

MISSISSIPPI STATE UNIVERSITY

DEPARTMENT OF ZOOLOGY  
P. O. DRAWER Z  
STATE COLLEGE, MISSISSIPPI 39762  
PHONE (601) 323-4321, EXT. 305

3 May 1967

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

Dear Mr. Fuhrmeister:

In my report of April 6, 1967 I presented ACh data for distressed fish collected at Eureka Bridge on March 26. You will recall that the specimens were quick-frozen on dry ice. The results were erratic and showed marked reductions in specific activity.

On the weekend of April 8-9 we collected bluegills at State College, Mississippi and took the living fish to Anniston. At Eureka Bridge we collected 5 bluegills and 3 additional fish belonging to the genus Lepomis. All of these fish (both State College and Choccolocco specimens) were placed on dry ice in an identical manner, returned to the lab, and tested for cholinesterase levels after one week (Table 1).

The samples tested were as follows:

Samples 1-8. Bluegills collected from Country Club Lake at State College, Mississippi, taken to Anniston, and frozen on dry ice.

Samples 9-13. Bluegills collected from Choccolocco Creek at Eureka Bridge and frozen on dry ice.

Samples 14-16. Other fish of the genus Lepomis collected from Choccolocco Creek and frozen on dry ice.

DSW 162405

STLCOPCB4040978

TABLE 1

| Sample No. | Brain Wt. (mg) | Optical Density | M ACh Hydrolyzed |      | Specific Activity | Average |
|------------|----------------|-----------------|------------------|------|-------------------|---------|
|            |                |                 | 20'              | 60'  |                   |         |
| 1          | 49.6           | .090            | 1.17             | 3.51 | 1.00              |         |
| 2          | 58.7           | .120            | 1.52             | 4.56 | 1.30              |         |
| 3          | 26.0           | .164            | 2.11             | 6.33 | 1.81              |         |
| 4          | 34.8           | .079            | 1.01             | 3.03 | 0.86              | 109.6   |
| 5          | 58.7           | .114            | 1.45             | 4.35 | 1.24              |         |
| 6          | 42.6           | .057            | 1.72             | 5.16 | 1.53              |         |
| 7          | 50.0           | .048            | 0.61             | 1.83 | 0.52              |         |
| 8          | 22.5           | .063            | 0.60             | 1.80 | 0.51              |         |
| 9          | 22.2           | .162            | 2.06             | 6.18 | 1.82              |         |
| 10         | 30.3           | .044            | 0.56             | 1.68 | 0.48              |         |
| 11         | 30.0           | .053            | 0.68             | 1.98 | 0.57              |         |
| 12         | 29.1           | .081            | 1.04             | 3.12 | 0.89              |         |
| 13         | 85.5           | .073            | 0.94             | 2.82 | 0.81              | 91.5    |
| 14         | 38.7           | .113            | 1.48             | 4.44 | 1.27              |         |
| 15         | 21.8           | .062            | 0.79             | 2.37 | 0.68              |         |
| 16         | 61.3           | .072            | 0.93             | 2.79 | 0.80              |         |

Substrates  
V. 100%

Substrates  
V. 100%

OTHER  
Pilot  
of  
Lipid  
Lipid  
Lipid

DSW 162406

Mr. L. C. Fahrmeister  
May 3, 1967

Page 3

Note that ACh levels for both groups of fish are low but comparable with those for frozen fish reported on April 6. Also, the results are erratic and contain some very low values for control fish. The most striking thing is that the average value for controls is much lower than those we have found previously using unfrozen fish.

If the foregoing test is valid, freezing whole fish has some adverse effect on ACh levels. Also, if this proves to be the case, the State of Alabama has no case. We are now planning an experiment in which the brains will be removed and frozen. It may be that freezing the entire fish is the source of the problem. Weiss recommends removing brains and freezing them in a small amount of butter. He claims that enzyme levels are unaffected by this procedure.

On April 29, fish which had been caged in Choccolocco Creek since March 4 were collected and brought back to State College. On April 30, the cholinesterase levels of these fish were determined. Each sample represents the brain of one fish (Table 2).

Samples 1-6. Fish caged in Choccolocco Creek  
above the Monsanto Chemical Plant (3 mi. E.  
Boiling Spring)

Samples 7-12. Fish caged in Choccolocco Creek  
downstream from the Monsanto Chemical Plant  
(Bridge on Hiway 109).

