A FINAL REPORT

Investigations of Certain Pesticide-Wildlife Relationships in the Choccolocco Creek Drainage

A Contract Between the

Monsanto Chemical Company and

Mississippi State University

(September 1, 1966 through August 31, 1967)

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DSW 162431

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INTRODUCTION

In the late summer of 1966, the Zoology Department of Mississippi State University entered into a contract with the Monsanto Chemical Company to investigate certain physical, chemical, and biological properties of the Choccolocco Creek Drainage in Alabama. The prime objective of the study was to assess the actual and potential impact of the Anniston Monsanto plant, which produces organophosphate insecticides, upon the ecology and welfare of the aquatic life inhabiting the stream system. A specific goal was to examine evidence and information related to the fish kill that occurred in Choccolocco Creek in March 1966

Specific aims and proposed procedures were outlined in the prepared agreement and in general, these were followed. In some cases preliminary findings suggested additional avenues of investigation or resulted in an alternative approach or change in emphasis.

SUMMARY OF INVESTIGATIONS AND RESULTS

Choccolocco Creek: This stream has its origins in runoff, predominantly from forested areas, and in ground waters reaching the surface as springs and seeps. Consequently, Choccolocco Creek is cold throughout the year and relatively free of fine sediments and suspended matter. A fairly steep gradient and swift flow over a predominantly rocky bottom results in the stream being well aerated. The downstream segment of the stream tends to become more sluggish, and is characterized by more suspended sediments, a mud bottom, and slightly less favorable oxygen and temperature conditions. During periods of heavy rainfall, the stream is subject to flooding and is characterized by extreme fluctuations in flow, both annually and over short periods, depending upon rainfall.

Known sources of pollution in Choccolocco Creek include industrial wastes in Snow Creek and Coldwater Creek, effluent from the Anniston Sewage Treatment Plant, and suspended sediments from agricultural lands. A favorable dilution factor appears to minimize the effects of these potential sources of pollution.

Water Quality: Water color is good in all areas excepting Coldwater Creek. The effluent of the Gypsum Plant frequently imparts a milky appearance to Coldwater Creek and favors the growth of excessive amounts of a slime fungus (perhaps *Fusarium* sp.).

Below the mouth of Snow Creek, Choccolocco Creek and especially its mud deposits have a distinctive odor traceable to Snow Creek. One of us claims to detect the odor of TCE at Jackson Shoals, presumably from the Army Ordinance Depot.

Table 1 is a summary of temperature, pH, and dissolved oxygen data collected at several sampling stations between January and August 1967 Although these data include some extreme values, especially from the Monsanto and Anniston waste treatment plants and Snow Creek, the water quality of Choccolocco Creek is well within the limits considered safe and beneficial for aquatic life.

Table 1. Means and ranges of temperature, pH and D.O. for several stations between January and August [illegible].
N = Sample size, M = Mean, R = Range.

Station	Temperature (°C) N	Temperature (°C) M	Temperature (°C) R	pH N	pH M	pH R	D. O. (ppm) Surface N	D. O. (ppm) Surface M	D. O. (ppm) Surface R	D. O. (ppm) Elbow N	D. O. (ppm) Elbow M	D. O. (ppm) Elbow R
Choccolocco Creek, Hwy #78 Bridge 4 mi. E. Boiling Springs	6	14.8	(8-21)	4	7.6	(7.4-7.8)	8	8.65	(6.5-11.4)	8	8.3	(6.7-11.2)
Choccolocco Creek, Hwy #231 Bridge		---			---		1	11.1		1	12.2	
Choccolocco Creek, Wood Bridge E. of mouth of Coldwater Creek	4	14.4	(8-23)	3	7.4	(7.2-7.7)	4	7.6	(4.5-10)	4	7.3	(4.2-9.7)
Choccolocco Creek, Hwy #93 Bridge	6	16.0	(9-23)	4	7.6	(7.4-8.0)	8	7.4	(5.2-10.0)	8	7.3	(5.1-9.8)
Choccolocco Creek, Hwy #77 Bridge	1	15.0		1	8.0		2	8.95	(8.9-9.0)	2	8.6	(8.5-8.8)
Monsanto Waste Treatment Plant	4	26.75	(17-34)	3	6.9	(6.2-7.6)	4	4.6	(2.5-6.5)		---	
Snow Creek, front ditch	4	28.25	(22-34)	3	6.0	(0.7-11.2)	4	2.0	(0.0-8.0)		---	
Anniston Sewage Treatment Plant	5	20.6	(17-28)	4	7.1	(6.8-7.3)	6	2.4	(0.0-4.8)		---	

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Bioassay Tests: The acute toxicity of several compounds to fish has been determined in static tests. The data are expressed as 36-hour median tolerated limits in Table 2. The results of a recent analysis of the Mississippi State University water supply used in bioassay tests are shown in Table 3.

