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TECHNICAL REPORT SUMMARY

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Division: Environmental Laboratory (EE & PC)	Dept. Number: 0222
Project: Fate of Fluorochemicals	Project Number: 9970612623
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Notebook Reference:	No. of Pages Including Coversheet: 25

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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

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.

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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 (*Ictalarus 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 *(Ictalarus punctatus)* were acclimated to the following test conditions for a minimum of 14 days: temperature, 21±1°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, tissuemized, 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).

RESULTS

The water solubility of FM3422 was 50 ppb (Veith-Comstock technique) coupled with the added observation that this chemical was slightly volatile (Mendel, 1977).

Using the method described by Chiou, et. al. (1977) the distribution coefficient for FM3422 was determined to be in excess of 10^5, in an n-octanol/water system. Values of this order of magnitude are indicative of the highly lipophilic nature of the test substance.

In consideration of the slightly volatile nature of FM3422, the test organisms were introduced into the newly filled experimental aquarium and sampling of both channel catfish and exposure water began fifteen minutes later.

Table 1a illustrates the mean concentrations of FM3422 in various layers of the exposure water. The test substance was quite evenly distributed throughout the aquarium as indicated by concentrations of 0.49, 0.54, and 0.52µℓ/ℓ of FM3422 in the top, middle and lower water layer of the aquarium. Since FM3422 was present as a suspension, excitability of the test organisms resulting in unusual disturbance of the bottom sand/FM3422 layer would contribute to the variability of mean concentration values as indicated by the magnitude of the standard error (Table 1a).

Upon analysis of the exposure water samples for FM3422, evidence was obtained that this material was being rapidly released into solution (Table 1b). These data suggest that concentrations approaching equilibrium values were attained within 1 hour of exposure.

Table 1a -- Mean Concentration of FM3422 Exposure Water: Experimental Period

Sampling Site	n	concentration[a] µℓ/ℓ
Top layer	15	.49±.06
Middle layer	15	.54±.06
Lower layer	15	.52±.04

[a]Mean concentration of exposure period ± S.E.

Table 1b -- Concentration (ppm) during Initial 4 Hours of test[a]

Sampling Site	15 min.	1 hour	2 hour	4 hour
Top layer	.19	.45	.43	.35
Middle layer	.31	.49	.47	.42
Lower layer	.23	.54	.47	.55

[a]Expressed as µℓ/ℓ

WHOLE FISH UPTAKE AND CLEARANCE

Uptake of the fluorochemical FM3422 by channel catfish has been tabulated (Table 2) and illustrated (Figs. 1, 2). During the first 24 hour exposure period, both Fish A and Fish B showed a rapid uptake of the test substance. This initial uptake rate may be attributable to movement of FM3422 across the integument with subsequent binding to lipophilic tissues. A slower rate of FM3422 uptake was noted for both organisms which was possibly accounted for by a decreased water/organism concentration gradient. No uptake plateau indicative of a steady-state was obtained following analysis

Table 2 -- FM3422 Uptake by Channel Catfish (*Ictalarus punctatus*) [a]

Time	Fish A	Fish B
15 min.	1	1
1 hour	4	4
2 hours	9	10
4 hours	16	29
8 hours	58	42
12 hours	62	60
16 hours	51	56
20 hours	61	74
24 hours	104	110
48 hours	132	213
72 hours	222	159
96 hours	190	257
120 hours	211	201
144 hours	320	279
168 hours	552	158

[a] Expressed as µg/g.

of FM 3422 content of those fish comprising the composite organism A (Figure 1). The 168-hour value for the test fluorochemical may be an outlier as these values encompassing the period from 72 - 120 hours suggest a plateau (steady state) at FM 3422 concentrations of ∿200 µg/g. A plateau effect was observed to occur with sample B, having an uptake value of approximately 220 µg/g FM 3422 (Figure 2). Concentrations of FM 3422 bioaccumulated by these test organisms were observed to vary independent of whole fish weight.

Clearance of FM 3422 by the channel catfish required in excess of 5 days (Table 3).

Table 3 -- FM3422 Clearance of *Ictalarus punctatus*[a]

Time	Whole Fish A	Whole Fish B
15 min.	306	319
1 hour	269	216
2 hours	252	309
4 hours	260	242
8 hours	260	242
12 hours	253	206
16 hours	235	177
20 hours	238	255
24 hours	237[b]	201
48 hours	-	190
72 hours	200	227
96 hours	136	226
120 hours	106	78

[a] Expressed as µg/g.
[b] Sample lost

TISSUE ANALYSIS: MUSCLE

Of the tissue analyzed for FM3422, the muscle layer of the channel catfish was found to possess the least propensity to bioaccumulate this fluorochemical (Table 4, Figure 3). A plateau effect was observed to occur in the concentration range of 100-200 µg/g (Table 4). Tissue weight was an independent variable, not affecting the bioaccumulation level of FM3422.

