

## SOME EFFECTS OF PESTICIDES ON MISSISSIPPI WATERS

By

C. Rex Bingham, Fisheries Biologist  
Mississippi Game & Fish Commission

## INTRODUCTION

Over the past twenty years much has been said, pro and con, about the effects of various pesticides on surface waters in Mississippi and the other Continental United States.

In past years reported fish kills, where insecticides were the prime suspect, have gone invalidated as the Mississippi Game & Fish Commission had not the capability to analyze for insecticides.

In certain lakes in the Yazoo Mississippi Delta repeated attempts to restock largemouth bass (*Micropterus salmoides*) has met with no success. Insecticide pollution was suspected of being responsible for the failure of the bass stocks to take.

In 1967 the Fisheries Division of the Mississippi Game & Fish Commission, supported by Federal D-J funds, undertook a two year insecticide surveillance of one of the aforementioned lakes (Wolf Lake). A Delta Lake, (Mossy Lake) that supports a good bass population, was selected for comparison.

The present account compares the insecticide levels found in the waters, sediments, and fish flesh from the two lakes during the 1968 period. It also compares the tolerance of bluegill from Mossy Lake and Wolf Lake to endrin, toxaphene, and DDT.

## METHODS &amp; MATERIALS

Five stations on Wolf Lake and three stations on Mossy Lake were arbitrarily established after an aerial flight to determine drainage patterns.

Water samples were collected at approximately two week intervals from surface and bottom levels. Surface water was collected in  $\frac{1}{2}$  gallon small mouth jugs that were pre-washed with redistilled acetone, followed by redistilled hexane. Bottom level water was collected in a stainless steel sewage sampler that had received identical treatment to the small mouth jugs. The bottom level water was immediately transferred to the  $\frac{1}{2}$  gallon jugs upon surfacing. An amount of water slightly in excess of 1 liter was collected in the field and adjusted to precisely 1 liter at the laboratory.

The water was extracted with a hexane-pet. ether mix (85-15%) for one hour in the jugs on a mechanical roller. The extract was removed from the water by transferring to separatory funnels and separating. The water was re-extracted twice with 25 mls. of the hexane-pet. ether mix and the extract combined. The extract was then washed with 20 mls. of pet. ether through 2" of anhydrous sodium sulfate that was prewashed with 20 mls. of

redistilled hexane. Extracts were collected in 250 mil. Kuderna-Danish evaporators and condensed to seven to nine mils. over a steam bath. They were then quantitatively transferred to 15 mil. graduated centrifuge tubes and evaporated precisely to 5 mils. with dry air.

Samples were chromatogrammed on a Micro-Tek 220 GLC equipped with electron capture detectors. Identification of insecticides was accomplished via multiple column (3% DC-200 on 100/120 mesh Gas Chrom-Q and 9% QF-1 on 100/120 mesh Gas Chrom-Q), partition coefficients, and survey of farmers and crop dusters of the area to determine insecticides being used. Quantitation was on the basis of purified standards received from various manufacturers and the Agriculture Research Service Laboratory at Gulfport, Mississippi.

Sediment samples were taken by scooping the top centimeter from bottom muds taken with an Ekman dredge. Five dredge loads were used as a representative sample. Samples were allowed to settle overnight and the excess water poured off. Samples were then placed on a paint shaker for a two minute period. A 100 grams subsample was taken for analysis and another 100 grams for drying.

Sediments were extracted with a hexane-isopropyl alcohol, (150-50) mils., mix after addition of 85 grams anhydrous sodium sulfate. They were extracted on mechanical rollers for a period of 4 hours. The extracts were then filtered through Whatman #40 filter paper into 500 mil. separatory funnels. They were then washed three times with volumes of water approximately equal to the volumes of extracts. Ten grams of anhydrous sodium sulfate and 2.5 grams of celite were added and the extracts filtered through 2" of sodium sulfate.

