# Use of Selected Freshwater Bivalves for Monitoring Organochlorine Pesticide Residues in Major Mississippi Stream Systems, 1972–73

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ABSTRACT

*Seven species of freshwater Pelecypoda,* Amblema costata, Corbicula manilensis, Elliptio crassidens, Lampsilis anadontoides, Lampsilis claibornensis, Megalonaias gigantea, *and* Plectomerus dombeyanus, *were collected and monitored for pesticide content during 1972 and 1973. Thirteen collection sites, representing five major river basins in the state of Mississippi, were sampled and compared. During the 24-month study, 26 water samples and 58 claim samples from the five river basins were analyzed. Individual samples weighed from 8 g to 20 g and consisted of 1–30 clams, depending on size. Residues of toxaphene and methyl parathion were found only in 1973 water samples. The study shows that freshwater clams are effective monitors of pesticide content. The tendency of clams to concentrate pesticides and their corresponding ability to eliminate them varies with species. Significant reductions in DDT and a corresponding buildup of* p,p'*-TDE were noted in 1973, following the limitations on the use of DDT and large-scale flooding throughout the state.*

## *Introduction*

The tendency of Pelecypoda to accumulate high body levels of pesticides from water containing very small amounts has been demonstrated under laboratory conditions (*2, 5, 7*). In addition, Butler (*2*) and Fikes (*5*) have shown that bivalves are able to eliminate virtually all of their body burden of pesticides when placed in pesticide-free water. Other researchers have investigated pesticide accumulations by marine and freshwater species following periods of spraying or from known pesticides outfalls (*1, 3, 4, 6*).

The present study evaluates the tendency of clams to accumulate pesticides and their ability to eliminate them under field conditions. It was felt that a comparison of 1972 and 1973 samples should reflect purging of high body concentrations as a result of either or both of these factors: the banning in December 1972 of the widespread use of DDT, and the extensive flooding of the state of Mississippi during Spring 1973.

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## *Sampling*

A survey of pesticide levels in selected species of freshwater clams in the major stream systems of the state of Mississippi was conducted in Summer 1972 in order to determine body concentrations of pesticides. All collections were made during periods of low stream flow (July through November). In 1973, clams were again collected from the same sites within respective river basins. Seven species of clams, *Amblema costata, Corbicula manilensis, Elliptio crassidens, Lampsilis anadontoides, Lampsilis claibornensis, Megalonaias gigantea,* and *Plectomerus dombeyanus,* were collected from the stream systems. Samples consisted of one to four species. Since species varied in size, sufficient numbers of individuals were collected to provide 8–20 g for extraction.

The 13 sampling sites are shown in Figure 1. Collections were made by hand from shallow water; in deeper areas, dip nets, dredges, and oyster tongs were used. Clams were refrigerated and returned to the laboratory in ice chests. Specimens were then identified, and their soft tissues were removed. Soft tissues were blotted on Whatman No. 1 filter paper to reduce excess water and were frozen at —15°C until extraction could be performed.

## *Analysis*

Pesticides were extracted from clams by a modification of the procedure outlined in *Analysis of Pesticides in the Aquatic Environment* (*8*). Each sample was combined with three times its weight of sodium sulfate and extracted twice with equal portions of redistilled hexane by blending 3 minutes each time. The procedure gave 92 percent recovery for the first extraction and 8 percent recovery for the second.

