

BIOACCUMULATION (ANALYTICAL ONLY)

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The 3M production lot number was 37.
The test sample is FC-143. It's purity was not sufficiently characterized, though current information indicates it is a mixture of 96.5 - 100% test substance and 0 - 3.5% C₆, C₇, and C₉ perfluoro analogue 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 (died in test) 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. One portion using ethyl acetate 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 and toluene extracted fish exposed to plant effluent contained no detectable amounts of ammonium perfluorooctanoate.

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., $\text{NH}_3\text{-N}$ 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: 5/25/00



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	☒ Closed	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.

cc: R.A. Prokop-236-3B
V. Pothapragada-236-3A
A.N. Welter-21-2W
R.L. Bohon-21-2W

Information Liaison
Initials: _____

5/1/79

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 ($\mu\text{g/g}$) 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.

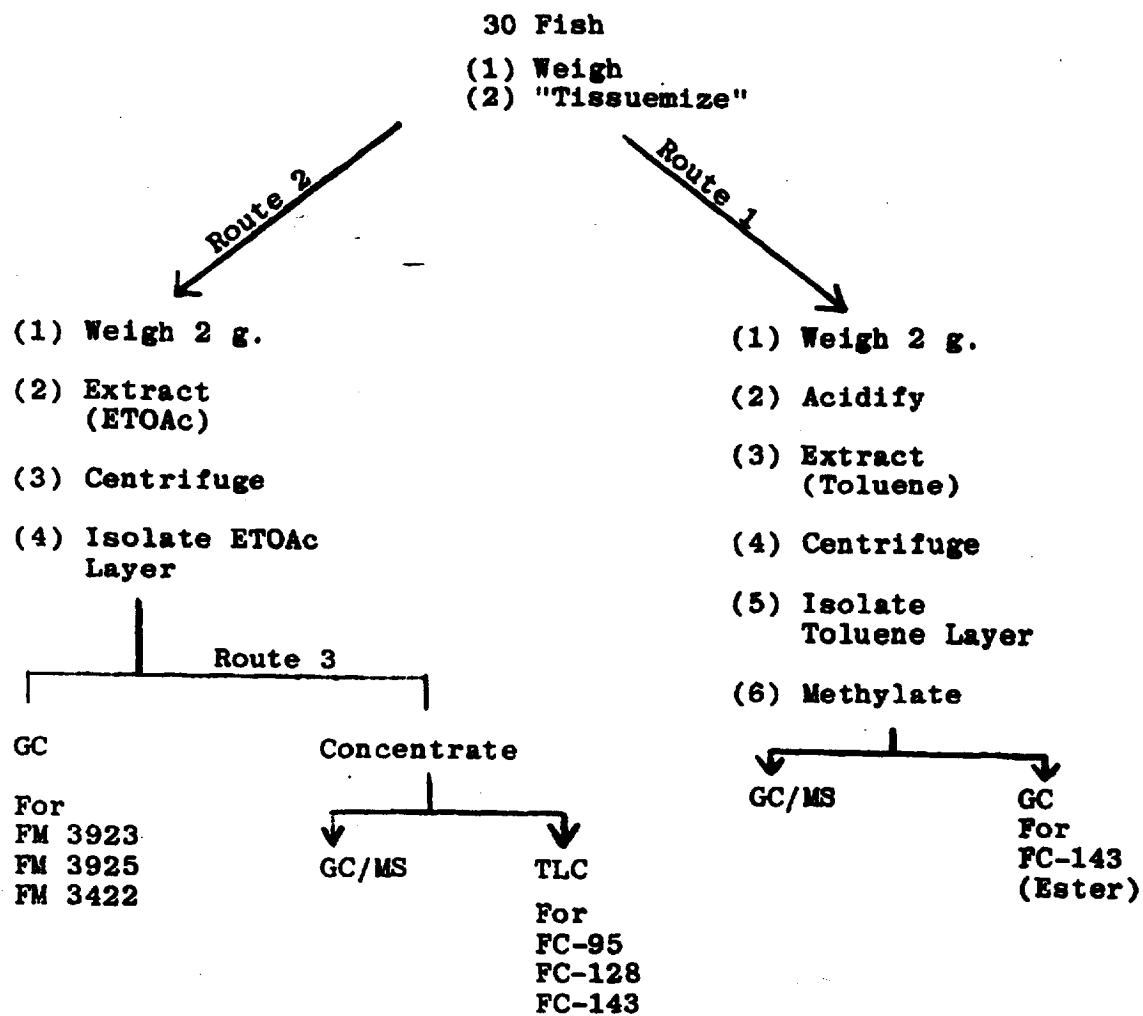
INTRODUCTION

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



5/1/79

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 Ni⁶³ 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 N₂ 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 Ni⁶³ 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.

5/1/79

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. Vraspin 5/21/79

Althaus 5/24/79

GAV/AM/cen

Attachment

CENTRAL ANALYTICAL LABORATORY

Report No. 7124

Date March 28, 1979

Subject: Decatur Fish Bloassy.

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 bloassy 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.

DISCUSSION

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 21-4918 mass spectrometer
Source: Chemical ionization
Mode: all samples were run in Electron Ionization, sample P.E. #2 was also run in Chemical Ionization with NH₃ 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 C₁₅ to at least C₂₄.

