Growth Response of Blue-Green Algae to Aldrin, Dieldrin Endrin and Their Metabolites

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It has been shown that certain chlorinated hydrocarbons inhibit the photosynthesis and growth of marine phytoplankton (1,2). Menzel et al. (1) reported that four species of marine phytoplankton vary in sensitivity to dieldrin, endrin, and DDT. Enough evidence has accumulated to show that these chlorinated hydrocarbons can be further converted in natural environments to form even more stable and sometimes more toxic residues, now referred to as "terminal residues" (3), than the parent compounds. For instance, photodieldrin is known to form from dieldrin in soil environments and on plant surfaces as a result of the action of sunlight (4,5) and microbial actions (6). To know the effects of any environmentally occurring substance on biological systems it is, therefore, imperative to study also the effects of its major conversion products in addition to the original compound. The purpose of this research is to test the effects of dieldrin, endrin, aldrin and five corresponding metabolites on the growth rates of two bacteria-free blue-green algal species.

The algae used in these experiments were Anacystis nidulans and Agmenellum quadruplicatum strain PR-6, hereinafter referred to as TX20 and PR-6 respectively. TX20 was obtained from the Laboratory of Algal Physiology, University of Texas at Austin, Texas. PR-6 is a marine isolate obtained from Dr. Chase Van Baalen of the University of Texas Marine Science Institute at Port Aransas, Texas. PR-6 was grown in Medium ASP-2 of Provasoli, McLaughlin, and Droop (7) as modified by Van Baalen (8). TX20 was grown in Medium Cg10, which is Medium C of Kratz and Myers (9) as modified by Van Baalen (10). The pH of all media was adjusted to 8.2 with 2N NaOH or HCl.

Cultures were grown at 39°C using the test tube method of Myers (11). Illumination was provided by four (two on each side) G.E. F20T12/CWX fluorescent lamps 9.0 cm from the tubes. Air enriched to 2% CO_2 (v/v), was continually bubbled through the culture tubes.

Growth of the liquid cultures was followed colorimetrically after the method of Kratz and Myers (9), using a Bausch and Lomb Spectronic 20 Colorimeter. Linearity between O.D. and cell concentration was verified. The growth rate of algae is expressed by the equation: $kt = \log (N_t/N_o)$, where k = growth rate constant, t = 24 hr., N_t = cell number at time t, N_o = cell number at time 0.

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The growth values reported are the specific growth rate constant, k, in $\log_{10}$ units/day. When k = 0.301, the generation time is one day. The sensitivity of the algae to the insecticide compounds was reflected by growth rate depression.

All growth rates (k values) were calculated from short-term experiments lasting 26 to 30 hr. Insecticides dissolved in ethanol were added directly to culture media just prior to inoculation (120,000 cells/ml). Control cultures containing the same amount of ethanol (0.01 ml to 0.1 ml 95% ethanol per 20 ml culture medium) gave k values similar to control cultures without ethanol. Although the insecticide solutions were not sterilized before adding to culture media, bacterial contamination was not a problem during the experiments. All insecticidal analogs used in this investigation have been chemically synthesized and purified (99%+) through recrystallization. The chemical nature of photodieldrin (6), metabolites F and G of dieldrin (12), photoaldrin and aldrin (4,5), and ketoendrin (13,14) has already been described in literature.

Comparison of k-values in Table 1 show an overall depression of growth rates in high concentrations (475 ppb, 950 ppb) of dieldrin and its metabolites. PR-6 exhibited less growth rate depression than TX20 to dieldrin, metabolite F, or photodieldrin. However, both PR-6 and TX20 seem similarly affected at 950 ppb metabolite G. Generally, TX20 appeared most sensitive to dieldrin, while PR-6 appeared equally sensitive to each of the four compounds.

Data in Table 2 indicate that both microorganisms are more tolerant of ketoendrin than endrin. Although much variation was encountered, mean growth rates of PR-6 demonstrated inhibition at all concentrations of endrin tested, whereas TX20 was affected only at high concentrations. While ketoendrin was less toxic to TX20, growth rates of PR-6 with ketoendrin were slightly higher than control cultures.

Results in Table 2 show that of the parent compounds tested aldrin had the least effect on either PR-6 or TX20. However, both had slightly lower growth rates in the presence of photoaldrin.

The data represent growth rates of experiments usually lasting 30-36 hr. It should be mentioned that TX20 cultures with 950 ppb insecticide demonstrated lag periods up to 12 hr preceeding exponential growth. However visual observations of final culture densities showed little difference when compared to control cultures. Reasons for this lag phenomenon are not understood but may involve growth dilution of inhibitory insecticide concentrations at cellular levels. Wurster (2) has shown that at 10 ppb DDT, photosynthesis by Skeletonema decreased as the cell concentration was reduced. Accumulation of extracellular products might also be associated with the lag, e.g. extracellular polypeptides of Anabaena cylindrica have been shown to reduce that microorganism's sensitivity to copper (15). It is also possible

TABLE 1.

Effect of dieldrin and dieldrin-metabolites on growth rate (k) of PR-6 and TX20. Control cultures: PR-6 K = 1.90±0.30, TX20 K = 2.06±0.15. Control cultures + 0.05 ml ethanol: PR-6 k = 1.88±0.27, TX20 k = 2.09±0.17. All values represent mean k of 3 to 5 replicate experiments.

ppb	Dieldrin		Metabolite F		Metabolite G		Photodieldrin	
	PR-6	TX20	PR-6	TX20	PR-6	TX20	PR-6	TX20
950	1.73±0.27	0.96±0.25	1.65±0.21	1.06±0.12	1.48±0.31	1.27±0.43	1.69±0.31	1.22±0.13
475	1.82±0.25	1.18±0.46	1.77±0.27	1.43±0.10	1.94±0.25	1.77±0.18	2.09±0.16	1.60±0.24
95	1.82±0.29	2.08±0.18	1.69±0.21	1.97±0.15	1.92±0.28	2.02±0.03	1.98±0.12	2.19±0.04
19	1.95±0.22	2.17±0.28	1.93±0.35	2.16±0.16	1.93±0.35	2.01±0.15	1.92±0.18	2.14±0.28
0.2	1.60±0.36	2.01±0.39	2.04±0.25	2.02±0.10	1.96±0.32	1.91±0.04	2.15±0.07	2.15±0.23