

3. *Report No. 2 (8/16/78) "Evaluation of the Bioconcentration Potential of FM 3422"*

This report covers new experiments based on the study in §1 (above), but using a shorter (!) exposure (to a sat'd. solution and under the same circumstances) and more frequent measurements only with channel catfish. However, the methods indicated for measurement of solubility and K_{ow} are fairly good and the basis for state-of-the-art protocols. Hence, I accept the value of solubility = 50 ppb and $\log K_{ow} = 5$. Further, with this very low solubility, even with the low measured v.p., one would expect a moderate Henry's Law constant leading to *volatility from water or a wetted surface*. On the other hand, the MW is 571, which puts it toward the upper part of the range of molecular sizes for which expected behavior is linear with respect to K_{ow} . That is, the molecular configuration may present some problems in crossing through the pores of the lipid bilayer of the several membranes.

- a. The report is very confusing when it expresses test agent conc. in "ul/l" and residues in "ug/g." The system was loaded at a rate of 0.5 g/L (500,000 ug/L)[end of para. 4, pg. 3] when the solubility is 50 ug/L (50 ppb), whereas you state that "0.5 ul/l = 500 ppb."
- b. The data in Tables 2 & 3 are shown for "Fish A" and "Fish B," as if these were sequential analyses of the same individuals. The text notes that four fish were removed at each time [para. 6, pg. 3]. Apparently, the sample was divided to provide 2 fish for whole body and 2 for tissues via being "tissuemized" (whatever that is). {It is interesting in this regard to note the problem in not having a protocol for dissection other than some QA/SOP in a book somewhere. For a number of years there were frequently significant differences between PCB residues in lake trout measured by Canadian scientists in comparison to those by NYS DEC scientists, although they both thought that they agreed on the same protocol. It turns out that there were two errors or differences. The Canadians only used the portion of the fillet above the median line for PCBs, whereas the U.S. group used the entire fillet minus the mid-line. Second, the Canadians removed material (fins, mid-line) by a rigid protocol, i.e., cutting a 6-mm notch to remove the mid-line and taking off the top 25 mm to remove the dorsal fin and associated fat. The DEC lab varied the fat removal so as to take off all fat by cutting in 1-5 mm more or less. These differences are very small, but reflect

the advisories given the public by separate agencies. Anyway, the U.S. values are much lower than those of the Canadians, even though the fish come from the same water.}

- c. The reporting and plotting of arbitrarily designated data ("Fish A" - Fig. 1 and "Fish B" - Fig. 2) is not very scientific. At least they should be on the same plot, as a range or recalculated as an average at time t . As an average value, the whole body residues were still climbing when the investigators terminated exposure. The avg. values for the times from 24 hrs on are 107, 172.5, 190.5, 223.5, 206, 299.5, and 355, implying a rate of "equilibrium" - approaching +20%/day! That is far from equilibrium. Further, although the general protocol resembles state-of-the-art, the small number of test organisms per time interval (should be ≥ 6) means that what the investigators see as an "outlier" may really be a typical point from a wide range of responses. Curiously, although the authors were concerned about the increasing uptake with weight in Report No. 1, they do not report body weight, lipid composition, or length (determinant of gill size) for the test subjects. The rate constants should be calculated and divided ($k_1/k_2 = BCF$), but the loss rate appears to accelerate with time. This may be a feature of the incomplete equilibrium, tissue distribution, or enterohepatic cycling via bile.
- d. By the time one gets around to the specific tissue distributions (which are expressed as up to 5 significant digits!), the authors are confusing individual and temporal variation with "plateaus" and "erratic" patterns. It is unlikely that 2 specimens at each time can yield comparable data unless there are criteria for age and size class. Reporting BCF by specific tissue, without attention to tissue lipid level is disingenuous or at least misleading.
- e. It is not clear how the authors have documented excretion via urine *per se*, since urine concentrations, conjugates, etc., were never measured. Presence of material in kidney merely reflects vascular perfusion. On the other hand, the tentative view of the importance of biliary excretion (already well established for lipophiles) is not understandable. Indeed, one might note that gall bladder distention could mean positive acceleration of biliary excretion (influenced by hepatic levels).
- f. The belief that the absence of toxic symptoms for the exposure regime used constitutes definition of this fluorocarbon as "non-toxic" is an abuse of the term, since (as Paracelsus said) 'all things are toxic and it is only a matter of dose which determines whether a chemical is a poison.' If the investigators were able to determine the N-Et FOSE alcohol as such, then it was not conjugated (statements about this route notwithstanding).

