Movement of Mirex from Sediment and Uptake by the Hogchoker, *Trinectes maculatus*

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Little information is available on the uptake of insecticides by benthic organisms. DERR and ZABIK (1972) determined that the absorption of DDE by the midge was related to levels of the insecticide in a contaminated environment. No attempt was made to differentiate between uptake from the sediment and from the water. NIMMO et al. (1971) described the absorption of polychlorinated biphenyls (PCB's) from sediment by crabs and shrimp. Because of probable feeding on the sediments by the organisms, no distinction could be made between absorption and ingestion of the PCB's.

Use of the proper test organism is necessary to determine the mode of uptake of an insecticide by an aquatic organism. Since many benthic dwellers are detritus feeders, it is often hard to distinguish between absorption and consumption of toxic materials. The hogchoker (Trinectes maculatus), however, is carnivorous (CASTAGNA, 1955) and ingestion of sediment is unlikely. Thus absorption of an insecticide from a contaminated substrate can be differentiated from consumption of insecticide-laden foods.

The purpose of this study included: (1) determination of the movement of mirex in a soil-water system, (2) measurement of the rate of absorption of mirex by a flatfish in this system, and (3) differentiation between uptake of mirex from the substrate and from the water.

Materials and Methods

Experimental Procedure

The experiment consisted of two 4 week tests, one utilizing static water conditions (static test), the other using constantly flowing water (flow-through test) of 30 ml/min. Nineteen liter aquaria were filled with 3.5 kg of coarse silica sand to simulate a sediment approximately 4 cm deep. Technical grade mirex (97%, Allied Chemical Company) was dissolved in hexane and mixed with sand to obtain test concentrations of about 5000, 1650 and 500 ppb (parts per billion) in 3.5 kg of sand. Control aquaria consisted of hexane without mirex mixed in the sand. Sediment in each tank was covered with 14 l. of water. Characteristics of the test water included: total hardness, 126 ppm (parts per million); pH, 7.8; dissolved oxygen, 7.0 ppm; and temperature, 24.9°C.

Juvenile hogchokers were collected from the Apalachicola River at Chattahoochee, Florida. No mirex was found in any of the

Bulletin of Environmental Contamination & Toxicology,
Vol. 14, No. 6 © 1975 by Springer-Verlag New York Inc.

test fish although muscle averaged 215 ppb DDE and liver averaged 556 ppb DDE. Fish were fed chopped earthworm (Eisenia foetida) every other day at the rate of 2% of the body weight. Trace amounts (less than 10 ppb) of DDT and DDE were present in the earthworms.

Pesticide Analysis

Sediment, water and tissue samples were taken on a weekly basis over a period of one month. Sediment samples of about 100 gm were air dryed on aluminum foil for 48 hrs and frozen. Water samples were stored in 1 liter glass jars and refrigerated at 6°C. Single muscle samples were obtained from each of two fish at each test concentration. Due to the small size of organs in the hogchoker, livers from two fish were pooled to make one sample.

Muscle, liver and earthworm samples were analyzed according to the micromethod of ENOS (1970). Samples were extracted with acetonitrile in a tissue grinder and the pesticide transferred to hexane. The extract was rinsed through a chromoflex column filled with activated florisil and topped with sodium sulfate. The column was eluted with 20 ml of 5% ethyl ether in hexane. Each elution was concentrated over a steam bath to 1-2 ml for injection. Sediment samples were extracted for 6 hours with 150 ml of 10% acetone in petroleum ether in a Soxhlet apparatus (MILLS et al. 1963). The extract was concentrated to 10 ml for injection. Water samples were extracted by shaking twice with 100 ml of hexane in a separatory funnel. Extracts were dried by rinsing over sodium sulfate and concentrated to about 1 ml.

Samples were analyzed on a Varian Aerograph series 2100 gas chromatograph with Ni^{63} and H^3 detectors. The Ni^{63} detector employed a 183 cm by 2 mm glass column packed with 6.4% OV 210 and 1.6% OV 17. Operating temperatures were: detector 270°C, oven 180°C, column 210°C and injector 225°C. The nitrogen gas flow rate was 27 ml/min. The tritium detector used a 183 cm by 2 mm glass column packed with 3% OV 101. Detector temperature was 275°C, oven 180°C, column 210°C and injector 225°C. The nitrogen gas flow rate was 70 ml/min. Standards were injected after every fourth sample. Every tenth sample was injected in both columns to verify identification and quantification of the insecticides.

