

BIOCONCENTRATION

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 of uncharacterized purity.

METHOD:

Method/guideline followed: 3M derived method outlined in the test procedure for determining the aquatic Bioconcentration Factor of N-EtFOSE alcohol.

Type: Static exposure with flow-through clearance phase.

GLP (Y/N): No

Year: 1977

Species: Bluegill (*Lepomis macrochirus*) and channel catfish (*Ictalurus punctatus*)

Supplier: Bluegill obtained from a private hatchery in Baltic, Ohio

Channel catfish obtained from a private hatchery in Lonoke, Arkansas

Analytical monitoring: Concentration of N-EtFOSE alcohol in water and fish by GC with electron capture; temperature

Length and weight:

Bluegill were in the range of 2.5-3 cm and weighed 0.5-1.0 grams

Channel catfish were 8-10 cm and weighed 5-10 grams

Exposure period: 21 days (Bluegill) & 14 days (Channel catfish)

Clearance period: 14 days for both species

Statistical methods: Not noted in report.

Test fish age: Not noted in report.

Loading: Not noted in report.

Pretreatment: None

Test conditions:

Dilution water: Carbon-filtered well water

Dilution water chemistry: Not included in report.

Stock and test solution preparation: The primary stock solution was prepared by dissolving 57 grams of N-EtFOSE alcohol in 50 ml of acetone, applying the solution to 10 pounds of 3.5 mm glass beads, evaporating off the acetone, and circulating carbon filtered well water through the glass beads. The system was designed to result in a final loading ratio of 0.5 grams per liter. Aerobic conditions were maintained throughout and the system was assumed to go to saturation after circulating for 3 weeks.

Exposure vessels: 30-gallon glass tanks

Number of replicates: One

Number of fish per replicate: Not noted in the report.
Number of concentrations: One plus a negative control
Water chemistry during the study:

Dissolved oxygen: >5 mg/L

Test temperature: 21±1°C

pH: Not noted in the report.

Photoperiod: 16 hours light and 8 hours dark with a 30 minute transition period.

Remarks field: Prior to exposure, and at the time of their transfer to test tanks, the fish did not exhibit any symptoms of disease or abnormalities of behavior and appearance. The fish were fed daily at a rate of 2 percent of their total body weight with a commercially available basic diet (Tetra Min.).

RESULTS

Analysis of the whole fish showed fluorochemical concentration ratios in fish to water of approximately 400:1. The study indicated that the N-EtFOSE alcohol concentration in the fish stabilized after 7 days. Fish having an initial concentration of 0.213 mg N-EtFOSE alcohol / g of fish placed in water free of the test substance showed a decrease of 0.012 mg test substance / g of fish in 14 days (wet weight).

CONCLUSIONS

No reliable conclusions can be derived from this study. Although the objective on the cover page indicates this report was to determine the extent of fluorochemical uptake and bioconcentration, it appears from the abstract on the cover page that the primary purpose was to develop a method for testing bioaccumulation with the investigation of bioaccumulation of fluorochemicals in fish being secondary. The study's objective is not detailed in the report body.

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

DATA QUALITY

Reliability: Klimisch ranking 3. There is a lack of description for the study's methodology. The vigorous method used to solubilize the test substance may have resulted in a supersaturated solution. The uptake and clearance of the test substance by both test organisms was quite rapid, leading one to question if perhaps test substance was sorbed to the fish rather than being taken up from solution. Test substance purity was not characterized. The initial water quality of the dilution water is missing. The test substance concentration used in the test is greater than the solubility. The recovery tank used appears to be the same for both species tested.

Third party review by Dr. James W. Gillett of Cornell University (observations included with report) also indicate a lack of reliability.

REFERENCES

3M Technical Report "Bioconcentration of FM 3422 in Bluegill Sunfish and in Channel Catfish" M. T. Elnabarawy, Project 9970612600, Fate of Fluorochemicals, Report Number 01, May 17, 1977

3M Technical Report "Aquatic Fate of A Fluorochemical: FM 3422." A. N. Welter, Project 9970512623, Fate of Fluorochemicals, Report Number 002, October 14, 1977

3M requested expert overview, "Bioaccumulation Studies", Dr. James Gillett, Cornell University, March 8, 1993

OTHER

Last changed: 5/18/00

TECHNICAL REPORT SUMMARY

Date
5/17/77

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)		Dept. Number 0222-78
Project Fate of Fluorochemicals		Project Number 9970612600
Report Title Bioconcentration of FM 3422 In Bluegill Sunfish and In Channel Catfish		Report Number 01
To A. N. Welter - 21-2W (58)		
Author(s) M. T. Elnabarawy - 2-3E		Employee Number(s) 46981
Notebook Reference 42669, Pages 17-26		No. of Pages Including Coversheet 5
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.)