Table 2.

Compound	Species	36 hr TL_m
TCE	Bluegills	60 ppm
TCE	Green sunfish	105 ppm
Parathion	Bluegills	65 ppb
Parathion	Mosquitofish	60 ppb
M. Parathion*	Bluegills	25 ppb
M. Parathion*	Mosquitofish	80 ppb
PNP	Bluegills	over 1000 ppb

* We recently discovered that a bottle labelled "Methyl Parathion" actually contained parathion when analyzed on the gas chromatograph. The sample was sent to us in response to a request for a sample of methyl parathion by American Cyanamid Co. I have reason to suspect that the bluegills and mosquitofish were actually tested on parathion, which may explain the discrepancy between our data and published toxicity data.

Table 3.

STATE CHEMICAL LABORATORY
Mississippi State University

Received 4/12/65 Reported 6/4/65 Analysis No. 359,511

Analysis of WATER (MISSISSIPPI STATE UNIVERSITY)

RESULTS

	PARTS PER MILLION
Silica(SiO_2)	11.34
Iron(Fe)	0.03
Aluminum(Al)	0.20
Calcium(Ca)	8.20
Magnesium(Mg)	1.69
Sodium(Na)	29.30
Potassium(K)	3.55
Carbonate Radical($CO_3^=$)	0.00
Bicarbonate Radical(HCO_3^-)	102.48
Sulfate Radical($SO_4^=$)	1.32
Nitrate Radical(NO_3^-)	0.66
Chloride Radical(Cl^-)	13.00
Nitrite Radical(NO_2^-)	0.04
Fluoride Radical(F^-)	0.10
Total	171.91
Less H_2CO_3 Equivalent of HCO_3^-	52.09
Total Solids by Analysis	119.82
Total Solids by Evaporation	114.40
Excess of Solids by Analysis	5.42
Ammonia Nitrogen	0.00
Albuminoid Nitrogen	0.00
Nitrate Nitrogen	0.15
Nitrite Nitrogen	0.01
Total Alkalinity as $CaCO_3$	84.04
Non-Carbonate Hardness	0.00
Total Hardness as $CaCO_3$ (Calculated)	27.93
Total Hardness as $CaCO_3$ (Soap Method)	26.00
Permanent Hardness as $CaCO_3$ (Soap Method)	18.20
Temporary Hardness as $CaCO_3$ (Soap Method)	7.80
pH	7.80
Color	None
Odor	None
Turbidity	None
Sediment	None
Taste	Normal
Free Carbon Dioxide	0.00
Total Acidity to Phenolphthalein, calculated as free carbon dioxide	1.00
Loss of Ignition	0.00
Iron(Fe) Content of Water Sample Taken from Sink Tap, Room 24, Hand Chem. Lab.	0.13

When a tributary of Snow Creek originating within the Monsanto Plant was found to be devoid of life, samples of water from the stream were used to bioassay bluegills. The fish lost equilibrium in the full strength water in 10 seconds and all died in less than 5 minutes. Bluegills survived less than 16 hours when bioassayed in 1 part Snow Creek water diluted with 300 parts tap water.

Further investigations showed unfavorable pH (HCl, H_3PO_4) and mercury to be the lethal agents. Both phosphoric acid and hydrochloric acid killed fish at a pH 3; fish survived 60 hours in both acids at pH 4. A concentration of 1 ppm $HgCl_2$ caused symptoms in bluegills in 2 hours and [illegible] minutes and killed the entire sample in 6 hours. Tests for synergistic action between parathion and mercury were negative.

When bluegills were caged for 48 hours in various places in Snow Creek and Choccolocco Creek, the lethal effects of Snow Creek were found to extend downstream only a few hundred yards.

Bluegills were caged in Choccolocco Creek for as long as 3 months Although these caged fish were forced to exist under crowded conditions in man-selected habitats, their survival in large numbers for weeks or months showed that Choccolocco Creek downstream from Snow Creek was not toxic, at least for the period of the experiments.

