Accumulation of Methoxychlor by Microorganisms Isolated from Aqueous Systems

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INTRODUCTION

Sorption of pesticides to microorganisms affects the distribution of these compounds within an aquatic system. The organisms can be consumed, and the sorbed pesticide can then move up the food chain or the organisms can die and become part of the sediment. The pesticides, then sediment bound, can be recycled to overlying waters through fall and spring inversions or through release from the sediment. They can also be anaerobically degraded within the sediment (MacRAE, 1967).

Accumulation of methoxychlor by bacteria has been studied (JOHNSON and KENNEDY, 1973) but to our knowledge accumulation by a variety of classes of microorganisms under similar conditions has not been investigated. Therefore, studies were undertaken to investigate the accumulation and desorption of methoxychlor by three types of microorganisms--bacteria, algae, and fungi--in aqueous solution.

MATERIALS AND METHODS

TEST MEDIUM

A saturated solution of methoxychlor was prepared by stirring the insecticide into basal salts medium (PAYNE and FEISAL, 1963) and sterilized by filtering through a sterile 0.22-micron Millipore filter. Dup-

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licate pesticide solutions with varying concentrations were prepared by aseptically diluting the filtrate with sterile basal salts medium.

MICROORGANISMS

The bacteria and fungi used in our studies were isolated from natural aquatic systems. *Flavobacterium harrisonii* was taken from a stream containing the effluent of a citrus processing plant, and *Bacillus subtilis* and *Aspergillus* sp. were taken from a stream containing the effluent of a chicken processing plant. The alga, *Chlorella pyrenoidosa* 395, was from the Starr collection, University of Indiana.

PROCEDURES

Inocula for the sorption studies were cultured as follows:

- Bacteria were incubated for 24 hours at 28°C in nutrient broth.
- Fungi were incubated for 72 hours at 28°C in basal salts medium containing glucose.
- Algae were incubated under continuous light (170 ft-c) for 200 hours at 15°C in Bensen and Fuller medium containing Hutner's trace elements (HUTNER, 1950).

Bacteria and algae were harvested by centrifugation and washed three times prior to inoculation in test medium. Liquid cultures of fungi were decanted and the liquid was replaced with basal salts medium containing the desired concentration of methoxychlor.

Dry weights of bacteria and algae were determined by transferring washed cells to tared beakers and drying in an oven at 90°C. Dry weights of fungi were determined by filtering the culture, first through tared prefilters, then through tared 0.22-micron

Nucleopore filters, and drying to a constant weight at 90°C.

The sorptive properties of the microorganisms were determined by incubating the microorganisms in basal salts medium with varying concentrations of methoxychlor (0.008-0.05 ppm) on a gyratory shaker at 28°C. High organism concentrations, ranging from 100 to 1000 mg (dry weight) per liter, were used to produce detectable amounts of sorption. The cultures of bacteria and algae were centrifuged at each sampling time and the pesticide remaining in the supernatant was measured by gas liquid chromatographic analysis of the organic solvent extract. The fungal cultures were removed from the shaker and allowed to settle for one minute, and samples of the supernatant in the flask were analyzed in the same way.

Supernatants of the cultures were extracted with 2,2,4-trimethylpentane (isooctane) and analyzed using a Tracor MT-220 gas liquid chromatograph equipped with a high temperature Nickel-63 electron capture detector. A one-meter glass column (4 mm ID) containing 80/100 mesh Gas Chrom Q with 3% silicone SE-30 liquid phase was used. Temperature settings were as follows: column, 210°C; detector, 240°C; and inlet, 195°C. Nitrogen carrier gas flow was 120 ml/minute.

No methoxychlor degradation products were detected using the described glc analysis. Hexane extracts of culture media were also analyzed for products by thin-layer chromatography using the procedures of KAPOOR *et al.* (1970). No products were detected during the experiments.

RESULTS AND DISCUSSION

Methoxychlor concentration decreased rapidly in the supernates of the bacterial and algal cultures,

reaching equilibrium within 30 minutes. The amount sorbed remained the same, within experimental error, at the 24-hour sampling time. Equilibrium was reached within 16 hours in the fungal system.

The time required for the systems to reach equilibrium was dependent upon the concentration of the pesticide and the concentration of the microorganisms. Equation (1) was used to describe this relationship

$$\frac{dS}{dt} = k_e MS \qquad (1)$$

where S is the concentration of the pesticide in water (mg/ℓ), M is the concentration of the microorganisms (mg/ℓ), and k_e is the rate constant (liter mg^{-1} hr^{-1}) for the equilibration of the system.

We were unable to gather sufficient data points to determine k_e because of the short equilibration time in the bacterial and algal systems. The processes of sampling, centrifuging, and extracting required a minimum of 10 minutes. Therefore, only a minimum value of k_e for each system was determined by substituting experimental values in equation (1). The minimum values ranged from 10^{-4} for *Aspergillus* sp. to 10^{-2} for *B. subtilis* and *C. pyrenoidosa* (TABLE 1).

The fungi formed small clumps while growing. Observed equilibration times were the same for clumps of 1 mm to 5 mm in diameter.

To determine if sorption of methoxychlor was mediated by a metabolic process, the insecticide was added to autoclaved cultures of bacteria, fungi, and algae. These cells sorbed as much as or a little more pesticide than the viable cells, indicating that it was not the result of a metabolically active process. Other researchers (KO and LOCKWOOD, 1968; and JOHNSON and KENNEDY, 1973) have reported similar findings for

bacteria and fungi in the presence of methoxychlor, DDT, and dieldrin.

