

Effects of Pesticides on *Euglena gracilis*. I. Growth Studies

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The growing concern over environmental contamination by pesticides is widespread. Recently, CHRISTIE, A. E. (1969), GREGORY, et. al. (1969) and MOORE, R. B. (1970) examined the effects of certain pesticides on photosynthetic micro-organisms which are ecologically important due to their position in the food web and their oxygen production.

The effects of two herbicides, five insecticides and two organic solvents on the growth of *Euglena gracilis* are reported here.

Methods and Materials

Euglena gracilis was grown in culture tubes containing 10 ml of *Euglena* medium (Difco) under constant illumination from fluorescent lamps at 25 C. Stock solutions of the pesticides, 2,4-D (2,4-Dichlorophenoxyacetic acid-99%), 2,4,5-T (2,4,5-Trichlorophenoxyacetic acid-99%), Aldrin AC-10 (1,2,3,4,10,10-Hexachloro-1,4,4a-dimethanonaphthalene-99.5%), DDT (Dichlorodiphenyltrichloroethane-100%), Malathion (0,0-Dimethylphosphorodithioate of mercaptosuccinate-95%), Parathion (0,0-Diethyl 0, p-nitrophenylphosphorothioate -99.7%), and Methoxychlor (2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane-recrystallized) were dissolved in either 95% ethanol or (DDT and Methoxychlor) in a 1:1 mixture of ethanol and acetone. Ethanol/acetone (1% v/v) was used as the control for DDT and Methoxychlor, ethanol (1% v/v) was used for all others.

The organisms were grown for one week in 250 ml flasks containing 100 ml of medium, counted with a hemacytometer and 9.0 ml aliquots distributed to culture tubes containing 1.0 ml of the pesticide solutions for a final concentration of 100, 50, 10, 5, or 1 ppm pesticide and 1.0% solvent. After 24 hours of exposure, the cells were again counted. Each experiment, which was repeated 5 or more times, included duplicate tubes of each pesticide, solvent and water controls.

Experiments in which organisms were exposed for 7 days were conducted in a similar fashion but only 3 pesticide concentrations (100, 50 and 10 ppm) were examined. Cell numbers were averaged for each series of experiments

and are reported in terms of percent of the appropriate control.

Results and Discussion

In table 1 are shown the results of exposing E. gracilis to pesticides and their solvents for 24 hours. Table 2 shows the effects of exposing the organisms for 7 days.

TABLE 1

Effects of 24 hour pesticide exposure on Euglena gracilis

Treatment	Concentration-ppm				
	100	50	10	5	1
	<u>Percent of appropriate control</u>				
Water	100	100	100	100	100
Ethanol	98.5	98.5	98.5	98.5	98.5
Ethanol/Acetone	98	98	98	98	98
2,4-D	74	84	104	104	115
2,4,5-T	61	76	102	96	99
Aldrin	83	88	94	96	108
DDT	81	76	105	109	89
Malathion	84	91	114	85	89
Parathion	93	98	101	92	84
Methoxychlor	83	82	105	118	106

TABLE 2

Effects of 7 day pesticide exposure to Euglena gracilis

Treatment	Concentration-ppm		
	100	50	10
	<u>Percent of appropriate control</u>		
Water	100	100	100
Ethanol	114	114	114
Ethanol/Acetone	123	123	123
2,4-D	78	103	161
2,4,5-T	67	120	161
Aldrin	160	148	181
DDT	281	331	286
Malathion	236	164	213
Parathion	165	217	177
Methoxychlor	265	293	216

One can readily see from the results of the 24 hour experiments that the solvents had little effect of the cells and that in most instances, 50 and 100 ppm of each pesticide depressed growth significantly with the herbicides 2,4-D and 2,4,5-T being the most inhibitory. Pesticide concentrations of 10 ppm or less stimulated

growth in most cases. In the long duration experiments of one week exposure, only the herbicides reduced growth and then only at the highest concentrations. All other pesticides and concentrations stimulated growth.

The insolubility of the pesticides (U.S. PUBLIC HEALTH SERVICE) except for 2,4-D and Malathion greatly reduces the possibility that concentrations examined in this study could ever occur in nature. The addition of organic solvents increases the solubility of the pesticides in most cases but DDT and Methoxychlor are still essentially insoluble.

The results from these experiments seem to indicate that pesticide killing of the photosynthetic micro-organism, E. gracilis, is not likely to occur in nature. After the preliminary experiments, it was observed that E. gracilis exposed to 50 and 100 ppm of 2,4-D or 2,4,5-T were morphologically altered. The majority of the cells were cyst-like after a short time and appeared to be dead. Therefore, a third series of experiments in which the treated cells were diluted 10 fold by transfer to pesticide free medium and cultivated for one week. Table 3 shows the results of one of these experiments. These data show that the cells were only temporarily changed and that recovery from high pesticide exposure is relatively rapid and complete.

TABLE 3

Recovery of Euglena gracilis
from high concentrations of pesticides

Treatment	Exposed 24 hr 100 ppm	Exposed 7 days 100 ppm	Exposed 24 hr	
			10 fold dilution	7 day recovery
Water control	9.1	115.0	17.0	
Ethanol	7.8	165.0	32.0	
Ethanol/Acetone	7.2	235.0	27.0	
2,4-D	8.0 rounded	17.0 rounded	19.0	normal
2,4,5-T	4.2 rounded	4.0 rounded	11.0	normal
Aldrin	10.2	80.0	13.0	
DDT	12.0	295.0	24.0	
Malathion	8.4	150.0	29.0	
Parathion	9.0	235.0	25.0	
Methoxychlor	7.0	130.0	24.0	

Original inoculum was 3.9×10^4 cells/ml.
Cell count $\times 10^4$

Respiratory experiments are now in progress to determine the effects of these and other pesticides on E. gracilis oxygen consumption.

Summary

The herbicides 2,4-D and 2,4,5-T were the most harmful to the photosynthetic microorganism Euglena gracilis. All of the test pesticides were harmful when concentrations of 50 to 100 ppm were used but the longer term effects, even at the high concentrations, were slight.

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