Depuration of the subject fluorochemical, FM3422, was delayed, approximately 50% of the material having cleared within four days (Figure 3 Table 5).

TISSUE ANALYSIS - BRAIN, GASTROINTESTINAL TRACT

Uptake of FM3422 by brain and the gastrointestinal tract was elevated due to the lipid nature of these materials (Table 6). Brain uptake of FM3422 continued throughout the experiment (Figure 4)

Table 4 -- FM3422 Uptake by *Ictalarus Punctatus* Muscle

Time	Muscle
Control	<0.1
15 min.	<0.1
1 hour	3
2 hours	5
4 hours	10
8 hours	22
12 hours	31
16 hours	38
20 hours	49
24 hours	61
48 hours	118
72 hours	127
96 hours	135
120 hours	153
144 hours	197
168 hours	147

[a] Expressed as µg/g.

Table 5 -- FM3422 Clearance of *Ictalarus puntatus* muscle[a]

Time	Muscle
15 min.	118
1 hour	127
2 hours	108
4 hours	128
8 hours	147
12 hours	148
16 hours	181
20 hours	117
24 hours	75
48 hours	102
72 hours	77
96 hours	69
120 hours	72

[a] Expressed as µg/g.

Table 6 -- FM3422 Uptake by *Ictalarus punctatus* brain tissue and the gastrointestinal tract of *Ictalrus punctatus*[a]

Time	Brain	Gastrointestinal Tract
Control	<0.1	<0.1
15 min.	2	<0.1
1 hour	8	6
2 hours	28	12
4 hours	50	41
8 hours	100	54
12 hours	115	110
16 hours	149	119
20 hours	240	206
24 hours	225	201
48 hours	352	230
72 hours	328	510
96 hours	606	533
120 hours	588	504
144 hours	695	688
168 hours	905	533

[a] Expressed as µg/g.

Table 7 -- FM3422 Clearance of Brain and the Gastrointestinal Tract of *Ictalarus punctatus*[a]

Time	Brain	Gastrointestinal Tract
15 min.	1152	281
1 hours	761	630
2 hours	712	325
4 hours	602	507
8 hours	511	302
12 hours	582	263
16 hours	618	1205
20 hours	559	805
24 hours	523	564
48 hours	586	264
72 hours	450	353
96 hours	423	390
120 hours	417	712

[a] Expressed as µg/g.

Table 8 -- FM 3422 Uptake by Liver, Kidney, and Gall Bladder of the Channel Catfish[a]

Time	Liver	Kidney	Gall Bladder
15 min.	<0.1	<0.1	<0.1
1 hour	11	<0.1	<0.1
2 hours	11	<0.1	<0.1
4 hours	31	37	<0.1
8 hours	67	59	<0.1
12 hours	89	77	158
16 hours	99	93	245
20 hours	161	149	271
24 hours	149	99	242
48 hours	329	189	1383
72 hours	261	200	1585
96 hours	481	359	1573
120 hours	469	315	2875
144 hours	384	349	3261
168 hours	561	452	13805

[a] Expressed as µg/g.

Table 9 -- FM 3422 Clearance by Liver, Kidney, and Gall Bladder of *Ictalarus punctatus*[a]

Time	Liver	Kidney	Gall Bladder
15 min.	504	274	1543
1 hour	650	567	4908
2 hours	758	439	1286
4 hours	779	447	5058
8 hours	507	390	3710
12 hours	446	559	4321
16 hours	728	515	4247
20 hours	420	365	2495
24 hours	443	259	3912
48 hours	575	351	2810
72 hours	316	203	1221
96 hours	76	182	1411
120 hours	507	285	6497

[a] Expressed as µg/g.

with no apparent plateau effect. At 168 h the concentration of FM3422 in this organ was equivalent to 905µg/g tissue. The brain tissue levels of FM3422 remained elevated upon completion of the clearance phase of this test; 417µg/g (Table 7, Figure 4).

The gastrointestinal tract uptake of FM3422 identified three phases: rapid and slow uptake components with a demonstrable plateau (Table 6, Figure 5). The first two phases persisted for 24 and 48 hours respectively while the plateau effect was minimally of 96 hours duration. As may be noted (Table 7 and Figure 5), this organ did not clear FM3422 readily, rather an inexplicable rise in organ content of FM3422 occurred during the latter stages of depuration.

