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Use of the Freshwater Clam *Corbicula manilensis* as a Monitor for Organochlorine Pesticides

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PHILLIPS (1978) has suggested the following criteria for a good in situ monitor of aquatic pollutants: 1) the organism should bioaccumulate environmental pollutants without mortality, 2) the organism should be sedentary to reflect conditions in the geographical area being studied, 3) the organism should be numerous, 4) the life span of the organism should be at least one year to enable it to measure seasonal variations in its environment, 5) the organism should be large enough to conveniently provide tissue for analysis, 6) the organism should be easily collected and relatively hardy. Bivalves meet these criteria and naturally occurring populations of these organisms have been widely used as indicators of overall water quality.

The Mussel Watch and the Pesticide Monitoring Program (BUTLER 1973, GOLDBERG 1975, GOLDBERG et al. 1978) have exploited bivalves' ability to concentrate several specific groups of pollutants including petroleum hydrocarbons (ANDERLINI et al. 1981, BOEHM + QUINN 1977), pesticides (BUTLER 1973, 1978), organic industrial wastes (BJORSETH et al. 1979), polychlorinated biphenyls (CALAMBOKIDIS et al. 1979), heavy metals (PHILLIPS 1977, 1978a) and radionuclides (NELSON 1962, SEYMOUR + NELSON 1977). The concentrations of these substances in bivalves reflect the average environmental levels but are conveniently measured because of bioconcentration.

Corbicula as a Monitor of Freshwater Quality. Among the bivalve species in American freshwaters is the Asiatic clam, Corbicula manilensis (MORTON 1979). First introduced four decades ago on the west coast, Corbicula has spread to the east coast, south to the Gulf of Mexico and as far north as Lake Erie. Its success is attributed to its pollution tolerance, its free-living larval form, its early maturation and its monoecious life cycle (BRITTON + MORTON 1979, SINCLAIR + ISOM 1963). Corbicula grows to three by four centimeters in size and has been reported as Corbicula manilensis, Corbicula leana (Prime), and Corbicula fluminea (Muller), all of which are probably the same species (BRITTON + MORTON 1979).

Corbicula offers several advantages as an organism for toxicity testing. It tolerates removal from water for reasonably long periods of time (BURRESS + CHANDLER 1976). It can easily be handled in the laboratory and it survives in aquaria without food

for periods of up to six months. Corbicula has already found limited use in toxicity testing (BURRESS + CHANDLER 1976) and has been suggested as a potential monitor of industrial effluent (FOSTER + BATES 1978).

In this study we have assayed the bioaccumulation of organochlorine pesticides by Corbicula taken from natural beds, incubated in an aquarium to permit self-cleaning (depuration) and temporarily implanted in cages in a river. Our intent was to explore the use of temporarily implanted Corbicula for the measurement of persistent pollutants in freshwater environments.

MATERIALS AND METHODS

A ponar dredge was used to collect Corbicula from in front of the cooling water intake of the coal-fired power plant on Lake Sangchris, Illinois. The clams were separated into different size classes, put in styrofoam coolers and transported to the laboratory. Depuration took place at 10°C in a 114-L aquarium fitted with an activated charcoal power filter; aquarium water was exchanged for tap water weekly over a 4-week period.

Rectangular cages were built of rolled aluminum 1.27-cm expanded mesh, having final dimensions of approximately 25.4 cm x 25.4 cm x 17.8 cm (WxLxD). Cages were anchored to the bottom of the Kaskaskia River near Tuscola, IL by a small mobile home anchor to which two nylon ropes had been attached. Each cage was attached to the ropes by self-closing spring clips. The cages were connected in two parallel rows of six cages. Six-hundred and sixty middle size clams, average weight 5.3 g, were weighed, measured and numbered on each valve. A vibrating tool for etching glass was used to mark the valves.

In each cage, 55 clams were placed in sand contained in a 20.3 cm x 20.3 cm aluminum baking pan. Before implantation it was determined that Corbicula were already present in the river and the necessary Illinois Department of Conservation permit was obtained. Duplicate cages were removed after 9, 18, 36, 60 and 72 days; only single cages were removed on days 2 and 4. A 3.8-L grab sample of river water was taken each time the cages were removed.

