Effects of Herbicides On Photosynthesis And Growth of Marine Unicellular Algae[1]

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INTRODUCTION

Little is known concerning effects of pesticides upon marine unicellular algae. Menzel *et al.* (5), Ukeles (6), and Wurster (7) showed that chlorinated hydrocarbon insecticides can inhibit growth and photosynthesis by these organisms, and Derby and Ruber (2) demonstrated inhibition of oxygen evolution by organophosphorus insecticides in four species. Ukeles (6) reported inhibition of growth of five species by urea herbicides. The study reported here was done to obtain knowledge of effects of several classes of herbicides upon growth and photosynthesis by four genera of marine unicellular algae.

MATERIALS AND METHODS

Culture of algae. Algae used were the chlorophytes *Chlorococcum* sp., and *Dunaliella tertiolecta* Butcher, and chrysophytes *Isochrysis galbana* Parke and *Phaeodactylum tricornutum* Bohlin. All were obtained from the collection of the Woods Hole Oceanographic Institution and maintained in axenic culture. They were grown and tested in a medium composed of artificial seawater[2] supplemented with trace elements and vitamins. The supplements were: 30 mg Na_2EDTA, 14 mg $FeCl_2 \cdot 6H_2O$, 34 mg H_3BO_3, 4 mg $MnCl_2 \cdot 4H_20$, 2 mg $ZnSO_4 \cdot 7H_2O$, 6 mg K_3PO_4, 100 mg $NaNO_3$, 40 mg $Na_2SiO_3 \cdot 9H_20$, 5 μg $CuSO_4$, 12 μg $CoCl_2$, 50 μg thiamine hydrochloride, 1 μg vitamin B_{12}, and 0.01 μg biotin per liter of medium. Salinity of the medium was 30 parts per thousand and the pH ranged from 7.9 to 8.1. The medium was sterilized by autoclaving for 15 min at 121 C. Growth was carried out at 20 C under 6,000 lux illumination from fluorescent tubes with alternating 12-hr periods of light and darkness. The cultures were not shaken.

The herbicides evaluated are listed in Table 1. A total of 30 formulations in 10 classes of herbicides were examined.

Evolution of oxygen. Algal cultures in the logarithmic phase of growth were centrifuged and resuspended in growth medium to an optical density of 0.100 at 525 $m\mu$. Concentrations of herbicides in the suspending medium ranged from 0 to those which inhibited evolution of oxygen by approximately 25, 50, 75, and 100%. Concentrations were calculated as ppm (parts per million) of the commercial product. From each cell suspension, 4.0 ml aliquots were taken and placed in reaction vessels and, after equilibration at 20 C for 30 min, oxygen evolution was measured at 10-min intervals for 90 min on a photosynthesis-model respirometer. Duplicate flasks were analyzed in each

TABLE 1. NAMES OF HERBICIDES USED IN STUDIES ON MARINE UNICELLULAR ALGAE

	Common name	Chemical name
Triazine	ametryne	2-(ethylamino)-4-(isopropylamino)-6-(methylthio)-s-triazine
	atrazine	2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine
	simazine	2-chloro-4-,6-bis (ethylamino)-s-triazine
	prometone	2,4-bis (isopropylamino)-6-methoxy-s-triazine
Urea	diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea
	neburon	1-butyl-3-(3,4-dichlorophenyl)-1-methylurea
	monuron	3-(p-chlorophenyl)-1,1-dimethylurea
	fenuron	1,1-dimethyl-3-phenylurea
Phenoxyacetic acid	2,4-D	(2,4-dichlorophenoxy)acetic acid
	2,4,5-T	(2,4,5-trichlorophenoxy)acetic acid
Propionic acid	silvex	2-(2,4,5-trichlorophenoxy)propionic acid
	dalapon	2,2-dichloropropionic acid
Picolinic acid	picloram + 2,4-D	4-amino-3,5,6-trichloropicolinic acid
Benzoic acid	chloramben	3-amino-2,5-dichlorobenzoic acid
Nitrile	dichlobenil	2,6-dichlorobenzonitrile
Phthalic	endothall	7-oxabicyclo [2.2.1] heptane-2,3-dicarboxylic acid
Dipyridylium	diquat	6,7-dihydrodipyrido [1,2-*a*:2′,1′—*c*]pyrazinediium ion
	paraquat	1,1′-dimethyl-4,4′-bipyridinium ion
Toluidine	trifluralin	*a,a,a*-trifluro-2,6-dinitro-N,N-dipropyl-*p*-toluidine

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[1] Contribution No. 130, Gulf Breeze Laboratory, Environmental Protection Agency.

