Diatom Diversity as a Function of Insecticidal Treatment with a Controlled-Release Formulation of Chlorpyrifos

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An abundance of information exists on the effects of various chemical complexes, including pesticides, upon the organisms which comprise the aquatic microflora, yet relatively little data have been developed on the effects of long-term exposure of these organisms to pesticides. Most of the literature has dealt with the immediate, often transitory effects of pesticides, following a fish kill or similar occurrence, and bioassays utilizing aquatic organisms are based on the establishment of LD or TL_m values at the end of a predetermined number of hours.

The work reported here was initiated to examine the "in situ" effects of various levels of chlorpyrifos, originating from the application of a controlled-release formulation used as a mosquito larvicide, on one component of the aquatic microflora, viz. the Bacillariophyceae.

Materials and Methods

Tests were conducted at the University of Arkansas Rice Branch Experiment Station located 11.3 km east of Stuttgart, Arkansas. Fifteen test plots were prepared using standard cultural practices for rice cultivation with all plots being identical in size, shape, and water depth. Treatments were made with a chlorinated polyethylene pelletized formulation of 10.6 percent chlorpyrifos so as to obtain 0.25, 0.5, 1.0, and 2.0 ppm in water based on a theoretical total initial release of all active ingredient (mean residue over entire test period was 0.0013 ppm, range 0.0009 to 0.0022 ppm, recovered from test plot water treated at the highest level, 2.0 ppm). Randomly selected treatments were replicated three times and three untreated plots served as controls.

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Bulletin of Environmental Contamination & Toxicology, Vol. 15, No. 5 © 1976 by Springer-Verlag New York Inc.

Diatometers were constructed of two 45.7 cm long, 2.5 cm X 2.5 cm untreated wooden stakes separated by braces of the same 2.5 cm X 2.5 cm material attached to the top ends and near the bottom ends so that the stakes were 7.6 cm apart. The stakes were grooved at 2.5 cm intervals on opposing interior surfaces so as to receive and firmly hold 10 standard 2.5 cm X 7.6 cm microscope slides.

The diatometers were loaded with 10 slides and four diatometers were placed side by side facing the same direction in each test plot at the time of treatment. Each diatometer was carefully pushed into the mud so that the lowest slide rested immediately above the bottom of the pool and water covered the top-most slide. Two diatometers were removed from each test plot and slides analyzed at the end of 6 weeks post-treatment and the remaining two in each test plot were removed and slides analyzed upon termination of the study (12 weeks).

The sequential comparison index (CAIRNS et al., 1968) was the determinative method used to obtain diversity estimates of diatom populations in treated plots versus untreated controls. In this method, the samples containing a random assortment of diatoms are systematically analyzed such that one organism is compared only with the previously observed organism. If the diatoms are similar, as determined by actual identification or according to similarity in shape and size, they, together, constitute a "run"; if they are different, each diatom constitutes a separate run. The greater the number of different species in the population, the greater the probability that the two organisms will be different and thus establish a new run; the result being that estimates of diversity are higher for those populations which have the greater number of runs.

Material containing the diatom frustules was washed from the microscope slides into 500 ml beakers. Extraneous organic matter was oxidized in an exhaust hood using potassium dichromate and 30 percent hydrogen peroxide. The reaction was allowed to continue to completion and 200 ml of distilled water was added. The samples were allowed to stand for 24 hours after which time the liquid was decanted and the remaining particulate matter resuspended. Two drops of the sample were placed on a coverslip, pre-heated on a slide warmer, and made to spread evenly over the coverslip by the addition of a few drops of glass distilled water. The slide warmer was then heated to 45°C and the samples were evaporated to dryness. The coverslips were then mounted on slides with Caedex® and allowed to set for 5 days.

Five transect lines were etched on the bottom of each slide with a diamond point stylus and under 500X magnification, 20 frustules were counted per transect, giving 100 counts per slide and 2000 counts per test plot (1000 per diatometer). The sequential comparison index (diversity) for each plot was calculated by dividing the number of runs by the total number of diatoms counted. The estimates of community diversity were then statistically analyzed to obtain indications of significant toxicity.

TABLE 1

Sequential comparison index (diversity) of diatoms following 6 and 12 weeks exposure to chlorpyrifos.

