3.27

測定方法
フロン113に
溶解させ、
KBr板にて測定

000603

A p p e n d i x — 2

Gas chromatograms (Analysis of test water)

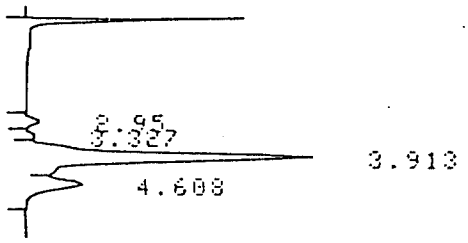
(17 pages in all)

Fig.1-1 Gas chromatogram (Analysis of test water)

Std.0.50 μg/ml

day 0

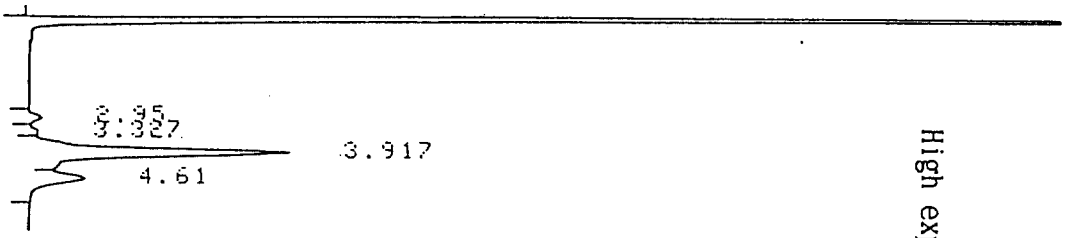
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.95	18728			2.3618
2	3.327	12342	V		1.5564
3	3.913	626699	V		79.0334
4	4.608	135186	V		17.0484
	TOTAL	792955			100

High exposure level

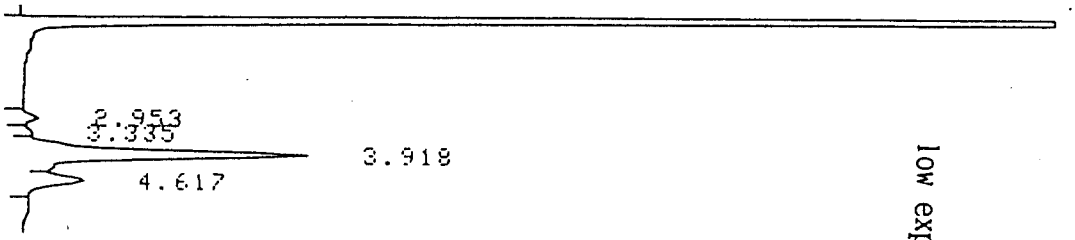
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.95	17757			2.458
2	3.327	12979	V		1.7967
3	3.917	560655	V		77.6097
4	4.61	131013	V		18.1357
	TOTAL	722403			100

low exposure level

START

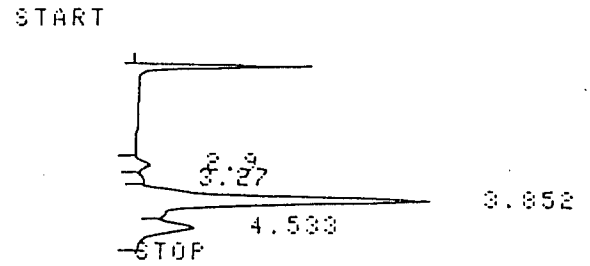


PKNO	TIME	AREA	MK	IDNO	CONC
1	2.953	18199			2.3672
2	3.335	12061			1.5688
3	3.918	609365	V		79.2617
4	4.617	129177	V		16.8023
	TOTAL	768802			100

Fig.1-2 Gas chromatogram (Analysis of test water)