Residue Analyses: A total of 59 water, fish and mud samples from various sites was analyzed with gas chromatography between September [illegible] and August 1967 (Table 4). Identifications were confirmed in many of the samples by thin-layer chromatography.

Table 4. Origins, types, and numbers of samples analyzed by gas chromatography.

Location	Number		
	Water	Fish	Mud
Choccolocco Creek - Hiway #78 Bridge (4 mi. E. Boiling Springs)	11	4	0
Anniston Sewage Treatment Plant	8	0	0
Monsanto Waste Treatment Plant	4	0	0
Snow Creek	5	0	0
Choccolocco Creek - Hiway #21 Bridge	1	0	0
Choccolocco Creek - Wood Bridge	4	2	0
Choccolocco Creek - Hiway #109 Bridge	1	1	0
Choccolocco Creek - Hiway #93 Bridge	3	3	0
Jackson Shoals	3	1	1
Eureka Bridge	2	3	0
Logan-Martin Reservoir (on Interstate #59)	1	0	0
Dry Creek at Hiway #202 Bridge	1	0	0

DDT, DDE, DDD and occasionally other chlorinated hydrocarbon insecticides were found in the low parts per billion range in nearly every water sample from Choccolocco Creek (both upstream and downstream from Anniston), the Anniston Sewage Treatment Plant, and from Logan-Martin Reservoir. Parathion occurred in trace quantities (less than 1 ppb) in some water samples. A maximum of 3 ppb parathion was found in a water sample for the Anniston Sewage Treatment Plant and 1.5 ppb parathion was the maximum found in a Choccolocco Creek sample.

Water samples from Snow Creek produced 12-20 major peaks on a gas chromatogram during a 30-minute elution time. Gas chromatographic analyses of fish, mud, and some water samples collected in Choccolocco Creek below the mouth of Snow Creek almost always showed over a dozen of these same peaks. Attempts to chromatograph mud samples were hopeless. These interfering compounds frustrated attempts to clean-up samples and often would have masked pesticides where retention times were similar. Table 5 illustrates the problem.

Table 5. A comparison of retention times (in minutes and seconds) for major peaks on chromatograms for samples of Snow Creek water, a bluegill caged in Choccolocco Creek at the Wood Bridge (upstream from the mouth of Coldwater Creek), and for several pesticide standards.

Snow Creek (Front Ditch)	Caged Bluegill (Elution A)*	Pesticide Standards
1. 3:00		
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; parathion and methyl parathion generally appear in Elution B.

Reversed pectoral fins: In their paper "The toxicity of organic phosphorus and chlorinated hydrocarbon insecticides to fish" (1960. Biological Problems in Water Pollution, 2nd Seminar) Henderson, Pickering, and Tarzwell stated that the pectoral and pelvic fins of fish affected by organic phosphorus compounds assumed an extreme forward position and that this was a characteristic response. Descriptions of distressed fish in the 1966 fish kill in Choccolocco Creek contain numerous references to reversed pectoral fins, loss of equilibrium, and a tetanic state.

Fish have been tested on more than 25 insecticides at Mississippi State University and a greater tendency for reversed pectorals in phosphates has not been noted. When reversed pectorals were observed in large numbers of dying fish at the Eureka Bridge in March 1967, at a time when the Monsanto Niran plant was not operating, a series of experiments were conducted to test the specificity of the reversed pectoral fin response. Fish were exposed to different concentrations of a large number of commercial insecticides, domestic chemicals, and physical stimuli. Some agents producing reversed pectorals were: toxaphene, DDT, parathion, malathion, high and low sudsing detergents, household bleach, hand soap, ammonia, "Sani-sol", salt, tobacco extracts, urine, gasoline, acetone, "HB-40", ethanol, high temperature shock, high and low temperatures, and electrical shock. In most cases, loss of equilibrium and tetanus preceded death. None of the compounds caused reversed pelvic fins. Documentary evidence of these tests was provided Monsanto in the forms of photographs.

Acetyl Cholinesterase Inhibition: A great deal of time has been devoted to various experiments concerning possible cholinesterase inhibiting effects of Choccolocco Creek waters. Furthermore, we have reexamined a number of practices employed in cholinesterase determinations to ascertain their validity and effects upon results obtained.

Acetyl cholinesterase levels have been determined for a) bluegills exposed to parathion and methyl parathion for short terms; b) bluegills caged in Choccolocco Creek upstream and downstream from Anniston; c) healthy, native bluegills seined from Choccolocco Creek above and below Anniston; and d) sick, native bluegills collected near the mouth of Choccolocco Creek during the fish kill of March 1967. These data provide no evidence suggesting chronic cholinesterase inhibition in Choccolocco Creek fishes.