The extracts were combined and filtered through Whatman No. 1 and No. 3 filter papers. The resulting extract was partitioned into acetonitrile and evaporated to dryness in a Kuderna-Danish concentrator over a steam bath. The residue was dissolved in 25 ml hexane

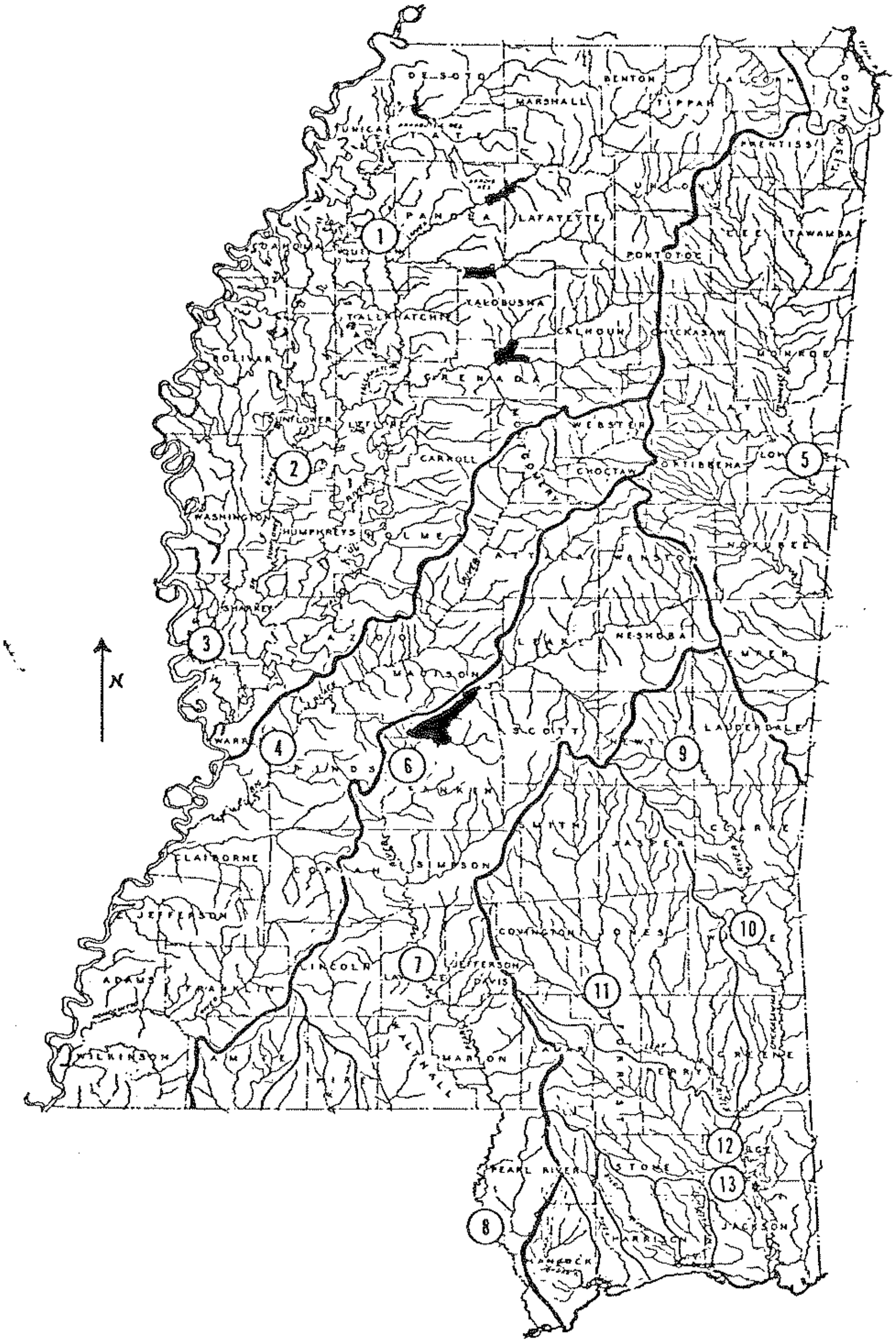


FIGURE 1. *Mississippi River Basin sampling sites, for monitoring pesticide residues in freshwater bivalves, 1972–73. Stations 1–3: Yazoo River Basin; Station 4: Big Black River Basin; Station 5: Tombigbee River Basin; Stations 6–8: Pearl River Basin; Stations 9–13: Pascagoula River Basin.*

and subjected to Florisil column cleanup: 4 inches $Na_2SO_4$ bottom layer, 10 g Florisil, 3 inches $Na_2SO_4$ top layer, ether–hexane (15 + 85) elution mixture.

