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Distribution of Mirex in an Experimental Estuarine Ecosystem[1]

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The purpose of these experiments was to determine the movement and accumulation of mirex in an experimental ecosystem by utilizing a method of introducing mirex that is representative of what occurs in the natural environment. Data derived from analyzing uptake and effects of pesticides are no more reliable than the success of attempts to duplicate natural field conditions. For example, if the mirex is introduced into water via carrier or solubilizer, synergistic or antagonistic effects cannot be discerned by the use of a carrier control. Since physico-chemical interaction between solubilizers and pesticides affects surface phenomena of the insoluble pesticide (SCHOOR 1975), transport across the first physiological barrier, the cell membrane, may also be affected. These interactions need not be defined, however, when using mirex leached from mirex bait (1.40 kg/ha) since this simulates what would occur in the environment. The only drawback to using such a leachate is the absence of precise control of mirex concentrations in the water.

Data on concentrations found in water are sparse, so it is difficult to choose an environmentally feasible, low concentration of mirex for laboratory exposures. SPENCE and MARKIN (1974) reported combined totals of sediment-bound and activated charcoal-adsorbed mirex ranging from 0.001 to 0.005 µg/L as long as 3 months after application of mirex bait (1.40 kg/ha) to freshwater ponds. After treatment of 250,000 ha of the upper watershed of the Tombigbee River in Mississippi with 330,000 kg of mirex bait, average concentrations of mirex found in the water throughout that year were approximately 0.001 µg/L, increasing to 0.03 µg/L during spring flood (Earl G. Alley, Mississippi State Chemical Laboratory, personal communication). The same concentrations were observed the following year after re-treatment of the area. Mirex, adsorbed to sorghum plants, was introduced to a model ecosystem and was apparently transported to the water by the fecal pellets of the salt marsh caterpillar, Estigmene acrea, that fed on the plants; water levels were found to be 0.018 µg/L after 33 days (METCALF et al. 1973). The authors did not state whether this residue represents the amount adsorbed by plankton in addition to that dissolved in the water itself.

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While data on environmental water levels of mirex are sparse, data on the uptake of mirex by organisms from water are well established (VAN VALIN et al. 1968, LUDKE et al. 1971, BAETCKE et al. 1972, BOOKHOUT et al. 1972, PRITCHARD et al. 1973). Uptake from low concentrations of mirex in water (0.22 µg/L measured) by blue crabs, Callinectes sapidus, has been shown, with highest accumulation of 31 µg/kg in 6 hr found in the hepatopancreas (SCHOOR 1974). Whole-body residues of mirex in blue crabs and grass shrimp, Palaemonetes pugio, after 28 days exposure to average concentrations between 0.03 and 0.16 µg/L in the water ranged from 20 to 590 µg/kg and from 90 to 2,400 µg/kg, respectively (TAGATZ et al. 1975); residues of mirex in the top layer of the sand ranged from 10 to 38 µg/kg.

METHODS and MATERIALS

The ecosystems and the introduction of mirex into the water have been described previously (TAGATZ 1976) so will only be summarized briefly. Static tests were conducted in glass-covered, 180-L glass aquaria maintained at 20 ± 1°C. Fluorescent lights provided 12-hr alternating light/dark cycles. Each aquarium contained 160 L of artificial seawater (distilled water plus Rila Marine Mix[2] at a salinity of 20 parts per thousand. Beach sand was used to provide a 4-cm layer of substratum in which 75 turtle grass plants, Thalassia testudinum, were planted to cover 40% of the bottom. Seventy-five adult grass shrimp, Palaemonetes vulgaris (30-35 mm, rostrum-telson length; 0.20 - 0.25 g each), were added. The systems were allowed to adjust under test conditions for one week. Two tanks contained all components except mirex (control), two tanks contained all components ("complete" system), and two tanks contained only water, shrimp, and mirex ("shrimp-only" system). Since the latter contained no natural food, shrimp were fed an amount of Biorell^{R} fish food (approximately 0.2 g) that was consumed within 2 hr.

Mirex was introduced into the tanks by air-lift columns (P.W. Borthwick, unpublished data, Gulf Breeze Environmental Research Laboratory, Gulf Breeze). Air introduced into the bottom of these columns swept water through the compartments containing the mirex bait, flushing the leached mirex into the aquarium. The columns were constructed to contain 0.63 g of mirex bait (0.3% mirex, 15% soybean oil adsorbed on 84.7% corncob grit).