Recovery rates were established on tissue, water and sediment by spiking triplicate samples with known quantities of mirex. Recovery ranged from 87 to 100+ % for tissue, 79 to 80% for water and 91% for sediment.

Results and Discussion

Uptake of mirex by the liver and muscle of fish showed a dose dependent (dose=initial sediment concentration) relationship. Regression lines for mirex in the tissues are listed in Table 1.

TABLE 1

Regression equations and significance of the slope from zero using a student's t-test.

tissue	test concentration[1]	regression line	$t=b/S_b$[2]	p[3]
STATIC				
muscle	556	Y=.41x - .34	4.61	<.01
muscle	1650	Y=.36x - .20	4.55	<.01
muscle	5000	Y=.94x - .65	14.03	<.01
liver	556	Y=.74x - .35		
liver	1650	Y=.90x - .34		
liver	5000	Y=2.25x-1.55		
FLOW-THROUGH				
muscle	543	Y=.34x - .06	3.68	<.01
muscle	1666	Y=.32x + .37	3.45	<.01
muscle	5000	Y=.82x - .43	8.83	<.01
liver	1666	Y=.93x - .20		
liver	5000	Y=1.42x- .73		

[1] ppb (parts per billion)
[2] b=slope, S_b=error of estimate/corrected sums of squares
[3] probability

Equations and data points are plotted in Figures 1 and 2. Slopes of the regression lines for muscle of all test concentrations differed significantly from zero ($p<.01$). Pooling of the livers to form one sample prevented any test of significance for the regression lines for mirex in liver. Residues of mirex in the two tissues were significantly related ($r=.892$, $p<.01$, static test; $r=.873$, $p<.01$, flow-through test).

A 2-way analysis of variance was calculated on paired muscle samples from the static and flow-through tests. Mean levels of mirex in muscle were significantly different ($p<.01$) with time and test concentration. Mean levels of mirex in liver were significantly different ($p<.05$) only with time of the flow-through test.

Figure 3 shows levels of mirex in water and sediment over the 4 weeks of each test. Mirex in the water was directly related to the initial dose of pesticide in the sediment. Concentrations of mirex in water reached equilibrium during the first week and declined over the following weeks. Due to increasing levels of ammonia, water in all aquaria of the static test was renewed at the beginning of the fourth week. Declining levels of mirex in

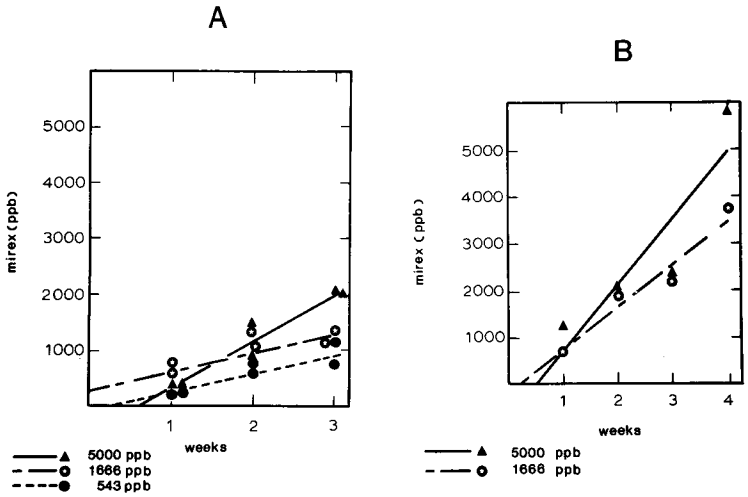

Figure 1. Regression lines for the uptake of mirex by the liver (A) and muscle (B) of fish from the static test.