Some specific findings derived from the cholinesterase investigations are as follows:

1. Bluegills caged in Choccolocco Creek at the Hiway #93 Bridge showed no significant inhibition after one month.
2. Native bluegills from Jackson Shoals actually had higher AChE levels than our control bluegills.
3. A large series of tests has revealed that freezing whole fish results in a reduction of measured cholinesterase activity. (Table 6).
4. A similar but less drastic reduction of measured AChE activity occurs when fish brains are frozen in buffer solution, as recommended by Weiss (Table 6).

5. The effects of freezing did not increase when fish were kept frozen for 2 months. (Table 6).
6. A 24-hr exposure to 25 ppb parathion solution resulted in 43% AChE inhibition. Freezing the fish for 8 days caused an additional decrease of 68%. The combination of parathion and freezing resulted in 86% inhibition.
7. Freezing dissected brains individually wrapped in aluminum foil does not cause a reduction in measured specific activity as does freezing whole fish or brains in buffer solution.
8. TCE is not a cholinesterase inhibitor.
9. Fish are able to survive and recover from AChE inhibition in excess of the 40-70% said to be usually lethal.

Table 6. Specific activities for 8-10 bluegills (2 brains/sample) after various treatments. A - Field collected; B - Whole fish frozen at below -60 C for 96 hr; C - Brains frozen at below -60 C in 2 ml buffer; D - Whole fish frozen 2 months at below -60 C; E - Brains frozen in buffer for 2 months at below -60 C.

		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.

<u>Faunal Survey</u>: Many hours of seining and observation have shown that Choccolocco Creek is well populated with a great variety of fishes. The fishing pressure on the stream, especially from Jackson Shoals downstream to Logan-Martin Reservoir, attests to this fact. It is a "law of nature" that fishermen do not fish where they do not make catches. We have checked creels and observed some nice catches of bluegills, crappie, and other species. Leopard frogs are very abundant along Choccolocco Creek and we have observed some turtles.

A preliminary survey of invertebrate life in Choccolocco Creek suggests that the stream is quite productive in terms of plankton, neuston, aquatic insects, crustaceans, annelids, fresh water clams, and snails. A downstream site yielded about 5 times as many snails as did a site upstream from Anniston, presumably a reflection of the additional fertility derived from the Anniston Sewage Treatment Plant and possibly the minerals for shell formation derived from the Gypsum Plant effluent.

It seems highly significant that backswimmers, a type of insect, inhabit the final settling tank at the Anniston Sewage Treatment Plant (which receives effluent from the Monsanto Treatment Facility).

<u>Fish Kills</u>: There is a disturbing amount of low-level mortality among Choccolocco Creek fishes and a few dead individuals can be found most anytime, especially from Jackson Shoals downstream. A further indication that some sublethal factor may be present is the apparently high incidence of diseased fish. Several fish were observed near Jackson Shoals that had large patches of fungus on them.

A major fish kill started several days prior to March 23 in Choccolocco Creek below Jackson Shoals and in Logan-Martin Reservoir, including areas upstream from the mouth of Choccolocco Creek. When the area was visited on March 25, vast numbers of fish were dying and wind-rows of them were washed up along the shores. Most of the dead fish were shad, which is probably the most abundant fish in the area, but great numbers of carp, bluegills, channel catfish, black bullheads, sauger, crappie, goldfish, red-eared sunfish, green sunfish, carp suckers, large-mouthed bass, and other species (especially minnows) were observed. These fish had normal cholinesterase activities and the Monsanto Niran plant had not operated since about March 5. The number of fish observed dying, those recently killed, and the large number that had been dead for several days would have exceeded the 56,200 counted in the 1966 kill. We estimated that a 75-ft segment of beach contained 1,000 dead fish.

Miscellaneous:

The project personnel have conducted literature searches and provided certain information requested by Monsanto.

CONCLUSIONS

1. The Monsanto waste treatment plant is effective. Once the effluent has been diluted in Choccolocco Creek, the resulting water quality parameters, at least those normally measured, are well within acceptable limits and the stream is abundantly populated with organisms of a great variety.

2. Although some pesticide residues occur in Choccolocco Creek, the concentrations do not appear sufficient to be biologically significant. In view of our problems in processing samples for chromatography, the decision not to purchase a phosphate detector for this project, may have been a bad one.