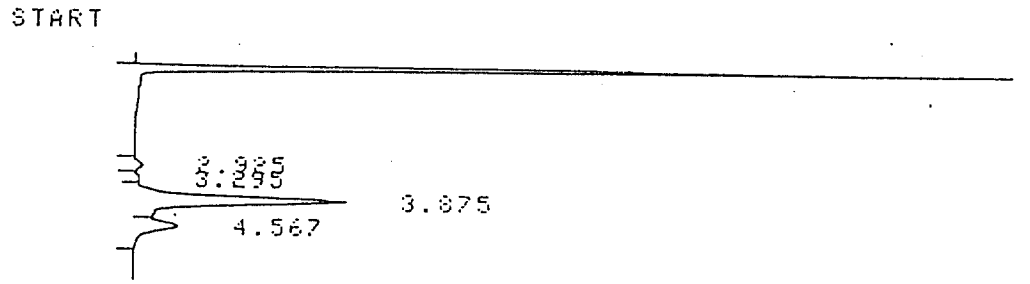
day 3

Std.0.50μg/ml



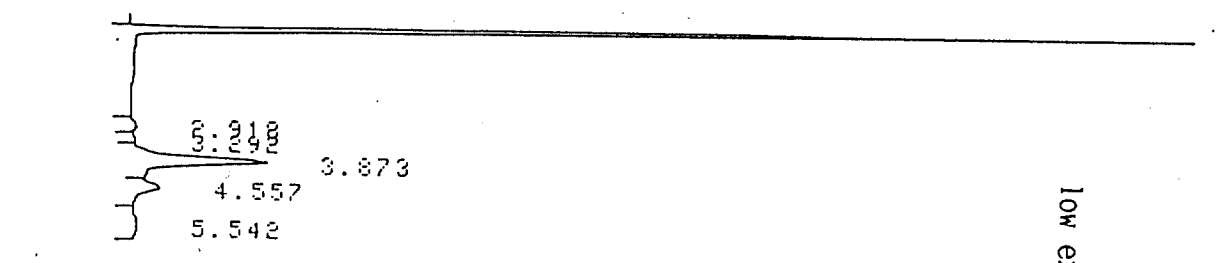
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.9	18925			2.3642
2	3.27	13281	V		1.6591
3	3.852	631636	V		78.9095
4	4.533	136615	V		17.0671
	TOTAL	800456			100

High exposure level



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.925	12801			2.23
2	3.295	9318	V		1.6232
3	3.875	443612	V		77.2803
4	4.567	108299	V		18.8665
	TOTAL	574030			100

low exposure level



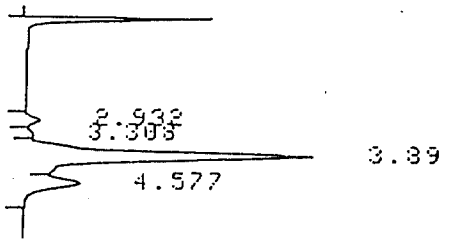
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.918	9094			2.32
2	3.292	5616			1.4326
3	3.873	291049	V		74.2463
4	4.557	68448	V		17.461
5	5.542	17797	V		4.5401
	TOTAL	392004			100

Fig.1-3 Gas chromatogram (Analysis of test water)

day 7

Std.0.50 μg/ml

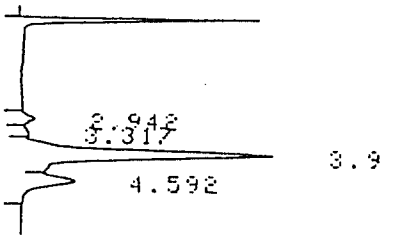
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.932	19308			2.438
2	3.308	13428	V		1.6956
3	3.89	625413	V		78.9728
4	4.577	133786	V		16.8935
	TOTAL	791934			100

High exposure level

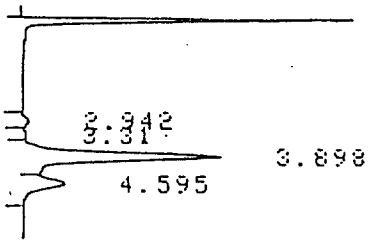
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.942	15751			2.2754
2	3.317	11039	V		1.5946
3	3.9	537225	V		77.6068
4	4.592	128225	V		18.5233
	TOTAL	692239			100

low exposure level

START



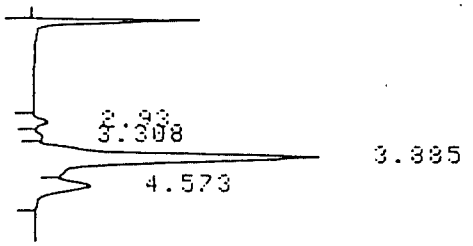
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.942	11288			2.1077
2	3.31	8453	V		1.5784
3	3.898	412616	V		77.047
4	4.595	103181	V		19.2669
	TOTAL	535538			100

Fig.1-4 Gas chromatogram (Analysis of test water)

day 10

Std.0.50μg/ml

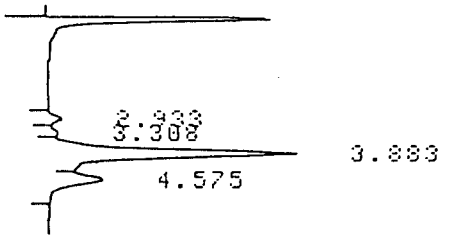
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.93	20002			2.5071
2	3.308	13275	V		1.6639
3	3.885	628548	V		78.7831
4	4.573	135996	V		17.0459
	TOTAL	797820			100

High exposure level

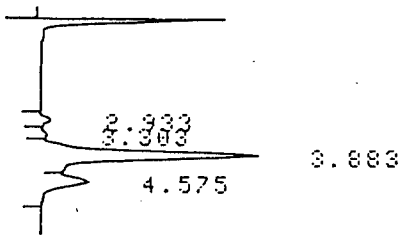
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.933	18308			2.5583
2	3.308	12432	V		1.7371
3	3.883	551308	V		77.038
4	4.575	133583	V		18.6665
	TOTAL	715630			100

low exposure level

START

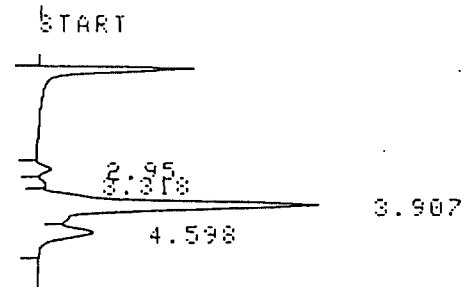


PKNO	TIME	AREA	MK	IDNO	CONC
1	2.933	15078			2.4128
2	3.303	10755	V		1.721
3	3.883	479951	V		76.8042
4	4.575	119119	V		19.062
	TOTAL	624903			100

