BIOCONCENTRATION

TEST SUBSTANCE

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

Remarks: 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% C_6, C_7, and C_9 perfluoro analogue compounds. The 3M production lot number 377.

METHOD:

Method/guideline followed: 3M derived preliminary investigation outlined in the Technical Report Summary. Investigation included method development for the analysis of fluoride by combustion.
Type: Static exposure with static clearance phase.
GLP (Y/N): No
Year: 1995
Species: Fathead minnow (*Pimephales promelas*)
Supplier: Aquatic Biosystems, Inc., Fort Collins, CO.
Analytical monitoring: Whole fish and test solutions were combusted in a modified organic halide analyzer (Dohrmann DX-2000). F^- activity in collection fluid was then measured with an ion-selective electrode connected to an Orion Research ion analyzer. The concentration of PFO was then calculated from the F^- ion concentration.
Length and weight: Not noted in the report.
Uptake period: 13 days
Depuration period: 15 days
Statistical methods: One-way analysis of variance and Tukey's HSD test (alpha = 0.05) were used to compare the average concentrations of PFO in tissues from PFO-exposed fish during the uptake and depuration experiment.
Test fish age: 64 days
Loading: 124.6 $\pm$ 20 mg fish (wet wt.) per liter test solution.
Pretreatment: Not noted.
Test conditions:

Dilution water: Carbon-filtered well water from 3M Well #2, St. Paul, MN

Dilution water chemistry:

hardness: 272 $\pm$ 8 mg/L as $CaCO_3$
alkalinity: 232 $\pm$ 8 mg/L as CaCO3
pH: 8.5 $\pm$ 0.1
Conductivity: 510 $\pm$ 14 µmhos/cm
Dissolved oxygen: 8.5 $\pm$ 0.3 mg/L

Stock and test solution preparation: The primary stock solution was prepared by dissolving solid PFO in dilution water to create a 1000 mg/L concentration. The nominal concentration of 25 mg/L was obtained by diluting an aliquot of the stock solution to 15 liters using dilution water.

Exposure vessels: 5 gallon cylindrical high density polyethylene tanks containing 15 liters of test solution.

Number of replicates: One

Number of fish per replicate: 30.

Number of concentrations: One plus blank control

Water chemistry during the study:

Dissolved oxygen: The DO concentration of the control dropped below 6.0 mg/L at 72 hours after the start of the test. Aeration was initiated at this point and continued throughout the test to maintain DO levels at or near 100% saturation.

Test temperature: 20±1°C

Average pH (control and test solutions): 8.2 ± 0.2

Photoperiod: 16 hours light and 8 hours dark.

Feeding: Fish were fed live brine shrimp every 48-hours beginning 24-hours after starting the test.

Sampling: Five fish were removed from each tank at 192 and 312 hours during the uptake phase and again 24, 96, 168, and 360 hours after being transferred to clean dilution water.

Remarks field: Fecal material / excess food was removed from tanks as needed. DO measurements were taken daily. Test solution pH was measured once more at the end of each experimental phase.

RESULTS

Concentration of PFO measured in test solutions.

Phase / Solution	Phase Time (hours)*	Average PFO Concentration (ug/mL)	Standard Deviation
UPTAKE			
Control	0	<1	-
	192	<1	-
	312	<1	-
25 ug/mL PFO	0	25.0	1.8
	192	25.5	2.2
	312	25.9	1.8
DEPURATION			
Control clearance tank solution	0	<1	-
	168	<1	-
	360	Not available	
25 ug/mL PFO exposed clearance tank	0	<1	-
	168	<1	-
	360	Not available	

* Elapsed time (hours) from the beginning of each experimental phase.

Average concentration of PFO (measured as F-) in *Pimephales promelas* tissues

Time (Hours)	PFO in Fish µg/g wet weight	Standard Deviation	Comparison Of Means **		
0	1.7	0.4			C
192	44.7	9.1	A		
312	46.7	5.8	A		
336 (24)*	19.9	5.1		B	
408 (96)	7.6	1.3			C
480 (168)	7.6	3.5			C
672 (360)	8.0	1.6			C

* Number in ( ) indicates elapsed time (hours) from beginning of depuration phase
** Means with the same letter are not significantly different at alpha = 0.05 by the Tukey-Kramer Honest Significant Difference test.