[2] From Rila Products, Teaneck, New Jersey. References to commercial products in this publication do not constitute endorsement by the Environmental Protection Agency.

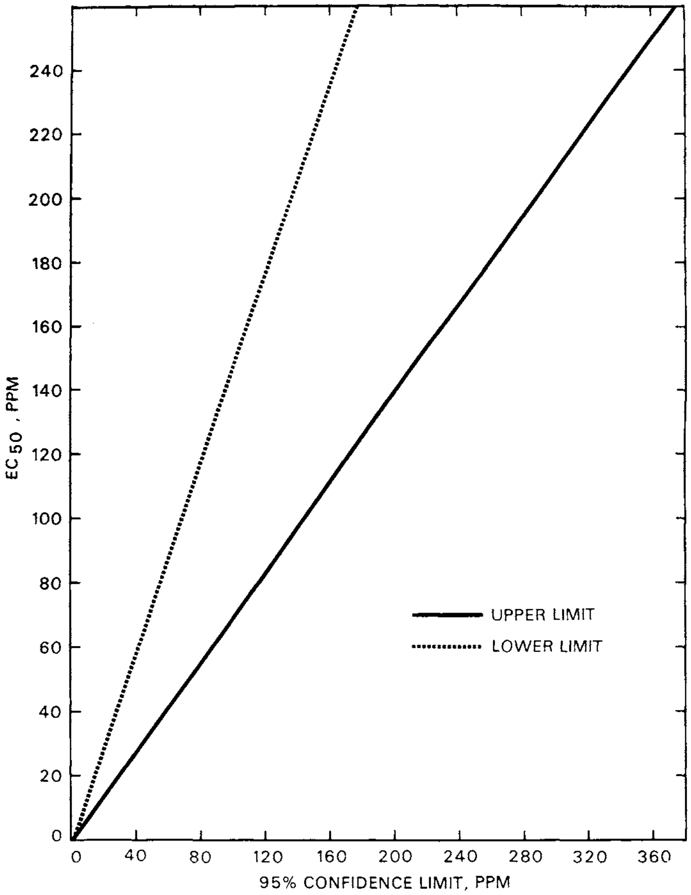

Figure 1.—Graph for derivation of 95% confidence limits of EC_{50} values for inhibition of oxygen evolution and growth of unicellular algae by herbicides.

test and each test was performed three times. Mean percentage inhibition after 90 min was calculated and EC_{50} values (effective concentration at which evolution of oxygen was 50% that of untreated cell suspensions) were calculated by straight-line graphical interpolation (1). The data were analyzed statistically by the method of Litchfield and Wilcoxon (4), which gives the 95% confidence limits of any median effective concentration. The 95% confidence intervals of EC_{50} values up to 260 ppm were plotted and are given in Figure 1. Values given as EC_{100} are the lowest concentrations which caused complete inhibition of oxygen evolution. These concentrations totally inhibited oxygen evolution in all tests, and confidence intervals were not calculated for them.

Growth. Growth experiments were carried out in optically matched test tubes. To 24 ml of medium that contained herbicide was added 1.0 ml of algal culture in the logarithmic phase of growth. Growth was measured spectrophotometrically at 525 mμ after 10 days. Statistical analyses were identical to those described above, and data are reported as concentrations of herbicides that reduced rate of growth by 50% (EC_{50}) and 100% (EC_{100}) of untreated cultures.

RESULTS AND DISCUSSION

Effects of herbicides on evolution of oxygen. Table 2 gives EC_{50} and EC_{100} values for the herbicides tested. The 95% confidence limits for all, except the combination of sodium and magnesium salts of dalapon (upper limit 23% above EC_{50}, lower limit 18% below EC_{50}) and the ammonium salt of chloramben (upper limit 25% above EC_{50}, lower limit 19% below EC_{50}), may be derived by extrapolation from Figure 1. The urea and triazine herbicides were the strongest inhibitors of oxygen evolution and diuron, neburon, and ametryne were the most toxic. The ureas and triazines are powerful inhibitors of the Hill reaction (3,8) and their effects were immediate on all genera, indicating that the herbicides were absorbed quickly by the marine algae. A similar effect with diuron on the freshwater alga *Chlorella pyrenoidosa* Chick was reported by Zweig (8).

Herbicide formulation affected oxygen evolution. For example, the technical acid of 2,4-D was more toxic than its butoxyethanol ester, and the technical acid of endothall was more toxic than its amino and dipotassium salts. A combination of the sodium and magnesium salts of dalapon was less toxic than the parent compound. However, the methyl ester of chloramben, formulated as emulsifiable concentrate, was more toxic than the technical acid and ammonium salt.