TISSUE ANALYSIS - Gallbladder

This organ demonstrated a delayed "uptake" of FM3422, detectable levels being initially noted at 12 hours exposure (Table 8). The "uptake" pattern differed from those of other organ systems being analyzed in that the concentration of fluorochemical increased throughout the exposure period. This organ showed an erratic clearance pattern, an initial depuration followed by increased levels of fluorochemical, a phenomenon which was observed throughout the remainder of the experiment (Table 9 Figure 6).

The remaining tissues tested exhibited similar levels of FM3422 uptake. In all cases a steady-state concentration could be calculated using the plateau method. Depuration data did not establish definitive evidence for the clearance of FM3422 by these tissues: gills, skeleton and skin. The liver did exhibit pronounced depuration of the test chemical followed by rapid uptake, a phenomenon associated with depuration data obtained for the gastrointestinal tract and the gall bladder.

Table 10 -- Compilation of Whole Fish and Organ System Bioconcentration Factors During Exposure to FM3422[a,b]

Sample	Weight[c] g	Bioconcentration Factor
Whole Fish #1	10.8±4.5	575
Whole Fish #2	11.5±2.4	406
Skeleton	11.8±2.3	501
Skin	2.9±.9	534
Gills	.64±.2	542
Liver	.51±.1	474
Kidney	.36±.1	709
Gastrointestinal	1.4±.4	1064
Gall Bladder	.03±.02	26548[d]
Brain	.19±.03	1344
Muscle	7.0±2.0	281

[a]BCF - calculated using plateau method
[b]Average water concentration of FM3422, 0.52 ppm
[c]Data expressed as mean ± S.D.
[d]Seven day BCF value, absense of plateau effect.

Table 11 -- Uptake Rate Constant of FM322 in *Ictalarus punctatus* (Channel Catfish)

Sample	Uptake rate constant composite	Rapid Phase	Slow Phase
Whole Fish A	3.2±1.3	5.7	2.7
Whole Fish B	6.4±3.1	8.6	3.9
Brain	7.7±2.4	9.3	5.1
Gills	5.4±3.3	7.3	2.7
Gastrointestinal Tract	6.0±2.4	7.3	4.4
Liver	4.5±2.3	6.3	3.6
Gall Bladder	82.1[a]	-	-
Kidney	4.0±1.4	5.4	3.0
Muscle	1.8±0.6[b]	-	-
Skin	6.7±3.2	8.9	3.6
Skeleton	3.1±1.0	3.5	2.4

[a] Uptake rate constant at 168 hours

[b] Values similar at all time periods

A tabulation of the bioconcentration factors (BCF) determined at an approximate steady state, using the plateau method, provided evidence demonstrating the lipophilicity of FM3422 (Table 10). Although the gall bladder possessed the highest BCF for comparative purposes these data can be ignored for reasons to be discussed. Of the remaining organ systems the propensity to bioaccumulate FM3422 was most prominent in the brain and gastrointestinal tract. The edible portion, muscle, minimally bioconcentrated FM3422.

A tabulation of uptake rate constants for whole fish and organ systems provides further evidence that the lipophilic nature of FM3422 does influence uptake rate (Table 11). Both brain and the gastrointestinal tract had high uptake rate constants. A second premise may be established that being the greater the surface area exposed to the material the greater the uptake rate, hence elevated uptake rate constants for both skin and gills. The former instance provides a physiological basis for uptake while in the latter case both mechanical and physiological mechanisms were operative.

Clearance rate constants could not be calculated due to data variability.

DISCUSSION

In these studies the principal mechanisms for initial uptake of FM3422 were the gills and integument. Substantiating evidence consisting of uptake rate constants which exceeded all other organ systems excepting brain, gastrointestinal tract and the gall bladder. The gill circulation functions as a transport system dispersing the fluorochemical to various sites in the body. The importance of this system in terms of both uptake and transport of foreign chemicals has been the

subject of recent papers by Granmo and Kollberg (1976) and Bass and Heath (1977).

Movement of FM3422 across the integument also contributes to the body burden of FM3422 based on uptake rate constants. Definition of the extent of this contribution cannot be determined as the exact transport pathway has not been established.

Our previous study (1977) did demonstrate the existence of a highly lipid layer underlying the skin which theoretically based on the lipophilicity of FM3422 should serve as a storage (binding) site for this chemical. Vascular perfusion of this area could serve to transport the fluorochemical to other sites in the body, however, the magnitude of perfusion is an unknown. Similarly, if one views the lipid layer as a binding site for FM3422, the nature of the binding becomes of relevance in determining the ease with which material may be transported elsewhere.

