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17 July 1967

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

Dear Mr. Fuhrmeister:

In view of continued evidence that freezing results in reduced cholinesterase activity, we decided to recheck the possibility that we were doing something wrong. This month Mr. Ludke and Mr. Gibson visited the U.S.D.I. Commercial Fisheries Laboratory at Gulf Breeze, Florida. They discussed and compared techniques with the personnel at the Laboratory who do routine cholinesterase tests. Most of their discussions at Gulf Breeze were with a man named Coppage.

Next, they accompanied Mr. Miller to Athens and again discussed techniques as employed in the Athens Laboratory. All of these discussions have indicated that cholinesterase procedures, while quite adaptable, are basically the same.

As a result of these visits, we have modified our procedures slightly but the modifications are of a nature that will reduce variability but presumably make little difference insofar as detecting inhibition is concerned.

Armed with all this newly acquired knowledge, we have repeated a test concerning the effects of freezing. On June 27th a number of bluegills were quick frozen and stored at a temperature below -40° C. On July 17th fresh fish from the same population and some of the frozen fish were used in cholinesterase tests in which each sample represents the brains of two fish. The results are shown in Table 1.

TABLE 1

	Sample No.	Brain Wt. (mg)	Optical Density	M Hydrolized 15 Min.	Specific Activity	Mean
Frozen:	1	89.3	.280	2.07	1.93	
	2	96.2	.251	1.85	2.15	
	3	95.4	.292	2.17	1.83	1.93
	4	101.7	.280	2.07	1.93	
	5	77.4	.300	2.17	1.83	
Control:	6	120.7	.225	1.68	2.32	
	7	115.0	.205	1.48	2.52	
	8	80.3	.210	1.57	2.43	2.36
	9	86.3	.239	1.68	2.32	
	10	97.3	.225	1.68	2.32	

An examination of these data reveals a 18-19% inhibition in the fish that were frozen. Note also that there is no overlap between the values for frozen and control fish.

In view of this test and all of the others that we have performed, it must be concluded that freezing does indeed reduce the level of cholinesterase activity in samples. If the fish samples collected at the time of the kill were handled correctly, as implied in Miller's report after his trip to Athens, then the entire matter of effects of freezing may become purely academic. I would still welcome a statement from Mr. Parker concerning the collection and storage of fish samples that were sent to the Southeastern Laboratory.

On June 6th, bluegills were placed in the various cages in Choccolocco Creek and within a few days we anticipate bringing some of them back to the University for cholinesterase tests. Also on June 6th water and tissue samples were collected for analysis. The water was extracted and analyzed as described in previous reports. Fish tissues were extracted with acetonitrile and cleaned up on florisil. The findings are as follows:

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	LOCATION	SAMPLE	PESTICIDES (ppb)
1.	Bridge - 4 miles E. Boiling Springs	Water (1 liter)	DDT (?) = .6 ppb DDE (?) = .4 ppb

Four significant peaks were noted--two with retention times identical to DDT and DDE. These peaks were not confirmed.

2.	Coosa Reservoir upstream from mouth of Choccolocco Creek	Water (1 liter)	DDT = 5.4 ppb DDE (?) = .7 ppb

Four peaks noted--two with retention times identical to DDT and DDE. DDT was confirmed by thin layer chromatography and partition coefficient.

3.	Wood Bridge - 0.5 miles upstream from mouth of Coldwater Creek	Water (1 liter)	DDE = trace

Eight peaks, one of which corresponds to DDE. No methyl parathion found.

4.	Hiway #93 Bridge	Caged Bluegill (8.41 g)	

Bluegill caged 2 months. Six significant peaks were found. Peak patterns were similar to those previously seen in Snow Creek extracts--but not nearly so concentrated as noted on other occasions. No retention times matched perfectly with pesticides.

5.	Snow Creek	Water (1 liter)	

Twenty peaks, similar to those found previously in Snow Creek extracts.

Analyses were made with a model 5360 Barber-Colman Pesticide Analyzer at the following operating conditions.

Column Oven Temp.	190° C.
Inj. temp.	220° C.
Detector temp.	205° C.

Gas flow approximately 150 ml./min. on a mixed polar column of SE-30 + QF-1 on Anakrom ABS.

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We have been running some bluegills in flow-thru systems and should have some information on that aspect soon.

Sincerely yours,

Denzel E. Ferguson
Professor of Zoology

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