In the laboratory, the clams were weighed and measured. The soft tissues of 50 clams from each cage were removed, put in aluminum foil, and weighed. Tissue samples were stored at -70°C until analysis. Water samples were extracted immediately with one 50-mL and two 25-mL portions of 15% (v/v) methylene chloride in hexane. The extracts were stored in screw capped flasks over 10 g anhydrous sodium sulfate at -70°C.

Pesticides were extracted from clam tissues and water extracts using the protocol of the Illinois Environmental Protection Agency for pesticide residues in fish (HURLEY 1973). This is a minor modification of the ARMOUR + BURKE (1970) and PORTER

et al. (1970) methods. This method uses petroleum ether and acetonitrile extractions to recover pesticides and to separate them from triglycerides, florisil chromatography to remove phthalate esters and deactivated silica gel chromatography to separate PCBs from pesticides.

Both water and tissue extracts were analyzed in a Hewlett-Packard 5730A Gas Chromatograph equipped with a ^{63}Ni electron-capture detector and a 3380A integrator/plotter. The carrier gas (5% methane - 95% argon) flowed through a 130 cm - 0.31 cm i.d. silanized glass column packed with 1.5% SP-2250/1.95% SP-2401 on 100-120 supelcoport (Supelco, Inc.) at 30 ml/min at 190°C. When peak resolution decreased the column was repacked.

Peaks were identified and quantified by reference to the retention times and areas of standard pesticides on standard curves prepared daily. Quantification of unknowns was based only on peak areas that fell within the linear portion of the standard curve. Unknowns with identifiable retention times sometimes occurred as small shoulders on large peaks but could not be quantified by the plotter-integrator. These peaks are not reported. A value of zero is reported when no peak was detected at the correct retention time.

The biological concentration factors (BCF) (KAPOOR et al. 1973) were calculated by dividing the pesticide concentration in fat by its concentration in water, both in units of ng/g. The values in Table 1 are based on the highest tissue levels that occurred over the 72-day experiment.

TABLE 1. Bioaccumulation of Pesticides in Clam Fat

	Sample (day)	Tissue (ng/g)	Water (ng/L)	Biological concentration ng/g fat / ng/g water
γ-chlordane	60	1,610	372	4,330
α-chlordane	60	1,520	316	4,810
Aldrin	60	830	NF[1]	13,390[2]
Dieldrin	60	3,150	891	3,540
Lindane	60	773	296	2,610
BHC	18	1,320	590	2,240
Heptachlor	60	510	NF[1]	10,630[2]
Heptachlor epoxide	60	2,050	880	2,330

1. Not found
2. This value was calculated by dividing the pesticide concentration in fat by the analytical detection limit. The detection limit for aldrin was 62 ng/L; for heptachlor it was 49 ng/L.

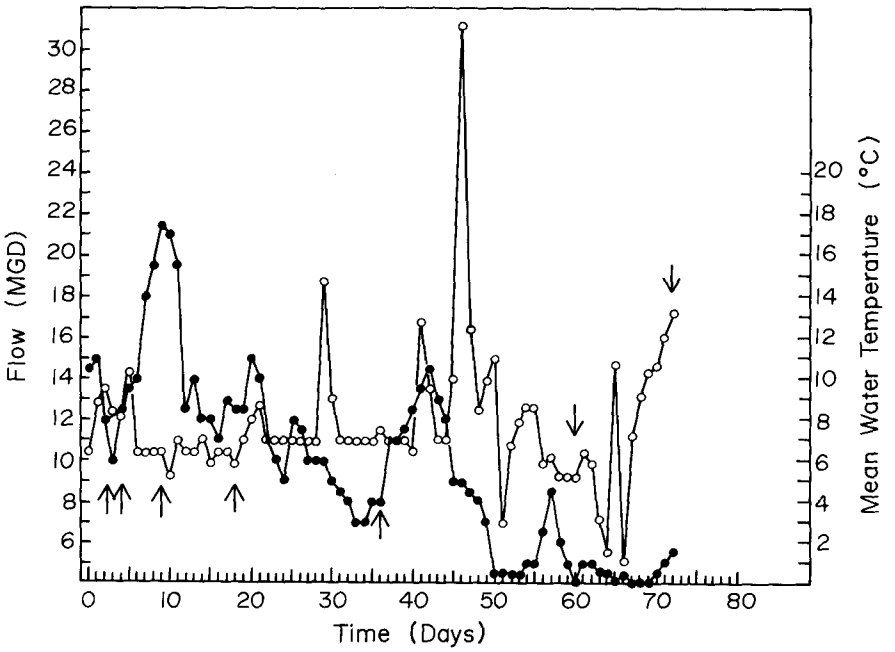

Figure 1. Twenty-four hour mean water flow (o) and water temperature (•) during the experiment. Day zero was October 11, 1979. The arrows indicate days on which clam cages were recovered from the river.