Water samples were also taken in conjunction with the clam samples. They were collected in glass jars which were sealed with aluminum foil-lined screw caps. Samples were refrigerated in ice chests and extracted upon return to the laboratory, as follows: One liter of unfiltered water was extracted with a 100-ml solution of 85 percent hexane and 15 percent petroleum ether by rolling 1 hr on a concentric rotator. The resulting extract was eluted through approximately 6 inch anhydrous $Na_2SO_4$ in a size 224 Chromaflex (Kontes, 1 inch ID) column using a mixture of hexane–ethyl ether (85 + 15). The extract was concentrated to 5 ml in a Kuderna-Danish apparatus over a steam bath.

All samples were analyzed on a Micro Tek 220 gas chromatograph equipped with $^{63}Ni$ electron-capture detector. Instrument parameters and operating conditions follow:

| | |
|---|---|
| Columns: | 6 ft × ¼ inch OD glass column packed with a mixture of 1.5 percent OV–17 and 1.95 percent QF–1 (1972 and 1973)<br>10 percent DC–200 (1972 only)<br>3 percent OV–1 (1973 only)<br>each on Chromosorb W (AW, DMCS, HP) |
| Temperatures: | injector 220°C<br>column 185–195°C<br>detector 260°C |
| Carrier gas: | prepurified nitrogen flowing at ca 60–80 ml/minute |

In 1973, a 3 percent OV–1 column was substituted for the 10 percent DC–200 because of its greater sensitivity and lower retention time.

Residues were qualitatively identified by comparison with a standard. Quantitative measurements were based on peak height and expressed in $\mu g/g$ (ppm) for clam samples and $\mu g$/liter (ppb) for water samples. Results were not corrected for recovery.

## *Results and Discussion*

Sites from which clams containing the highest levels of pesticides were taken corresponded with areas of greatest agricultural usage. Highest detectable levels of toxaphene and DDT and its metabolites were found in clams from sampling sites within the Yazoo, Big Black, and Tombigbee River Basins (Fig. 1). Watersheds of these basins are within the most highly exploited agricultural areas of Mississippi (Statistical Reporting Service, U.S. Department of Agriculture, 1973, personal communication). Pesticide residues were also detected in clams from the northern areas of the Pearl River Basin which drains farmlands. Clams from the Pascagoula River Basin contained the lowest levels of pesticides (Table 1).

Pesticide residues were not found in water samples collected during 1972. Residues of toxaphene and/or methyl parathion were detected in water samples collected during 1973 from sites within the Yazoo, Big Black, and Tombigbee River Basins (Table 2). Detectable levels of toxaphene and methyl parathion in water samples taken in 1973 reflect in part the increased use of these pesticides, following the limits imposed on use of DDT. The extreme insolubility of DDT in water may have precluded its detection in trace amounts in the 1972 samples.

Overall pesticide residues in clams from sites within the Yazoo River Basin were less in 1973 than in 1972 (Table 1). With respect to DDT, there were large-scale reductions of both *o,p′*-DDT and *p,p′*-DDT in clams. Residues of *p,p′*-TDE, a first-order breakdown product of DDT, remained constant or changed only slightly in 1973. Increases of *p,p′*-TDE coincided with subsequent decreases in *p,p′*-DDT levels. Residues of *p,p′*-DDE declined slightly or remained constant. Decrease in DDT, an increase in TDE, and an overall elimination of pesticides indicates that the clams may be metabolizing the DDT. This purging would have been accomplished when the entrance of DDT into the streams was reduced. Limited spraying or increased dilution would have caused such a reduction.