These samples were too dilute to show toxaphene. They will be passed through floisil, condensed and rechromatogrammed to establish toxaphene residue levels.

No further cleanup was required and samples were chromatogrammed as described for water samples. Calculation was on the basis of 150 mils. hexane and no recovery factor was used.

Fish were collected with small mesh seine and electrical seine. Fish were, either frozen in the field with dry ice, or brought live to the laboratory and placed in a refrigerator freezer. Frozen small fish were prepared by removing the head, viscera, and skin.

Extraction and cleanups were accomplished as per Training Course Manual, Analysis of Pesticides in the Aquatic Environment, U. S. Department of the Interior, January, 1968.

Fish samples were chromatogrammed similar to water samples.

Data for DDT and its metabolites found in water were totaled and the average per lake established for each sampling date. The averages, sum of squares, and variances are shown in Table 1. Toxaphene data were treated in the same manner for the dates that it occurred in samples and is contained in Table 2. Methyl parathion is presented in like manner in Table 3. Table 4. contains similar data for DDT + metabolites found in sediments.

Bluegill measuring from 1 to 3 inches were collected in small mesh seines and held in laboratory well water (pH 7.3, hardness 47 mg/l  $\text{CaCO}_3$ ) for at least 24 hours prior to bioassay.

Technical grade endrin, DDT, and toxaphene were prepared as 1% solutions in acetone. Desired solution concentrations were obtained by adding acetone, not to exceed 2 ml/l final solution, and tap water to final concentrations.

Test fish were held in insecticide solutions until death or conclusion of test after 36 hours. Mortality was recorded every 2 hours and dead fish removed. All tests were run at room temperature  $70 \pm 2$  °F.

## RESULTS AND DISCUSSION

DDT and its metabolites, TDE and DDE, were found in Wolf Lake on every sampling date. On four sampling dates none were found in Mossy Lake. The average amounts of DDT and metabolites are much higher in Wolf Lake except on two dates, 9-17-68 and 12-11-68. On these dates abnormally high amounts were obtained at one station on Mossy Lake. This station was located at the mouth of a cotton field drainage ditch.

Toxaphene was found in Wolf Lake water on 10 of 15 sampling dates and only one of the 15 in Mossy Lake. The toxaphene level is much higher in Wolf Lake than Mossy Lake.

Methyl parathion appeared in both lakes on two dates, 9-5-68 and 9-17-68. Planes were spraying in both areas on these dates.

Both, toxaphene and DDT, reached a peak in April and a low in August on Wolf Lake. The highest amount of toxaphene found in Wolf Lake occurred 9-15-68, while planes were spraying. As the variance indicates, sampling points were far from uniform in concentration on this date.

The general trend for DDT in both lakes was low to medium in February and early March, peaking in the spring months, falling off in the summer and rising again in the fall. This trend is also observed for toxaphene in Wolf Lake.

Sediment samples show Wolf Lake to be much higher in DDT than Mossy Lake (Table 4.).

The flesh of nine bluegill from Mossy Lake averaged 1.013 ppm DDT,  $s^2=.4930$ . That of eight bluegill from Wolf Lake averaged 5.041 ppm DDT,  $s^2=1.7520$ . Toxaphene is not compared from the sediments or fish flesh as this portion of the project is incomplete.

Bluegill bioassays gave  $\text{LD}_{50}$  of 15 ppb for Mossy Lake vs 300 ppb for Wolf Lake in endrin, 25 ppb DDT for Mossy Lake vs 30 ppb DDT for Wolf Lake, and 20 ppb toxaphene for Mossy Lake vs 100 ppb toxaphene for Wolf Lake.

Two hundred thousand largemouth bass (1-4" length) were placed in Wolf Lake May 16-25, 1968. None were recovered during bi-weekly seining operations with small mesh seines and several electrical seining operations.

