MISSISSIPPI STATE UNIVERSITY

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

8 February 1967

cc PAPAGEORGE
CARDER
LETSY
WP DUNLAP
MILLER
COLEY

Mr. L. C. Fuhrmeister
Technical Services Superintendent
Monsanto Chemical Company
Anniston, Alabama 36201

Dear Mr. Fuhrmeister:

Enclosed is a description of our findings since the last progress report.

We have been unable to demonstrate any reason why large fish kills should occur in Choccolocco Creek.

I received your notice of Mr. Carder's visit. Since I teach two classes on Thursday morning, I am hoping that it will be convenient for me to meet with you on Friday the 24th at 8 AM. Should this be impossible, I will attempt to make arrangements for Thursday.

Sincerely,

Denzel E. Ferguson
Professor of Zoology

Enclosure

DEF:wb

DSW 162370

TOWOLDMON0054693

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Progress Report - January

On January 24 dissolved oxygen measurements were taken at several sampling sites by the Winkler Method. Water temperatures and pH-values were recorded at the same time. These data are summarized below:

Location	Time	Water Temp. (°C)	pH	D. O. (ppm) Surface	D. O. (ppm) Elbow Depth
1. Choccolocco Creek: Bridge - 4 mi. E. of Boiling Springs	11 A.M.	14	7.8	10.8	10.0
2. Anniston Sewage Treatment Plant: (Final tank)*	12 A.M.	21	7.2	3.8	**
3. Monsanto Sewage Treatment Facility - at outfall to city.	2 P.M.	31	7.6	4.7	**
4. Snow Creek - outfall from Monsanto Plant (Front ditch at fence)	2:30 P.M.	34	0.7	None Detected	**
5. Choccolocco Creek at Hiway 93 Bridge	3:30 P.M.	14.5	7.5	8.5	8.5

* The basin contained a large amount of foam.

** Not measured.

The D. O. values for the Choccolocco Creek sites are well within safe limits. Barring adverse effects of detergents and low nocturnal D.O.-values fish might even survive in the Anniston Sewage Treatment Plant. Although the front ditch from the Monsanto Plant contained no measurable D.O. and had a very low pH, (Our tests have previously shown that bluegills die in 20 minutes at pH 3.0) both pH and D.O. are normal at the downstream Choccolocco Creek site (Hiway 93 Bridge).

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We have completed preliminary studies to determine possible synergistic action of mercury and parathion. On January 18, four 15-gallon aquaria were prepared with the following test solutions: 1) 20 liters of 100 ppb $HgCl_2$ solution; 2) 20 liters of 100 ppb parathion; 3) 20 liters of solution in which both $HgCl_2$ and parathion were present at 100 ppb concentrations; 4) 20 liters of tap water as a control. Each aquarium was stocked with 25 medium-sized green sunfish (Lepomis cyanellus).

The following table shows the number of survivors.

	100 ppb $HgCl_2$	100 ppb Parathion	Mixture 100 ppb $HgCl_2$ and Parathion	Tap water Control
No. Fish	25	25	25	25
12 hours	25	23	24	25
24 hours	25	18	23	25
36 hours	25	15	18	25
60 hours	25	1	2	25

On February 3, the test was repeated using 20 green sunfish in each aquarium but 200 ppb $HgCl_2$. The numbers of survivors were as follows:

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	100 ppb $HgCl_2$	100 ppb Parathion	Mixture 100 ppb $HgCl_2$ and Parathion	Tap water Control
No. Fish	20	20	20	20
12 hours	20	20	20	20
24 hours	20	20	20	20
36 hours	20	19	20	20
48 hours	20	15	20	20
60 hours	20	13	16	20
72 hours	20	10	13	20

These findings provide no evidence of synergistic activity. On the contrary, it appears possible that mercury may exert a slight antagonistic effect on parathion activity. We plan to repeat these studies using bluegills.

On February 2, bluegills were collected from Country Club Lake. This fish population is used to stock cages in Choccolocco Creek. Three aquaria were prepared, one containing 25 ppb parathion, a second containing 100 ppb parathion, and another as a tap water control. Twelve fish were placed in each aquarium for 12 hours, after which the cholinesterase levels of these fish were determined. Each sample in this test represents the brains of two fishes.

Samples

2-5 are fish exposed to 100 ppb parathion
6-10 are fish exposed to 25 ppb parathion
11-15 are control fish.