Fluorochemical
Biology/Activity
screening
(bioaccumulation)
(Fish)
EE & PC - Div.

CURRENT OBJECTIVE:

The purpose of this pilot study was to determine the extent of fluorochemical (FM 3422) uptake and/or bioconcentration by Bluegill sunfish (Lepomis macrochirus) and Channel catfish (Ictalurus punctatus).

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.

A modified technique is proposed for monitoring fluorochemicals in suspect aquatic environments by whole fish or tissue analysis. Bluegill sunfish (Lepomis macrochirus) and Channel catfish (Ictalurus punctatus) contained concentrations of FM 3422 greater than those found in their water environment, achieving ratios of approximately 400:1. Concentrations of FM 3422 by these fish species had also plateaued within seven days of exposure. When whole fish (Lepomis macrochirus) were analyzed for FM 3422 uptake following a two-minute exposure in the test tank, an insignificant uptake was noted (.0006 mg/g).

Bioconcentration studies are useful to qualitatively monitor fluorochemicals in water. Whole fish or tissue analysis from fish exposed to fluorochemicals in their environment may prove to be a useful tool in evaluating the mobility of these chemicals in an aquatic environment.

Experimental:

A sample of FM 3422 (57 grams dissolved in 50 ml acetone) was applied to 10 pounds of $3\frac{1}{2}$ mm glass beads. The glass beads were then placed under the hood and the acetone was allowed to evaporate over a 48-hour period. The FM 3422 coated beads were spread evenly in an all-glass 30-gallon tank (114 liters), forming a layer of approximately 2 cm above the undergravel filter. The test tank was filled to its capacity with carbon-filtered well water. Chemical composition of the water is available upon request. The amount of test compound applied was designed to give a final loading ratio of 0.5 grams per liter. For 3 weeks and under continuous aerobic conditions (aeration was maintained at all times), this dynamic system generated saturated water solution of FM 3422 without organic solvents. Under identical test conditions, a control tank was set up in a similar manner containing no FM 3422.

After the 3-week period of aerobic aging, test fish were introduced into the tanks (an assumption was made that equilibrium in the system had been reached). Fish used in this experiment were obtained from private hatcheries:

	<u>Bluegill sunfish</u> <u>(<i>Lepomis macrochirus</i>)</u>	<u>Channel catfish</u> <u>(<i>Ictalurus punctatus</i>)</u>
Source:	Baltic, Ohio	Lonoke, Arkansas
Size:	2.5-3 cm	8-10 cm
Weight:	.5-1.0 grams	5-10 grams

The fish were held and cared for in adequately aerated water (dissolved oxygen was greater than 5 mg/l). The fish were acclimated to test conditions; water temperature was maintained at room temperature $70 \pm 2^\circ$ F. ($21 \pm 1^\circ$ C). A photoperiod of 16-hour light and 8-hour dark was provided with a 30-minute transition period. The fish were fed daily at a rate of 2 percent of their total body weight with a commercially available basic diet (Tetra Min). Prior to exposure, and at the time of their transfer to test tanks, the fish did not exhibit any symptoms of disease or abnormalities of behavior and appearance.

A criterion for sampling was set up. On various days of increasing periods of exposure, representative water samples at various depths were collected and fish were sacrificed at random. At the end of the exposure period, remaining fish were transferred to a clean aquarium, which was continuously filled with fresh water (a flow-through system) to determine the clearance (recovery) rate. At sacrifice, the total fresh body weight was recorded. Some channel catfish were dissected, and various parts were removed and retained for analysis.

Results:

Extracts of water samples and of sacrificed fish were analyzed for FM 3422 concentrations by the GC technique with an electron capture detector. Extractions and GC analytical techniques have been performed in-house under the direct supervision of A. Mendel. Fish-to-water concentration ratios of FM 3422 were also calculated. Detailed description of this work is attached. Additional information on this project can be found in M. T. Elnabarawy's technical notebook, No. 42669, pp. 17-26.

