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Mr. L. C. Fuhrmeister
Technical Services Superintendent
Monsanto Chemical Company
Anniston, Alabama 36202

Dear Mr. Fuhrmeister:

We have been having problems! High water in Choccolocco Creek hindered attempts to replace fish in our cages until last weekend (Jan. 15). Also, water samples collected during high water failed to provide useful information concerning microflora. These analyses were done by our Microbiology Department and will be repeated when the stream flow returns to normal.

The most vexing problem has been the appearance of a large peak on gas chromatograms that coincides with parathion--this peak was found in samples from all of our stations. Initially, we thought it to be parathion. However, when we found it upstream from Anniston and because of its peculiar behavior on thin-layer plates, we decided that it was not parathion. Yet, the peak masked the actual parathion peak (if any) and prevented quantitation of parathion in samples. After rechecking all equipment, reagents, and other materials used to process samples, we finally discovered the source of the mysterious peak. It seems that hexane stored in polyethylene squeeze bottles picks up at least three contaminants, one of which has the exact same retention time as parathion. This has been a most exasperating experience.

The following samples were analyzed with the problem peak present, and we quantitated the peak as though it were parathion.

DATE	LOCALITY	SAMPLE	INSECTICIDES
10/10/66	Bridge on Hiway 78 - 3 mi. East of Boiling Springs	750 ml water	DDT - 0.4 ppb DDD - trace DDE - 0.4 ppb 3 unknown peaks

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DATE	LOCALITY	SAMPLE	INSECTICIDES
10/10/66	Anniston Sewage Treatment Plant	750 ml water	DDT - 0.2 ppb DDD - 0.2 ppb DDE - 0.3 ppb Methyl Parathion - 0.7 ppb 2 unknown peaks
10/10/66	Choccolocco Creek ½ mi. upstream from mouth of Coldwater Creek.	750 ml water	DDT - 0.8 ppb DDD - trace DDE - 0.8 ppb BHC - trace Methyl Parathion - 1.1 ppb 3 unknown peaks
10/10/66	Bridge on Hiway 109	Bluegill (1.01 g)	

About 15 tall peaks, many of which interfere with pesticides. One peak corresponds with methyl parathion (13.550 ppm) and another with malathion (10.560 ppm). Many of these large peaks correspond exactly with peaks noted in mud samples collected downstream from Anniston and with those in water samples from Snow Creek at the Monsanto Plant.

We believe that a number of materials that enter Snow Creek from the Monsanto Plant are being accumulated by fish in Choccolocco Creek. Presumably these substances are non-toxic.

10/24/66	Choccolocco Creek ½ mi. upstream from mouth of Coldwater Creek.	Bluegill (3.29 g) (Starkville fish - caged).	

At least 15 peaks and high levels of interference. Some had retention times similar to parathion, DDE, DDT, malathion, and methyl parathion.

12/10/66	Bridge on Hiway 78 - 3 mi. East of Bioling Springs.	1000 ml water	BHC - trace DDT - 2.3 ppb DDE - 4.0 ppb Malathion - 3.5 ppb Parathion* - 12.9 ppb

* This peak is the one mentioned earlier. If parathion is actually present, it is masked by the contaminant.

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DATE	LOCALITY	SAMPLE	INSECTICIDES
12/10/66	Anniston Sewage Treatment Plant	1000 ml water	DDT - 2.0 ppb DDD - 0.3 ppb DDE - 1.8 ppb BHC - 0.2 ppb Malathion - 9.0 ppb Parathion* - 26.8 ppb 2 unknown peaks

* The contaminant peak.

All water samples were extracted with chloroform. Tissue sample were extracted with Hexane/Isopropanol (3:1). All samples were cleaned up on florisil columns and evaporated to an appropriate concentration for analysis. Analysis was made on 10% DC-200 on Anakrom ABS.

We have reason to believe that our values reported for BHC may be unreliable since we encounter a fair amount of interference in this area.

The following data are derived from tests of cholinesterase activity in 6 bluegills cage one month in Choccolocco Creek at the Hiway 93 bridge. Originally the fish were collected from Country Club Lake near Starkville. Six control fish from Country Club Lake were tested for comparison.

DATE	TREATMENT	BRAIN WT. (mg)	μ M ACh HYDROLYZED 20' at 25° C	SPECIFIC ACTIVITY
12/20/66	Caged	34	1.40	1.20
12/20/66	Caged	36	1.40	1.20
12/20/66	Caged	37	1.70	1.46
12/20/66	Caged	40	1.70	1.46
12/20/66	Caged	44	2.20	1.89
12/20/66	Caged	46	2.00	1.71
12/20/66	Control	29	1.50	1.29
12/20/66	Control	36	2.90	2.49
12/20/66	Control	37	2.25	1.93
12/20/66	Control	42	1.35	1.16
12/20/66	Control	43	1.80	1.54
12/20/66	Control	43	1.80	1.54

Control: Mean - 1.66
Range - 1.20 - 1.89 ⟶

Caged: Mean - 1.49
Range - 1.16 - 2.49

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Although the means suggest a 10.24% inhibition in the caged fish, this difference is not significant at the 5% level when tested with a t-test. However, the t-value (2.501) barely missed being significant ($t = 2.571$ with 5 d.f.), in spite of the inadequate sample size.

A preliminary literature search has revealed several reports of enzyme inhibition by mercury, especially some of the enzymes involved in degradation of phosphate insecticides (See R. D. O'brien. 1960. Toxic phosphorous esters. Academic Press, N. Y. p. 119). We have some studies underway regarding possible synergistic or additive effects of mercury and parathion. We are not yet ready to report our findings.

In conclusion, it is obvious that all our data are incomplete. A number of materials originating at the Monsanto Plant (via Snow Creek) are showing up in fish and mud samples taken downstream from Anniston. We hope to make a trip to Anniston soon and bring chromatograms to illustrate this phenomenon. We have not been able to detect parathion in Choccolocco Creek. Studies of possible cholinesterase inhibition in caged fish hold promise but are still incomplete.

Preliminary flow-through studies and an investigation of the effects of mercury are just now getting started.

Sincerely yours,

Denzel E. Ferguson
Professor of Zoology

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