Biological concentration factors ranged from 2,240 to 13,390 (Table 1). The BCF of related compounds (heptachlor and heptachlor epoxide or aldrin and dieldrin) differed, the more polar epoxides being bioconcentrated to a lesser degree than the parent compounds. There was remarkably little apparent fluctuation in the concentration of pesticides in water, the exception being the peak in lindane and BHC seen on day 4 (Figure 2). This was attributed to the constant flow of the river on the days that the grab water samples were taken (Figure 1). The Kaskaskia River above the implantation site drains an agricultural watershed, and pesticide contamination comes primarily from runoff and soil leaching.

The peaks of lindane and BHC on day 4 did not cause discernable corresponding peaks in the clam fat. Pesticide accumulation by bivalves is a dynamic process that balances uptake from water and release from fat. The rate of each of these processes is affected by the concentration of pesticides in water and in fat, and probably follows simple first-order kinetics of uptake and of loss. Presumably, a net flux of zero i.e., an equal uptake and release, is obtained if the water concentration remains constant for a sufficient period of time. Short episodes of high pesticide

RESULTS AND DISCUSSION

Implanted Clams. The clams exhibited good health by increases in their average length and width during the first two weeks. The average width increased 0.030 cm; the length increased 0.024 cm. After the first two weeks, the length and width did not increase further. The mean weight increased 0.070 g above the initial weight during the first 36 days and did not change thereafter. The changes in shell dimension were completed by day 18, in contrast to weight gain.

Increases in the average clam weight were expected. The average change in fat per g of soft tissue showed some unexpected variations. This value increased quickly from 25.3 mg to 28.4 mg during the first 9 days after implantation and then showed a slower rate of increase reaching 29.5 mg on the 36th day. The rapid initial gain was expected since the animals had been starved for four weeks during the laboratory depuration. After day 36, the amount of clam fat per g of soft tissue dropped to 26.4 mg. This loss of fat was not accompanied by an overall weight loss but did correspond to a period of severe low water temperatures, Figure 1. In fact the mean temperature for the 12 days between the last two samples was 0.6°C and the lowest mean water temperatures of the experiment were recorded during this period.

Overall, the initial changes in dimensions, total weight and fat content were expected of starved clams placed into an environment in which they could thrive. The drop in fat per gram of soft tissue during the 12 days of very cold water temperatures suggests a survival metabolism of energy reserves; alternatively it might be accounted for by a release of larvae, as has been noted previously in populations of Corbicula in Lake Sangchris during this time of year (LARIMORE + TRANQUILLI 1977). (The lipid content of most bivalves will increase before spawning and will decline during and after spawning (PHILLIPS 1978b).) Changes in the fat content are important since they should influence the amount of lipophilic pesticides bioconcentrated by a clam.

Pesticide Analysis. Eight pesticides, aldrin, dieldrin, hexachlorocyclohexane (δ-BHC), lindane, α-chlordane, γ-chlordane, heptachlor and heptachlor epoxide, were bioconcentrated in clam fat. Figure 2 shows typical results. All of the pesticides showed similar features in their pattern of uptake. Pesticide concentrations were either constant or decreased slightly during the first nine days. Between day 9 and day 18 there was a pronounced uptake which was followed by a period of relatively stable pesticide concentrations in most cases. Pesticides that did not show stable concentrations after day 18 were aldrin and dieldrin, which continued to increase, and BHC, which decreased. The concentrations of all of the pesticides except lindane and heptachlor epoxide fell during the final sampling interval, the period noted earlier for fat loss and very low water temperatures.