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 fluorochemicals (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 bioassay 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

Analytical From Fish Bioconcentration Studies

PROJECT NO. 9470612000

3M TECHNICAL NOTEBOOK NO.

41947 21

Subject: Bioaccumulation of FM-3422 in Channel Catfish

Date: 1/15/76

Object:

Reference:

A significant problem with this work is that no where was it demonstrated that solvent extracts of fish tissue were quantitative.
 SARC- 3-4-83

Three groups of 5 channel catfish were exposed for 32 days to Control water, 400ml acetone on marbles, and 16g FM-3422 + 400ml Acetone sprayed on marbles in 16L Tank.

The fish after - 32 day exposure were dried and wt taken

Control
3.95g

Acetone only
4.65g

FM-3422 + Acetone
4.77g.

The three (5 lot) groups were digested with ~25ml 20% KOH at 50°C for 1 hr. The three digested samples were then diluted to ~50ml and extracted with 10ml N-Octanol.
G.Ced for FM-3422

50ml aliquots of the tank water was extracted with 10ml octanol also for G.C. analysis,

All samples G.Ced see FC 1/19/76 to yield

Water - mg/l FM-3422
Control - Nil

Acetone - Nil

FM-3422 - 0.17mg/L

Fish - mg/g - dried fish
Control - 0.0015mg/g

Acetone - 0.0006mg/g

FM-3422 - 0.161mg/g

Signature

E. V. Vesper

Date:

3/8/78

PROJECT NO. 99 706 12600

3M TECHNICAL NOTEBOOK NO.

PAGE

Subject:

41947 23
Date: 2/18 - 2/20/76

Object:

Reference: LR 3114 was to determine the uptake of FM-3422 by channel catfish in at 1000 ppm tank (static) 33 day exposure. Verify the fish test. Page 21

Samples submitted - Test Water (1000 ppm) Fresh gutted wt.

Fish were gutted - { Fish ① - 3.20g
Fish ② - 3.46g
Fish ③ - 2.58g

Test water sample was extracted with 10ml Oct per 10ml H₂O

Fish were digested in 10ml each of 20% KOH by heating to ~50°C for 1 hr. Then extracted with 10ml Oct.

Extracts G.C. See integrator reports 043-048

to yield Test Water 4.86 mg/l

Fish ① - 0.31 mg/g - Fresh gutted wt.

Fish ② - 0.24 mg/g - " " "

Fish ③ - 0.46 mg/g - " " "

The test water was filtered through a Millipore 0.22µ filter and 50ml water extracted with 2ml Oct. G.C. integrator report 049-051 to yield 0.011 mg/l.