Discussion:

Throughout the experiment, all fish appeared generally healthy and active in both test and control tanks. During periods of exposure, 5 bluegill sunfish died (2 from test tank and 3 from control tank). The mortality was most likely due to physical injury and was not considered to be test compound related. Water was added periodically to supplement the loss due to sampling and evaporation.

Concentrations of FM 3422 in both fish species had reached a plateau by 7 days of exposure.

Measured concentrations of FM 3422 in the viscera illustrated the importance of fish size on bioaccumulation (the test compound tends to bio-concentrate in higher ratios in larger fish).

After transferring the remaining bluegill sunfish to the recovery tank, and before introducing channel catfish, 5 pounds of sea-sand (washed and ignited) were evenly dispersed on the bottom covering the FM 3422 coated beads. Analysis of water samples taken before and after adding the sand showed no change in FM 3422 concentration.

Whole fish analysis of 3 bluegill sunfish after a two-minute dip in the test tank showed insignificant uptake (.0006 mg/g).

If you have any questions, please contact me on 3-9186.

MTE/cel

Attachments

Bluegill Sunfish (Lepomis macrochirus)

Treatment	FM 3422 Concentrations		Fish-to-Water Ratio
<u>Uptake (exposure days):</u>	<u>In Fish (mg/g)*</u>	<u>In Water (ppm)**</u>	
0	No background	.329+ .003 (air-off)	
8	.118	.290+ .005 (air-off)	407:1
14	.117	.428 (air-off)	273:1
21	.213+ .005	.580 (air-off)	367:1
<u>Clearance (Recovery days):</u>			
0	.213		
7	.007		
14	.012		

* Values are means and standard deviations from the analysis of 3 (whole) fish.

** Values are means and standard deviations from the analysis of 3 water samples representing depths of 36, 20 and 6 cm from bottom.

Channel Catfish (Ictalurus punctatus)

Treatment	FM 3422 Concentrations		Fish-to-Water Ratio
<u>Uptake (exposure days):</u>	<u>In Fish (mg/g)*</u>	<u>In Water (ppm)**</u>	
0	No background	.340 (air-off)	
7	.128 $\pm$.01	.325 (air-off) 1.075 (air-on)	394:1 119:1
14	.100	.546 $\pm$.02 (air-off) .620 $\pm$.05 (air-on)	183:1 161:1
<u>Clearance (Recovery days):</u>			
0	.100		
7	.023		
14	.003		

* Values are means from the analysis of 2 (whole) fish.

** Values are means and standard deviations from the analysis of 3 water samples representing depths of 36, 20 and 6 cm from bottom.



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TECHNICAL REPORT SUMMARY

Date
10/14/77

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)	Dept. Number	0222
Project	Fate of Fluorochemicals	Project Number	9970612623
Report Title	Aquatic Fate of A Fluorochemical: FM 3422	Report Number	F 102
To	D. L. Bacon		
Author(s)	A. N. Welter <i>ANW</i>	Employee Number(s)	09362
Notebook Reference	No. of Pages Including Coversheet		9

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.)EE & PC - Div.
Fluorochemicals
(Aquatic)
Toxicity
(Bioconcentration)

CURRENT OBJECTIVE:

Progress 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.

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INTRODUCTION

The subject compound (FM 3422) was selected for testing based on its importance as an intermediate in the synthesis of other commercially important fluorochemicals. These compounds represent a major commitment by the Commercial Chemicals Division. With increasing governmental regulations pertaining to the influence of chemicals on the environment, consideration of the environmental impact of this chemical class was mandated. Furthermore, since FM 3422 might qualify as a 3M "critical chemical," extensive laboratory investigations were performed to assess its possible environmental impact.

Physicochemical data have been utilized to predict the behavior of chemicals in the environment in the absence of experimental data. Available physicochemical data for FM 3422 include the following (1):₅ water solubility - 0.05 ppm, partition coefficient in n-octanol/water system - $>10^5$. These data would suggest that FM 3422 would be persistent, relatively insoluble in water and possess lipophilic properties. FM 3422 was nontoxic within its solubility limits when submitted for aquatic toxicity determinations.

It is the purpose of this report to present data relative to the bioconcentration potential, uptake and clearance rates of FM 3422 in either the bluegill (*Lepomis macrochirus*) and/or the channel catfish (*Ictalurus punctatus*).