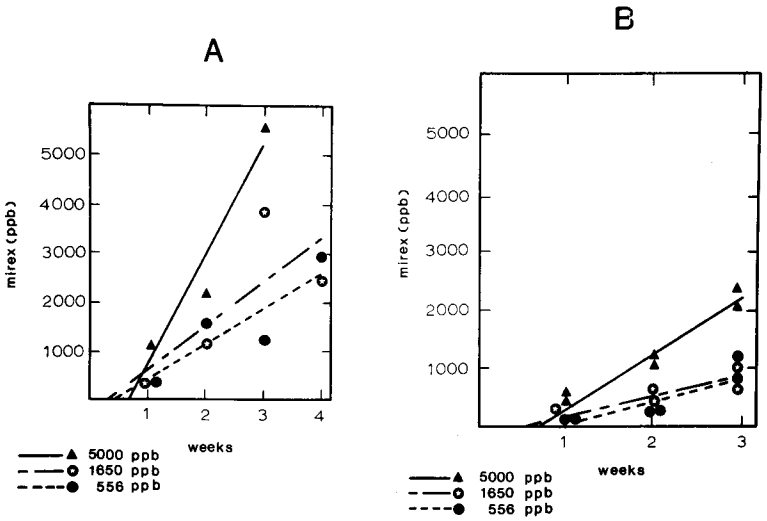

Figure 2. Regression lines for the uptake of mirex by the muscle (A) and liver (B) of fish from the flow-through test.

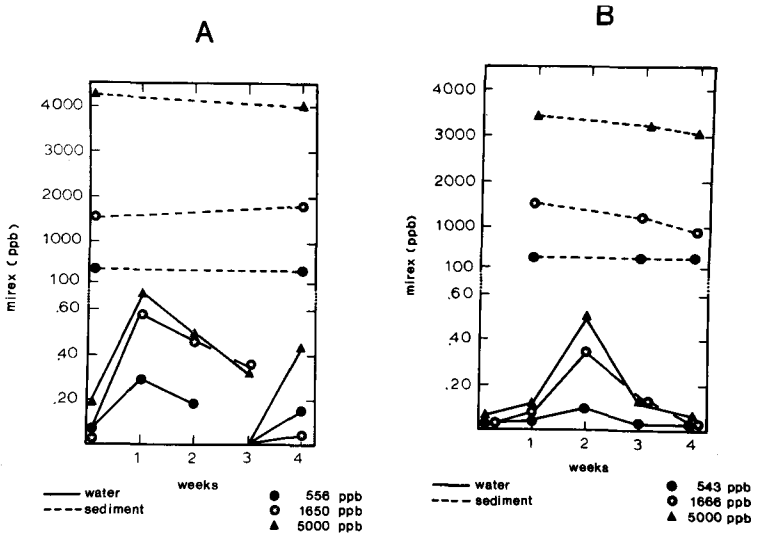

Figure 3. Levels of mirex in water and sediment from the static (A) and flow-through (B) tests.

the flow-through test could be attributed to the removal of the constantly flowing water. The static test, however, showed a similar decrease of mirex in solution. Previous research had demonstrated a marked decrease of pesticide in solution when fish are introduced to a static-pesticide system (GAKSTATTER and WEISS 1967; HOLDEN 1962). Thus absorption and retention of mirex from the water by the hogchoker probably caused the reduction of mirex in the water of the static test. Absorption of mirex by the fish in conjunction with the dilution of the flowing water probably caused the decline of mirex in the water of the flow-through test.

Increased adsorption of chlorinated hydrocarbon insecticides on soils and sediments can be proportional to increased organic content and decreased grain size of the soil (BOWMAN et al. 1965; LOTSE et al. 1968). The silica sand used in the experiments had a large grain size and low organic content. Desorption of mirex from sediment occurred, with the insecticide either dissolved in the water, absorbed or metabolized by the fish, codistilled with water or adsorbed to the sides of the aquaria. Only a small amount of the total mirex was desorbed from the sediment of the static test (Figure 3A). By the end of the fourth week, 40-45% of the mirex had leached from the sediment of the flow-through system (Figure 3B).