Signature

M. D. V. Vreijer

Date:

3/8/78

PROJECT NO. 9970612600

3M TECHNICAL NOTEBOOK NO.

Subject:

Date:

41947 24
2/23 - 2/25/77

Object:

Reference:

LR 3114 - The second part of the uptake bioassay was the removal of the fish from the FM-3422 tank and placement of them into ~~the~~ a tank of clean H₂O to determine the cleaning effect if any.

Samples submitted - Control Water

	Recovery tank water	Fresh gutted wt.
Control Fish #1		4.63g
Control Fish #2		4.80g
Exposed fish #1		3.90g
Exposed Fish #2		3.66g

Water samples extracted 2ml/10ml Oct/H₂O

Fish digest as earlier and extracted with 10ml Oct.

Sample ext. G Ced Ing reports - 061-067 to

yield Control Water - <0.01mg/l FM-3422

Recovery Water - 0.033mg/l "

Fish
C-1 - 0.00067mg/g "

C-2 0.00097mg/g "

F-1 0.029mg/g "

F-2 0.031mg/g "

Signature

M. D. Vraspir

Date:

3/8/78

PROJECT NO. 9970612600

3M TECHNICAL NOTEBOOK NO.

41947 25

PAGE

Subject:

Date: 2/73 - 2/24/76

Object:

Reference:

Some question was raised to the validity of the 1ml/50ml Oct/H₂O extraction ratios used to obtain the lower detection limits.

A. 0.5mg/l soln of FM-3422 in H₂O was made by dispersing the FM-3422 via acetone

1st, 2nd, & 3rd extractions were made with the 1ml/50ml ratio G.C. - Int. reports - 052-057

The yield was 1st - ~~84%~~

2nd ~~13%~~

3rd 2%

Total = 0.438mg/l

100% recovery
in 35ccum for
1:50 ml extract of
H₂O

Same procedure was tried on a sat water soln of FM-3422. G.C. int. reports 058-059

To yield 1st ~~86%~~ 89% 7.5mg/l

2nd ~~72%~~ 10% 0.84mg/l

3rd 1% 0.06mg/l

Total = 8.4mg/l

Signature

E.O. Vesper

Date:

3/8/78

Subject:

Date:

41947 41
3/8/77

Object:

Reference:

3 samples were drawn from the FM-3422 fish tank. These samples were taken at the same levels as the initial samples and will be analyzed for FM-3422

Samples ① ^{Day 8} ~~Fish~~ ② ^{Day 8} ~~Fish~~ ③ ^{Day 8} ~~Fish~~

Mo gave me 2 digested bluegill fish samples

Day 8 Control fish 1.15g Exposed Fish 0.977g.

Water samples and fish samples were taken on 3/14/77 - 14 days of exposure.

Control 3/14/77 - 3 fish wt. = 1.439g.

Exposed 3/14/77 - 3 fish wt. = 1.172g.

Fish samples were extracted with 2 - 10ml portions of ethyl Acetate and then the 2 - 10ml aliquots were diluted to 25ml. Digested fish sample were pH adjusted with H₂SO₄ conc. to pH 7-8

Fish samples GC. Integrator reports - 051 - 054

Results: Control fish - No background

	Total wt. fresh fish	Total FM-3422 mg	FM-3422 mg/g fish
Exposed Fish 8 days 3/8/77 -	0.977g	0.115mg	0.118 mg/g fish

Exposed Fish 14 days 3/14/77 -	1.172g	0.137mg	0.117 mg/g fish
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See page 54 for 21 day Exposure.

Signature

[Signature]

Date:

3/21/77

M.T. Shokri

3/20/77

4. *Report No. 14 (5/1/79) "Analysis of Fluorochemicals in Bluegill Fish"*

Although this report offers some detailed explanation of analytical procedures, the sample sources and data are presented in a very marginalized manner. It gives the impression that des-(hydroxyethyl)ation of N-Et FOSE alcohol occurs in the environment.