Fig.1-5 Gas chromatogram (Analysis of test water)

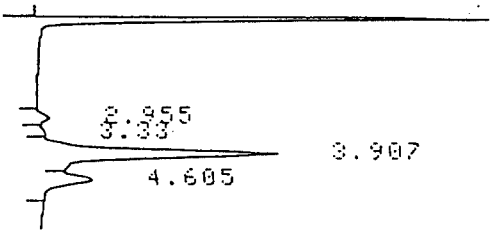
day 14

Std.0.50μg/ml



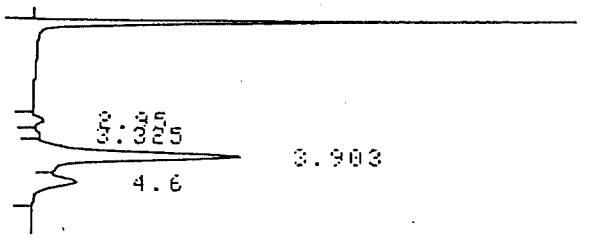
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.95	19573			2.4931
2	3.318	12944	V		1.6487
3	3.907	618105	V		78.728
4	4.598	134492	V		17.1302
	TOTAL	785115			100

High exposure level



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.955	16633			2.4338
2	3.33	11652	V		1.7049
3	3.907	526234	V		76.9982
4	4.605	128918	V		18.8632
	TOTAL	683437			100

low exposure level



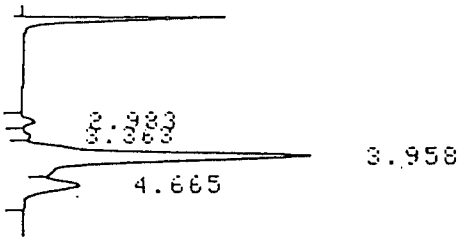
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.95	14798			2.4802
2	3.325	11091	V		1.8588
3	3.903	459129	V		76.9487
4	4.6	111650	V		18.7123
	TOTAL	596669			100

Fig.1-6 Gas chromatogram (Analysis of test water)

day 17

Std.0.50 μg/ml

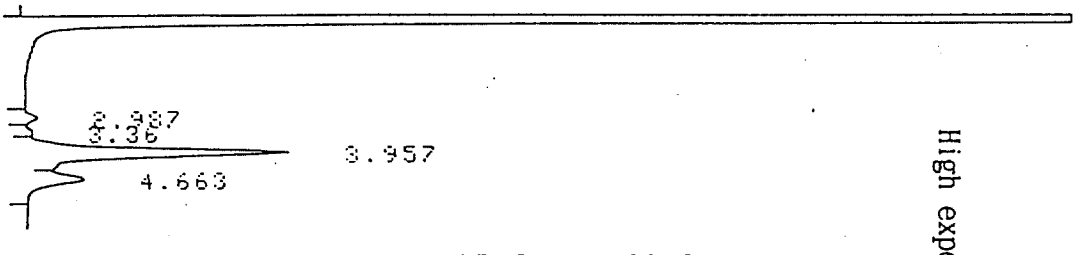
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.983	18752			2.2682
2	3.363	13121	V		1.587
3	3.958	651281	V		78.7756
4	4.665	143601	V		17.3692
	TOTAL	826754			100

High exposure level

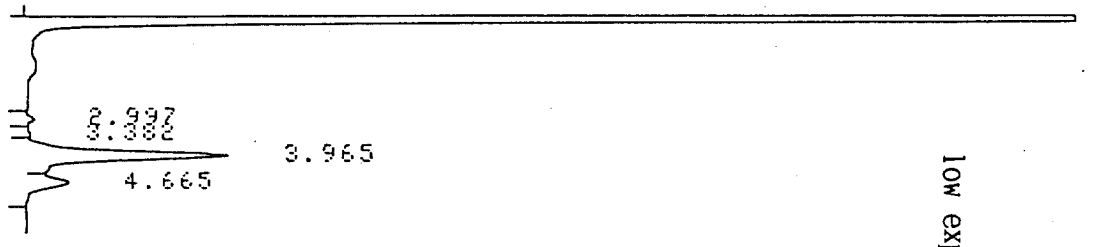
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.987	17961			2.3229
2	3.36	12491	V		1.6154
3	3.957	596662	V		77.1664
4	4.663	146100	V		18.8952
	TOTAL	773214			100

low exposure level

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.997	12765			2.1484
2	3.382	9348	V		1.5733
3	3.965	459404	V		77.3195
4	4.665	112646	V		18.9588
	TOTAL	594163			100