It was demonstrated previously in our laboratory that the more highly lipid-containing organs tended to bioconcentrate the subject fluorochemical more readily. This finding has been substantiated by the present study. Transport of FM3422 to the brain and gastrointestinal tract by the circulatory system of the fish seems self-evident as these organs are highly vascular. Evidence obtained in this study suggests that the subject chemical is bound tightly as it does not depurate appreciably. By implication a similar situation may exist at the oil layer/integument interface, thereby reducing the overall contribution of the integument as a source of fluorochemical for other organs in the body.

Organs contributing to the digestive process of *Ictalarus punctatus*

presented an interesting profile. The gall bladder, serving as a reservoir for bile manufactured by the liver, possessed the greatest concentration of fluorochemical observed in these studies. When samples were obtained for FM3422 analysis, distention of the gall bladder was observed, indicating that at our sampling time we were in reality analyzing a concentrated sample of bile. It is also probable that this organ participates in the excretion of FM3422 from the body. The composition of bile is such that given the lipophilicity of FM 3422, binding sites, complexation and/or conjugation of this fluorochemical to biliary components is a distinct possibility. Contraction of the gall bladder with the resultant emptying of its contents into the gastrointestinal tract may account for the sporadic nature of the clearance curve for this organ.

One may view the sequence of events occuring in the depuration phase of this study as it relates to the digestive system in the following manner: FM3422 is cleared at a measurable rate by various organs, being transported to the liver by the circulatory system. In the liver, FM3422 either binds, complexes and/or becomes a conjugate of newly manufactured bile. The bile is then stored in the gall bladder being expressed into the duodenal area of the intestine. This material may then be either excreted within fecal matter and/or some FM3422 may undergo re-uptake for bodily distribution.

Excretion of FM3422 is not solely via fecal matter as a similar scenario can be described for renal clearance wherein elevated levels of FM3422 are maintained through the first 72 hours of depuration.

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 (*Ictalarus 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 µg/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.

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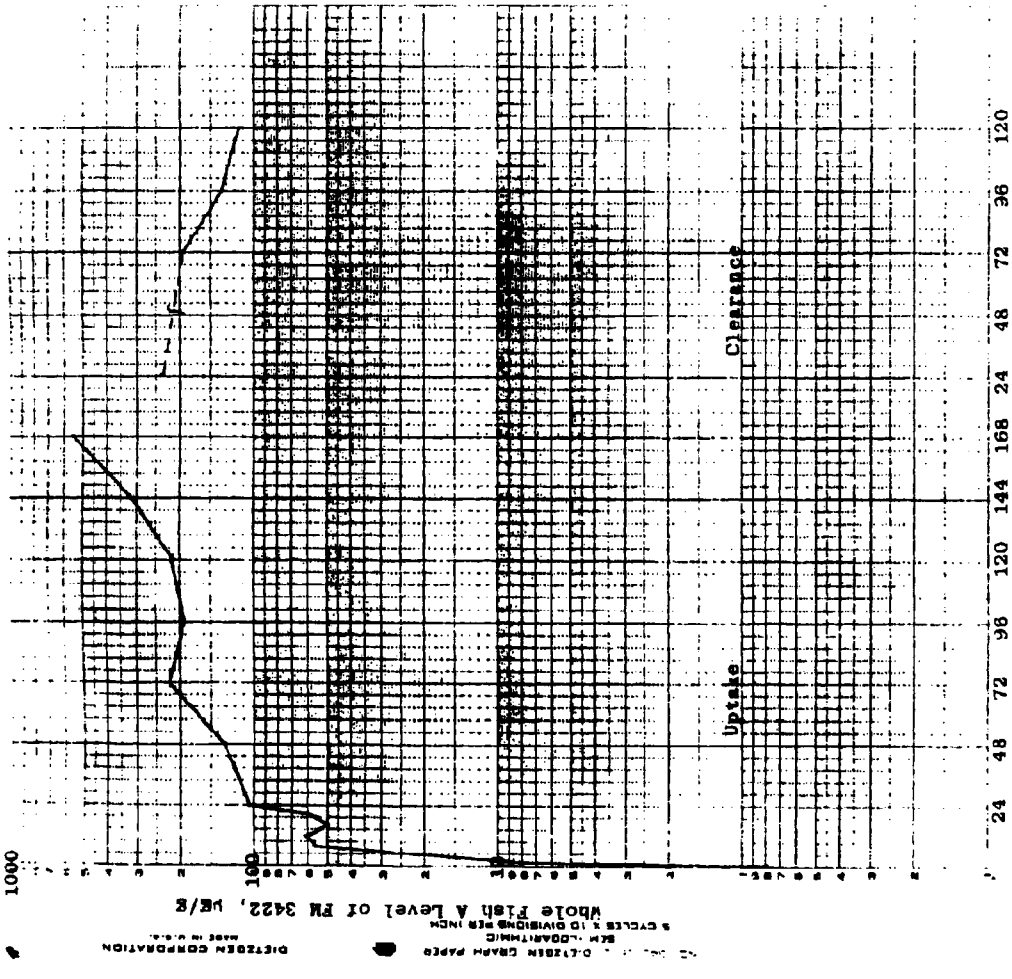

Figure 1 - Composite of designated Whole Fish A, one organism per data point, concentration of FM 3422 in water (C_w) equals 0.52 ppm. Slope Intercept y equals 1.511.

