Differential Responses of Marine Phytoplankton to Herbicides: Oxygen Evolution

by TERRENCE A. HOLLISTER
Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory, Sabine Island
Gulf Breeze, Fla. 32561
and
GERALD E. WALSH
Associate Laboratory of the National Environmental Research Center
Corvallis, Ore.

INTRODUCTION

Marine unicellular algae vary in their responses to a variety of toxicants, including chlorinated hydrocarbon insecticides (UKELES 1962, MENZEL et al. 1970), organophosphate insecticides (DERBY and RUBER 1970), and fungicides (UKELES 1962). Little is known, however, about toxicities of herbicides to marine unicellular algae. Responses of four algal species to 30 herbicidal formulations have been reported (WALSH 1972) and the urea and triazine herbicides were the most toxic. Four urea herbicides also caused depression of the carbohydrate contents of six species of algae, and effect was directly proportional to salinity of the growth medium (WALSH and GROW 1971).

The work reported here was done to learn if marine unicellular algae differ in their responses to herbicides. We tested 18 species against the substituted ureas, neburon and diuron, and the triazines, atrazine and ametryne.

MATERIALS AND METHODS

The algae were obtained from the culture collections of the Woods Hole Oceanographic Institution, Scripps Institution of Oceanography, and Indiana University. All were maintained and tested in a growth medium composed of artificial seawater [2] supplemented with trace elements and vitamins. The supplements, per liter of medium, were: 30 mg Na_2EDTA, 14 mg $FeCl_2 \cdot 6H_2O$, 34 mg H_3BO_3, 4 mg $MnCl_2 \cdot 4H_2O$, 2 mg $ZnSO_4 \cdot 7H_2O$, 6 mg K_3PO_4, 100 mg $NaNO_3$, 40 mg $Na_2SiO_3 \cdot 9H_2O$, 5µg $CuSO_4$, 12 µg $CoCl_2$, 50 µg thiamine hydrochloride, 1 µg vitamin B_{12}, and 0.01 µg biotin. Salinity was 30 parts per thousand and the pH ranged between 7.9 and 8.1. The medium was sterilized by autoclaving for 15 minutes at 121°C.

Five ml of stock algae were inoculated into 100 ml of growth medium and incubated at 20°C under 6,000 lux illumination from fluorescent tubes with alternating 12-hour periods of light and

[1] Contribution No. 159 from the Gulf Breeze Laboratory.

[2] From Rila Products, Teaneck, New Jersey. Mention of commercial products does not constitute endorsement by the Environmental Protection Agency.

Bulletin of Environmental Contamination & Toxicology,
Vol. 9, No. 5 © 1973 by Springer-Verlag New York Inc.

darkness for three days. Then, the cultures were centrifuged gently and resuspended in growth medium to an optical density of 0.100 at 525 mμ on a Fisher electrophotometer. The algal cultures were not axenic.

Effects of herbicides were measured as inhibition of oxygen evolution. Both ureas and triazines inhibit photosynthesis and move quickly into the cells (ZWEIG 1969) and the concentrations required for inhibition of both growth and photosynthesis are the same (WALSH 1972).

Concentrations of herbicides in the suspending media ranged from zero to those which inhibited evolution of oxygen by approximately 25, 50, and 75%. Concentrations were calculated as parts per billion (ppb) of the technical preparation. From each cell suspension, 5.0 ml were placed in reaction vessels of a Gilson photosynthesis model differential respirometer. After equilibrating at 20°C for 30 minutes, oxygen evolution was measured for 60 minutes with a CO_2 buffer in the well of the reaction vessel (UMBREIT et al. 1964). Duplicate flasks were analyzed and each test was performed three times.

All data were subjected to statistical analysis. Mean percentage inhibition after 60 minutes was calculated and EC50 values (effective concentrations at which evolution of oxygen was 50% that of untreated cell suspensions) were calculated by the least squares method. Concentrations were converted to logarithms and responses to probits and the standard error obtained for each series of tests.

RESULTS AND DISCUSSION

The EC50 values for the four herbicides and 18 algal species tested are shown in Table 1 and summarized by family in Table 2. Atrazine was the least toxic; ametryne, neburon, and diuron were approximately equal in toxicity. Species of the family Bacillariophyceae were generally the least sensitive, requiring as much as 5.8 times more ametryne to reduce oxygen evolution by 50% than did species of the other families.