A second and possibly better explanation for the variations in observed levels of DDT and its metabolites would be runoff. It is well known that runoff is the strongest contributor of pesticides to streams which drain farmland. During the present two-year study, clams apparently assimilated greater amounts of TDE and lesser amounts of DDT as the levels entering the streams changed.

Flooding of the Mississippi River during Spring 1973, as well as limitations on DDT use, probably influenced these fluctuations of residues in clams throughout the Yazoo River Basin. Sampling sites within this basin are located near the area flooded and also drain more farmland than do any of the previously mentioned basins.

Toxaphene residues in clams were lower at most sites in 1973; however, residues in water increased. Water samples were taken near the surface, and increases in residues are probably the result of local spraying rather than runoff.

Reductions of all metabolites of DDT were observed at the sampling site within the Big Black River Basin. Levels of *p,p′*-TDE decreased, from 0.36 ppm to 0.09 ppm. Decrease in *p,p′*-DDT levels were even greater, from 0.67 ppm to 0.06 ppm; therefore, the same pattern was followed. The single sampling site from this

TABLE 1. *Comparison of 1972 and 1973 organochlorine residues in bivalves from major stream systems of the state of Mississippi*

| | RESIDUES µg/g | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CHLORDANE | | ENDRIN | | TOXAPHENE | | p,p'-DDE | | p,p'-TDE | | p,p'-DDT | | o,p'-DDT | | ΣDDT | |
| YAZOO BASIN: | 1972 | 1973 | 1972 | 1973 | 1972 | 1973 | 1972 | 1973 | 1972 | 1973 | 1972 | 1973 | 1972 | 1973 | 1972 | 1973 |
| COLDWATER RIVER (QUITMAN COUNTY—R10W, T28N, 35) | | | | | | | | | | | | | | | | |
| *Corbicula manilensis* | ND | ND | ND | ND | 2.87 | 1.39 | 0.25 | 0.10 | 0.15 | 0.14 | 0.54 | 0.05 | 0.13 | ND | 1.07 | 0.28 |
| *Lampsilis anadontoides* | ND | ND | ND | ND | TR[1] | 0.48 | 0.08 | 0.04 | 0.06 | 0.04 | 0.11 | 0.02 | ND | ND | 0.25 | 0.10 |
| SUNFLOWER RIVER (SUNFLOWER COUNTY—R4W, T18N, 8) | | | | | | | | | | | | | | | | |
| *Amblema costata* | ND | ND | ND | ND | 4.02 | 3.65 | 0.21 | 0.24 | 0.26 | 0.33 | 0.11 | ND | 0.53 | 0.22 | 1.11 | 179 |
| *Lampsilis anadontoides* | ND | ND | ND | ND | 4.86 | 1.51 | 0.28 | 0.12 | 0.27 | 0.16 | 0.22 | ND | 1.00 | 0.05 | 1.78 | 0.31 |
| *Plectomerus dombeyanus* | ND | ND | ND | ND | 1.01 | 0.75 | 0.05 | 0.10 | 0.10 | 0.14 | ND | ND | 0.08 | 0.03 | 0.23 | 0.28 |
| STEEL BAYOU (ISSAQUENA COUNTY—R8W, T10N, 12) | | | | | | | | | | | | | | | | |
| *Amblema costata* | ND | ND | ND | ND | 1.09 | 0.72 | 0.12 | 0.18 | 0.13 | 0.16 | ND | ND | 0.07 | 0.01 | 0.32 | 0.26 |
| *Corbicula manilensis* | ND | ND | ND | ND | 7.68 | 3.62 | 0.39 | 0.23 | 0.40 | 0.49 | ND | ND | 0.53 | 0.10 | 1.32 | 0.81 |
| *Lampsilis anadontoides* | ND | ND | ND | ND | 1.93 | 1.11 | 0.18 | 0.11 | 0.21 | 0.21 | ND | ND | 0.12 | 0.04 | 0.51 | 0.35 |
| *Plectomerus dombeyanus* | ND | ND | ND | ND | 0.84 | 0.62 | 0.08 | 0.08 | 0.10 | 0.14 | ND | ND | 0.07 | 0.04 | 0.25 | 0.25 |
| BIG BLACK RIVER BASIN: | | | | | | | | | | | | | | | | |