TECHNICAL REPORT SUMMARY

Date
August 16, 19

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 Evaluation of the Bioconcentration Potential of FM 3422		Report Number 2
To D. L. Bacon		
Author(s) A. N. Welter		Employee Number(s) 09362
Notebook Reference		No. of Pages Including Coversheet 25
SECURITY ▶	<input type="checkbox"/> Open (Company Confidential)	<input checked="" type="checkbox"/> Closed (Special Authorization)
	3M CHEMICAL REGISTRY ▶	
	New Chemicals Reported <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	

KEYWORDS:

(Select terms from 3M Thesaurus. Suggest other applicable terms.)

EE & PC - Div.

Fluorochemicals
(Aquatic)

Toxicity
(Bioconcentration)

CURRENT OBJECTIVE:

Progress Report

1. Evaluate Bioconcentration Potential of FM 3422.
2. Determine uptake and clearance rates of FM 3422 in whole fish and/or tissue.
3. Determine excretory pathway for FM 3422.

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.

cc: DLB
RLB
MTE
AM
R.Prokop-236-3B
EAR
SKW

Information Liaison
Initials: SKW

006251

INTRODUCTION

Living organisms possess the ability to concentrate and accumulate high concentrations of lipophilic organic compounds either directly from their environment or from their food source, a phenomenon which is well documented (Burnett, 1971; Gustafson, 1970). This ability to bioconcentrate seemingly non-toxic substances might well pose a hazard for the ultimate predator species.

This investigation was conducted to determine whether a fluorochemical, FM3422, does bioconcentrate in organisms and if so, does the material depurate rapidly. Selective organ systems were analyzed for the subject fluorochemical as potential uptake sites.

Since the aquatic environment serves as a primary mode of entry for FM3422 into the environment, this study was conducted using the channel catfish (*Ictalurus punctatus*) as the test species.

The subject fluorochemical, FM3422 is a white granular material, molecular weight-571, possessing physico-chemical properties which suggest that this material may bioconcentrate. Thus, the material was found to be relatively water insoluble and possessed a high distribution coefficient, data which is indicative of a highly lipophilic molecule. This profile is generally accepted as that of a substance which will tend to bioconcentrate.

METHODS

The hydrophobicity of FM3422 precluded a simplistic determination of its water solubility. Use of the Veith-Comstock technique

(1975) wherein water in a constant-level reservoir was continuously saturated with FM3422 by circulating the water through a bed of inert substrate impregnated with FM3422. Samples for analytical analysis were obtained via a sampling port. Replicate studies were performed.

The distribution coefficient of FM3422 in n-octanol/water was determined using the methods described by Chiou et. al. (1977) and Fujita et. al. (1964).

Channel catfish (*Ictalurus punctatus*) were acclimated to the following test conditions for a minimum of 14 days: temperature, $21 \pm 1^{\circ}\text{C}$, 16-hour light and 8-hour dark photoperiod with a 30 minute transition period and were fed daily (Tetra Min).

FM3422, previously impregnated on 3.5 mm glass beads was placed in a 114 l aquarium, forming a layer approximately 2 cm above the gravel filter which was then covered by sand. The test tank was then filled with carbon-filtered well water. This system under continuous aerobic conditions generated a saturated water solution of FM3422, having a final loading ratio of 0.5g/l.

Based on a previous study (1977) which indicated rapid uptake and depuration rates for FM3422 in this species, the bioconcentration test was modified so that the uptake and clearance periods were seven and five days respectively.