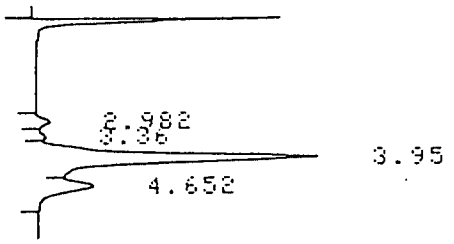
Fig.1-7 Gas chromatogram (Analysis of test water)

day 21

Std.0.50 μg/ml

START

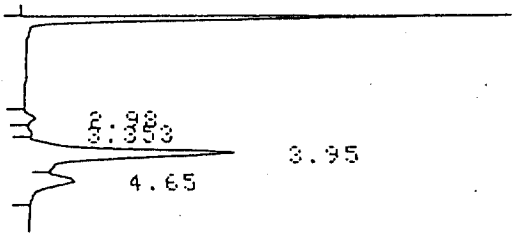
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.982	18998			2.2975
2	3.36	13311	V		1.6098
3	3.95	651927	V		78.8394
4	4.652	142669	V		17.2533
	TOTAL	826905			100

High exposure level

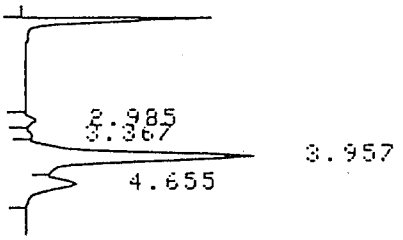
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.98	14537			2.3224
2	3.353	10426	V		1.6656
3	3.95	483481	V		77.2387
4	4.65	117513	V		18.7733
	TOTAL	625957			100

low exposure level

START



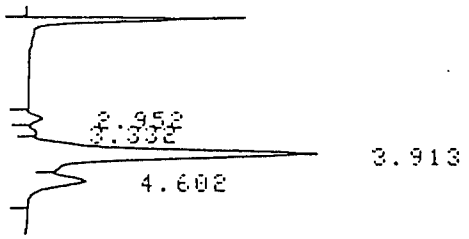
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.985	15518			2.2609
2	3.367	11180	V		1.6289
3	3.957	529472	V		77.1397
4	4.655	130210	V		18.9705
	TOTAL	686380			100

Fig.1-8 Gas chromatogram (Analysis of test water)

day 24

Std.0.50 μg/ml

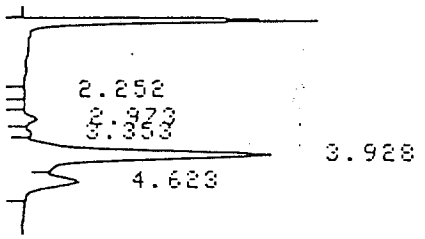
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.952	20893			2.4306
2	3.332	13828	V		1.6087
3	3.913	675116	V		78.5387
4	4.602	149759	V		17.422
	TOTAL	859596			100

High exposure level

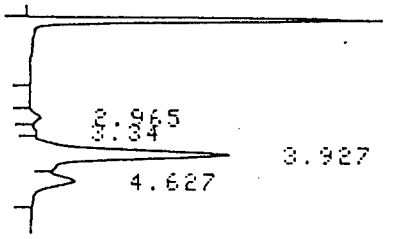
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.973	19175			2.6038
2	3.353	11557	V		1.5693
3	3.928	567817	V		77.1053
4	4.623	137869	V		18.7216
	TOTAL	736417			100

low exposure level

START



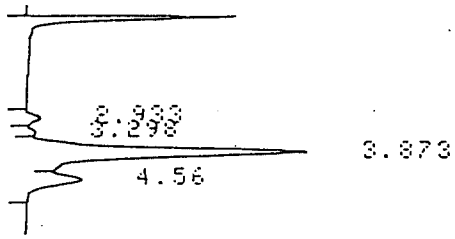
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.965	13667			2.2947
2	3.34	9585	V		1.6094
3	3.927	457676	V		76.848
4	4.627	114633	V		19.2479
	TOTAL	595561			100

Fig.1-9 Gas chromatogram (Analysis of test water)

day 28

Std.0.50μg/ml

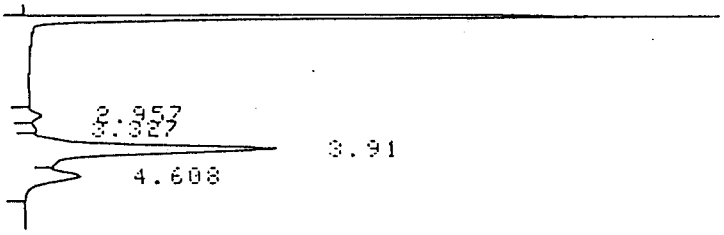
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.933	21055			2.5425
2	3.298	14268	V		1.7229
3	3.873	648705	V		78.3328
4	4.56	144111	V		17.4018
	TOTAL	828140			100

