

# **A Gravity-Flow Column to Provide Pesticide-Laden Water for Aquatic Bioassays**

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Traditionally, chemicals having a low solubility in water have been dissolved in water miscible solvents (e.g. acetone, ethanol, polyethylene glycol) before introduction into bioassay water. These solvents act as carriers or dispersants, and allow exposure of test animals to relatively high concentrations of the toxicant. Toxicity of the solvent must be considered as well as the toxic test chemical and solvent. Concentration of solvent should be several orders of magnitude less than that which is toxic to the test animal (PARRISH, personal communication).

It is often desirable to avoid the use of a solvent, and a pesticide typically applied as a dust, wettable powder, granule, or bait could be tested as formulated using the method described here.

A column containing granular pesticide, bait, or inert material coated with pesticide may be utilized to achieve realistic concentrations of pesticides in assay water without using a solvent. Column systems have produced good results in several pesticide bioassay experiments. CHADWICK and KIIGEMAGI (1968) showed that water from a glass column packed with sand coated with technical dieldrin contained fairly constant amounts of the toxicant for a period of five months after an initial leaching period. GRAJECER (1968), used a similar elution column that contained gravel charged with endrin. JOHNSON (1967) utilized a glass elution column containing endrin coated sand to provide concentrated stock solution to a special serial-dilution apparatus. Our report shows that mirex can be introduced into flow-through aquatic bioassay systems without a solvent by means of a gravity-flow column containing mirex bait.

## Materials and Methods

Mirex is a chlorinated hydrocarbon insecticide formulated in bait which consists of corn cob grits (84.7 percent) impregnated with soybean oil (15.0 percent) containing mirex (0.3 percent).

Columns (FIGURE 1) were designed to hold three layers of mirex bait. An outer glass tube (50 cm length x 100 mm O.D.) with

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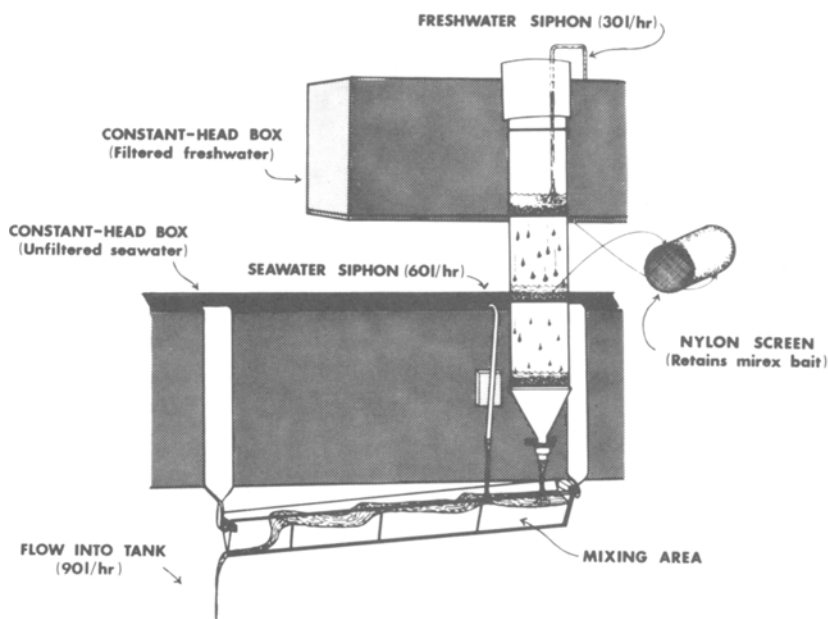


FIGURE 1. Gravity-flow Column

a glass powder funnel cemented to the bottom served as a holder for three inner glass tubes (15 cm length x 90 mm O.D.). Nylon monofilament screen (0.84 mm mesh) cemented to the bottom of each inner tube retained the bait.

The bait was soaked for 24 hours in fresh water to allow swelling before being placed in the columns. Filtered tap water siphoned from a constant head box was percolated through three layers of mirex bait (150 grams total). During a 4 to 8-day conditioning period, column effluent bypassed tanks to avoid introduction of excessive amounts of mirex into the bioassay system. Mirex concentrations in column effluents, initially high, diminished and became more consistent after this conditioning period.