| BIG BLACK RIVER (WARREN COUNTY—R5W, T6N, 22) | | | | | | | | | | | | | | | | |
| *Corbicula manilensis* | ND | ND | ND | ND | 4.43 | 3.78 | 0.27 | 0.07 | 0.36 | 0.09 | 0.10 | ND | 0.67 | 0.06 | 1.40 | 0.22 |
| *Lampsilis anadontoides* | ND | ND | ND | ND | TR[1] | 1.01 | 0.04 | 0.02 | 0.05 | 0.03 | ND | ND | 0.06 | 0.02 | 0.14 | 0.06 |
| TOMBIGEE RIVER BASIN: | | | | | | | | | | | | | | | | |
| TOMBIGBEE RIVER (LOWNDES COUNTY—R18W, T18S, 17) | | | | | | | | | | | | | | | | |
| *Megalonaias gigantea* | ND | ND | ND | TR[1] | 0.92 | 0.22 | 0.08 | 0.04 | 0.06 | 0.02 | 0.08 | 0.03 | ND | ND | 0.22 | 0.10 |
| PEARL RIVER BASIN: | | | | | | | | | | | | | | | | |
| PEARL RIVER (HINDS COUNTY—R1E, T6N, 36) | | | | | | | | | | | | | | | | |
| *Amblema costata* | 0.01 | ND | ND | ND | ND | 0.13 | 0.05 | TR[2] | 0.02 | TR[2] | TR[2] | TR[2] | ND | ND | 0.07 | TR[2] |
| *Lampsilis anadontoides* | 0.01 | ND | ND | ND | ND | TR[1] | 0.03 | TR[2] | TR[2] | TR[2] | ND | ND | ND | ND | 0.03 | TR[2] |
| *Plectomerus dombeyanus* | TR[2] | ND | ND | ND | ND | 0.13 | 0.02 | 0.01 | TR[2] | TR[2] | ND | ND | ND | ND | 0.02 | 0.01 |
| *Corbicula manilensis* | ND | ND | ND | ND | ND | TR[1] | 0.01 | TR[2] | ND | TR[2] | ND | ND | ND | ND | 0.01 | TR[2] |
| *Lampsilis anadontoides* | ND | ND | ND | ND | ND | TR[1] | ND | TR[2] | ND | TR[2] | ND | ND | ND | ND | ND | TR[2] |
| PEARL RIVER (PEARL RIVER COUNTY—R18W, T5S, 29) | | | | | | | | | | | | | | | | |
| *Corbicula manilensis* | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| PASCAGOULA RIVER BASIN: | | | | | | | | | | | | | | | | |
| CHUNKY RIVER (NEWTON COUNTY—R13E, T6N, 36) | | | | | | | | | | | | | | | | |
| *Corbicula manilensis* | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| *Elliptio crassidens* | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| *Lampsilis anadontoides* | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| CHICKASAWHAY RIVER (WAYNE COUNTY—R7W, T8N, 10) | | | | | | | | | | | | | | | | |
| *Corbicula manilensis* | ND | ND | ND | ND | ND | TR[1] | ND | TR[2] | ND | TR[2] | ND | TR[2] | ND | ND | ND | TR[2] |
| *Elliptio crassidens* | ND | ND | ND | ND | ND | 0.30 | ND | TR[2] | ND | TR[2] | ND | 0.03 | ND | ND | ND | 0.03 |
| LEAF RIVER (JONES COUNTY—R13W, T6N, 33) | | | | | | | | | | | | | | | | |
| *Corbicula manilensis* | ND | ND | ND | ND | ND | TR[1] | 0.04 | 0.02 | 0.05 | 0.01 | 0.05 | 0.02 | ND | ND | 0.13 | 0.05 |
| *Lampsilis anadontoides* | ND | ND | ND | ND | ND | TR[1] | 0.01 | 0.01 | TR[2] | TR[2] | TR[2] | TR[2] | ND | ND | 0.01 | 0.01 |
| PASCAGOULA RIVER (GEORGE COUNTY—R8W, T2S, 23) | | | | | | | | | | | | | | | | |
| *Corbicula manilensis* | ND | ND | ND | ND | ND | ND | TR[2] | TR[2] | TR[2] | TR[2] | ND | ND | ND | ND | TR[2] | TR[2] |
| *Lampsilis anadontoides* | ND | ND | ND | ND | ND | ND | ND | TR[2] | ND | TR[2] | ND | TR[2] | ND | ND | ND | TR[2] |
| *Plectomerus dombeyanus* | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| BLACK CREEK (GEORGE COUNTY—R8W, T3S, 14) | | | | | | | | | | | | | | | | |
| *Lampsilis claibornensis* | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |

