Progress Report (February 8 - March 1)

Between February 18 and 24, 14 bluegills from our State College population were kept in 4 ppb parathion in an aquarium. The insecticide solution was changed daily. Fourteen bluegills were kept in tap water and served as controls. By February 25, 6 of the treated fish were dead and all controls were alive.

On February 26, cholinesterase activities were determined for the 8 surviving treated fish and for 10 control fish. At the same time, AChE activity was measured in 8 bluegills that had been caged in Choccolocco Creek (4 mi. E. Boiling Spring) upstream from Anniston. These fish had been caged in the creek since November 20, 1966. (All caged bluegills at sites downstream from Anniston were dead.) The results of these AChE determinations are given in Table 1.

Table 1.

Sample	Brain Weight*	Optical Density	μM AChE Hydrolyzed 20'	60'	Specific Activity**	Average	Percent Inhibition
Tapwater Control	93.2 mg	.215	2.11	6.33	1.81		
Tapwater Control	81.8 mg	.179	1.76	5.28	1.51		
Tapwater Control	90.5 mg	.218	2.16	6.48	1.85	1.64	
Tapwater Control	95.9 mg	.171	1.69	5.07	1.45		
Tapwater Control	98.4 mg	.188	1.85	5.55	1.58		
Parathion Treated	87.1 mg	.011	0.12	0.36	0.10		
Parathion Treated	100.4 mg	.003	0.04	0.12	0.03	.12	93%
Parathion Treated	103.6 mg	.003	0.04	0.12	0.03		
Parathion Treated	116.6 mg	.035	0.36	1.08	0.31		
Caged in C. Creek	115.1 mg	.189	2.05	6.15	1.76		
Caged in C. Creek	96.1 mg	.178	1.75	5.25	1.50		
Caged in C. Creek	106.4 mg	.155	1.53	4.59	1.31	1.45	
Caged in C. Creek	97.3 mg	.144	1.43	4.29	1.23		

* Each value is based on a pooled sample of two brains.
** Specific Activity = μM AChE hydrolyzed/hour/mg brain tissue.

0110101

HARTOLDMON0004709

In general, the AChE levels in the controls conform to those we have reported previously. Probably, the lower values for the caged controls are not significantly different from the tapwater controls. The high level of inhibition in the treated fish is rather surprising. In short-term exposures, fish usually die when AChE inhibition reaches 30 - 60%, however, the levels we found have been reported in the literature. Weiss (1964) exposed bluegills to 1 ppb parathion and obtained as much as 77% inhibition in 14 days. Also, he noted up to 90% inhibition in 10 ppb parathion after 6 days.

It is clear that very low concentrations and long exposures can be quite harmful. In the next few weeks, we intend to concentrate much of our efforts on this aspect of the problem, especially as it pertains to fishes in the lower portions of Choccolocco Creek.

The following water-quality data were collected in February:

Table 2.

February 11, 1967

Location	Time	pH	Temp (°C)	D. O. (ppm) Surface	D. O. (ppm) Elbow Depth
Choccolocco Creek - 4 mi. E. Boiling Spring	9:00	7.3	9	11.4	11.0
Anniston Sewage Treatment Plant	10:00	6.9	19	0.9	---
Choccolocco Creek - Flat Bridge	11:00	7.4	9.5	10.4	9.7
Choccolocco Creek - Hiway #93 Bridge	1 PM	7.2	10	10.2	9.6
Monsanto Treatment Plant (Outflow to City)	12 Noon	7.0	25.5	6.7	---
Front Ditch	12:20	6.5	21	8.0	---

(Cont.)

HARTOLDMON0004710

February 24, 1967

Location	Time	pH	Temp (°C)	D. O. (ppm) Surface	D. O. (ppm) Elbow Depth
Choccolocco Creek - 4 mi. E. Boiling Spring	9:30	7.5	8	11.4	11.2
Anniston Sewage Treatment Plant	10:30	6.8	17	3.1	---
Choccolocco Creek - Flat Bridge	11:30	7.3	8	10.0	9.7
Choccolocco Creek - Hiway #93 Bridge	12:40	7.4	9	10.0	9.8
Monsanto Treatment Plant	1:30	7.0	25	4.7	---
Front Ditch	2:30	6.2	25	None detectable	

The following samples were collected February 11, 1967 and analyzed on GLC:

Table 3.

Location	Sample	Results
Choccolocco Creek - 4 mi. E. Boiling Spring	Water	DDE 1 ppb * DDT Trace DDD Trace Lindane Trace Malathion Trace 4 unknown peaks
Choccolocco Creek - 4 mi. E. Boiling Spring	Caged Bluegill	DDE 90 ppb DDT 80 ppb Lindane 25 ppb 1 unknown

HARTOLDMON0004711

Location	Sample	Results
Choccolocco Creek - Flat bridge (upstream from mouth of Coldwater Creek)	Water	DDT Trace DDE Trace Heptachlor Epoxide Trace 7 unknowns

* Less than 1 ppb is reported as a trace.

A bluegill caged at the flat bridge location and water from the front ditch were collected on February 11. At least 14 major peaks appear in the front ditch water sample and ten peaks having similar retention times appear in the caged bluegills. Several of the peaks have retention times very similar to pesticides. The retention times for the major peaks in these samples are compared with those of several pesticides in Table 4.

Table 4.

Retention Times (in Minutes and Seconds) for Peaks in:

Snow Creek (Front Ditch)	Caged Bluegill	Pesticide Standards
1. 3:00	(Elution A)*	
2. 3:55		
3. 4:25		
4. 5:05	1. 5:00	5:08 = Methyl Parathion
5. 6:25	2. 6:15	6:20 = Malathion
6. 7:25	3. 7:15	7:10 = Parathion
7. 7:50	4. 7:40	
8. 9:25	5. 9:20	9:15 = Heptachlor Epoxide
9. 11:10	6. 11:00	
10. 14:00	7. 13:45	13:30 = DDE
11. 16:50	8. 16:30	17:10 = DDD
12. 19:40	9. 19:25	
13. 21:25		
14. ?	10. 22:55	22:15 = DDT

*. Elution A contains most chlorinated hydrocarbons; phosphates generally come out in Elution B.

HARTOLDMON0004712

All retention times are based on the zenith of peak height and estimates of time are subject to an error of about ± 5 seconds. It is apparent that these unknown peaks will mask or interfere with peaks of pesticides having similar retention times. In many cases, fortification of the sample with pesticide standards and decreasing the rate of carrier gas flow failed to separate pesticide peaks from these unknowns.

We are certain that several of the peaks noted in the caged fish originate in Snow Creek. In addition to similar retention times, peak width, shoulders, and other characteristics are often similar.

Peak #3 in the bluegill resembles parathion but appears in the first clean-up elution from the column. Parathion should be in the "B" wash.

We include the above information to indicate the difficulty that is encountered in attempting to identify pesticides in samples with GLC. I discussed it at our February 24 conference.

Fish samples were extracted with acetonitrile and water samples were extracted by the chloroform procedure. Both methods are standard procedures recommended in the FWS Guide to the Analysis of Pesticide Residues. A Barber-Coleman 5360 Pesticide Analyzer and a 10% Dow-200 on 60/80 Anakrom [illegible] column was used.

0110105

HARTOLDMON0004713