The Effects of Mirex and Methoxychlor on the Growth and Productivity of *Chlorella pyrenoidosa*

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Introduction

Mirex and methoxychlor are both chlorinated hydrocarbon insecticides which have experienced heavy usage in parts of the United States. This paper reports some of the effects exerted by these two insecticides on the unicellular green alga, Chlorella pyrenoidosa.

Mirex (dodecachloro-octahydro-1,2,4-metheno-2H cyclobuta (cd) pentalene) is a 10-carbon, condensed-ring polycyclic hydrocarbon with all valence points chlorinated (U.S.D.A. 1972). Little research has been conducted on the effects of mirex on photosynthesizing organisms (CASSISTA AND KRICHER 1973; KRICHER AND CASSISTA 1972). Recent studies (KAISER 1974; TEN NOEVER de BRAUW and INGEN 1973) have indicated that mirex is quite stable and of widespread occurance.

Methoxychlor (1,1,1-trichloro-2,2,bis-(4-methoxyphenyl) ethane) is generally quite similar in structure to DDT (CORBETT 1974). Compared with DDT, methoxychlor has a very high elimination rate, low toxicity, and low accumulation rate in organisms (JOHANSSON 1971). Essentially no literature exists on possible effects of methoxychlor on photosynthesizing organisms.

Methods

Cultures of Chlorella pyrenoidosa were obtained from the American Type Culture Collection (Strain #7516). Sterile cultures were maintained and experiments performed in liquid Bristol's medium (STEIN 1973). All stock and experimental cultures were kept on a rotary shaker under continuous illumination by fluorescent light of 165 foot-candles. Pesticides, when added, were introduced into the medium in the organic solvent acetone.

Chlorella censuses were made directly with a Spencer Bright-Line Beubauer hemacytometer, and by percent transmittance measured on a Spectronic 20 (wavelength 680 nm). Pearson r correlation coefficients indicated that cell counts and % transmittance were highly inversely correlated (r = -.88). Primary productivity (rate of carbon

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fixation) was determined using C-14 labeled sodium bicarbonate (5 microcuries/ampoule). Counting was done on a Unilux I Liquid Scintillation System using Permablend^{Tm} II in toluene. Productivity was expressed as mg C/m^3/hr.

Results and Discussion

An experiment was performed to determine the effects of mirex, methoxychlor, and these two insecticides in combination on the population growth of Chlorella. There were two replicates within each of the pesticide treatments, three untreated controls, and one control containing 1% acetone. All pesticide concentrations were 100 ppb. Variability between replicates was consistently quite low (coefficient of variability < 12%). For comparative purposes, data are presented as ratios of control mean transmittance readings to treatment mean transmittance readings (Table I).

TABLE I

Ratios of Control Mean to Treatment Means

Hour	Mirex	Methoxychlor	Mirex + Methoxy	Acetone control
8	1.00	1.03	1.00	0.99
20	0.96	0.97	0.98	0.97
32	0.97	0.96	0.99	0.98
44	0.88	0.84	0.91	0.91
56	0.89	0.82	0.91	0.87
68	0.89	0.81	0.95	0.92
92	0.77	0.72	0.85	0.85
116	0.73	0.71	0.81	0.84
140	0.70	0.71	0.83	0.84
164	0.79	0.81	0.96	0.98

Ratios show little difference between the control and the four treatments until 44 hours. At this time, population density in all treatments dropped below the control. The acetone control population was depressed from 44 to 140 hours. This indicates that a 1% acetone concentration is probably somewhat toxic to Chlorella cells. As time progressed, the populations appeared to recover. Observations of cells exposed to 1% acetone revealed extensive lysis.

Since all other treatments contained 1% acetone, comparisons will be made between the pesticide samples and acetone control. Populations treated with 100 ppb mirex were 8% lower than the acetone control at 92 hours. By the end of the experiment, the mean mirex population density was 19% lower than the acetone control (Table I). Populations treated with 100 ppb methoxychlor were 17% lower than the acetone control when the experiment was ended (Table I). The populations exposed to mirex and methoxychlor in

combination at a total concentration of 100 ppb showed little deviation from the acetone control (Table I). Thus, it appears that in combination, each at 50 ppb, there was no effect on population growth.

These results indicate that mirex and methoxychlor independently affected Chlorella population growth at a concentration of 100 ppb. Each pesticide probably affected a different physiological mechanism that either killed the cells or inhibited cell division. If the two chemicals affected the same physiological process, one would expect that a combination of the two at a total concentration of 100 ppb would be equally as effective as the two pesticides were separately at 100 ppb. However, it appears that 50 ppb of each pesticide was below the toxicity threshold for Chlorella.

Two experiments were performed to evaluate pesticide effects on gross productivity of Chlorella. Each experiment consisted of three replicates of a control, a control plus 0.1% acetone, and a pesticide treatment plus 0.1% acetone. Both pesticides were introduced at 1 ppm. Chlorella populations of approximately 1×10^6 were allowed to grow for 70 hours in the presence of the pesticide before productivity was determined. At 70 hours, Chlorella populations were slightly but not significantly lower in the acetone and treatment cultures.

Results indicate that acetone, at a concentration of 0.1%, may exert a depressing effect on Chlorella productivity (Table II). Cultures containing both acetone and 1 ppm pesticide exhibited only slightly lowered productivities than the cultures exposed only to acetone (Table II).

TABLE II

Mean productivity ($mgC/m^3/hr$) $\pm$ one standard deviation

	Methoxychlor	Mirex
Control	1609 ± 51	1593 ± 86
Acetone	1415 ± 223	1311 ± 113
Ace. + Pest.	1140 ± 95	1290 ± 196

Since populations in the treatment cultures were slightly depressed, the lowered productivity in the treatment cultures may have been a simple function of lowered population size.

The results indicate that neither mirex nor methoxychlor dramatically affects the population growth or productivity of Chlorella pyrenoidosa.

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