METHODS

M. T. Elnabarawy has recently described the standard methods utilized in the Environmental Laboratory relative to the acclimation period, care of aquatic organisms and method of chemical exposure of these organisms (2).

Specific protocols for the determination of bioconcentration factors (BCF), uptake and clearance rates used in this study follow: Bluegill BCF were determined at the 8, 14 and 21 days of exposure to FM 3422. Clearance values were evaluated on the 7 and 14 days of depuration. Channel catfish BCF were obtained at the 7th and 14th day of exposure with clearance values being determined after identical periods of depuration. Bioconcentration factors for specific organs of the channel catfish were determined after either one or four weeks of exposure to the test fluorochemical. Water samples were obtained at three different levels within the experimental tank on those days when fish samples were obtained. The analytical techniques used for the determination of FM 3422 levels were those routinely used by the Environmental Laboratory and will be the subject of a report (1).

RESULTS

Bioconcentration factors, uptake and clearance rates were monitored in both the bluegill (*Lepomis macrochirus*) and channel catfish (*Ictalurus punctatus*) at varying time intervals during exposure to 0.5 g/l FM 3422 in the aquatic environment as well as during depuration (Tables 1, 2, Figures 1, 2).

Graphic representation of these data indicates that a rapid uptake of FM 3422 by both organisms had occurred (Figs. 1, 2). The initial sampling period (Days 7 and 8) values indicate that a steady state has been attained at some earlier time period. Of greater importance was the rapid clearance of the test material from both organisms which was quite apparent after seven days of depuration. The channel catfish cleared FM 3422 somewhat more completely than did the bluegills.

Bioconcentration factors and clearance values determined for whole organs were quite similar and seemingly independent of exposure periods (Table 1). The elevated fluorochemical C_w values observed in the channel catfish experiments were due to a single series of outlier values. When these values were ignored, fluorochemical concentrations achieved in both experimental tanks were identical, 0.4 ppm.

In the channel catfish, the more lipophilic organs bioconcentrate FM 3422 to a greater degree than the relatively lipid-free materials (Table 2). Thus, the oil layer obtained from the skin and the viscera (gut) possessed the highest fluorochemical bioconcentration factors following one week of exposure to FM 3422. Brain tissue, which was analyzed only following four weeks of exposure to the fluorochemical showed similar elevated BCF's. The gills had attained high levels of FM 3422 at both exposure periods, which is probably indicative of a large surface area available for binding. The remaining organs which were tested - muscle, skin, skeleton - achieved similar levels of FM 3422. These values for BCF were approximately one order of magnitude less than that found for the more lipophilic materials.

DISCUSSION

In these studies, uptake of FM 3422 by the bluegills and channel catfish probably occurred via the gills, oral and/or cutaneous routes. Transient exposure, < 2 min., of fish to a fluorochemical-aquatic environment did demonstrate uptake of this fluorochemical. Uptake may result from the penetration of the lipophilic gill epithelium by this lipophilic molecule. During long-term (days) FM 3422 exposure, the fluorochemical could enter the gill circulation and thence be transported to various sites within the organism. Granmo and Kollberg (3) have discussed the uptake mechanisms of nonionic surfactants in the cod. In their studies, it was demonstrated that rapid uptake of this chemical by the gills had occurred and that blood was the principal transport medium to the various tissues/organs where deposition occurred. Bass and Heath (4) postulated that the gills may be damaged by exposure to toxic materials resulting in tissue hypoxemia which culminated in death of the test organism. Bass et al (5), in a subsequent paper, conclusively demonstrated that hyperplasia with lamellar fusion of gill filaments and edema did occur in the presence of a toxicant. The resulting hypoxic condition was due to impaired respiratory gas transport. These papers stress the importance of the gills in the uptake of foreign chemicals while also enumerating potential lesions which may result, manifesting themselves as a toxic response.

Oral uptake of FM 3422 (feeding) would afford a direct route for the absorption of this fluorochemical by the gastrointestinal tract (gut). Movement of the fluorochemical into the intestinal circulation would result in the transport of this chemical by the blood to other organs of the body; liver, gall bladder, mesentery, etc. Circulation of the fluorochemical would then result in the selective deposition of this material in the more lipophilic tissues of the organism. It has been suggested, Chiou et al (6) that toxicity associated with

exposure to lipophilic materials may be the result of the long-term slow release of the chemical or a metabolic product into the circulation of the organism.