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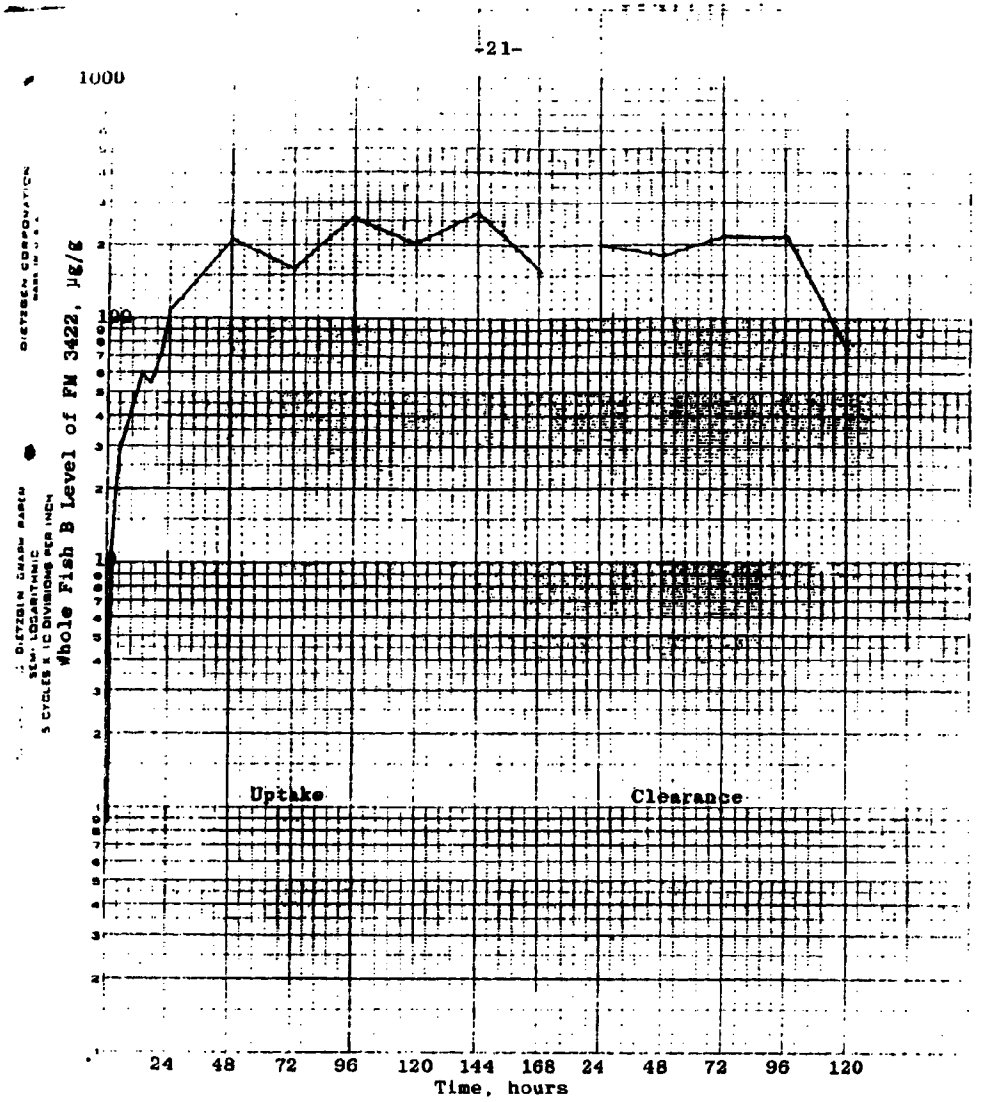

Figure 2 - Composite of designated Whole Fish B, one organism per data point. C_w = 0.52 ppm. Slope Intercept y equals 1.699.