High exposure level

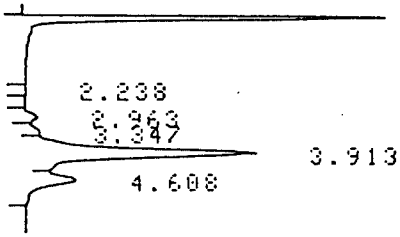
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.957	18743			2.5062
2	3.327	12935	V		1.7297
3	3.91	575386	V		76.9388
4	4.608	140785	V		18.8253
	TOTAL	747850			100

low exposure level

START



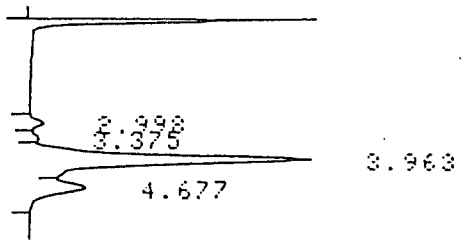
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.963	18682			2.5926
2	3.347	25742	V		3.5724
3	3.913	548095	V		76.0652
4	4.608	128042	V		17.7697
	TOTAL	720560			100

Fig. 1-10 Gas chromatogram (Analysis of test water)

day 31

Std. 0.50 μg/ml

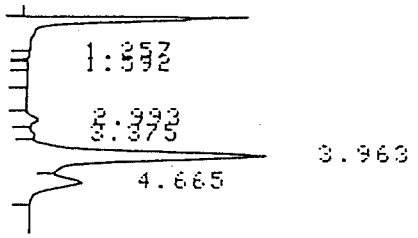
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.998	19746			2.3422
2	3.375	13661	V		1.6204
3	3.963	662568	V		78.5922
4	4.677	147071	V		17.4452
	TOTAL	843045			100

High exposure level

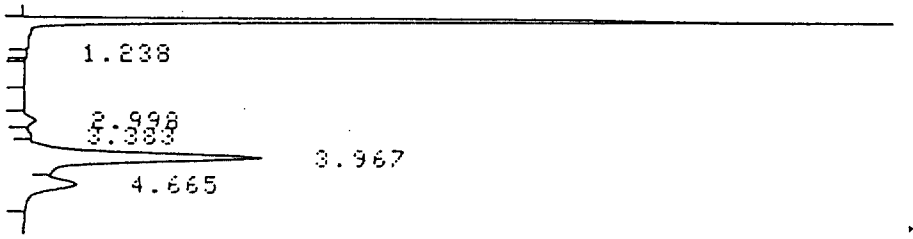
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.993	16703			2.3366
2	3.375	11146	V		1.5593
3	3.963	552060	V		77.2272
4	4.665	134942	V		18.8769
	TOTAL	714852			100

low exposure level

START



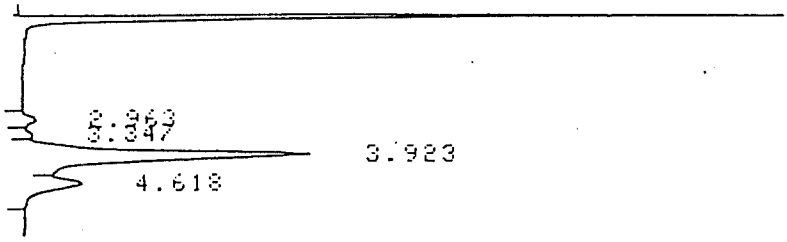
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.998	17037			2.3687
2	3.383	12302	V		1.7103
3	3.967	552129	V		76.7607
4	4.665	137817	V		19.1603
	TOTAL	719286			100

Fig.1-11 Gas chromatogram (Analysis of test water)

day 35

Std.0.50 μg/ml

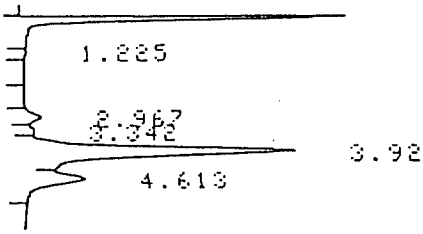
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.963	19804			2.2919
2	3.347	13140	V		1.5207
3	3.923	678328	V		78.5019
4	4.618	152820	V		17.6856
	TOTAL	864091			100

High exposure level

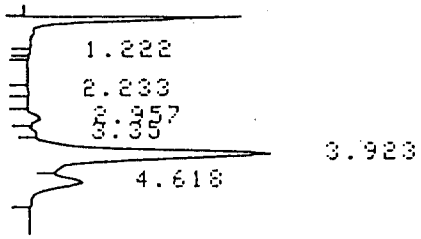
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.967	21191			2.6104
2	3.342	14378	V		1.7712
3	3.92	625275	V		77.026
4	4.613	150928	V		18.5924
	TOTAL	811772			100

low exposure level

START



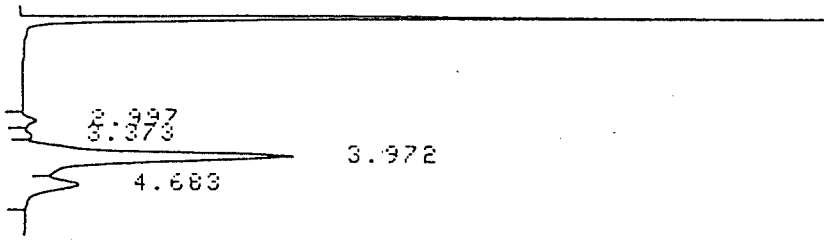
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.957	16649			2.2839
2	3.35	11240	V		1.5419
3	3.923	569179	V		78.0794
4	4.618	131906	V		18.0948
	TOTAL	728974			100