Percutaneous penetration of FM 3422 may explain the bioaccumulation of this material in the oil layer of the skin. It may be speculated that FM 3422 may remain localized in this area with a subsequent leaching effect resulting in its eventual clearance.

In these pilot studies, we did not quantitate uptake rates of FM 3422 either in the intact organism or specific organ systems. However, the data did indicate that in the intact organism a steady state (intake=output) had been attained in less than seven days. Uptake rates observed are a function of metabolic rate, age, weight, water temperature, feeding habits, etc. These variables must be controlled when replicating these experiments. Clearance (elimination) rates were not determined during the initial days of depuration; therefore, it can only be stated that FM 3422 was cleared in <7 days. Of interest would be the determination of clearance rates at the earlier time intervals in an effort to ascertain whether or not these values would indicate the presence of a two-phase system. The initial rapid phase indicative of the elimination of free or loosely bound fluorochemical may be followed by a slower phase which would be presumptive evidence for the release of tightly bound material.

It should be noted that in this study uptake and clearance values were only determined in intact organisms rather than utilizing the specific organ technique. It would appear that this latter method should be utilized in an effort to determine whether or not FM 3422 is also rapidly cleared from the more lipophilic organs.

One may speculate that bioconcentration, per se, may be due to a multiplicity of chemical-receptor interactions. In receptor theory several types of receptor attachments have been identified. For example, the material may bind to the receptor in stable or labile fashion, or the material may be found in the free state. Of importance would be the determination of whether or not binding associated with FM 3422 may be reversible. This latter hypothesis may approximate the true situation, inasmuch as FM 3422 was rapidly cleared. If clearance were prolonged, this would be indicative of an irreversible binding. This pharmacological tool may well have application in assessing applicable mechanisms and modes of chemical uptake and clearance by aquatic organisms.

In these pilot studies, we have demonstrated that FM 3422 does:

- 1) Bioconcentrate in lipophilic organs achieving levels of fluorochemical approximately one order of magnitude greater than those found in relatively less lipophilic organs.
- 2) Attain a steady state within 7 days.
- 3) Clear rapidly, channel catfish > bluegill.

In the absence of toxic signs and considering the relatively rapid clearance of the fluorochemical by the test organisms one may assume that this material was nontoxic under the conditions employed in the foregoing experiments.

BIBLIOGRAPHY

- (1) Mendel, A. Personal communication. Report in progress.
- (2) Technical Report, May 17, 1977, M. T. Elnabarawy to A. N. Welter entitled: "Bioconcentration of FM 3422 in Bluegill Sunfish and in Channel Catfish."
- (3) Granmo, A. and S. Kollberg: Uptake Pathways and Elimination of a Nonionic Surfactant in Cod (*Gadus Morrhea* L.), Water Research 10:189-194, 1976.
- (4) Bass, Michael L. and Alan G. Heath: Cardiovascular and Respiratory Changes in Rainbow Trout, *Salmo gairdneri*, Exposed Intermittently to Chlorine, Water Research 11: 497-502, 1977.
- (5) Bass, Michael L. Charles T. Berry, Jr., and Allan G. Heath: Histopathological Effects of Intermittent Chlorine Expose on Bluegill (*Lepomis macrochirus*) and Rainbow trout (*Salmo gairdneri*), Water Research 11: 731-735, 1977.
- (6) Chiou, Cary T., Virgil H. Freed, David W. Schmedding and Rodger L. Kohnert: Partition Coefficients and Bioaccumulation of Selected Organic Chemicals, Environmental Sci. and Tech. 11: 475-478, 1977.