Following the introduction of the channel catfish into the experimental and control aquaria samples of 4 channel catfish and three water samples, top, middle and bottom layer, for FM3422 uptake analysis were obtained at the following time periods: control,

prior to introduction, 15 minutes, one hour, two hours, four hours, eight hours, twelve hours, sixteen hours, twenty hours, twenty-four hours, and at twenty-four hour intervals for a period of seven days. The channel catfish were then removed to clearance tanks for two hours and finally transferred to a flow-through system having flow rates through the test chambers of four water volumes per 24 hours. A sampling regimen identical to that of the uptake phase of this study was immediately initiated and terminated after five days.

Individual (whole fish, n=2) and pooled tissue samples (n=2) were weighed, tissue-mixed, refrigerated and stored in ethyl acetate prior to the analytical determination for FM3422. Whole fish were blotted before the weighing procedure. The pooled samples consisted of the following tissue: brain, gills, liver, gall bladder, kidney, gastrointestinal tract, skin, muscle and skeleton.

Tissue and water samples were analyzed for FM3422 using a Hewlett-Packard model 5713 gas chromatograph equipped with an electron capture detector (Ni^{63}). Specifications and operating conditions were as follows: stainless steel column-length-6 feet. x 1/8"; column packing and support-10% Carbowax 20 Mon Chromosorb-W acid washed 60/80 mesh; operating temperature-isothermal 180°C, detector-300°C; carrier gas-5% methane in argon. Recovery rate was above 90%.

Statistical treatment of the data utilized the 3M Trac system, MINITAB II program. Bioconcentration factors and uptake rate constants were calculated based on formulae proposed by an ASTM committee developing a standard method for conducting bioconcentration studies with fish (1977).

The edible portion of the channel catfish, the muscle fillet, did not bioaccumulate FM3422 to an appreciable extent substantiating our previous observation for this tissue.

In general, no adverse signs were observed during the duration of the experiment attesting to the non-toxic nature of this chemical under the conditions employed in this test.

CONCLUSIONS

This study of the ability of the channel catfish (*Ictalurus punctatus*) to bioconcentrate the fluorochemical FM3422 has led to the following conclusions:

1. Both lipophilic organs and organs possessing a large surface area at the water/organ interface possess the highest uptake rate constants.
2. The brain and gastrointestinal tract have BCF's in excess of 10^3 due to the lipophilic nature of the subject fluorochemical.
3. The gall bladder due to its storage function showed the highest concentration of FM3422 on a $\mu\text{g/g}$ basis.
4. Whole fish or organ weight was an independent variable, not a determinant in the bioconcentration of FM3422.
5. Excretion of FM3422 consists of both urinary and fecal pathways.
6. Clearance of FM3422 by components of the digestive system contributes to the erratic nature of the depuration curves.
7. FM3422 at the exposure concentration did not elicit signs of toxicity, therefore, under the conditions of this experiment, this fluorochemical can be considered non-toxic.

006268

BIBLIOGRAPHY

1. Bass, Michael L. and Alan G. Heath. Water Research 11: 497-502, 1977.
2. Burnett, R. Science 174:606, 1971.
3. Chiou, C. T., Freed, V. H., Schmedding, D. W., and Kohnert, R. L. Env. Sci. Tech. 11:475, 1977.
4. Eaton, John - Chairman, ASTM Draft Guidelines, 1977.
5. Elnabarawy, M. T. Technical Report, 1977.
6. Fujita, T., Iwasa, J., Hansch, C. J. Am. Chem. Soc. 86:5175, 1964.
7. Granmo, A. and S. Kollberg. Water Research 10:189, 1976.
8. Gustafson, C. G. Env. Sci. Tech. 4:814, 1970.
9. Mendel, A. Technical Report, 1977.
10. Veith, G. D. and Comstock, V. M. J. Fish Res. Board Can. 32:1849, 1975.
11. Welter, A. N. Technical Report, 1977.