With some of the chemicals, notably diquat, paraquat, endothall salts, a combination of picloram and 2,4-D, and 44.5% trifluralin, it was impossible to determine EC values due to color imparted to the cell suspensions by the herbicides. Also, crystals of trifluralin were present in concentrations of 250 ppm and above.

Effects of herbicides on autotrophic growth. EC_{50} and EC_{100} values for effects of herbicides upon growth of algae are presented in Table 3. The 95% confidence limits for EC_{50} values may be derived by extrapolation from Figure 1.

As in the short-term oxygen inhibition studies, the urea and triazine herbicides were most toxic to growth, and EC values were similar to those for growth inhibition except for a 4% granular formulation of simazine and 25% emulsifiable solution of prometone. Less of these two formulations was required for inhibition of growth than for inhibition of oxygen evolution. Similarly, among the other compounds, less herbicide was required for inhibition of growth than for inhibition of oxygen evolution, except for 2,4-D, 2,4,5-T, dalapon, and the ammonium and methyl ester formulations of chloramben. For example, only slight inhibition of oxygen evolution by diquat was noted at concentrations up to 5,000 ppm, where density of color precluded further tests. However, in growth studies, EC_{50} values for the four genera of algae ranged between 15 and 200 ppm, whereas EC_{100} values were between 25 and 500 ppm. Conversely, higher concentrations of the methyl ester of chloramben (emulsifiable concentrate) were required to inhibit growth than to inhibit oxygen evolution. This may have been related to exposure of algae to the carrier during shaking in the respirometer, whereas during static growth experiments carrier settled to the bottoms of the tubes.

When interspecific differences in toxicity of any given herbicide occurred, *D. tertiolecta* was most often the most resistant species. Similarly, Menzel *et al.* (5) reported that *D. tertiolecta* was the most resistant species when tested with DDT (1,1,1-Trichloro-2,2-bis (*p*-chlorophenyl) ethane), dieldren (1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene), and endrin (1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octhahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene). A notable exception was the relative resistance of *Chlorococcum* to diquat and paraquat (Table 3).

In addition to these differences in resistance among species, the chrysophytes *I. galbana* and *P. tricornutum*

TABLE 2. CONCENTRATIONS OF HERBICIDES (PPM) THAT DECREASED OXYGEN EVOLUTION OF MARINE UNICELLULAR ALGAE BY 50% (EC_{50}) AND 100% (EC_{100}).

Herbicide	Formulation	*Chlorococcum* sp.		*D. tertiolecta*		*I. galbana*		*P. tricornutum*	
		EC_{50}	EC_{100}	EC_{50}	EC_{100}	EC_{50}	EC_{100}	EC_{50}	EC_{100}
Ametryne	Tech. acid[1]	0.02	0.05	0.04	0.10	0.01	0.08	0.01	0.08
Ametryne	80% W.P.[2]	0.05	0.10	0.06	0.20	0.01	0.04	0.04	0.08
Atrazine	Tech. acid	0.10	0.40	0.30	0.70	0.10	0.20	0.10	0.20
Atrazine	80% W.P.	0.40	0.80	0.60	1.00	0.20	0.50	0.20	0.60
Simazine	Tech. acid	2.5	4.0	4.0	6.0	0.60	1.0	0.60	1.5
Simazine	4% G[3]	50	100	50	100	10	50	10	50
Prometone	Tech. acid	0.40	1.0	2.0	3.5	1.0	2.5	0.10	0.40
Prometone	25% E.S.[4]	5.0	15	15	25	3.5	18	3.0	18
Diuron	Tech. acid	0.02	0.05	0.01	0.03	0.01	0.02	0.01	0.02
Neburon	Tech. acid	0.02	0.04	0.02	0.04	0.02	0.04	0.04	0.09
Monuron	Tech. acid	0.10	0.20	0.09	0.16	0.10	0.17	0.09	0.17
Fenuron	Tech. acid	2.0	3.2	1.2	3.0	1.2	2.0	1.2	2.7
2,4-D	Tech. acid	60	90	50	90	60	85	60	95
2,4-D	Butoxyethanol ester	100	200	100	400	100	300	200	300
2,4,5-T	Tech. acid	150	200	150	225	50	150	75	100
Silvex	Tech. acid	250	375	200	300	250	400	225	300
Dalapon	Tech. acid	25	50	25	50	40	55	25	35
Dalapon	Na and Mg salts	2,250	4,000	2,500	4,250	2,250	4,000	2,500	4,000
Picloram + 2,4-D	......	>2,000	......	>2,000	......	100	275	>2,000	......
Chloramben	Tech. acid	115	150	150	175	100	175	100	150
Chloramben	Ammonium salt	2,225	5,500	2,750	5,500	1,500	2,500	3,000	5,500
Chloramben	Methyl ester	2.0	3.7	1.7	3.0	1.5	2.5	2.7	5.0
Dichlobenil	Tech. acid	90	300	125	300	100	300	150	250
Endothall	Tech. acid	100	300	425	900	60	400	75	150
Endothall	Amine salt	>1,000	......	>1,000	......	>1,000	......	>1,000	......
Endothall	Dipotassium salt	>5,000	......	>5,000	......	>5,000	......	>5,000	......
Diquat	Dibromide	>5,000	......	>5,000	......	>5,000	......	>5,000	......
Paraquat	Dichloride	>5,000	......	2,500	>5,000	5,000	>5,000	3,500	>5,000
Trifluralin	Tech. acid	>500	......	>500	......	>500	......	>500	......
Trifluralin	44.5% E.S.	>100	......	>100	......	>100	......	>100	......