Fig. 1-12 Gas chromatogram (Analysis of test water)

day 38

Std. 0.50 μg/ml

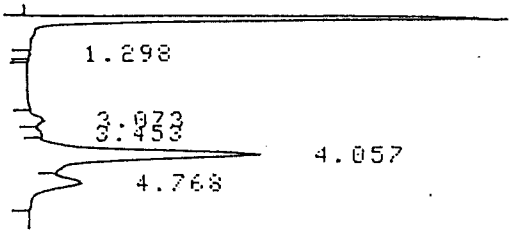
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.997	18649			2.2743
2	3.373	13448	V		1.6401
3	3.972	643757	V		78.5081
4	4.683	144133	V		17.5775
	TOTAL	819988			100

High exposure level

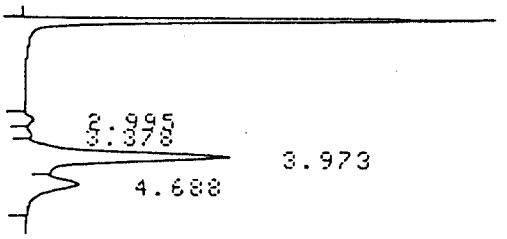
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.073	20305			2.7173
2	3.453	17773	V		2.3784
3	4.057	569616	V		76.2262
4	4.768	139577	V		18.6782
	TOTAL	747271			100

low exposure level

START



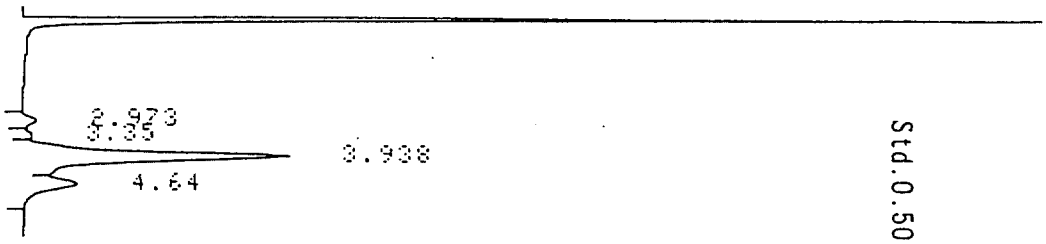
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.995	13573			2.053
2	3.378	10671	V		1.614
3	3.973	479125	V		72.4686
4	4.688	157779	V		23.8644
	TOTAL	661148			100

Fig.1-13 Gas chromatogram (Analysis of test water)

day 42

Std.0.50 μg/ml

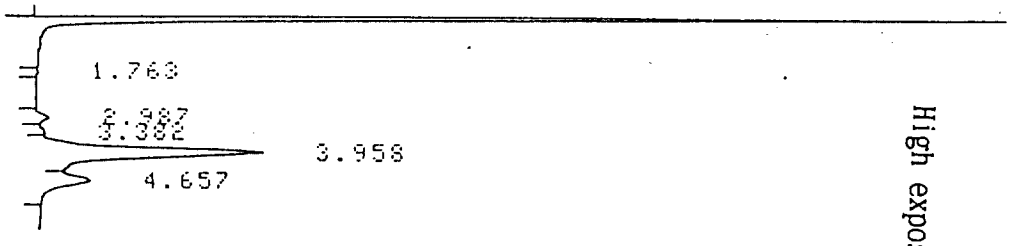
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.973	19073			2.3741
2	3.35	13235	V		1.6475
3	3.938	633159	V		78.8126
4	4.64	137906	V		17.1658
	TOTAL	803373			100

High exposure level

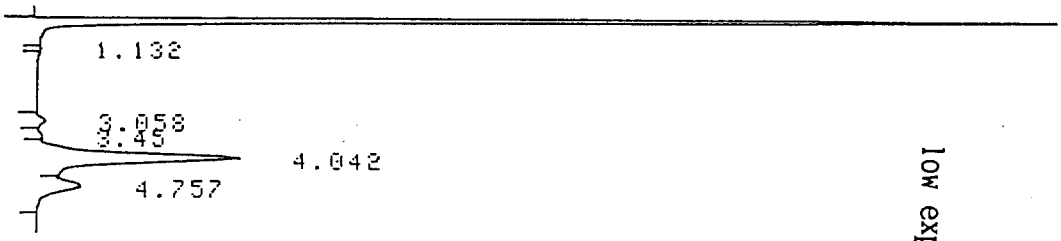
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.987	16879			2.3965
2	3.382	12686	V		1.8012
3	3.958	539450	V		76.5898
4	4.657	135321	V		19.2125
	TOTAL	704337			100

low exposure level

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.058	14151			2.21
2	3.45	10364	V		1.6186
3	4.042	494294	V		77.1962
4	4.757	121500	V		18.9752
	TOTAL	640308			100