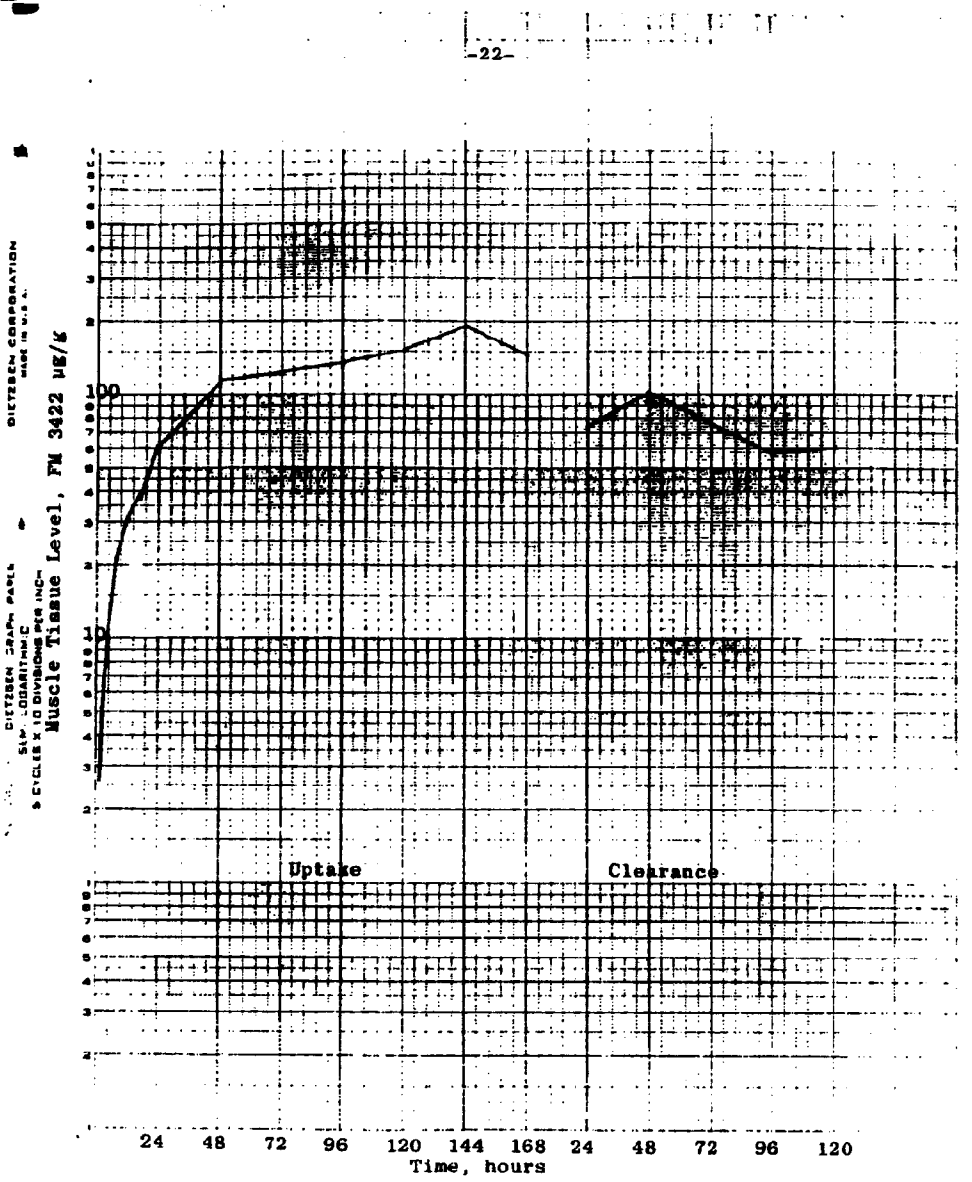

Figure 3 - Muscle Composite Data - 2 tissue analyses per point.
C_w = 0.52 ppm. Slope Intercept y = 1.225.