3. Snow Creek is a potential source of future legal problems. The stream does not support life and contains many materials that accumulate in water, fish, and muds downstream. Although there is no evidence that these materials are harmful to fish, their presence constitutes damaging evidence of pollution. The argument that these compounds impart undesirable palatability qualities to Choccolocco Creek fish would be very convincing and probably easy to prove.

4. Choccolocco Creek fish populations are subject to continued low-level mortality and periodic massive die-offs. There are reports of fish kills in Dry Creek; Coldwater Creek is highly polluted. Monsanto needs to monitor the biological effects of its effluents as a protection against future accusations. (See Recommendations).

5. An attempt to investigate a fish kill after it has happened is futile, however some pertinent information has emerged from the studies.

    a. The number and variety of fish in Choccolocco Creek at the present time suggests that the 1966 kill had no adverse long-time effects.

b. Reversed pectoral fins are not a specific symptom of phosphate poisoning.

c. Massive fish kills occur in Choccolocco Creek and the Martin-Logan Reservoir when the Monsanto Plant is not in operation.

d. Freezing samples of whole fish serves to reduce measured AChE levels as expressed in specific activity of brain tissues. The enzyme is not affected directly. Although we do not know the source of the discrepancy, we suspect that the effect may be due to a combination of factors such as altered weight relationships, qualitative differences in brains that are removed fresh or frozen, or possibly due to the introduction of some anti-cholinesteritic material.

e. There is a considerable body of evidence disputing the generalization that a 40-70% AChE inhibition is generally lethal.

RECOMMENDATIONS

1. Do not release untreated waste in the future!
2. Clean-up Snow Creek.
3. In the event of a future fish kill, collect samples of fish and water and immediately call in a qualified consultant.
4. A qualified biologist should inspect Choccolocco Creek periodically, perhaps every other month, to document the status quo in the event of another fish kill.

5. Monsanto should monitor Choccolocco Creek, preferably near the Hiway #21 Bridge. A population of mosquitofish of known parathion tolerances and AChE characteristics should be established in a conveniently located pond. Samples of 10-20 fish from this population should be caged in the Creek and checked daily. The fish should be replaced weekly. Any marked die-off should be analyzed for insecticide residues and the brains of survivors checked for cholinesterase level. Some individual deaths could be expected and ignored.

APPENDIX

The following are some criticisms of the evidence presented by the State in its case against the Monsanto Company:

1. Control fish used in the AChE tests were collected a) from a different location than were the moribund fish, b) from a different population of fish, c) from a different environment (i.e., swift, clear, cold water, higher D. O., rocky bottom vs. impounded, muddy, warmer water, lower D. O., muddy bottom), d) at a different time.

AChE levels may differ in the same species of fish from one pond to another. Furthermore, it is conceivable that the control fish were inhibited at the time of the fish kill. For example, C8 and C9 (Table 4, Southeast Water Laboratory Report) are about the same size but C9 had 25% less activity. If C9 were collected April 6 and C8 on April 25, it would be very significant. Were collection dates and specimens kept separate? The point is that these data do not constitute a valid comparison in terms of a "scientifically controlled" experiment.

2. Table 4 of the Southeast Water Laboratory Report contains some obvious errors and discrepancies. The number of samples is too small to form an adequate basis for sweeping conclusions, especially in a suit involving $225,000.00.

Items:

a) Control and moribund bluegills were run on different days. The significance of this becomes apparent when the data for control long ears are compared. Those tested on April 28 had a mean of 1.54, but those tested on April 29 had a mean of 1.27 (an average of 18% less). Moribund long ears run on April 28 averaged .55 but those done on April 29 averaged only .27, which is over 50% less. If the fish were from the same sample, the variation suggests bad technique as does the fact that the difference in specific activity is about .27 in both cases. Such data are certainly suspect as to reliability

b) There are some serious discrepancies in the weights given in Table 4 of the report. For example, compare the weights of C5 and C6 (control bluegills).

	Total Wt. (g)	Length	Brain Wt. (mg)
C5	152.1	6.8	108.1
C6	83.5	5.5	213.0

C5 weighed almost twice as much, yet had a brain weight almost 50% smaller. This could not be! Fish and fish brains grow in an orderly, predictable manner.