The calculated 312 hour BCF for PFO is 1.8 using the equation BCF = C_a / C_w where C_a is the equilibrium concentration of the test substance in the fish and C_w is the equilibrium concentration of the test substance in the water to which the organism is exposed.

CONCLUSIONS

No definitive conclusions can be derived from this study. General observations can be made.

PFO concentrations increased in the fish tissues over the course of exposure. The PFO in fish tissue appeared to be directly related to the concentration of PFO in the test solution.

While concentrations decreased in the whole fish samples over the period of the clearance phase, the time required for complete removal is longer than the 15 days used in the test.

The concentration of PFO in test solutions remained constant during the static exposure experiment.

Relative to the control treatment, the survival and growth of the test fish exposed to PFO were not adversely affected during the experiment.

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

DATA QUALITY

Reliability: Klimisch ranking 2. This study meets the criteria for quality testing, however, there was no definitive analysis of the compound in either the fish or the test solutions. It was measured indirectly as F^- ion. This would not allow for the determination of degradation or metabolism byproducts and could yield falsely high readings. There was also no consideration for the possibility that the test substance may have been sorbed to the surface of the fish rather than residing internally, thus possibly overestimating the BCF. The study lacks characterization of the test substance purity.

Third party review by Dr. John Giesy of Giesy Ecotoxicology, Inc. (observations included with report) verifies the studies strengths and weaknesses.

REFERENCES

3M Technical Report "Assessment of the Bioaccumulative Properties of Ammonium Perfluorooctanate: Static" R.D. Howell, J.D. Johnson, J.B. Drake, R.D. Youngblom , May 31, 1995

3M requested expert overview, "Bioaccumulative Properties of Ammonium Perfluorooctanoate: Static Fish Test", Dr. John P. Geisy of Geisy Ecotoxicology, Inc., March 20, 1995

OTHER

Last changed: 5/24/00

Technical Report Summary

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To: Patent & Technical Communications Services - 201-2C-12

Report Summary must be typewritten. Guidelines are on reverse side.

3M

Document Number:	Department Number: 0222	Project Number:	Report Number (Place additional D.N.s in Abstract area):
To: D. L. BACON			Date Typed: 05/31/95
Author(s): R. D. HOWELL, J. D. JOHNSON, J. B. DRAKE, R. D. YOUNGBLOM			Employee Number(s): 295864 / 7288
Division/Department: ENVIRONMENTAL TECHNOLOGY & SERVICES	CC: 01	Loc: 089	Organization Code:
Project Description: METHOD DEVELOPMENT			
Report Title: Assessment of the Bioaccumulative Properties of Ammonium Perfluorooctanate: Static			Period Covered: May 1994 - May 1995

Keywords

Bioaccumulation; Ammonium perfluorooctanoate; fish; static fish test

Project Objective & Report Abstract

Procedures are described that were used for obtaining laboratory data for the uptake and depuration of ammonium perfluorooctanoate (PFO) by the fathead minnow, Pimephales promelas, from dilution water using a static exposure technique. These procedures were intended to provide background information on the bioaccumulation of PFO in fish.

Report Type

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- ☐ Management SUMmary
- ☐ TRP Trip or Field Report
- ☐ FACtory Experiment
- ☐ ENGineering
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- ☐ TECh. Service
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☐ Open Report and Summary ☒ Closed Report - Open Summary

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ASSESSMENT OF THE BIOACCUMULATIVE PROPERTIES OF AMMONIUM PERFLUOROOCTANOATE: STATIC FISH TEST

ABSTRACT:

Procedures are described that were used for obtaining laboratory data for the uptake and depuration of ammonium perfluorooctanoate (PFO) by the fathead minnow, *Pimephales promelas*, from dilution water using a static exposure technique. These procedures were intended to provide background information on the bioaccumulation of PFO in fish.

OBJECTIVES:

To accomplish a preliminary investigation of PFO uptake and depuration in fish. This investigation included method development for analysis of fluorine by combustion.

METHODS:

Summary of Practice

The uptake and depuration test was conducted using a static (test solutions were not renewed) exposure system. During the uptake phase of the experiment, fish were exposed for 13 days to dilution water to which a selected concentration of PFO had been added. Following the uptake phase, fish were transferred to dilution water which did not contain added PFO for a 15 day depuration phase. A control treatment, in which fish were exposed to dilution water to which PFO had not been added, was included with the test. The control provided a measure of the acceptability of the test by giving an indication of the quality of the test fish, and the suitability of the dilution water, food, test conditions, and handling procedures.