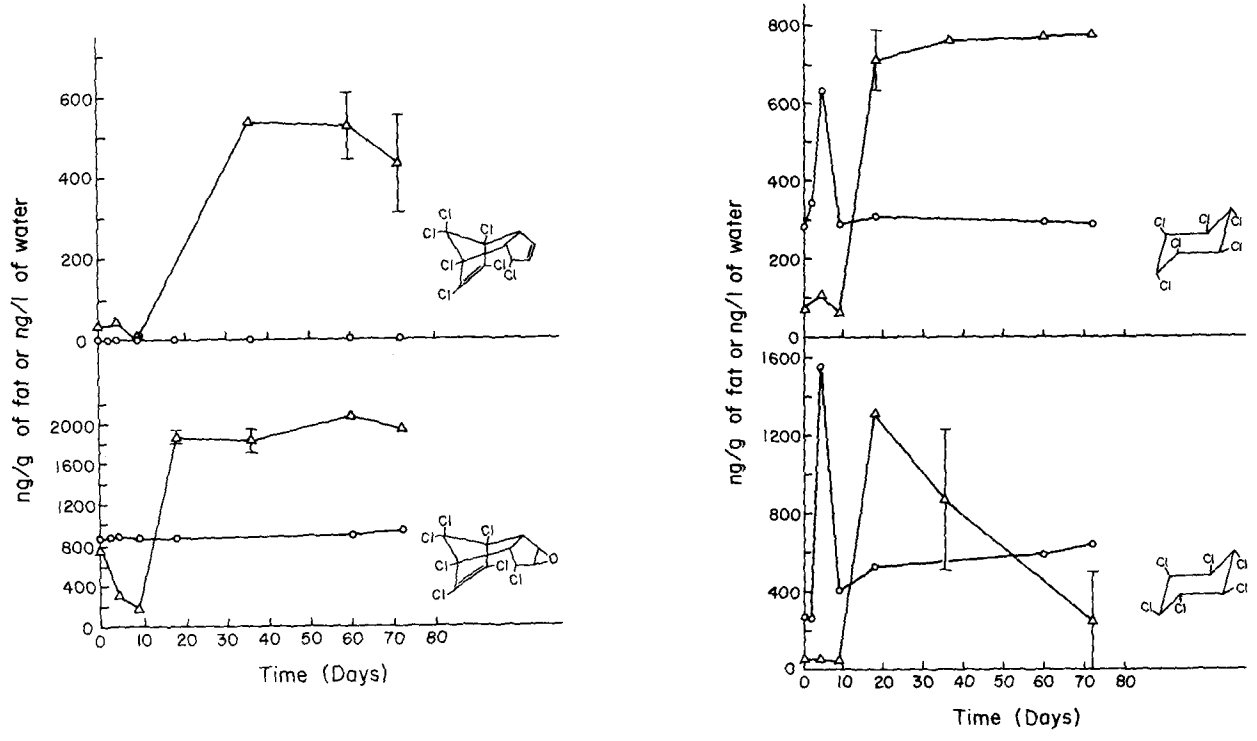

Figure 2. The concentrations of heptachlor (top left), heptachlor epoxide (bottom left), lindane (top right) and benzene hexachloride (bottom right) in water (o) and in clam fat (Δ) are shown versus duration of implantation in the river. Bars show the pesticide concentrations in duplicate cages.

concentration such as that on day 4, may not induce discernable increases of the pesticide in fat if the exposure period is brief.

From the pattern of pesticide uptake for the pesticides whose water concentrations seemed invariant (γ-chlordane, dieldrin, heptachlor epoxide), a period of at least 18 days was required before pesticides in the clam fat came to equilibrium with the pesticide in water. Similar kinetics were seen in a study using the freshwater clam, Amblema plicata, in a flow-through chamber (FIKES + TUBB 1972). This clam biomagnified dieldrin 2,700-fold from a 20 ppt solution after a two-week exposure. From our study it was not possible to tell whether the 18-day period was needed to achieve a balance between uptake and release, or whether it was primarily needed for stressed clams to regain a healthy nutritional state. The latter interpretation is suggested because all of the pesticides were accumulated after the initial burst of fat deposition and simultaneously with weight gain. Fat deposition and pesticide uptake should be coincident instead of sequential if uptake was merely a physical process independent of the organism's nutritional state. The minimum period for the fat of a healthy clam to reflect the levels of pesticide in its environment may therefore be less than 18 days.

We have demonstrated the feasibility of temporarily implanting Corbicula manilensis in cages in an aquatic environment and determining pesticide biomagnification by this bivalve. This is a first step toward using caged Corbicula to monitor persistent, lipophilic substances in water. Further study of the kinetics, level and reproducibility of ecological magnification, or the influence of nutritional state and of the kinds of compounds accumulated by Corbicula will advance the development of this monitoring method.

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