Sorption of methoxychlor to microorganisms may be represented by the empirically derived equation of Freundlich (POINKE and CHESTER, 1973)

$$\frac{x}{m} = kc_e^{1/n} \qquad (2)$$

where x is the amount (mg) of pesticide sorbed to the microorganisms; m is the dry weight (mg) of the organisms; c_e is the concentration (mg/ℓ) of pesticide in the medium at equilibrium; and k and 1/n are constants. The constant 1/n was determined from the slope of log-log plots of x/m as a function of c_e. Since in our systems 1/n was near unity, the equation may be simplified to

$$k = \frac{x/m}{c_e} \qquad (3)$$

The slopes of the arithmetic plots of x/m versus c_e yield the values of k. The arithmetic plots (Figure 1) and values of 1/n and k (TABLE 1) were obtained using the least squares statistical computer program, MLAB, developed at the National Institutes of Health (KNOTT and REECE, 1972).

In systems in which 1/n is close to unity, k corresponds to the distribution coefficient, K_d, with a correction factor of 10^6 to account for the different units used to obtain k. K_d is a ratio of the amount of pesticide sorbed to the microorganisms, in mg/mg, to the concentration of pesticide in water, in mg/mg; k however is calculated using units of mg/ℓ for the concentration of pesticide in water. Distribution coefficients serve as useful indices for comparing the degree of sorption by various classes of microorganisms.

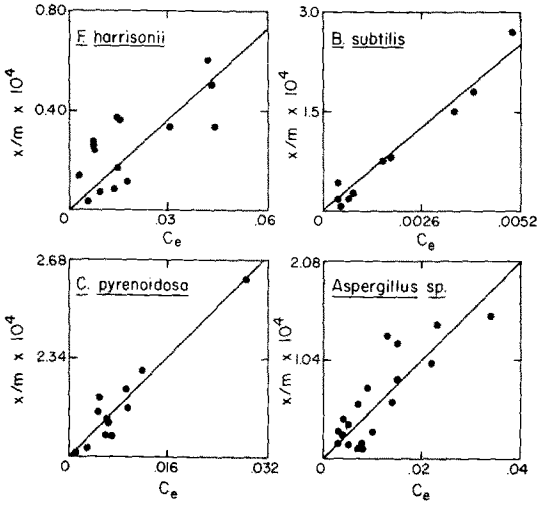

Figure 1. Sorption of methoxychlor by bacteria, algae, and fungi.

TABLE 1
Values for Sorption of Methoxychlor

Organism	k_e (Minimum) (liter mg^{-1} hr^{-1})*	$\frac{1}{n}$	k (liter mg^{-1})**
B. subtilis	$(2.4 \pm 1.8) \times 10^{-2}$	1.2	0.048 ± 0.0022
F. harrisonii	$(4.2 \pm 4.0) \times 10^{-3}$	0.81	0.0012 ± 0.00015
Aspergillus sp.	$(1.3 \pm 0.75) \times 10^{-4}$	0.91	0.0052 ± 0.00043
C. pyrenoidosa	$(1.2 \pm 1.0) \times 10^{-2}$	0.99	0.0084 ± 0.00052

*mg = mg organisms
**mg = mg methoxychlor

The values of K_d for the four organisms are all within an order of magnitude. VANCE and DRUMMOND (1969), KING *et al.* (1969), and JOHNSON and KENNEDY (1973), in their sorption studies with algae and bacteria using dieldrin, endrin, DDT, aldrin, methoxychlor, and lindane, found K_d values consistent with our data.

One explanation for sorption has been postulated. WARE and ROAN (1970), in their review of interactions of pesticides with aquatic microorganisms, suggested that lipid soluble pesticides, like methoxychlor, would sorb more strongly to surfaces containing relatively larger amounts of lipid material. SHIN *et al.* (1970), on the other hand, studied adsorption of DDT by various soil fractions. In their investigations, treating the soil with ether and ethanol for removal of lipoidal materials increased the adsorption of DDT to the soil, suggesting that other components played a larger role in the sorption than the lipoidal materials.

Also, if a microcapsule surrounds the cells, it would affect the sorption of the pesticide. At the present time, therefore, no clear explanation of the factors that control microbial sorption of non-ionic pesticides exists. Studies with various fractions of the cell walls of microorganisms, similar to those of SHIN *et al.* with soil fractions, would help to clarify the role of the various components (*e.g.*, proteins, lipids) in the sorption process.

Microorganisms that have sorbed pesticides can release or desorb these compounds as the organisms move to aqueous environments containing little or no pesticide. The pesticides are then redistributed in the system. To determine whether or not the organisms desorb methoxychlor, bacteria and fungi that had reached equilibrium in media containing methoxychlor were harvested and immersed in media containing no

pesticide. The supernatant was sampled and analyzed for methoxychlor. Equilibrium was achieved within the same period of time as in the sorption experiments and the distribution coefficients were the same. Our findings agree with those of HANCE (1969), who reported that soil systems in which desorption equilibrium was achieved in less than 24 hours were those in which the Freundlich constant, 1/n, was approximately one.

To test our distribution coefficients for methoxychlor on microorganisms in natural water, a water sample (pH 6.9) was collected from a river near High Shoals, Georgia, and centrifuged; half of the supernatant was then replaced with distilled water containing methoxychlor. The resulting concentration of methoxychlor in the system was 0.008 ppm. Algae (*Scenedesmus* sp. and *Chlorella* sp.), protozoa, and bacteria were present, as determined by direct microscopic examination. No fungi were observed. The system equilibrated within 45 minutes and the value of k calculated from equation (3) was 0.0037. This value falls within the range of values found for the four microorganisms in laboratory media. Although only one field site was tested it seems to indicate that laboratory data can give a fair first approximation of the sorption by a mixed natural population. More field investigations are needed before definite conclusions can be drawn.

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