During both phases of the experiment, fish and water samples were removed periodically from test chambers and analyzed for PFO.

Apparatus

Facilities - All fish tests were conducted at the 3M Environmental Laboratory in St. Paul, MN. Proper test conditions (temperature, photoperiod, and ventilation) were maintained with the use of a controlled environment chamber (REVCO Scientific Inc., Asheville, NC).

Test Chambers - Fish and test solutions were contained in 5 gallon cylindrical high density polyethylene tanks (Nalge CO., Rochester, NY).

Dilution Water

Source - Dilution water was obtained in the 3M Environmental laboratory from an on-site well (3M Well #2, St. Paul, MN).

Treatment - Dilution water was carbon-filtered prior to collection using a commercial filtration system (Culligan Water Conditioner, Culligan International CO., Northbrook, IL). Before use, dilution water was aerated for at least 12 hours in order to bring the pH and concentrations of dissolved oxygen and other gases into equilibrium with air.

Characterization - Carbon-filtered, aerated well water had the following characteristics (MEAN ± S.D.): pH, 8.5 ± 0.1; dissolved oxygen, 8.5 ± 0.3 mg/L; conductivity, 510 ± 14 µmhos/cm; alkalinity, 232 ± 8 mg/L as $CaCO_3$, hardness, 272 ± 8 mg/L as $CaCO_3$.

Test Material

General - Commercial PFO (Specialty Chemicals Division, 3M Company, St. Paul, MN, Lot #377) was used for the test.

Acute Toxicity - The *P. promelas* 96 hour LC_{50} for PFO was determined to be 766 mg/L (3M Environmental Laboratory, unpublished data).

Test Concentrations - Fish were exposed to dilution water containing a nominal concentration of 25 mg/L PFO during the uptake phase of the test. An earlier study (3M Environmental Laboratory, unpublished data), indicated that while this concentration did not adversely affect *P. promelas*, it was great enough to allow for the accurate measurement of PFO in test solutions and fish tissues.

Stock Solutions - 1000 mg/L PFO stock solutions were prepared by dissolving solid PFO in dilution water.

Test Organism

Species - The freshwater fathead minnow, *Pimephales promelas*, was used as the test organism.

Source - Test fish were obtained from an external bioassay supply laboratory (Aquatic Biosystems, Inc., Fort Collins, CO).

Care and Handling - Fish were maintained at the 3M Environmental Laboratory in 50 gallon flow-through tanks

(Frigid Units, Inc., Toledo, OH) containing carbon-filtered dilution water. Fish were allowed to acclimate to the dilution water for at least 12 days before being used for testing. The concentration of dissolved oxygen in the holding tanks was maintained near 100% saturation by continuous aeration. Fish were fed live brine shrimp and/or TetraMin flake food (TetraWerke, Germany). Water temperature remained relatively constant at approximately 14 C.

Age - All fish were 64 days old at the start of the test.

Procedure

Summary Of Test Parameters

* Test temperature: 20 ± 1 C
* Photoperiod: 16 h:8 h (light:dark)
* Test solution volume: 15 L
* Test solution renewal: none
* Nominal test concentrations (uptake): 0.0 (Control) and 25 mg/L PFO
* No. replicates per test concentration: 1
* No. organisms per test concentration: 30
* Length of uptake phase: 312 h (13 days)
* Length of depuration phase: 360 h (15 days)
* Feeding: fish were fed live bring shrimp

Uptake/Depuration Experiment

Description - The uptake/depuration experiment involved exposing *P. promelas* under static conditions to dilution water to which PFO had been added. The uptake phase was conducted for a period of 312 h (13 days). After 312 h, the fish were transferred to dilution water to which PFO had not been added. This portion of the experiment, the depuration phase, lasted for 360 hours (15 days). Fish and test solution samples were taken at the beginning and end of each phase, and periodically throughout each of the experimental phases.

Test Set-Up (Uptake) - Two test tanks were filled with carbon-filtered, aerated dilution water. One tank was dosed with the appropriate volume of 1000 mg/L PFO stock solution to produce 15 L of solution at a nominal concentration of 25 mg/L PFO. The second tank contained only dilution water and served as a control. Dissolved oxygen and pH were measured in the test solutions. A sample of test solution was taken from each tank and stored at 4 C in 6 mL polypropylene scintillation vials (Wheaton Scientific, Millville, NJ) until needed for PFO analysis.