Treatment	0.25 ppm Plots*		0.50 ppm Plots*		1.0 ppm Plots*		2.0 ppm Plots*		Control Plots*	
Slide Number	Sample No.† 1	2	Sample No. 1	2	Sample No. 1	2	Sample No. 1	2	Sample No. 1	2
1	0.76	0	0.61	0	0.77	0	0.69	0	0.61	0
2	0.84	0	0.59	0	0.82	0	0.65	0	0.57	0.83
3	0.76	0	0.81	0	0.48	0	0.68	0	0.75	0.84
4	0.78	0	0.85	0	0.75	0	0.73	0	0.68	0.84
5	0.81	0	0.58	0	0.70	0	0.73	0	0.65	0.87
6	0.77	0.64	0.71	0	0.75	0	0.75	0	0.70	0.84
7	0.81	0.63	0.74	0.45	0.36	0	0.79	0	0.70	0.85
8	0.78	0.87	0.78	0.70	0.68	0.80	0.75	0	0.67	0.88
9	0.79	0.86	0.92	0.85	0.73	0.89	0.40	0	0.77	0.90
10	0.68	0.89	0.62	0.86	0.82	0.89	0.79	0.41	0.81	0.89
Mean Diversity	.778	.389	.721	.286	.686	.258	.696	.041	.691	.774
S_D	.042	.419	.119	.387	.148	.416	.113	.129	.072	.273
S^2	.0018	.1756	.0142	.1482	.0222	.1731	.0128	.0166	.0052	.0745

* Mean of 3 Test Plots.
† Sample 1 index determined at 6 weeks post-treatment; Sample 2 index determined at 12 weeks post-treatment.

Results and Discussion

Changes observed in the diversity of diatom populations have long been used as indicators of the presence of toxic substances (WILLIAMS, 1964; PATRICK, 1973). The feasibility of using "in situ" diatom populations, collected on glass slides and analyzed by the sequential comparison index method as a measure of water quality, has been clearly demonstrated (HOHN, 1959); the higher the diversity of a population, the better the water quality, or as in this study, higher diversity was interpreted to imply lower toxicity. Observations made during the course of this study tend to reinforce these previous findings.

Estimates of mean diversity between treatment levels following 6 weeks of exposure to the chlorpyrifos formulation did not vary substantially (Table 1). The highest diversity was observed in those plots treated at 0.25 ppm followed in decreasing order by those plots treated at 0.5 ppm, 2.0 ppm, the controls, and 1.0 ppm. A Student-t test comparing between-treatment diversity estimates indicated significant differences (.05 level) only between the 0.25 ppm treated plots and the untreated control plots. Conversely, the estimates of mean diversity following 12 weeks of exposure were highest in the control plots followed in decreasing order by those plots treated at 0.25 ppm, 0.5 ppm, 1.0 ppm, and 2.0 ppm. In all plots receiving treatment, estimates of diversity decreased during the 6-week to 12-week period. Within the control plots, however, the mean diversity increased from 0.691 at week 6 to 0.774 at week 12 (Table 1). A Student-t test to determine the presence of significance within a treatment level indicated that diversity estimates made at week 6 were all significantly different (.05 level) from those made at week 12 with the exception of the controls.

No increase in the degradation rate of chloropyrifos was observed over the 12-week period, nor did the diatom population have any noticeable effects upon the larvicidal activity of the insecticide. It is unknown whether or not the diatoms accumulated chlorpyrifos within the system or if any of the insecticide was physically adsorbed onto the frustule surfaces as is the tendency with DDT and certain other persistent insecticides (HOFFMAN and DROOZ, 1953). However; the small quantities of chlorpyrifos used for effective mosquito control (0.0015 ppm) coupled with the relatively rapid degradation rate of the organo-phosphates probably precludes an excessive accumulation within, or adsorption upon the diatom frustules.

Summary and Conclusions

Following treatment with a controlled-release formulation of chlorpyrifos, no substantial differences between diversity estimates were evident for 6 post-treatment weeks for both treated and control plots. By post-treatment week 12, significant decreases in diversity estimates occurred in treated plots, suggesting that a directly proportional relationship exists between

chlorpyrifos concentrations and reduced diatom colonization through time. Comparatively, diatom colonization progressed "normally" and populations proceeded toward "maturity" in the control plots as indicated by the increase in the diversity estimates between post-treatment weeks 6 and 12. Any adverse effects due to the insecticidal treatment, however, were not considered to be environmentally deleterious in light of the restricted type of habitat (rice culture) in which the chlorpyrifos was used.

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