Grass shrimp collected for analysis were dissected under a microscope; the hepatopancreas, exoskeleton, muscle, and head of three shrimp combined to make one sample (control and exposed). The plant samples consisted of waxy, green leaves and detrital part of leaves. Extractions of mirex was by the method of SCHOOR (1973).

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[2]Mention of commercial products or trade names does not constitute endorsement by the U.S. Environmental Protection Agency.

Samples of wet sand (control and exposed) were extracted in a separatory funnel three times with 100 ml each of hexane, and final concentrations of mirex were based on the air-dry weight of the sand. All samples were reduced to appropriate volumes under a gentle stream of air and analyzed without clean up. The recovery of mirex from all components analyzed ranged from 90 to 100%. The individual mirex concentrations in the components of two replicate tanks were averaged and the significant figures (two) determined by the analytical error which was 10% maximally.

A Hewlett-Packard Model 5700 gas chromatograph with a ^{63}Ni electron-capture detector was used for quantitative determination of mirex. An OV-101 column (2% OV-101 on Gas Chrom Q, 100-120 mesh) was operated at 195°C with the detector at 300°C and the argon/methane (10:1) carrier gas at a flow-rate of 60 ml/min. Since the behavior of this detector was non-linear at low concentrations of mirex, a standard curve was prepared for compensation. The limits for quantitation of mirex were preselected at: (a) 2.0-ml-minimum reduced-extract volume; (b) 5.0-µl injected volume and; (c) 20-mm-minimum peak height. An injection of 5 µl of 5 pg/µl mirex resulted in a peak height of 100 mm at the most sensitive electrometer setting (xl). Under these conditions, a 1-g sample would have a detection limit of 2 µg/kg; a 1-L water sample, 0.003 µg/L.

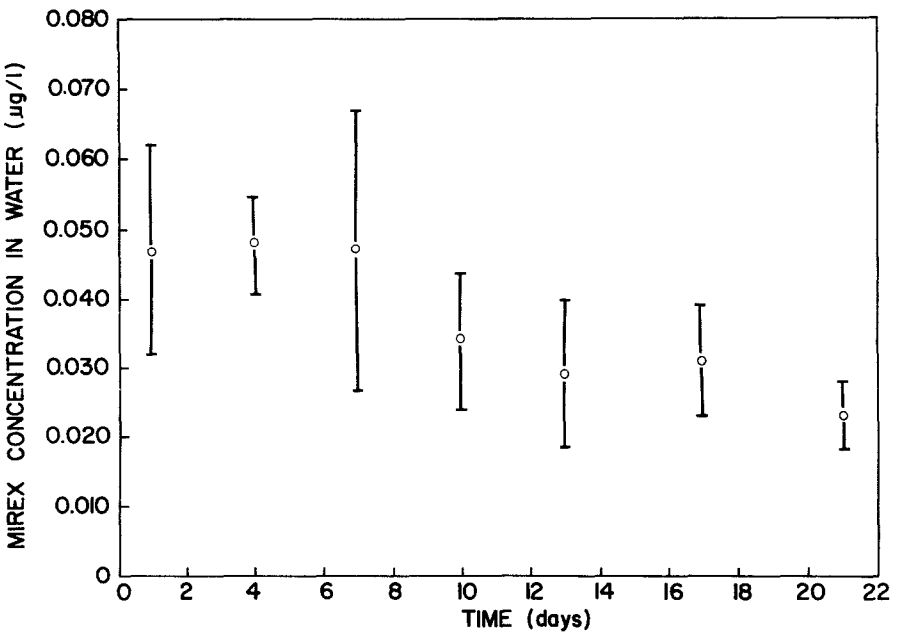

Figure 1. Average mirex concentration and SD's in water of eight similarly conducted tests.

RESULTS and DISCUSSION

The data in Figure 1 show the range of mirex residues that can be expected in seawater when using the described delivery system; the averages and SD's of eight similarly conducted tests are shown. The amounts of mirex taken up by both systems are shown in Table I. No mirex residues were found in any control samples. Since the actual exposure concentrations were not the same for both systems, the values in parentheses were calculated from hypothetically equal water concentrations of mirex in both systems. The minor adjustment helped to clarify the trends shown in the table. The mirex levels in the water include the mirex adsorbed to algae and other particulates. The actual concentration of mirex in the water was determined by centrifugation (SCHOOR 1975) at 250,000 x g; 0.003 µg/L of mirex remained in the water. When a 1-L water sample that contained 0.003 µ/L of mirex was filtered through a 450-nm filter, all of the mirex was removed from the water. The mirex was most likely adsorbed to the filter as occurred with the polychlorinated biphenyls (SCHOOR 1975).