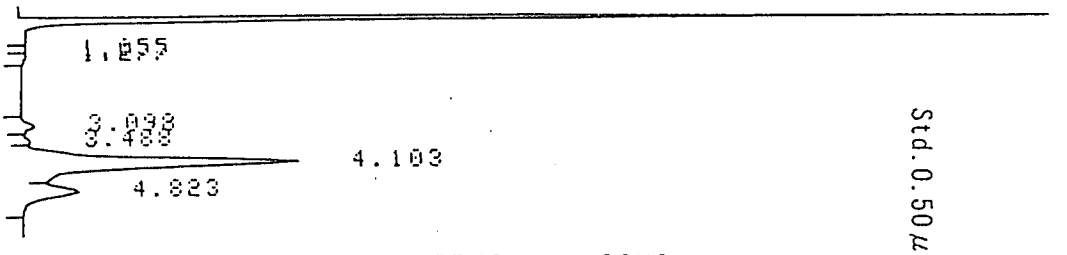
000617

Fig. 1-14 Gas chromatogram (Analysis of test water)

day 45

Std. 0.50 μg/ml

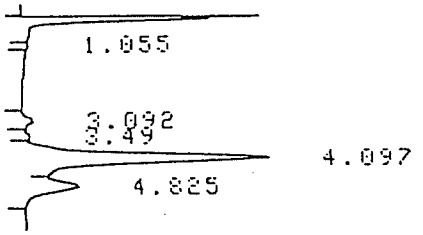
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.098	19202			2.2843
2	3.488	12900	V		1.5346
3	4.103	663595	V		78.9425
4	4.823	144909	V		17.2387
	TOTAL	840606			100

High exposure level

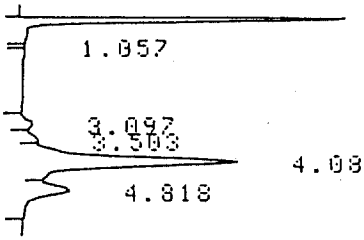
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.092	18955			2.4487
2	3.49	12769	V		1.6496
3	4.097	594606	V		76.815
4	4.825	147745	V		19.0867
	TOTAL	774075			100

low exposure level

START



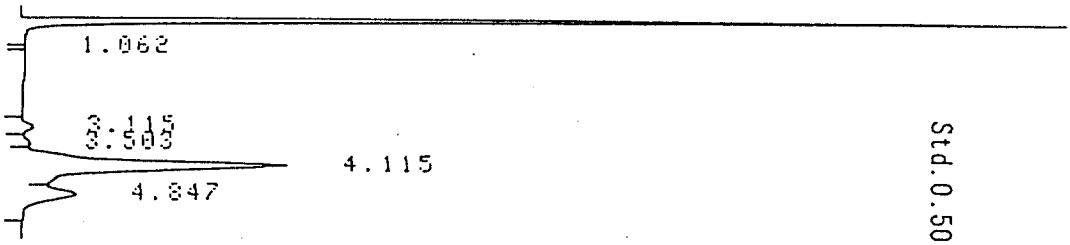
PKNO	TIME	AREA	MK	IDNO	CONC
1	3.097	19555			2.7398
2	3.503	31894	V		4.4685
3	4.087	537210	V		75.2666
4	4.818	125084	V		17.525
	TOTAL	713742			100

Fig.1-15 Gas chromatogram (Analysis of test water)

day 49

Std.0.50 μg/ml

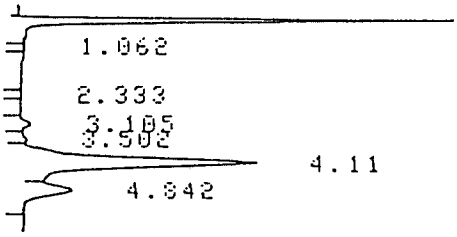
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.115	18136			2.1992
2	3.503	15023	V		1.8218
3	4.115	645311	V		78.2513
4	4.847	146194	V		17.7277
	TOTAL	824665			100

High exposure level

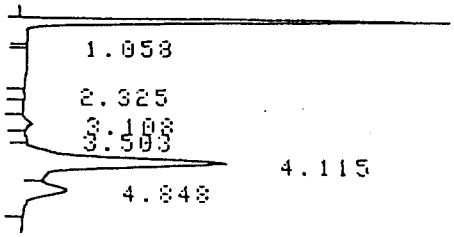
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.105	15066			2.0726
2	3.502	10678	V		1.469
3	4.11	566263	V		77.9018
4	4.842	134887	V		18.5566
	TOTAL	726893			100

low exposure level

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.108	12808			2.0383
2	3.503	9152	V		1.4564
3	4.115	488226	V		77.6958
4	4.848	118196	V		18.8095
	TOTAL	628382			100

