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15 August 1967

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

Dear Mr. Fuhrmeister:

The following is a brief report of some of our recent findings:

On June 8 we collected a large number of bluegills from a pond near State College. Some of the freshly caught fish were tested (2 brains per sample) for cholinesterase activity (A, Table 1). Half of the remaining fish were frozen whole and the brains of the other half were removed and frozen in 2 ml of phosphate buffer. After 96 hr, half of the frozen whole fish (B, Table 1) and half of the frozen brains in buffer solution (C, Table 1) were used to determine cholinesterase activity, each sample consisting of 2 brains.

The remaining frozen whole fish (D, Table 1) and frozen brains in buffer solution (E, Table 1) were tested for cholinesterase activity on August 7 after being stored in a deep freeze (below -60 C) for 2 months.

The data in Table 1 illustrate that freezing whole fish for 96 hr results in a 57% inhibition compared with that of the living controls. The specific activities of brains frozen for 96 hr in phosphate buffer solution exhibit a 37% inhibition.

After 2 months, the values are very similar and no further inhibition is evident. Apparently time is not a significant factor. It is interesting that the values in A do not overlap any of those in B, C, D or E; also those for B and D do not overlap with those of C and E.

Table 1. Specific Activity

| Sample | Group: A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | 3.02 | 1.21 | 2.06 | 0.98 | 1.78 |
| 2 | 3.19 | 1.37 | 1.73 | 1.14 | 1.96* |
| 3 | 2.74 | 1.27 | 2.14 | 1.32 | 1.81 |
| 4 | 2.64 | 1.20 | 1.44 | 1.05 | 1.89* |
| 5 | --- | --- | --- | 1.14 | 2.06 |
| Mean | 2.90 | 1.27 | 1.84 | 1.12 | 1.90 |

* Based on 1 brain; all others represent two brains.

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A large collection of bluegills was obtained from Sheely Hills pond on August 8. About one-third of the fish were maintained alive in the laboratory and tested the following day for cholinesterase activity (A, Table 2). The remaining fish were placed in 25 ppb parathion for 24 hr. Half of the parathion-exposed fish were then tested (B, Table 2); the other half of the parathion-exposed fish were individually wrapped in aluminum foil, frozen whole, and tested for cholinesterase activity on August 11 (C, Table 2). All tests involved 1 brain per sample.

The 24-hr exposure to 25 ppb parathion caused a 43% reduction in cholinesterase activity compared with the controls. Freezing caused an additional decrease of 68%. The combination of parathion exposure and freezing resulted in 86% inhibition. There is no overlap between values in the three sets of data.

Table 2.

| Sample | Group: | Specific Activity A | B | C |
|---|---|---|---|---|
| 1 | | 2.52 | 1.20 | 0.95 |
| 2 | | 2.45 | 1.25 | 0.61 |
| 3 | | 2.30 | 1.21 | 0.95 |
| 4 | | 2.15 | 1.13 | 0.91 |
| 5 | | 2.25 | 1.41 | 0.06 |
| 6 | | 2.38 | 1.05 | 0.30 |
| 7 | | 2.13 | 1.20 | 0.26 |
| 8 | | 2.30 | 1.09 | 0.30 |
| 9 | | 2.38 | 1.13 | 0.16 |
| 10 | | 2.15 | 1.45 | 0.53 |
| 11 | | 2.08 | 1.44 | 0.00 |
| 12 | | 1.93 | 1.30 | 0.48 |
| 13 | | 2.33 | 1.35 | 0.19 |
| 14 | | 2.15 | 1.44 | 0.00 |
| 15 | | 2.03 | 1.41 | --- |
| Mean | | 2.23 | 1.27 | 0.41 |

On August 9 bluegills were obtained from a back-water area of Choccolocco Creek near the Hiway 78 bridge east of Boiling Springs (A, Table 3). Two fish were frozen whole on dry ice at time of collection (B, Table 3). On the same day, bluegills were obtained from Choccolocco Creek at Jackson Shoals (C, Table 3) and two fish were frozen whole on dry ice (D, Table 3). All fish (living and frozen) were tested for cholinesterase activity on August 9 using one brain per sample (Table 3).



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Table 3.

| Sample | Group: | Specific Activity A | B | C | D |
|---|---|---|---|---|---|
| 1 | | 2.45 | 1.40 | 2.18 | 1.28 |
| 2 | | 2.33 | 0.54 | 1.98 | 1.33 |
| 3 | | 2.47 | | 1.93 | |
| 4 | | 2.43 | | 2.30* | |
| 5 | | 2.14 | | 1.78* | |
| 6 | | 1.78 | | 2.38 | |
| 7 | | 2.20 | | | |
| Mean | | 2.26 | 0.87 | 2.09 | 1.305 |

* Green sunfish; all others are bluegills.

The slight inhibition indicated in the Jackson Shoals fish is probably not significant since the ranges overlap and the two habitats were not strictly comparable. A decrease in cholinesterase activity attributable to freezing is again apparent in the few frozen fish (B & D).

Below are a few dissolved oxygen readings:

| | 6-28-67 | 7-27-67 |
|---|---|---|
| Hiway 78 Bridge - (Choccolocco Creek) E. of Boiling Springs | 8.2 ppm (surface)<br>7.0 ppm (elbow depth) | 7.0 (surface)<br>6.7 (elbow depth) |
| Outflow - Anniston Sewage Treatment Plant | 2.6 ppm (surface) | |
| Hiway 93 Bridge - Choccolocco Creek | 6.1 ppm (surface)<br>5.5 ppm (elbow depth) | 7.0 (surface)<br>7.0 (elbow depth) |
| Hiway 231 Bridge - Choccolocco Creek | 11.1 ppm (surface<br>12.2 ppm (elbow depth) | |

On August 9, we noted that Choccolocco Creek was crystal clear at the Hiway 93 bridge but murky and turbid at Jackson Shoals, only 3 or 4 miles downstream. Do you people know of any source of contamination between these two sites? We observed 2 or 3 dead or dying fish at Jackson Shoals but there were plenty of live ones too.

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We have a survey of invertebrates in Choccolocco Creek underway at the present time and the results will be included in the final report in early September.

We have prepared a set of instructions for determining cholinesterase activities in fish brains. I am enclosing a copy for your use.

Sincerely yours,

Denzel E. Ferguson
Professor of Zoology

Enclosure

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