LEGEND

Figure 1 - Abscissa in days, Bluegill Data

Uptake indicates exposure period to FM 3422

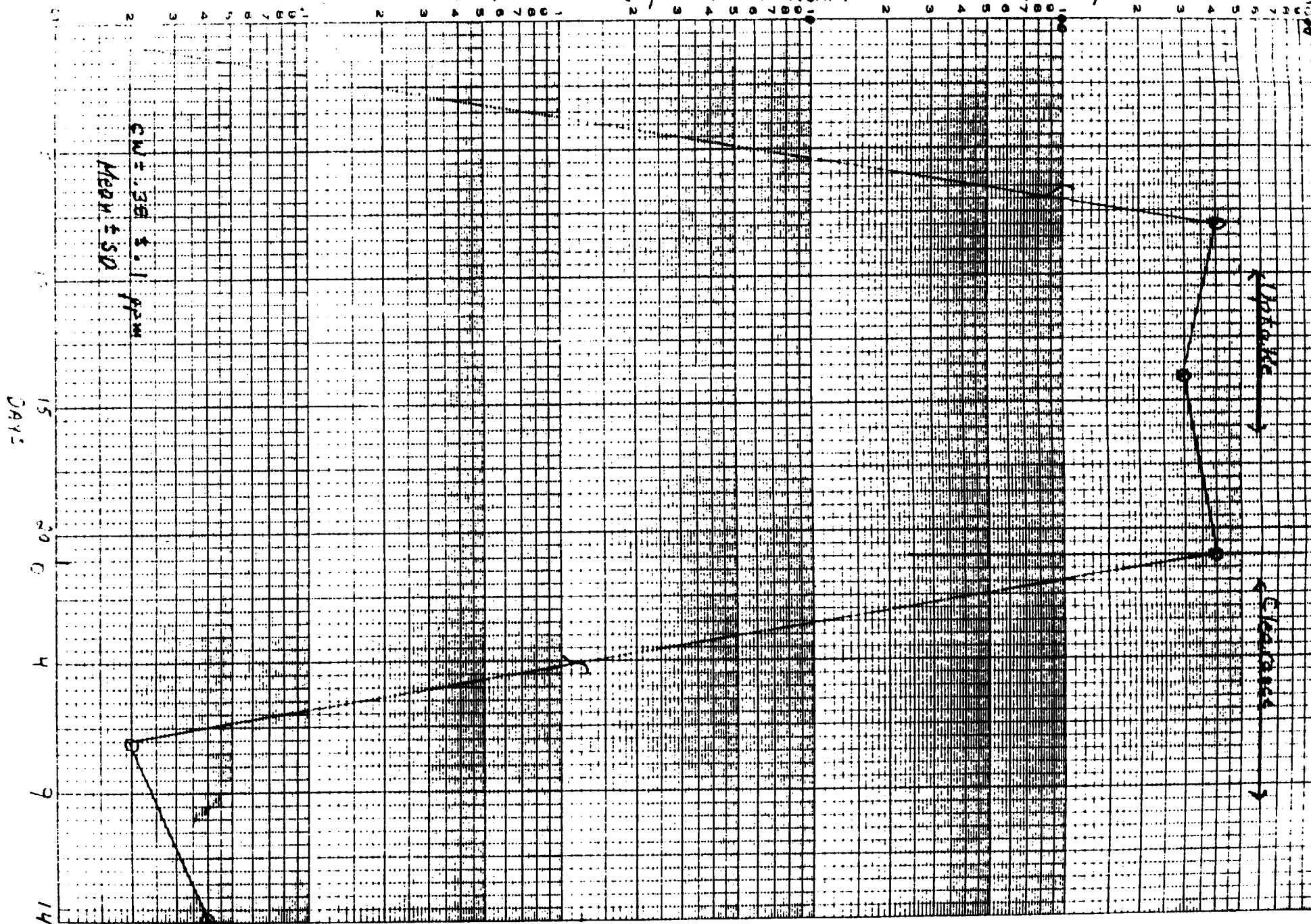
Clearance indicates days of depuration

C_w value indicates concentration of fluorochemical in water

X indicates absence of intermediate values, hence the line segment is arbitrary.

Figure 2 - Legend as for Figure 1, Channel Catfish Data.

