c.c. C. C. Lang

2 June 1967

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

Dear Mr. Fuhrmeister:

In response to your request, I have prepared a bibliography of scientific literature related to parathion-fish relationships. I have included only the major references used in our work plus a few others that seem most pertinent. If this list is not satisfactory, please advise me.

We have completed an experiment designed to show the effects of freezing upon the results of acetylcholinesterase tests. Briefly, we compared cholinesterase levels in fresh fish, brains frozen in buffer (as recommended by Weiss), and brains of frozen whole fish (as used by the SE Lab).

On May 16, 1967 we obtained a large number of bluegills from Country Club Lake, the source of our local bluegills for the Monsanto project. The fish were taken to the laboratory and observed overnight to make certain that all were healthy and unharmed by the collecting operation.

On May 17 a sample of 12 fish was selected and used in cholinesterase tests. The brains of two fish were pooled for each test (Group A, Table 1).

At the same time the brains were removed from a second series of 12 fish (2 brains per sample), placed in 2 ml of phosphate buffer, quick frozen, on stored at -22°F. On May 25 these samples were tested for cholinesterase activity (Group B, Table 1).

Also, on May 17, a third group of 12 fish was frozen whole and kept at -22°F. On May 25, they were thawed, their brains removed, and 2-brain samples were tested (Group C, Table 1).

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Finally, on May 25, 1967 a sample of 10 fresh fish were obtained from Country Club Lake and tested (Group D, Table 1). These and the two types of frozen samples were alternated in the testing procedure.

An inspection of the Table 1 shows that freezing has had an effect upon the data, as we had reported previously. These findings are contrary to all reports in the literature. We plan to repeat this test and freeze the samples for various periods of time. If the enzyme deteriorates with time, the State's case will be damaged, since they collected "sick fish" several weeks prior to collecting controls.

Table 1

Acetylcholinesterase levels in samples containing brains of two bluegills. A. Field-collected May 16, 1967; B. Brains frozen in buffer; C. Whole fish frozen; D. Field-collected May 25, 1967. Samples B, C, and D were done alternately at the same time.

	SPECIFIC ACTIVITY			
SAMPLE	GROUP: A	B	C	D
1	3.13	1.89	1.54	2.58
2	2.96	1.97	1.63	2.58
3	2.79	1.84	1.54	2.79
4	2.61	2.03	1.50	2.74
5	2.83	1.67	2.40*	2.83
6	3.81	(lost)	(lost)	
MEAN	3.02	1.88	1.75	2.70

* Based on one brain of an extra large fish. Note that the average is 1.55 when this sample is omitted.

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Sometime prior to our meeting on June 13, I will send you a summary of our work thus far.

Sincerely yours,

Denzel E. Ferguson
Professor of Zoology

DEF:wb

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Literature Related to Effects of

Parathion on Fishes

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Rudd, R. L. and R. E. Genelly. 1956. Pesticides: Their use and toxicity in relation to wildlife. Calif. Dept. of Fish and Game. Bulletin No. 7. 209 p.

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Briedenbach, A. W., et al. 1966. The identification and measurement of chlorinated hydrocarbon pesticides in surface waters. U. S. Dept. of Interior, Federal Water Pollution Control Administration. 70 p.

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Weiss, C. M. and J. H. Gakstatter. 1965. The decay of anticholinesterase activity of organic phosphorus insecticides on storage in waters of different PH. Proc. Second International Water Pollution Research Conference, Tokyo 1964. pp. 83-102.

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Weiss, C. M. 1959. Response of fish to sub-lethal exposures of organic phosphorus insecticides. Sewage and Industrial Wastes. pp. 580-593.

Weiss, C. M. 1958. The determination of cholinesterase in the brain tissue of three species of fresh water fish and its inactivation in vivo. Ecology. 39(2): 194-199.

Williams, A. K. and C. R. Sova. 1966. Acetylcholinesterase levels in brains of fishes from polluted waters. Bull. Environ. Contamination and Toxicol. 1: 198-204.

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Doudoroff, P. and C. E. Warren. 1965. Dissolved oxygen requirements of fishes. Biological Problems in Water Pollution. Pub. Health Service Publ. No. 999-WP-22.

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