Thirty *P. promelas* were added to each of the test tanks. Ten additional fish were sacrificed and their wet weights were

determined in order to calculate loading rate. Each of the ten fish was placed in a separate 15 mL polypropylene centrifuge tube (Corning Inc., Corning, NY) and stored at -25 C until analyzed for background PFO concentration.

Test Set-Up (Depuration) - Following the uptake phase of the experiment, remaining fish were transferred to two test tanks containing only 15 L carbon-filtered, aerated dilution water. Dissolved oxygen and pH were measured in each tank following transfer of fish, and test solution samples were taken from each tank for PFO analysis.

Test Maintenance - Dissolved oxygen measurements in the test tanks were taken daily during both phases of the test. If the dissolved oxygen concentration in either of the test tanks fell below 6.0 mg/L, both tanks were gently aerated for the remainder of the test. Fish were fed live brine shrimp 24, 72, 120, 168, 240, 288, 336, 384, 432, 480, 528 and 576 h after starting the test. Fecal material/excess food was removed from tanks with a disposable glass pipette as needed. Test solution pH was measured once more at the end of each experimental phase.

Test Sampling - Five fish were removed from each tank at 192 and 312 h during the uptake phase and again 24, 96, 168 and 360 h after being transferred to clean dilution water. Fish were sacrificed by freezing in liquid nitrogen. Fish were allowed to thaw, and wet weights were determined. Each fish was placed in a separate 15 mL polypropylene centrifuge tube and stored at -25 C until needed for analysis. Test solution samples were taken at each fish sampling time and stored in 6 mL polypropylene scintillation vials at 4 C until needed for analysis.

Analysis of PFO

Whole fish and test solutions were combusted in a modified organic halide analyzer (Dohrmann DX-2000, Rosemount Analytical Inc., Santa Clara, CA), and F^- activity in collection fluid was measured with an ion-selective electrode connected to an ion analyzer (Orion Research Inc., Boston, MA). The concentration of PFO in whole fish tissue and test solutions was calculated from the measured concentrations of F^- ion.

Data Analysis

At each sampling point, the weight of PFO-exposed fish was compared to that of control fish using a t-test (alpha = 0.05).

One-way analysis of variance and Tukey's HSD test (alpha = 0.05) were used to compare the average concentrations of PFO

in tissues from PFO-exposed fish during the uptake and depuration experiment.

RESULTS:

Test Organism Loading Rate

The loading rate of *P. promelas* was 124.6 ± 20 mg fish (wet wt.) per liter of test solution.

Water Quality Characteristics

Measured water quality parameters remained within acceptable levels during the static experiment. The average pH of both the control and PFO test solutions was 8.2 ± 0.2 for the entire test. The dissolved oxygen concentration of the control solution dropped below 6.0 mg/L 72 h after starting the test. Therefore, aeration of the test solutions was initiated at 72 h, and continued throughout the remainder of the experiment. With aeration, the dissolved oxygen levels in both test solutions remained at or near 100% saturation.

Concentration Of PFO In Test Solutions

The concentration of PFO in test solutions remained constant during the static exposure experiment. During the uptake phase, the measured concentration of PFO in spiked dilution water was consistent at approximately 25 mg/L (Table 1). In all other test solutions, during either phase, PFO concentrations were always less than 1.0 mg/L.

Survival And Growth Of Test Fish

Relative to the control treatment, the survival and growth of *P. promelas* exposed to PFO were not adversely affected during the experiment. The percent survival of the control fish and the PFO-exposed fish were 97 and 93, respectively. In addition, the average wet weight of PFO-exposed *P. promelas* did not differ significantly from the average wet weight of control fish at any of the sampling points (Table 2, Figure 1).

Concentration Of PFO In Test Fish

Uptake and Depuration

The concentration of PFO measured in fish tissue was directly related to the concentration of PFO in test solution. At the beginning of the uptake phase, the PFO concentration in fish tissue was < 1.0 µg/g (wet wt.). After 192 and 312 h of

exposure to PFO in dilution water, the average concentration of PFO in fish tissue was 44.7 and 46.7 µg/g (wet wt.), respectively (Table 3, Figure 2). The concentration of PFO in fish did not differ signficantly between 192 and 312 h of exposure, but was significantly greater at both of these sampling times than at 0 h. 24 h after being transferred to clean water, PFO-exposed fish had an average PFO tissue concentration of 19.9 µg/g (wet wt.). At 96 h post-exposure, the concentration of PFO in PFO-exposed fish had decreased to approximately 8 µg/g (wet wt.), and remained relatively constant until test termination at 360 h (cumulative test time = 672 h). The concentration of PFO in fish tissue at 96, 168 and 360 h post-exposure did not differ significantly from that measured at test start (0 h).