[1]Technical acid; [2]Wettable powder; [3]Granular formulation; [4]Emulsifiable solution.

TABLE 3. CONCENTRATIONS OF HERBICIDES (PPM) THAT DECREASED GROWTH OF MARINE UNICELLULAR ALGAE BY 50% (EC_{50}) AND 100% (EC_{100}).

Herbicide	Formulation	*Chlorococcum* sp.		*D. tertiolecta*		*I. galbana*		*P. tricornutum*	
		EC_{50}	EC_{100}	EC_{50}	EC_{100}	EC_{50}	EC_{100}	EC_{50}	EC_{100}
Ametryne	Tech. acid[1]	0.01	0.05	0.04	0.10	0.01	0.05	0.02	0.05
Ametryne	80% W.P.[2]	0.01	0.05	0.02	0.05	0.01	0.05	0.05	0.08
Atrazine	Tech. acid	0.10	0.50	0.30	1.20	0.10	0.20	0.20	0.50
Atrazine	80% W.P.	0.10	0.50	0.40	1.50	0.10	0.20	0.20	0.50
Simazine	Tech. acid	2.0	7.5	5.0	8.0	0.50	1.0	0.50	1.0
Simazine	4% G[3]	2.5	10	20	50	5.0	25	2.0	10
Prometone	Tech. acid	0.50	0.75	1.5	4.5	1.0	2.5	0.25	1.0
Prometone	25% E.S.[4]	1.5	2.5	5.0	13	0.50	1.0	2.0	5.0
Diuron	Tech. acid	0.01	0.03	0.02	0.05	0.01	0.03	0.01	0.03
Neburon	Tech. acid	0.03	0.07	0.04	0.08	0.03	0.07	0.03	0.06
Monuron	Tech. acid	0.10	0.25	0.15	0.25	0.13	0.28	0.10	0.25
Fenuron	Tech. acid	0.75	2.0	1.5	2.5	0.75	2.0	0.75	2.0
2,4-D	Tech. acid	50	75	75	100	50	75	50	75
2,4-D	Butoxyethanol ester	75	150	75	300	75	250	150	250
2,4,5-T	Tech. acid	100	175	125	200	50	125	50	75
Silvex	Tech. acid	25	50	25	50	5.0	20	5.0	20
Dalapon	Tech. acid	50	100	100	175	20	35	25	35
Dalapon	Na and Mg salts	500	1,000	400	800	500	750	500	750
Picloram + 2,4-D	......	100	200	1,250	2,500	50	75	100	200
Chloramben	Tech. acid	50	125	50	125	15	50	25	100
Chloramben	Ammonium salt	4,000	5,000	4,000	5,000	3,500	4,000	3,000	4,000
Chloramben	Methyl ester	2.5	5.0	5.0	7.5	5.0	7.5	5.0	7.5
Dichlobenil	Tech. acid	60	75	60	75	60	75	25	50
Endothall	Tech. acid	50	85	50	95	25	50	15	25
Endothall	Amine salt	300	750	450	750	225	500	250	500
Endothall	Dipotassium salt	1,500	5,500	1,500	5,000	3,000	5,000	500	1,000
Diquat	Dibromide	200	500	30	45	15	25	15	25
Paraquat	Dichloride	50	75	20	35	5.0	15	10	20
Trifluralin	Tech. acid	2.5	10	5.0	10	2.5	10	2.5	10
Trifluralin	44.5% E.S.	2.5	5.0	2.5	5.0	2.5	5.0	5.0	10

[1]Technical acid; [2]Wettable powder; [3]Granular formulation; [4]Emulsifiable solution

were more susceptible than the chlorophytes to silvex, dalapon, diquat, paraquat, and endothall technical acid.

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