## CONCLUSIONS

DDT and toxaphene are having a subtle but drastic effect upon our aquatic environment. Bass and crappie are virtually absent in many waters where they flourished previous to the advent and widespread intensive use of long lived insecticides. Fishes that are found in such waters show a tolerance that reflect the extent to which those waters have been affected by such insecticides. Massive fish kills have been observed to be produced by both, quick kill, and long lived insecticides. Fishes and other lower forms of life have a marvelous reproductive power and potential for reestablishment, if the environment does not remain inhibitive.

Though insecticides are unquestionably necessary to our modern way of life, long lived insecticide residues are causing portions of life to either disappear or evolve to an extent that may render them harmful to man and other consumers.

## ACKNOWLEDGMENTS

The research was supported by the Mississippi Game & Fish Commission through a Federal Aid D-J Project. The assistance of Messrs. Robert Seater, Thad Brooks, Walter Parker, Bobby Gunter, Jack Herring and Mrs. Clara Johnston is acknowledged.

I am obligated to Mr. Barry O. Freeman and Harry Barkley for proof reading.

TABLE 1.

## MOSSY LAKE WATER - DDT + METABOLITES

| Date     | Av. ppb | SS     | s <sup>2</sup> |
|----------|---------|--------|----------------|
| 2-22-68  | .000    | .0000  | .0000          |
| 3-7-68   | .000    | .0000  | .0000          |
| 3-19-68  | .120    | .0049  | .0010          |
| 4-15-68  | .090    | .0192  | .0038          |
| 5-1-68   | .092    | .0185  | .0037          |
| 5-21-68  | .095    | .0250  | .0050          |
| 6-4-68   | .006    | .0007  | .0001          |
| 6-18-68  | .020    | .0024  | .0005          |
| 7-9-68   | .000    | .0000  | .0000          |
| 7-23-68  | .005    | .0007  | .0001          |
| 8-19-68  | .000    | .0000  | .0000          |
| 9-5-68   | .007    | .0005  | .0001          |
| 9-17-68  | .125    | .1000  | .0200          |
| 10-16-68 | .023    | .0033  | .0006          |
| 12-11-68 | .262    | 1.0827 | .2165          |

## WOLF LAKE WATER - DDT + METABOLITES

| Date     | Av. ppb | SS    | s <sup>2</sup> |
|----------|---------|-------|----------------|
| 2-22-68  | .164    | .0192 | .0021          |
| 3-7-68   | .198    | .6096 | .0677          |
| 3-19-68  | .371    | .1153 | .0128          |
| 4-15-68  | .502    | .2102 | .0233          |
| 5-1-68   | .364    | .1188 | .0132          |
| 5-21-68  | .463    | .0842 | .0105          |
| 6-4-68   | .467    | .3970 | .0441          |
| 6-18-68  | .296    | .2806 | .0312          |
| 7-9-68   | .142    | .3094 | .0344          |
| 7-23-68  | .231    | .1733 | .0217          |
| 8-6-68   | .066    | .0336 | .0037          |
| 8-19-68  | .026    | .0008 | .0001          |
| 9-5-68   | .174    | .1204 | .0134          |
| 9-17-68  | .101    | .1459 | .0162          |
| 10-16-68 | .089    | .0073 | .0008          |
| 12-11-68 | .102    | .0094 | .0010          |



TABLE 2.

## WOLF LAKE WATER - TOXAPHENE

| Date     | Av. ppb | SS     | s <sup>2</sup> |
|----------|---------|--------|----------------|
| 4-15-68  | 1.437   | 5.659  | .629           |
| 5-1-68   | .598    | 5.821  | .647           |
| 5-21-68  | .982    | 3.162  | .395           |
| 6-4-68   | .463    | .865   | .096           |
| 6-18-68  | .472    | 6.039  | .671           |
| 7-9-68   | .150    | .806   | .090           |
| 7-23-68  | .277    | 20.972 | 2.621          |
| 9-5-68   | 2.707   | 87.005 | 9.667          |
| 9-17-68  | .863    | 27.840 | 3.093          |
| 12-11-68 | 1.594   | .634   | .070           |