Bioconcentration Factor

The relationship between exposure to PFO and the amount of PFO in the tissue of *P. promelas* was described by a bioconcentration factor (BCF). Bioconcentration refers to the increase of a pollutant concentration from water when passing directly into an aquatic species (Moriarty 1983). The BCF was defined as:

$BCF = C_a/C_w$

where, C_a = equilibrium concentration of a pollutant in the aquatic organism, and C_w = equilibrium concentration of a pollutant in water to which the organism is exposed. Because the concentrations of PFO in test solution and fish tissue did not change between 192 and 312 h, the assumption was made that an apparent equilibrium, or steady state, had been reached in the static exposure system. Therefore, the *P. promelas* 312 h apparent steady-state BCF (BCF_{312h}) for PFO was calculated as the concentration of PFO in fish tissue at 312 h divided by the concentration of PFO in test solution at 312 h, or

BCF_{312h} = 46.7 µg/g (wet wt.)/25.9 µg/mL = 1.8.

REFERENCES:

Moriarty, F. 1983. Ecotoxicology: The Study of Pollutants in Ecosystems. Academic Press, Inc., Orlando, FL, 233 p. 233.

FIGURE 1. Average wet weights of Control group and PFO-exposed *P. promelas* sampled throughout Uptake/Depuration experiment.