Each of three tanks received filtered tap water that had passed through 150 grams of mirex bait. Three tanks received effluent from columns that contained 150 grams of control bait (with all components except insecticide). To simulate mixing in an estuary, column effluent (30 liters/hour) and unfiltered seawater (60 liters/hour) were mixed in a V-shaped trough that

emptied into each tank (FIGURE 1).

Biweekly water samples were taken directly from the tanks and extracted with petroleum ether. Extracts were dried with anhydrous sodium sulfate and evaporated to an appropriate volume for identification and measurement by electron-capture gas chromatography. The limit of detection for mirex in water samples was 0.010 parts per billion (ppb, micrograms/liter).

Range, mean, and standard error values for each experiment are shown in Tables 1 to 3. Randomized block analysis of variance and the Newman-Keuls range test (HICKS, 1973) were used to detect significant differences in mirex concentrations among tanks and experiments. Linear regression analysis was utilized to detect significant variation within individual tanks during each 28-day experiment.

### Results and Discussion

In May 1973, a 28-day flowing-seawater experiment was completed in six 2.44 m - diameter fiberglass tanks. Mirex residues (Table 1) in treated tank water varied between <0.010 and 0.125 ppb over the 28-day period.

TABLE 1

Mirex concentration (parts per billion) in tank water during first 28-day simulated estuary experiment, April-May 1973 ( n = 9 for each tank).

TANK	1	2	3
Range	<0.010-0.091	<0.010-0.110	0.014-0.125
Mean	0.03	0.04	0.04
Standard error	0.01	0.01	0.01
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Tank water temperature	23.1° C (range: 19.3 to 25.2)		
Salinity	13.2‰ (range: 10 to 18)		
Conditioning period	8 days		

A second 28-day experiment was conducted in July-August 1973. Concentrations of the pesticide in treated tanks ranged between 0.032 and 0.52  $\mu\text{g}/\ell$ . Data in Table 2 indicate that the columns delivered higher concentrations of mirex than in the first experiment.

TABLE 2

Mirex concentration (parts per billion) in tank water during second 28-day experiment, July-August 1973 (n = 9 for each tank).

TANK	1	2	3
Range	0.032-0.36	0.043-0.23	0.053-0.52
Mean	0.10	0.10	0.16
Standard error	0.03	0.02	0.05
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Tank water temperature	29.8°C (range: 28.0 to 30.8)		
Salinity	15.7 ‰ (range: 14 to 18)		
Conditioning period	4 days		

Although mirex concentrations in water samples fluctuated, residues differed by less than one order of magnitude.

In October-November 1973, a third experiment was conducted (Table 3). Mirex residues (0.013 to 0.23  $\mu\text{g}/\ell$ ) were somewhat lower than for the summer experiment.

TABLE 3

Mirex concentration (parts per billion) in tank water during third 28-day simulated estuary experiment, October-November, 1973 (n = 9 for each tank).

TANK	1	2	3
Range	0.029-0.20	0.029-0.23	0.013-0.12
Mean	0.07	0.06	0.04
Standard error	0.02	0.02	0.01
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Tank water temperature	23.4°C (range: 17.0 to 27.0)		
Salinity	17.5 ‰ (range: 15 to 19)		
Conditioning period	6 days		

## CONCLUSIONS

Concentrations of mirex among individual tanks in each test were not statistically different at the 5-percent significance level; whereas, differences in mirex concentrations in tank water among experiments were significant. Paired comparisons indicated statistical differences between the first and second, and the second and third experiments, but not between the first and third experiment. These differences in mean mirex concentrations in tank water may have been caused by seasonal variations in water temperature. Fluctuations in the mirex concentrations within individual tanks were not significant.

In its present state of development, the described gravity-flow column is being utilized in seasonal tests to deliver mirex-laden water to determine toxicity and uptake of mirex by several animal species in an artificial estuarine ecosystem.

## REFERENCES

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