NOTE: ND = None detected.
[1] Less than 0.1 µg/g for toxaphene.
[2] Less than 0.01 µg/g for DDT and metabolites.

TABLE 2. *Pesticide residues in water samples taken concurrently with Pelecypoda samples from major stream systems in the state of Mississippi, 1973*

| Sampling site | Organo-chlorines | Residues, µg/liter (ppb) |
|---|---|---|
| Yazoo River Basin: | | |
| Coldwater River (Quitman County—R10W, T28N, 35) | Toxaphene | 0.46 |
| | p,p'-DDE | 0.01 |
| Sunflower River (Sunflower County—R4W, T18N, 8) | Toxaphene | 0.44 |
| Steele Bayou (Issaquena County—R8W, T10N, 12) | Toxaphene | 2.11 |
| | Methyl parathion | 0.25 |
| Big Black River Basin: | | |
| Big Black River (Warren County—R5W, T6N, 22) | Toxaphene | 1.42 |
| | Methyl parathion | 0.46 |
| Tombigbee River Basin: | | |
| Tombigbee River (Lowndes County—R18W, T18S, 17) | Methyl parathion | 0.08 |
| Pearl River Basin: | | |
| Three sampling sites | None detected | |
| Pascagoula River Basin: | | |
| Five sampling sites | None detected | |

basin is the lower end of the more highly agricultural, or Delta, region of Mississippi.

The single species collected from the Tombigbee River Basin, *M. gigantea*—a large species of clam whose soft parts weigh in excess of 300 g in mature adults—showed reduced levels of all pesticides except endrin between 1972 and 1973 (Table 1). Trace amounts of endrin were found in this species in 1973 but not in 1972. The Tombigbee River Basin drains away from the Mississippi River, thus agricultural lands are not as extensive as around the Yazoo and Big Black Basins. As anticipated in this large species from the Tombigbee River Basin, DDT and toxaphene levels were lower in 1972. Also, its residues were proportionately less reduced by 1973 than the residues in other, smaller species from the Delta basins. Since 300 g of tissue was too large an amount to be extracted by this procedure, 20 g of tissue consisting of approximately equal amounts from the gill, mantle, foot, and visceral mass were extracted. Reported levels may have varied slightly because of differences in the amounts of tissues extracted.

In the Pearl River Basin, 1973 residues of DDT and its metabolites in clams and water also decreased in areas where detectable levels had been found the previous year. Toxaphene residues in clams increased slightly (Table 1).

This Pearl River Basin, which is located south of the Delta region and east of the Mississippi River, receives fewer pesticides than the Yazoo, Big Black, or Tombigbee River Basins. The increase in toxaphene residues, although slight, is further evidence of the tendency of clams to reflect local fluctuations since no increases in toxaphene were observed in the flooded areas. Chlordane was found in clams in 1972 at one site on the Pearl River near Jackson, Mississippi (Fig. 1, No. 6), but was not found in 1973. Chlordane residues were very low and no residues of chlordane were detected in any other clam or water samples throughout the state.