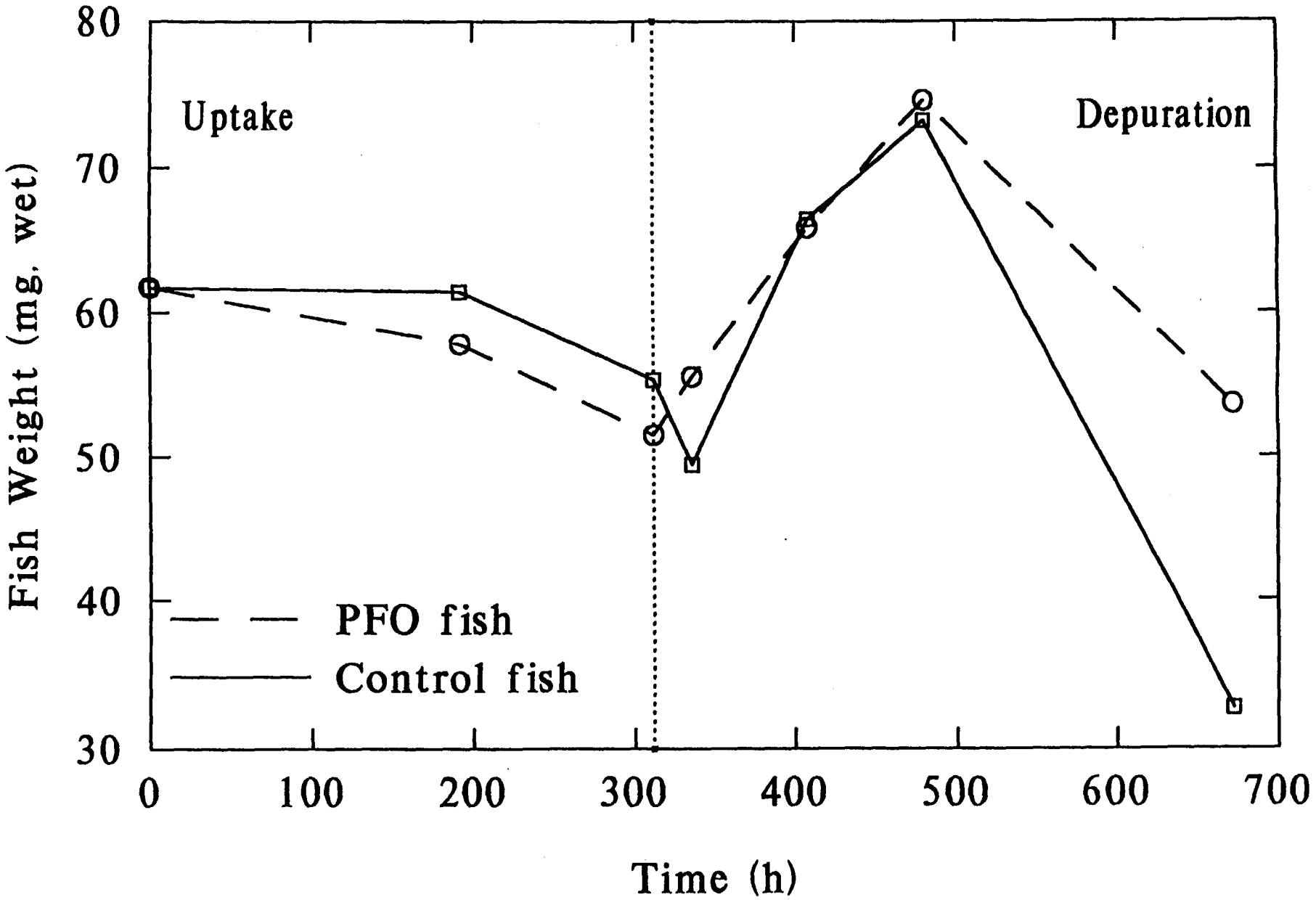

FIGURE 2. Uptake and depuration of PFO by *P. promelas*

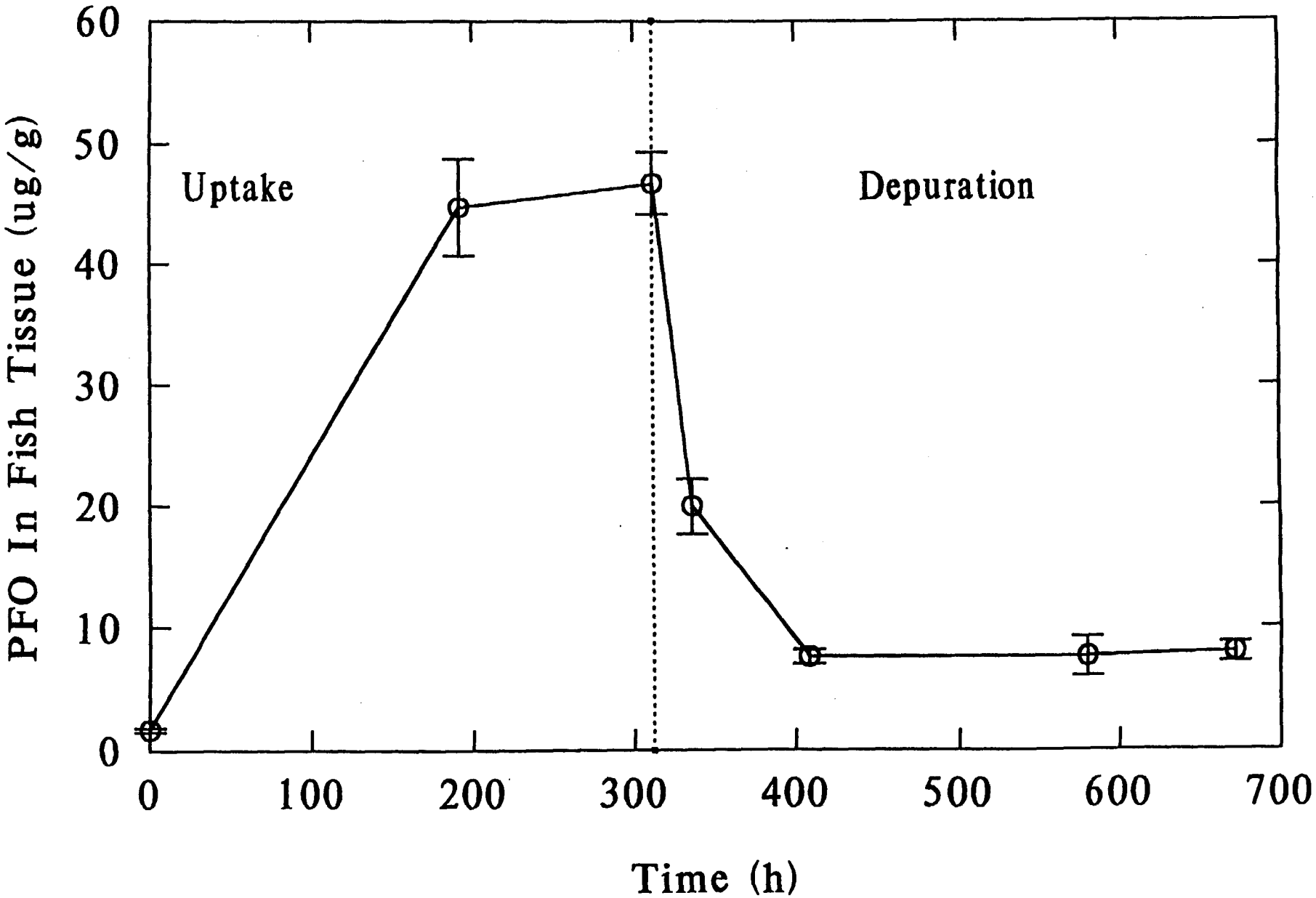

TABLE 1. Concentration of PFO measured in test solutions.

Phase/ Solution	Phase Time (h)*	Avg. PFO Conc. (µg/mL)	Std. Dev.
UPTAKE			
Control	0	< 1	-
	192	< 1	-
	312	< 1	-
25 µg/mL (N) PFO	0	25.0	1.8
	192	25.5	2.2
	312	25.9	1.8
DEPURATION			
Control	0	< 1	-
	168	< 1	-
	360		
	0	< 1	-
	168	< 1	-
	360		

* Elapsed time (h) from the beginning of each experimental phase.

** Test solution containing PFO-exposed fish.

TABLE 2. Average wet weights of Control group and PFO-Exposed *P. promelas*.

Time (h)	Control Fish		PFO-Exposed Fish		P§
	Average Wet Wt. (g)	Std. Dev.	Average Wet Wt. (g)	Std. Dev.	
0	61.7	5.2	61.7	5.2	n.s.·
192	61.4	20.8	57.8	13.4	n.s.
312	55.3	11.7	51.5	42.0	n.s.
336 (24)*	49.4	13.9	55.5	22.4	n.s.
408 (96)	66.4	7.3	65.8	22.4	n.s.
480 (168)	73.2	44.4	74.6	55.8	n.s.
672 (360)	32.7	16.8	53.6	16.9	n.s.

* Number in ( ) indicates elapsed time (h) from beginning of depuration phase.

§ Results of t-test (a=0.05) comparing wet weights of control and PFO-exposed fish sampled at the same time.

· Not Significant.

TABLE 3. Average concentration of PFO (measured as F-) in *P. promelas* tissues.

Time (h)	PFO In Fish (µg/g, wet wt.)	Std. Dev.	Comparison Of Means**		
0	1.7	0.4			C
192	44.7	9.1	A		
312	46.7	5.8	A		
336 (24)*	19.9	5.1		B	
408 (96)	7.6	1.3			C
480 (168)	7.6	3.5			C
672 (360)	8.0	1.6			C

* Number in ( ) indicates elapsed time (h) from beginning of depuration phase.