The hepatopancreas was the only living component in which the concentration of mirex increased during the testing period. This phenomenon is not reflected in the whole-body residues, since the amount of mirex in the hepatopancreas is too small to alter whole-body residues. However, based on the weight of hepatopancreas, the concentration becomes rather large. Therefore, regarding toxicological effects on the organisms, accumulation of mirex in specific tissue should be emphasized rather than whole-body residues, which are more appropriate for trophic level effects. The residues of mirex found on the exoskeleton cannot be attributed solely to adsorption on the chitin; the exoskeleton of the shrimp in the "shrimp-only" system would have had to contain at least the same amounts as the system with the plants and sand. The residues in the "head" represent a combination of material adsorbed and present in the gills and the brain. The low and constant amounts detected in the turtle grass were likely due to adsorption. The amounts found on the sand are surprisingly low; however, there appears to be a linear increase in the amount adsorbed, suggesting the possibility that sand is a reservoir.

Table II represents the amounts of mirex contained in the various compartments of the two systems. The residues in the shrimp are about the same for both systems, at least after the first 7 days exposure. Before that time, however, shrimp in the experimental ecosystem accumulated mirex more quickly than those exposed to mirex water only. Possible reasons for the leveling off of the residues (except hepatopancreas) after seven days of exposure could be (1) metabolism of mirex; (2) no further uptake (some quasi-equilibrium); or (3) excretion at a controlled rate. There is, however, little precedent for metabolism of mirex to any great degree, and a quasi-equilibrium between shrimp and water seems unlikely because of continued input. Since the shrimp continue to take up mirex in the hepatopancreas, tissues like muscle or exoskeleton may have specific binding sites that, once filled,

TABLE I

Concentrations of mirex in components of two experimental systems.

Exposure (days)	System[b]	Mirex concentration[a] (µg/kg) Shrimp: Hepato-pancreas	Shrimp: Exo-skeleton	Shrimp: Muscle	Shrimp: Head	Shrimp: Whole-body residues	Thalassia: Detritus	Thalassia: Leaves	Water
1	C	280	120	40	110	100	17	8	0.12
	S	50(70)[c]	40(60)	20(30)	80(110)	40(50)			0.88(0.12)
4	C	370	200	90	200	160	28	18	0.057
	S	240(140)	100(60)	30(20)	100(60)	100(60)			0.10(0.057)
7	C	360	320	180	280	260	27	17	0.031
	S	580(250)	300(130)	120(50)	270(120)	230(100)			0.073(0.031)
10	C	500	180	60	130	140	31	13	0.030
	S	740(380)	230(120)	260(60)	260(140)	220(110)			0.058(0.030)
13	C	650	150	80	120	130	29	15	0.018
	S	880(510)	210(120)	140(80)	150(90)	200(120)			0.031(0.018)

[a]Values are averages of two replicate tanks.
[b]C = Complete system (Shrimp, Thalassia, sand);
S = Shrimp-only system
[c]Values in parentheses are mirex concentrations from hypothetically equal water concentrations of mirex in both systems.

TABLE II

Amounts of mirex in various compartments of two experimental systems.

Exposure (days)	Amounts of mirex (µg)[a]						
	Complete System					Shrimp-only system	
	Shrimp	Thalassia	Sand	Total	Water	Shrimp	Water
1	1.0	1.4	8.4	11	20	0.4	15
4	2.0	2.4	18	22	9.1	1.1	16
7	2.6	2.2	14	19	4.9	2.0	12
10	1.6	2.3	31	35	4.7	2.6	9.2
13	2.1	1.7	43	47	2.9	2.5	5.0

[a]Values are averages of two replicate tanks and represent the amounts of mirex remaining in each component.

excrete any excess (SCHOOR 1973). This explanation could also hold for the observations of TAGATZ et al. (1975) who found whole-body residues in Palaemonetes pugio to range from 90 to 320 µg/kg under almost identical physical conditions after a 28-day exposure to average concentrations of mirex of 0.04 µg/L.

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