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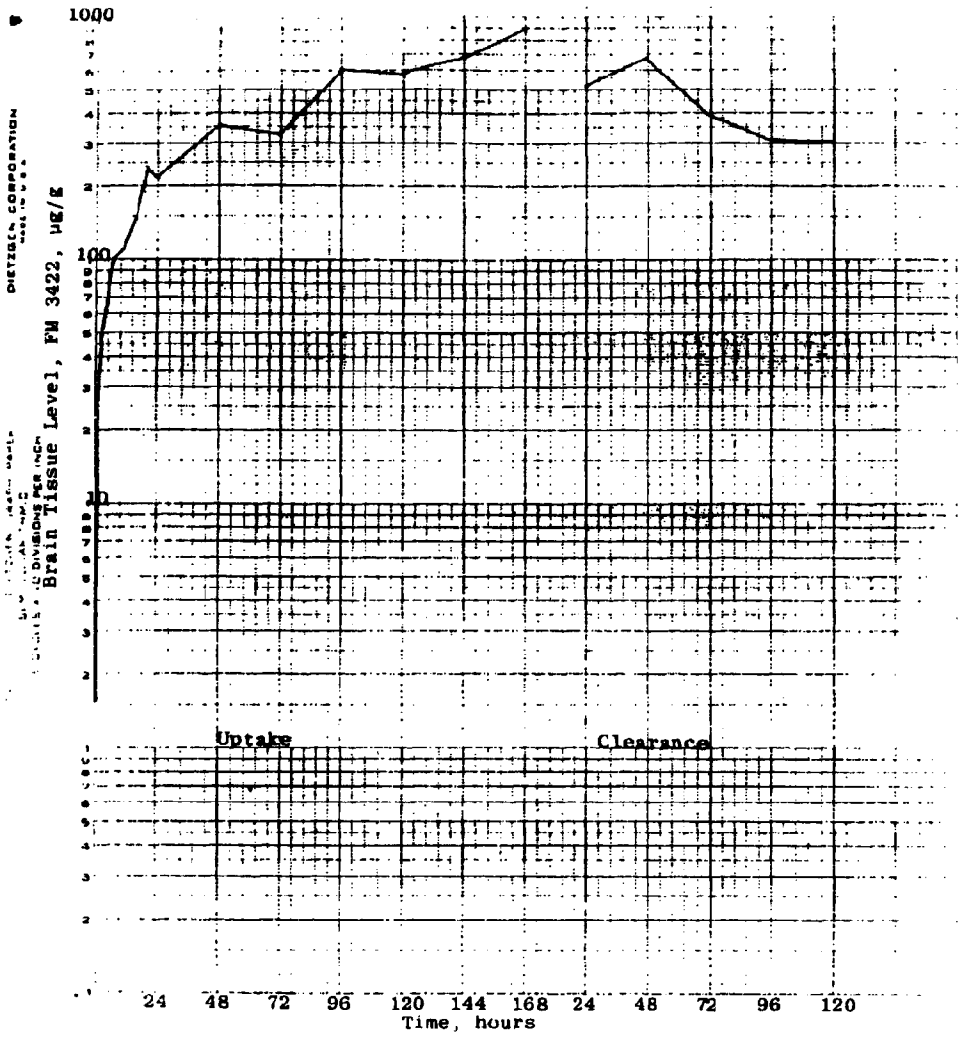

Figure 4 - Composite Data of Brain Tissue - 2 tissue analyses per point. C_w = 0.52 ppm. Slope Intercept y = 2.357.