## MOSSY LAKE WATER - TOXAPHENE

| Date     | Av. ppb | SS   | s <sup>2</sup> |
|----------|---------|------|----------------|
| 4-15-68  | .000    | .000 | .000           |
| 5-1-68   | .000    | .000 | .000           |
| 5-21-68  | .000    | .000 | .000           |
| 6-4-68   | .000    | .000 | .000           |
| 6-18-68  | .000    | .000 | .000           |
| 7-9-68   | .000    | .000 | .000           |
| 7-23-68  | .000    | .000 | .000           |
| 9-5-68   | .000    | .000 | .000           |
| 9-17-68  | .000    | .000 | .000           |
| 12-11-68 | .380    | .018 | .004           |

TABLE 3.

## WOLF LAKE WATER - METHYL PARATHION

| Date    | Av. ppb | SS       | s <sup>2</sup> |
|---------|---------|----------|----------------|
| 9-5-68  | 2.700   | 393.1600 | 43.6844        |
| 9-17-68 | .245    | .4581    | .0509          |

## MOSSY LAKE WATER - METHYL PARATHION

| Date    | Av. ppb | SS       | s <sup>2</sup> |
|---------|---------|----------|----------------|
| 9-5-68  | 5.262   | 512.1013 | 128.0253       |
| 9-17-68 | .133    | .2781    | .0556          |

TABLE 4.

## WOLF LAKE SEDIMENT - DDT + METABOLITES

| Date    |     | Av. ppm | SS     | s <sup>2</sup> |
|---------|-----|---------|--------|----------------|
| 2-7-68  | Wet | .563    | .0865  | .0216          |
|         | Dry | 1.859   | 1.5639 | .3910          |
| 6-4-68  | Wet | .497    | .0158  | .0040          |
|         | Dry | 1.828   | .2791  | .0698          |
| 9-3-68  | Wet | .894    | .2194  | .0548          |
|         | Dry | 3.254   | 9.0528 | 2.2632         |
| 9-25-68 | Wet | .513    | .1371  | .0457          |
|         | Dry | 2.100   | 6.7677 | 2.2559         |

## MOSSY LAKE SEDIMENT - DDT + METABOLITES

| Date    |     | Av. ppm | SS    | s <sup>2</sup> |
|---------|-----|---------|-------|----------------|
| 2-7-68  | Wet | .091    | .0158 | .0079          |
|         | Dry | .421    | .3423 | .1711          |
| 6-4-68  | Wet | .105    | .0049 | .0024          |
|         | Dry | .580    | .0410 | .0205          |
| 9-3-68  | Wet | .094    | .0038 | .0019          |
|         | Dry | .578    | .2774 | .1387          |
| 9-25-68 | Wet | .100    | .6053 | .0026          |
|         | Dry | .552    | .0720 | .0360          |



## BIBLIOGRAPHY

- Bingham, C. Rex, 1966. The Effects of Dual Combinations of Four Commonly-Used Insecticides on Susceptible and Resistant Mosquitofish. Thesis.
- Ferguson, Denzel E. and Bingham, C. Rex, 1966. The Effects of Combinations of Insecticides on Susceptible and Resistant Mosquitofish.
- Ferguson, Denzel E., undated. Characteristics and Significance of Resistance to Insecticides in Fishes.
- Ferguson, Denzel E., 1967. The Ecological Consequences of Pesticide Resistance in Fishes.
- Ferguson, Denzel, and Ludke, Larry J., undated. Insecticide-Resistant Fishes: A Potential Hazard to Consumers.
- Ferguson, Denzel E. and Bingham, C. Rex, 1966. Endrin Resistance in the Yellow Bullhead, *Ictalurus natalis*.
- O'Brien, R. O., 1967. Insecticides Action and Metabolism.
- Steel, Robert G. D., 1960. Principles and Procedures of Statistics.