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

Dear Mr. Fuhrmeister:

I was shocked and grieved to learn about Gene Coley. His loss is going to make our efforts considerably more difficult.

Last Friday one of my students went to Anniston to bring home the last of our caged fish and to restock the cages. During the night the water in the plastic containers froze and he lost 90 bluegills that were to have been placed in the cages. On Saturday he found that all caged fish below Anniston were dead; those upstream were alive.

I had made plans to bring two students over this Saturday and Mr. Coley was to have shown us where we might seine bluegills during the weekend. We are coming anyway. The combination of a plant shut-down and the loss of our caged fishes necessitates that we obtain native fish immediately if we are to learn anything about acetyl-cholinesterase levels. We hope to collect some dead fish for mercury analyses at the same time.

I am enclosing a summary of some recent findings which may be of interest to you.

Sincerely yours,

Denzel E. Ferguson
Denzel E. Ferguson
Professor of Zoology

DEF:wb

DSW 162377

STLCOPCB4040952

Progress Report (February 8 - March 1)

Between February 18 and 24, 14 bluegills from our State College population were kept in 4 ppb parathion in an aquarium. The insecticide solution was changed daily. Fourteen bluegills were kept in tap water and served as controls. By February 25, 6 of the treated fish were dead and all controls were alive.

On February 26, cholinesterase activities were determined for the 8 surviving treated fish and for 10 control fish. At the same time, AChE activity was measured in 8 bluegills that had been caged in Choccolocco Creek (4 mi. E. Boiling Spring) upstream from Anniston. These fish had been caged in the creek since November 20, 1966. (All caged bluegills at sites downstream from Anniston were dead.) The results of these AChE determinations are given in Table 1.

Table 1.

Sample	Brain Weight*	Optical Density	μ M AChE Hydrolyzed		Specific Activity**	Average	Percent Inhibition
			20'	60'			
Tapwater Control	93.2 mg	.215	2.11	6.33	1.81		
Tapwater Control	81.8 mg	.179	1.76	5.28	1.51		
Tapwater Control	90.5 mg	.218	2.16	6.48	1.85	1.64	
Tapwater Control	95.9 mg	.171	1.69	5.07	1.45		
Tapwater Control	98.4 mg	.188	1.85	5.55	1.58		
Parathion Treated	87.1 mg	.011	0.12	0.36	0.10		
Parathion Treated	100.4 mg	.003	0.04	0.12	0.03	.12	93%
Parathion Treated	103.6 mg	.003	0.04	0.12	0.03		
Parathion Treated	116.6 mg	.035	0.36	1.08	0.31		
Caged in C. Creek	115.1 mg	.189	2.05	6.15	1.76		
Caged in C. Creek	96.1 mg	.178	1.75	5.25	1.50		
Caged in C. Creek	106.4 mg	.155	1.53	4.59	1.31	1.45	
Caged in C. Creek	97.3 mg	.144	1.43	4.29	1.23		

* Each value is based on a pooled sample of two brains.

** Specific Activity = μ M AChE hydrolyzed/hour/mg brain tissue.

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<u>Location</u>	<u>Sample</u>	<u>Results</u>
Choccolocco Creek - Flat bridge (upstream from mouth of Coldwater Creek)	Water	DDT Trace DDE Trace Heptachlor Epoxide Trace 7 unknowns

* Less than 1 ppb is reported as a trace.

A bluegill caged at the flat bridge location and water from the front ditch were collected on February 11. At least 14 major peaks appear in the front ditch water sample and ten peaks having similar retention times appear in the caged bluegills. Several of the peaks have retention times very similar to pesticides. The retention times for the major peaks in these samples are compared with those of several pesticides in Table 4.

Table 4.

Retention Times (in Minutes and Seconds) for Peaks in:

<u>Snow Creek (Front Ditch)</u>	<u>Caged Bluegill</u>	<u>Pesticide Standards</u>
1. 3:00	(Elution A)*	
2. 3:55		
3. 4:25		
4. 5:05	1. 5:00	5:08 = Methyl Parathion
5. 6:25	2. 6:15	6:20 = Malathion
6. 7:25	3. 7:15	7:10 = Parathion
7. 7:50	4. 7:40	
8. 9:25	5. 9:20	9:15 = Heptachlor Epoxide
9. 11:10	6. 11:00	
10. 14:00	7. 13:45	13:30 = DDE
11. 16:50	8. 16:30	17:10 = DDD
12. 19:40	9. 19:25	
13. 21:25		
14. ?	10. 22:55	22:15 = DDT

*. Elution A contains most chlorinated hydrocarbons; phosphates generally come out in Elution B.

All retention times are based on the zenith of peak height and estimates of time are subject to an error of about ± 5 seconds. It is apparent that these unknown peaks will mask or interfere with peaks of pesticides having similar retention times. In many cases, fortification of the sample with pesticide standards and decreasing the rate of carrier gas flow failed to separate pesticide peaks from these unknowns.

We are certain that several of the peaks noted in the caged fish originate in Snow Creek. In addition to similar retention times, peak width, shoulders, and other characteristics are often similar.

Peak #3 in the bluegill resembles parathion but appears in the first clean-up elution from the column. Parathion should be in the "3" wash.

We include the above information to indicate the difficulty that is encountered in attempting to identify pesticides in samples with GLC. I discussed it at our February 24 conference.

Fish samples were extracted with acetonitrile and water samples were extracted by the chloroform procedure. Both methods are standard procedures recommended in the FHS Guide to the Analysis of Pesticide Residues. A Barber-Colman 5360 Pesticide Analyzer and a 10% Dow-200 on 60/80 Anakron III column was used.

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