BIOCONCENTRATION FACTOR (UPTAKE) AND CLEARANCE OF FM 3422, E1027, 11



BIOCONCENTRATION FACTOR (UPPER) AND CLEARANCE OF FM3412, CHANNEL CATFISH

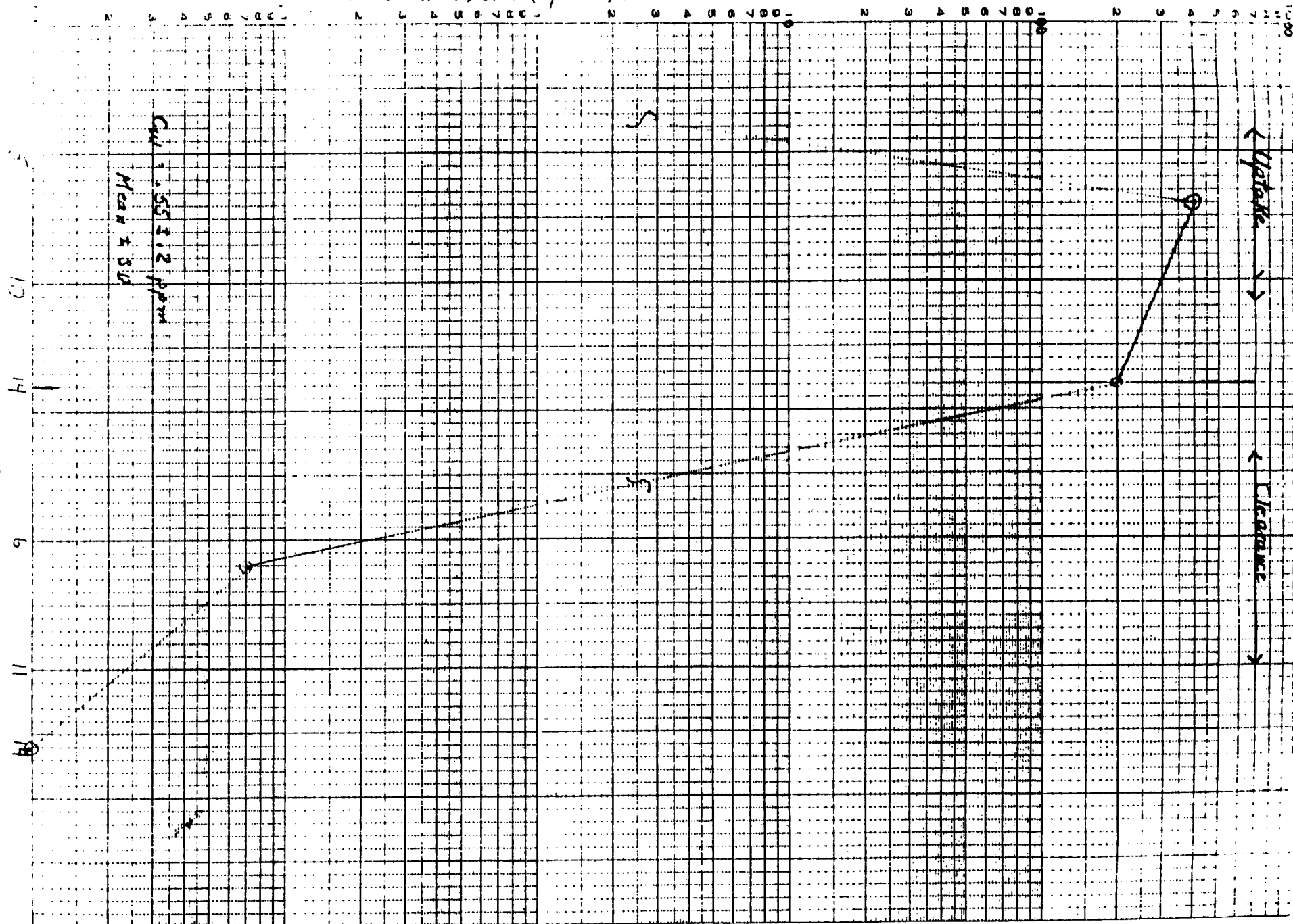


TABLE 1 FM 3422: BIOCONCENTRATION FACTORS AT VARYING
EXPOSURE AND CLEARANCE TIMES: BLUEGILLS AND
CHANNEL CATFISH ^{a,b}

	<u>BIOCONCENTRATION FACTOR</u>			<u>CLEARANCE</u>	
<u>Organism</u>	<u>D A Y S</u>				
	<u>8</u>	<u>14</u>	<u>21</u>	<u>7</u>	<u>14</u>
Bluegills ^b	4×10^2	3×10^2	4×10^2	2×10^{-2}	4×10^{-2}
	<u>D A Y S</u>				
	<u>7</u>	<u>14</u>		<u>7</u>	<u>14</u>
Channel catfish ^c	4×10^2	2.10^2		7×10^{-2}	9×10^{-3}

^a Values are rounded to nearest hundredth.

^b $C_w = .4 \pm .1$ ppm, Mean $\pm$ SD Concentration of FM 3422 in water.

^c $C_w = .6 \pm .2$ ppm, Mean $\pm$ SD Concentration of FM 3422 in water.