DSW 162407

TABLE 2

| Sample No. | Brain Wt. (mg) | Optical Density | $\mu$ M ACh Hydrolyzed 20' | Hydrolyzed 60' | Specific Activity | Average* |
|------------|----------------|-----------------|----------------------------|----------------|-------------------|----------|
| 1          | 56.8           | .235            | 3.01                       | 9.03           | 2.58              |          |
| 2          | 55.0           | .165            | 2.12                       | 6.36           | 1.82              |          |
| 3          | 43.2           | .170            | 2.18                       | 6.54           | 1.87              | 198.7    |
| 4          | 53.1           | .220            | 2.82                       | 8.46           | 2.40              |          |
| 5          | 55.0           | .145            | 1.87                       | 5.61           | 1.60              |          |
| 6          | 4.10           | .150            | 1.93                       | 5.79           | 1.65              |          |
| 7          | 36.6           | .205            | 2.63                       | 7.89           | 2.25              |          |
| 8          | 45.6           | .225            | 2.89                       | 8.67           | 2.48              |          |
| 9          | 45.3           | .180            | 2.31                       | 6.93           | 1.97              | 220.3    |
| 10         | 47.2           | .205            | 2.62                       | 7.86           | 2.25              |          |
| 11         | 46.4           | .230            | 2.94                       | 8.82           | 2.52              |          |
| 12         | 56.7           | .160            | 2.05                       | 6.15           | 1.75              |          |

\* Procedure in this test departed from usual in that the brain mixture was allowed to set 4 hours rather than the usual 3. The effect was a slight increase in the average activity.

DSW 162408

Mr. L. C. Fuhrmeister  
May 3, 1967

Page 5

The following data refer to GLC analyses of samples collected  
March 26, 1967.

| <u>Location</u>            | <u>Sample</u>                                  | <u>Results</u>                                                                                                                                                                                                    |
|----------------------------|------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Monsanto Clean-Up<br>Plant | 1,000 ml water                                 | No methyl parathion or<br>parathion peaks present.<br>Two peaks found which did<br>not correspond with<br>pesticides.                                                                                             |
| Eureka Bridge              | Gold fish liver<br>and spleen<br>(3.77 g).     | Many peaks present (at<br>least 17 within 25 min.<br>from injection). High<br>degree of interference<br>with pesticide standards.<br>No methyl parathion peak<br>present.                                         |
| Eureka Bridge              | Whole shad<br>(20.82 g)                        | DDT = .904 ppm<br>DDD = .544 ppm<br>DDE = .536 ppm<br>Run on GLC. No inter-<br>ference and confirmed<br>with thin-layer chromatography.<br>All fishes run were showing<br>symptoms of sickness when<br>collected. |
| Eureka Bridge              | Shad (whole<br>body minus tail).<br>(21.93 g). | Many peaks (as in gold fish<br>liver and spleen). At least<br>20 interfering peaks within<br>35 minutes from injection.                                                                                           |

---

The following data refer to GLC analyses of samples collected  
April 9, 1967.

| <u>Location</u>                 | <u>Sample</u>  | <u>Results</u>                                                                                                                   |
|---------------------------------|----------------|----------------------------------------------------------------------------------------------------------------------------------|
| 50 ft. west of<br>Eureka Bridge | 1,000 ml water | DDT = trace*<br>DDE = trace*<br>Lindane = trace*<br>Plus about 5 other peaks<br>which do not match pesticide<br>retention times. |

---

\* Trace = less than .1 ppb

DSW 162409

STLCOPCB4040982

Mr. L. C. Fuhrmeister  
May 3, 1967

Page 6

| <u>Location</u>                         | <u>Sample</u>  | <u>Results</u>                                              |
|-----------------------------------------|----------------|-------------------------------------------------------------|
| North of inlet west<br>of Eureka Bridge | 800 ml water   | 8 peaks.<br>DDT = .3 ppb<br>DDE = .3 ppb<br>Lindane = trace |
| Under bridge at<br>Jackson Shoals       | 1,000 ml water | DDE = .2 ppb<br>DDT = .4 ppb<br>Also 6 extraneous<br>peaks. |

---

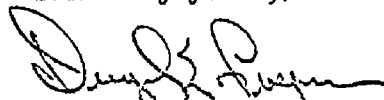
Pesticides (standards) analyzed for:

- (1) Lindane
- (2) Methyl parathion
- (3) Heptachlor Epoxide
- (4) Heptachlor
- (5) Parathion
- (6) DDT
- (7) DDD
- (8) DDE
- (9) Malathion

We did not take DO, pH, and temperatures in April since these values have remained constant and well within safe limits.

We hope soon to have a more definitive answer concerning the effects of freezing whole fish on enzyme activity.

Sincerely yours,

  
Denzel E. Ferguson  
Professor of Zoology

DEF:wb

DSW 162410