** Means with the same letter are not significantly different at alpha=0.05 by the Tukey-Kramer Honest Significant Difference test.

Appendix

Comments from Dr. John Giesy, Michigan State University

March 20, 1995

Dr. Robert Howell
3-M Corp.
Environ. Lab. Building 2-3E-09
P.O. Box 3331
St. Paul, MN 55113

Dear Robert:

I have reviewed the report entitled *Assessment of the Bioaccumulative Properties of Ammonium Perfluorooctanoate: Static Fish Test,* which prepared by James Drake. I found no fatal errors in the experimental design, methods, data interpretation or conclusions drawn from this study.

I have attached a short report which contains my specific comments on the study that was conducted and suggestions for future studies.

Please contact me if you have additional questions.

Sincerely,

John P. Giesy, Ph.D.

enc: Report

Report on an Experiment:

Bioaccumulative Properties of Ammonium Perfluorooctanoate: Static Fish Test,
which was prepared by James Drake.

By:
John P[redacted], Ph.D.
Principal Scientist
Giesy Ecotoxicology, Inc.

March 20, 1995

Dr. Robert L. Howell
3-M Corp.
Environ. Lab. Building 2-3E-09
P.O. Box 3331
St. Paul, MN 55113

Comments:

General:

1) The set of experiments was well designed and there are no fatal errors in logic, experimental design, methods selected, statistics applied or conclusions drawn.

2) The report is well written and the results clearly presented.

3) I agree with the conclusions that there is little bioaccumulation of perfluorooctanoate (FFO) by the fathead minnow.