Static and flow-through methods were used to obtain different concentrations of mirex in water while utilizing similar levels of mirex in sediment. Levels of mirex in the water of the flow-through test were considerably less than that of the static test (Figure 3). Mirex levels and rates of accumulation in muscle from the flow-through test, however, were similar to that of the static test (Figures 1 and 2). Expressed as partition coefficients, bioconcentration of mirex in muscle was approximately 3800X (static test) and 10,400X (flow-through test). With the exception of the slow dilution of the water (turn over time: 40 hrs) and

subsequent leaching of mirex from sediment, experimental conditions of the flow-through test were similar to that of the static test. Thus absorption of mirex from water may not accurately describe the accumulation of mirex by the tissues of the fish.

The unique relationship of the hogchoker to the sediment suggests the possible absorption of mirex from the substrate. The fish is seldom found swimming and cannot remain stationary within the water column. The fish usually rests upon or is buried within the top layer of sediment. Thus, due to the proximity of the hogchoker to the sediment, mirex bound to the sand is available to the fish. Figure 4A implies that a substantial amount of mirex might have been absorbed by the fish from the sediment of the static test. Approximately .003 and .007 mg of mirex was lost from the water of the 1650 and 5000 ppb test tanks, respectively, during the second and third weeks of the static test. The fish gained about .012 mg (1650 ppb test tank) and .024 mg

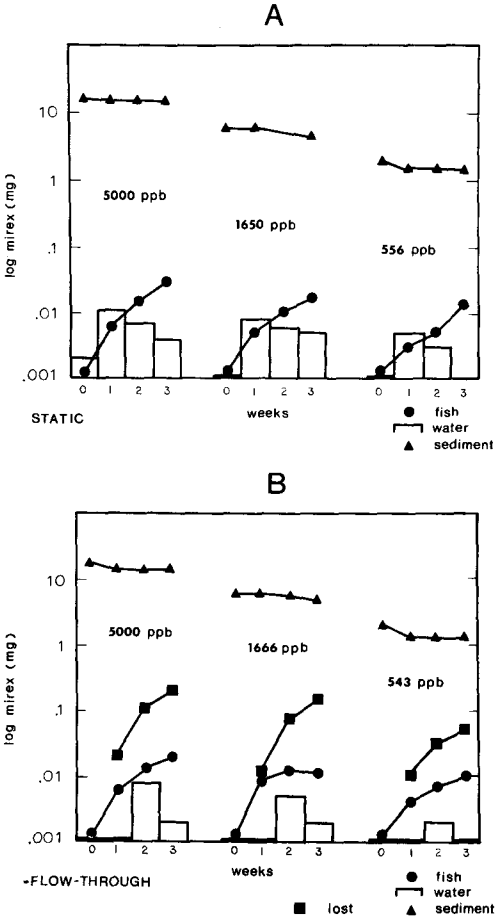

Figure 4. Accumulation of mirex (mg) in the fish, water and sediment and lost to the constant flow system for the static (A) and flow-through (B) tests.

(5000 ppb test tank) during the same two week period. Thus the fish gained more than 3X the amount of mirex lost from the water of the static system. A considerable amount of mirex in the fish, therefore, was obtained from a source other than the water. This source was, in all probability, the sediment. Because significant quantities of mirex were leached from the sediment and lost to the outside in the flow-through test (Figure 4B), movement of mirex from sediment and water to fish could not be calculated. The fish in this system, however, probably absorbed a significant amount of mirex from the sediment.

Summary

Mirex contaminated sediments of the static test retained virtually all of the insecticide over a 4 week period. About 40% of the initial concentration was lost from the sediment under constant flow conditions. Mirex in water was directly related to levels of mirex in sediment. Mirex in water reached an equilibrium during the first week and declined over the following 3 weeks. Uptake of mirex by tissues showed a dose dependent relationship. Accumulation of the insecticide increased over time and did not appear to reach an equilibrium. Residues in the muscle of fish increased significantly with time ($p < .01$) and test concentration ($p < .01$). Mirex in the liver of fish increased significantly with time ($p < .05$) of the flow-through test. Declining levels of mirex in water indicated mirex was absorbed from the water by the fish. Considerably more mirex, however, was gained by the fish than was lost from the water. Thus the fish probably absorbed a significant amount of mirex from the sediments.

Acknowledgments

The authors thank C. Cripe for his assistance in the pesticide analysis and the Florida Sea Grant Program, NOAA (R/EA-1) for financial support.

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