TABLE 2 BIOCONCENTRATION FACTOR OF CHANNEL
CATFISH ORGANS AT VARYING EXPOSURE
PERIODS OF FM 3422

<u>Organ</u>	<u>BIOCONCENTRATION FACTOR</u>	
	<u>1-Week Exposure ^a</u>	<u>4-Week Exposure ^c</u>
Muscle	3×10^2	3×10^2
Viscera (Gut)	2×10^3 7×10^2	3×10^3
Gills	7×10^2 1×10^3	1.6×10^3
Skin and Skeleton	3×10^2 5×10^2	
Skin		9×10^2
Skeleton		5×10^2
Oil Layer (Skin)	3×10^3 (1) ^b	
Brain		10^3

^a Specimens obtained from 2 channel catfish

^b Single analysis

^c Analysis of (1) channel catfish

1. *Report No. 1 (5/17/77) "Bioconcentration of FM3422 in Bluegill Sunfish and in Channel Catfish" and Progress Report No. 2 (10/14/77) "Fate of Fluorochemicals"*

A modified (from what?) exposure (?) technique was used to estimate BCF to bluegill and channel catfish (whole body) and particular tissues following exposure to a supersaturated solution (suspension) of FM 3422 (N-Et FOSE alcohol). Experimental detail are lacking in many areas for which there are text statements and conclusions. Even for its time this set of experiments was only marginally useful, at best. Problems include the following:

- a. The character and source of the FM 3422 is never stated, particularly with regard to purity; similarly, the assay technique is undocumented as to precision and sensitivity for the purposes to which it was applied.
- b. The material taken up and bioaccumulated was never identified as the nominal toxicant to which the fish were exposed, as far as readers are concerned. Could it be a metabolite and/or bound residue?
- c. Geo. Chapman and Chuck Warren at Oregon State Univ. had demonstrated the utility of saturated glass bead delivery in a flow-through system in the early 1970's. In the course of those experiments they were able to demonstrate the presence of a very much more highly toxic component of tech. dieldrin which was not the nominal compound (HEOD). Their method was to saturate the glass beads with tech. dieldrin by removal of acetone solvent in the flash evaporator, then place the beads in a large column over glass wool and a portion of clean beads. The water-jacketed column was brought to experimental temperature and portions of the water stream assayed for dieldrin (as HEOD) by extraction and GC/EC. A compound with lower EC response than HEOD for its toxicity (or much more toxic for its EC response) came off the column during the equilibration phase (1-2 weeks), but was not detectable by 3-4 weeks when the HEOD concentration was stabilized (it remained constant for ca. 10 weeks). Use of the Brungs-Mount diluter with this saturated solution produced consistent results if the diluter apparatus was equilibrated with the test solution. The point is that the column can generate suspensions and solutions of changing composition and toxicity, as well as concentration. The static system employed in these papers cannot equilibrate in the same sense as does a flow-through system.
- d. The authors used the "air-off" concentrations as that for the BCF calculation, although they clearly had a problem with values of FM 3422 15-300% (!?) higher with "air-on." The latter was probably the "well aerated" water to which test fish were exposed. This underlines the extensive problem supersaturation creates.
- e. It is likely that the values reported have little to do with bioaccumulation or even uptake per se, at least as expositied within the reports. Whole body residues might include entrapped material (colloidal particles in the gills); tissue residues depend upon not only correct dissection, but also protection from surficial contamination.
- f. The number of fish constituting a sample, the number of fish per tank, the size (length, weight) of each fish, and whether measured fish were live or among the dead removed all are alluded to but never detailed by the authors. Nevertheless, they proceed to note that larger fish accumulate more FM 3422 than smaller fish and that some (3) of the controls died. Heavier fish might have a higher percentage of fat than younger, leaner fish. It would be useful to know if the test subjects had about the normal lipid content.
- g. It is not clear why fish were dipped for 2 min, then analyzed. The experiment is without much meaning with respect to protection, toxicity analysis, etc. I know of no database including such a measurement for comparison with other toxicants.
- h. The cited value for K_{ow} appears in error; it may involve mis-measurement.

- i. Some of the tissues show no increase in BCF between 1 and 4 weeks; others (gills and gut) increase dramatically. The data in tissues do not support the assumption that a true equilibrium is reached between the fish and its environment or within the fish between tissues.