Specific:

1) The use of the fathead minnow as a model species in initial experiments is appropriate and is useful in demonstrating that there is little bioaccumulation potential of PFO. However, this experiment does not address the issue of accumulation via food, by this species or larger predatory fishes. Some consideration should be made of feeding fatheads contaminated with PFO to larger fishes in a longer exposure.

2) The exposure period seems to be adequate to establish that the fish had attained a steady state concentration (SSC) from external exposure. However, in future experiments it would be useful to conduct the exposure at least one sampling period (4-5 days) longer to establish more definitively that SSC had been achieved.

3) The results of the study indicate that the bioaccumulation factor is approximately 1.8. While this value indicates that there is little accumulation of PFO, the true bioaccumulation factor is probably even less. I think that most of the PFO that was accumulated by the fish was due to surface sorption. Thus, the study probably overestimates the true BCF. Based on the data presented, the true BCF is probably a factor of eight (8) or nine (9) less than the reported value. I would suggest that in any future studies that the fish be rinsed for approximately four hours in clean flowing water before they are placed into the clean water to follow depuration.

While the accumulation potential seems relative small, relative to other halogenated hydrocarbons, it is not unexpected, based on the structure of the compound, and similar to the BCF expected for detergents.

The current study addressed only a single exposure concentration. The fact that there was accumulation of PFO into the fishes which was not rapidly depurated indicates that there is accumulation into a tightly bound pool. The fact that the concentration of PFO in that pool did not seem to increase during the experiment indicates that this pool had become saturated. If so, this indicates that based on a pharmaco-kinetic assessment that the BCF could be biased by the use of a single, relatively great exposure concentration. The existence of the tightly bound pool suggests that there are two depuration components, one very rapid and a second from which there seems to be no depuration at all during the duration of the study. If this is so, then exposure to lesser concentrations of PFO for longer periods of time would possible result in the same maximum bound PFO. Thus the BCF, which is the ratio of PFO in the fish to that in the water would be greater. Thus, the question is, are the pharmaco-kinetic constants true constants, which are independent of exposure concentration. This was not considered in the present study. For instance, if the fish were exposed to a concentration one hundredth as great for a longer period of time, but still reached the same total body burden,

the BCF for PFO accumulation by the fathead minnows would be one hundred (100) times as great. Future studies should include an assessment of the pharmaco-kinetic constants dependance on duration and intensity of exposure.

4) The duration of the depuration phase was adequate to establish that the fish had reached a concentration (5 mg/kg, ww) that seemed to be tightly bound in or on the fish and that further depuration was unlikely. The accumulation of as much as 5 ppm is not a small amount of compound, if the exposure concentrations are at all relevant for expected environmental concentrations. This result raises the question of where the PFO is associated with the organism. Is it surface-bound or bound in the internal tissues of the fish? If so, which tissues are the targets for accumulation?

5) While the experiment was not conducted to assess toxicity of PFO, the results indicate that PFO is not acutely toxic to fathead minnows. This is similar to results with PFO in other studies and consistent with the median tolerance limit LC-50 of over 700 mg/L.

The mechanism of action of perfluorinated compounds, which is often peroxisome proliferation, is a more chronic effect and any adverse effects due this mechanism of action would not be expected in a short-term study. Thus, the chronic toxicity of PFO to fish, especially predatory fished, remains an open question. Potential effects on long-term survival, growth and reproduction were not addressed in the bioaccumulation study with fathead minnows.

7) The use of a static system seems justified since actual concentrations were measured and there was no degradation of PFO and a constant exposure concentration was maintained. Future studies with smaller concentrations should consider the use of a flow-through system, which is the preferred methodology in definitive studies of bioaccumulation.

Potential future studies:

1) Disposition of PFO in fish tissues. I would suggest a radio-tracer study followed by whole-organism autoradiography.

2) The fact that there seems to be irreversible binding of PFO into the fatheads, indicates that there could be considerable accumulation, especially from the diet of predatory fishes. The current study only addressed bioaccumulation from water. A dietary study or biomagnification study is indicated.

3) Chronic effects of exposure to PFO on reproduction of fathead minnows. This is a relatively straight-forward study to conduct and would give needed information on the potential for chronic effects on survival, growth and fecundity.

4) The potential for long-term, chronic effects was not addressed in this study or any others. The potential for long-term biomagnification and effects could be assessed in a longer-term feeding study with predatory fishes.