Also, if one plots total fish weight against brain weight, it is obvious that several of the brain weights are comparatively too small and in error. Compare C3 and C4 with C5 and C8, e.g.,

	Total Wt. (g)	Brain Wt. (mg)
C3	27.2	110.5
C4	32.4	107.6
C5	152.1	108.1
C8	114.8	108.7

The brain weights are about the same but C3 and C4 weigh only 1/4 or 1/5 as much. These data are erroneous. One wonders about the entire Table when these obvious errors appear.

c) Weiss (1959. Response of fish to sub-lethal exposures of organic phosphorus insecticides. Sewage and Industrial Wastes, p. 583 - Fig. 1) shows a direct correlation between brain weight and specific activity in four species of fish, including bluegills. In general, the smaller the brain weight or fish size, the higher the specific activity. This relationship has been confirmed in other studies, including our own.

The brain weights and specific activities for control bluegills in Table 4 of the SWL report fail to show any orderly relationship. For example compare the following:

Sample	Brain Wt. (mg)	Specific Activity
C1	65.2	1.73
C2	72.7	1.78
C3	110.5	1.74
C5	108.1	1.83
C8	108.7	1.85

Also, note that C7 and C8 are both 6 inches in length, yet they represent the upper and lower extremes in specific activity for the entire sample, despite the fact that fish tested ranged from 2.8 to 6.8 inches in length.

The apparently random nature of these findings and the absence of basic relationships makes them unacceptable as scientific evidence.

d) The report mentions that 40-70% inhibition is lethal. The long ear #19 was alive (but sick) when collected yet showed 98% inhibition. Weiss (1961) reports an initial inhibition of 80% in fish that survived. These fish continued to exhibit 60% inhibition after 30 days. Weiss also reports a case where he obtained 95% inhibition and subsequent recovery of the fish.

e) The SWL report stresses the absence of overlap between data for moribund and control fish. We find no overlap between data for fish frozen whole and those from the same sample run fresh. A statement from Parker concerning collection of fish could be very instructive.

f) Were any control fish captured with electric shockers? If so, how does this method affect AChE levels?

3. In Table 1 (SWL Report), samples 6 and 5a were reported as "negative" and 18 ppb, respectively, yet they are from the same locality. Samples 1 and 1' were collected only 10 feet apart, but differed by 23 ppb. Samples 6, 3, 1' and 5 were collected within a 3-mile segment of impounded water and contained 0, 51, 15 and 50 ppb, respectively. How could anyone base a $225,000 judgement on such variable and flimsy data? If a graduate student were to turn in such data, he would flunk the course.

   It should be noted that only a single sample was taken at the upstream sites. Thus, samples 4 and 10 were "negative", but so was sample 6. The point is that 2 or 3 samples should have been taken at the sites where samples 4 and 10 were collected. Based on the variation mentioned above, additional samples might have contained 50 ppb!

4. Reversed pectoral fins are not a specific symptom of phosphate poisoning.

5. Massive fish kills occur when the Niran plant is down. All species are affected.

6. Fish kills are reported in Dry Creek.

7. There are many cholinesterase inhibitors.

8. The SWL reported no other insecticides in the water samples, but reported parathion in the 2 ppb range. Our analyses and those of the Southern Testing and Research Lab. show other

insecticides to be present, although in small amounts. The point is that the SWL may not have really looked for other possible causes for the fish kill.

9. No parathion was reported in fish samples by SWL. In view of the variation and discrepancies in their water analyses and AChE data, only the recovery of parathion from dead fish could constitute positive proof of parathion poisoning.

10. A few goldfish were included among the 56,200 fish counted in the 1966 kill. Goldfish have a 24-96 hr TL_m of 2.7 ppm parathion. This concentration would have killed all game fish and exceeds the 2.0 ppm report by the Anniston Sewage Treatment Plant for effluent received from Monsanto. The Anniston Sewage Plant claims the concentration to have been 0.79 ppm (by the Averill-Norris Method, which probably over estimates) when they released it to Choccolocco Creek. Given the benefit of dilution, uptake by other organisms, decomposition, etc., it is inconceivable that goldfish would be killed.

The estimates of fish killed are statistically and biologically unsound. If concentrations of 50 ppb parathion occurred in lower Choccolocco Creek and in Logan-Martin Reservoir in March 1966, it would have indeed killed thousands of fish. However, the kill of March 1967 also killed thousands of fish and was attributed to disease. Only an analysis of fish for parathion content or a careful histopathological examination could distinguish between the two causes of death. Such analyses were not made and I am convinced that many of the dead fish in the 1967 kill would have been included among those counted in 1966.

11. Several other questions were raised in my communication of March 28, 1967.