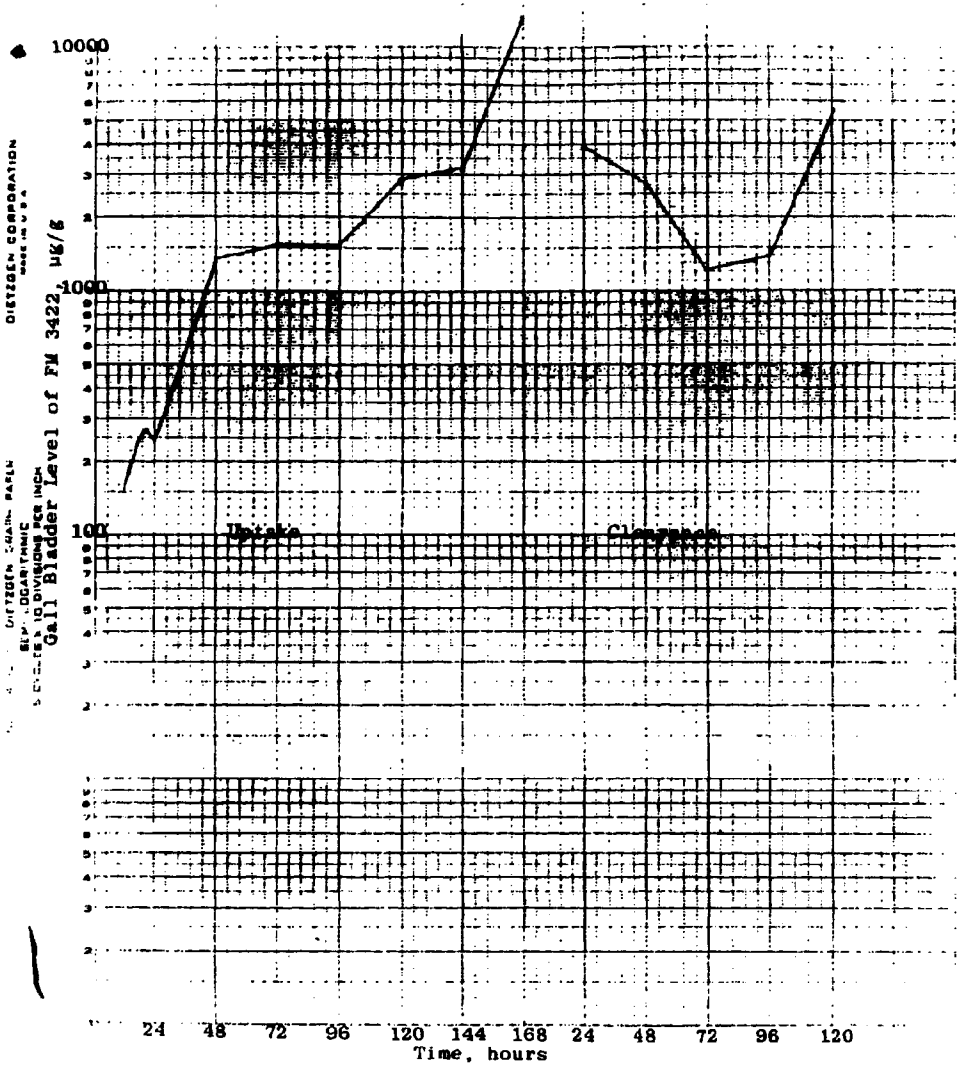

Figure 5 - Composite Data of Gastrointestinal Tract - 2 tissue analyses per point. C_w = 0.52 ppm. Slope Intercept y = 2.153.

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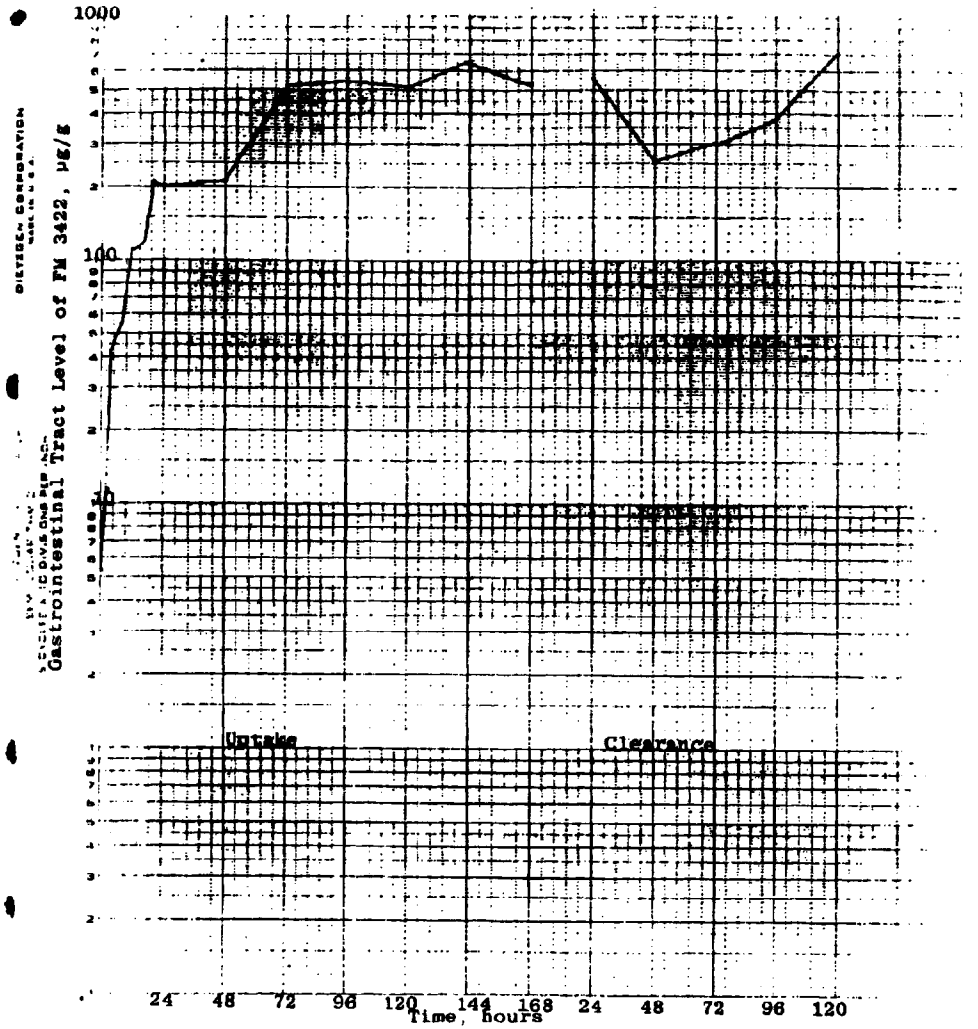

Figure 6 - Composite Data of two gall bladder analyses per point. C_w = 0.52 ppm. Slope Intercept y = 1.364.