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The results of these tests are shown in the following table:

Sample No.	Brain Wt.	Optical Density	μM ACh Hydrolyzed 20'	60'	*Specific Activity	Average	Percent Inhibition
2	76.7 mg	.060	0.95	2.85	0.81		
3	91.4 mg	.100	1.36	4.08	1.16	.72	56%
4	76.0 mg	.010	0.44	1.32	0.39		
5	96.5 mg	.025	0.60	1.80	0.51		
6	76.2 mg	.050	0.85	2.55	0.73		
7	76.7 mg	.070	1.05	3.15	0.90		
8	70.6 mg	.055	0.90	2.70	0.77	.82	50%
9	77.5 mg	.045	0.80	2.40	0.68		
10	72.5 mg	.080	1.16	3.48	0.99		
11	83.1 mg	.145	1.81	5.43	1.55		
12	81.4 mg	.120	1.57	4.71	1.34		
13	80.6 mg	.145	1.81	5.43	1.55	1.63	
14	86.2 mg	.165	2.01	6.03	1.72		
15	76.5 mg	.195	2.32	6.96	1.99		

* Specific Activity μM ACh hydrolyzed/hour/mg brain weight

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On January 25, 1967 water samples were collected for gas chromatographic analyses. At the same time, bluegills that had been caged 2 weeks in Choccolocco Creek upstream and downstream from Anniston were collected for analyses. The findings are shown in the following table (water samples are given in ppb; fish in ppm):

Locality	Sample	Pesticides
Choccolocco Creek Bridge - 4 miles E. Boiling Springs	Water (1000 ml)	DDE 0.2 ppb (3 unidentified peaks, one very close to malathion)
Choccolocco Creek Bridge - 4 miles E. Boiling Springs	2 Bluegills (8.58 g)	DDE 0.143 ppm (3 unidentified peaks matching those in control fish from the State College population)
Anniston Sewage Treatment Plant	Water (1000 ml)	DDT 1.7 ppb Parathion 3.0 ppb Heptachlor Epoxide ? Trace 7 unidentified peaks
Monsanto Treatment Facility (at outfall to city)	Water (1000 ml)	Extremely high background after clean-up on florisil. High EC response, even after dilution of the sample, prevented analysis.
Snow Creek (Front Ditch at outfall from Monsanto Plant)	Water (1000 ml)	11 unidentified peaks, several having retention times similar to pesticides. Some of these same peaks reappear in fish caged downstream.
Choccolocco Creek at Hiway 93 Bridge	Water (1000 ml)	DDT 0.3 ppb DDE Trace DDD 0.2 ppb Parathion 1.5 ppb 3 unidentified peaks, one very close to methyl parathion.
Choccolocco Creek at Hiway 93 Bridge	2 Bluegills (8.47 g)	DDT 0.451 ppm DDE 0.253 ppm Parathion 0.354 ppm Malathion ? 4.251 ppm Ten unidentified peaks, 5 of which match specific peaks in Snow Creek.

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Chromatographic analyses were done on a 10% Dow-200 column with a 5% QF-1 polar column used as a back-up. In certain cases (Malathion and Heptachlor Epoxide) interference was encountered and the identifications are tentative. The parathion reported in the bluegill tissues (Hiway 93 Bridge) did not exactly match the standard and eluted from the clean-up column early. However, clear separation was made with the parathion peak in the two water samples reported. Several peaks extracted from Snow Creek have similar retention times as pesticide standards. Certain peaks in Snow Creek appear in bluegills caged downstream. Identification of these peaks is beyond our present capabilities.

Conclusions

The data reported show no adverse environmental conditions in Choccolocco Creek or in the Anniston and Monsanto treatment facilities. Snow Creek continues to be the only environment unfit for aquatic life, however, there is no evidence that these undesirable effects alter Choccolocco Creek at downstream sites. Several materials in Snow Creek appear to accumulate in fishes caged downstream, but the biological properties of these compounds are unknown.

We are accumulating background data for assessing possible cholinesterase inhibition in caged fish. Survival of caged fish for relatively long periods of time suggests that the factor or factors causing fish kills in Choccolocco Creek may not be operative at all times, at least on a lethal level. When rains stop and stream-flow decreases, we hope to study native fishes and determine possible effects of cumulative long-term sublethal factors.

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