Results of analysis of clams from the Pascagoula River Basin were similar to the Pearl River Basin results (Table 1). At Stations 10 and 12 (Fig. 1) slight increases in both toxaphene and DDT and its metabolites were recorded. These increases were between 0.005 µg/g and 0.01 µg/g in all but one sample (Table 1). On the Leaf River, Station 11, a decrease in DDT levels was observed between 1972 and 1973. This basin is the farthest from the Delta region, and residues were so low that no other trends in pesticide changes could be determined between 1972 and 1973.

A comparison of these increases and decreases in DDT residues in clams shows that *C. manilensis* is the best indicator of change. In every instance in 1972, *C. manilensis* contained the highest levels of DDT and toxaphene. In 1973, *C. manilensis* again showed the most substantial reductions (Table 1). This imported species, which is taxonomically, anatomically, and physiologically different from the Unionid species tested, might have a greater tendency to concentrate pesticides and a correspondingly greater ability to eliminate them.

## *Conclusions*

Results indicate that freshwater clams are of value in reflecting changes in pesticide levels in streams. In areas where pesticides have been used for many years, once-a-year sampling programs may be sufficient to reflect changes in extremely residual pesticides, such as DDT. At all sampling sites within the Delta region of Mississippi, large reductions, not elimination, of DDT and its metabolites were recorded in clams between 1972 and 1973. In other areas of the state where pesticides were not expected because of lack of large agricultural operations, none were found; therefore the greatest contributor of pesticides to the streams of Mississippi is agricultural usage.

Although it is an indirect means of assessment, monitoring pesticides in freshwater clams may provide additional information on residue occurrence. Analysis of water is not reflective of biological concentrations.

Different species of clams also seem to have varying tendencies to concentrate and varying abilities to eliminate pesticides. *C. manilensis* is apparently the best indicator of the freshwater species sampled.

## LITERATURE CITED


(1) *Bedford, J. W., E. W. Roelofs, and M. J. Zabik. 1968.* The freshwater mussel as a biological monitor of pesticides in a lotic environment. Limnol. Oceanogr. 13 (issue?):118–126.

(2) *Butler, P. A. 1969.* Pesticides in the sea. Pages 513–516 in The Encyclopedia of Marine Resources, Frank E. Firth, Ed., Van Nostrand-Reinhold Co., New York, N.Y.

(3) *Check, R. M., and M. T. Canario, Jr. 1972.* Residues of chlorinated hydrocarbon pesticides in the northern quahog (hard-shell clam) *Mercenaria mercenaria,* 1968–1969. Pestic. Monit. J., 6(3):229–230.

(4) *Dimond, J. B., A. S. Getchell, and J. A. Blease. 1971.* Accumulation and persistence of DDT in a lotic ecosystem. J. Fish. Res. Board Can. 28(issue?):1877–1882.

(5) *Fikes, M. H. 1971.* The uptake, retention, and release of dieldrin in the gills of the naiad mollusk, *Amblema plicata* (Say, 1817). Masters thesis, Ohio State University, Columbus, Ohio, 21 pp.

(6) *Foehrenbach, J., G. Mahmood, and D. Sullivan. 1971.* Chlorinated hydrocarbon residues in shellfish (Pelecypoda) from estuaries of Long Island, New York. Pestic. Monit. J. 5(3):242–247.

(7) *Petrocelli, S. R., A. R. Hanks, and J. W. Anderson. 1973.* Uptake and accumulation of an organochlorine insecticide (dieldrin) by an estuarine mollusc, *Rangia cuneata.* Bull. Environ. Contam. Toxicol. 10(5):315–320.

(8) *U.S. Department of the Interior. 1968.* Federal Water Pollution Control Administration, Training Program. Analysis of Pesticides in the Aquatic Environment, 1968, Cincinnati, Ohio.