Wide variations occured in response to the toxicants among the individual species of the families Chlorophyceae, Bacillariophyceae, and Chrysophyceae. A measure of the range of responses among species was calculated by derivation of the ratio of the highest EC50 to the lowest EC50. The ratio, here called the "Difference Factor", is given for each family and herbicide in Table 2. Difference Factors were greatest in the Bacillariophyceae, being as high as 11.9 for neburon-treated algae. In that case, the EC50 for Cyclotella nana was 11 ppb, whereas for Nitzschia (Indiana strain 684) it was 131 ppb.

TABLE 1. EC50 (ppb) of neburon, diuron, atrazine, and ametryne on oxygen evolution by marine unicellular algae. Standard errors (SE) were derived by unweighted probit analysis.

Family / Species	Neburon		Diuron		Atrazine		Ametryne	
	EC50	SE	EC50	SE	EC50	SE	EC50	SE
Chlorophyceae								
Chlamydomonas sp.	37	5	37	3	60	8	41	5
Dunaliella tertiolecta	10	3	10	3	159	18	40	6
Platymonas sp.	12	5	17	3	102	8	24	4
Chlorella sp.	22	3	19	2	143	8	32	3
Neochloris sp.	39	6	28	5	82	7	36	7
Chlorococcum sp.	20	3	20	4	80	7	10	3
Bacillariophyceae								
Thalassiosira fluviatilis	108	9	95	10	110	19	58	7
Navicula inserta	124	11	93	12	460	15	97	9
Amphora exigua	82	5	31	4	300	21	26	4
Achnanthes brevipes	23	4	24	1	93	11	19	1
Stauroneis amphoroides	17	3	31	2	348	67	65	11
Cyclotella nana	11	4	39	7	84	19	55	8
Nitzschia closterium	120	13	50	6	287	68	62	6
Nitzschia (Ind. 684)	131	9	169	17	434	84	135	11
Chrysophyceae								
Monochrysis lutheri	12	4	18	3	77	23	14	4
Isochrysis galbana	20	5	10	3	100	17	10	4
Phaeodactylum tricornutum	40	7	10	3	100	19	10	5
Rhodophyceae								
Porphyridium cruentum	24	4	24	3	79	9	35	4

TABLE 2. Average EC50 values (ppb) for four herbicides and four families of marine unicellular algae. The Difference Factor (DF) is the ratio of the highest to the lowest EC50 among the algal species.

Family	Number of Species Tested	Neburon		Diuron		Atrazine		Ametryne	
		EC50	DF	EC50	DF	EC50	DF	EC50	DF
Chlorophyceae	6	23	3.9	22	3.7	104	2.6	31	4.1
Bacillariophyceae	8	77	11.9	67	7.0	265	5.5	65	7.1
Chrysophyceae	3	24	3.3	13	1.8	92	1.3	11	1.4
Rhodophyceae	1	24	-	24	-	79	-	35	-

These data show that when bioassay analyses are conducted for effects of herbicides on marine unicellular algae, two factors are particularly important: (1) the response in relation to familial taxonomic position, and (2) the wide range of responses by individual species within a given family. It is necessary, therefore, to use several species from each of several families in algal bioassay studies to obtain realistic data concerning effects of herbicides on algae.

ACKNOWLEDGEMENT

We thank E. I. DuPont de Nemours and Co. for providing the urea herbicides, and Geigy Agricultural Chemicals for providing the triazine herbicides used in these experiments.

REFERENCES

DERBY, S. B. (SLEEPER) and E. RUBER. Bull. Environ. Contam. Toxicol. 5:553-558, (1970).

MENZEL, D. W., J. ANDERSON, and A. RANDTKE. Science 167: 1724-1726, (1970).

UKELES, R. Appl. Microbiol. 10:532-537, (1962).

UMBREIT, W. W., R. H. BURRIS, and J. F. STAUFFER. Manometric Techniques, 4th ed., Burgess Pub. Co., Minneapolis, Minn., 305 pp. (1964).

WALSH, G. E. Hyacinth Control J. 10:45-48, (1972).

WALSH, G. E. and T. E. GROW. Weed Sci. 19:568-570, (1971).

ZWEIG, G. Residue Rev. 25:69-79, (1969).