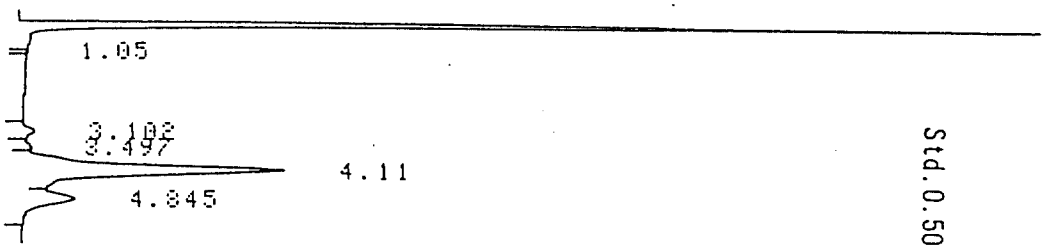
000619

Fig. 1-16 Gas chromatogram (Analysis of test water)

day 52

Std. 0.50 μg/ml

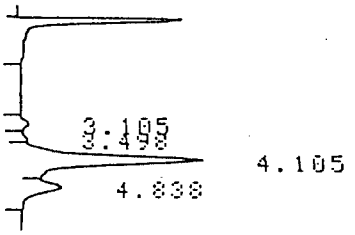
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.102	16532			2.0554
2	3.497	12395	V		1.541
3	4.11	636203	V		79.0993
4	4.845	139180	V		17.3043
	TOTAL	804309			100

High exposure level

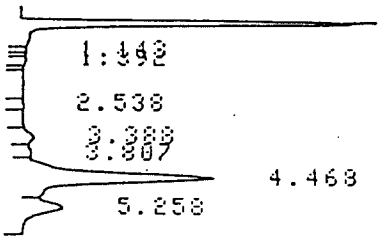
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.105	11329			2.0103
2	3.498	7764	V		1.3776
3	4.105	441513	V		78.3407
4	4.838	102974	V		18.2714
	TOTAL	563580			100

low exposure level

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.388	15406			2.4839
2	3.807	13754	V		2.2176
3	4.468	479148	V		77.2548
4	5.258	111910	V		18.0437
	TOTAL	620218			100

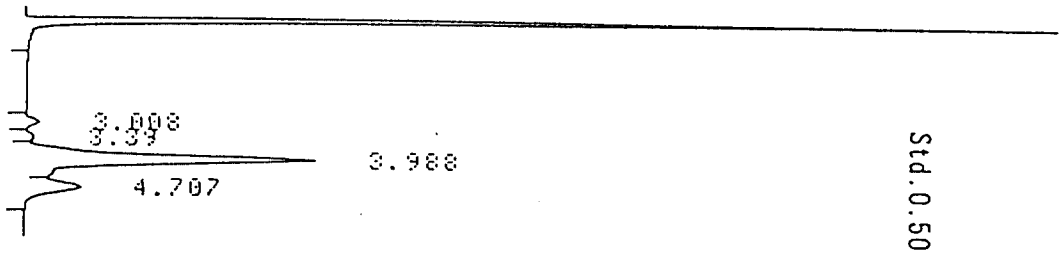
000620

Fig.1-17 Gas chromatogram (Analysis of test water)

day 56

Std.0.50μg/ml

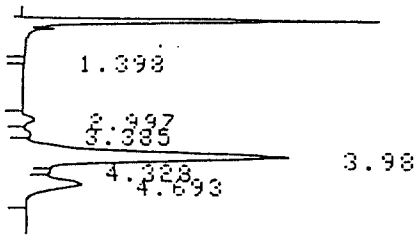
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.008	17698			2.1876
2	3.39	11850	V		1.4648
3	3.988	642753	V		79.4501
4	4.707	136700	V		16.8974
	TOTAL	809001			100

High exposure level

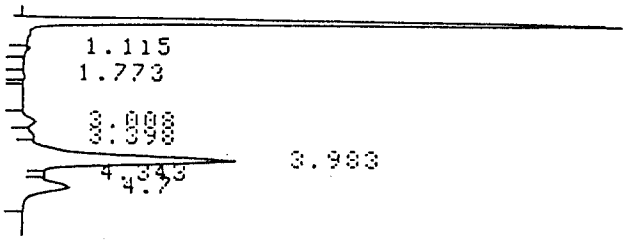
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.997	17988			2.3522
2	3.385	13222	V		1.729
3	3.98	557714	V		72.9303
4	4.328	32223	V		4.2137
5	4.693	143575	V		18.7747
	TOTAL	764721			100

low exposure level

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.008	20549			3.2061
2	3.398	20365	V		3.1773
3	3.983	482711	SV		75.3127
4	4.7	117317	V		18.3039
	TOTAL	640942			100

000621