Primary Production: Depression of Oxygen Evolution in Algal Cultures by Organophosphorus Insecticides

by SYLVIA B. (SLEEPER) DERBY and ERNEST RUBER
Biology Department, Northeastern University
Boston, Mass.

Introduction

The side-effects of insecticides have become a matter of great public concern during the last decade. Several workers have studied effects of pesticides on algae but generally, this area has had less attention than that of aquatic invertebrates, birds and fishes. Ukeles (1) used changed optical density after ten to fourteen days as an index of growth. The organophosphates TEPP and Dipterex were not inhibitory at the levels around 50 parts per million (ppm). Sevin (a carbamate) was not inhibitory to 3 species at 1 ppm but was at 10 ppm; two species Phaeodactylum tricornutum and Monochrysis lutheri were affected at 1 ppm, the former was affected even at 0.1 ppm. Three chlorinated hydrocarbons were also studied: Lindane which was not inhibitory at 7.5 ppm except again to P. tricornutum (5 ppm) and M. lutheri (7.5 ppm); DDT did not affect growth at the highest concentration used (0.6 ppm) except for M. lutheri; toxaphene was the most toxic showing effects beginning at 0.15 ppm and descending to 0.001 ppm for M. lutheri.

Butler (2) using the ^{14}C method for a four-hour exposure period reported DDT at 1 ppm reducing productivity of a "natural community" by 77%, reducing that of Platymonas sp. by 24% and not affecting Dunaliella euchlora. Baytex (an organophosphate) scarcely affected the "natural community" but reduced productivity of both algae by about 50%.

Ruber and Ferrigno (3) used the light-dark bottle Winkler system with 6 hour incubations in the field for a week before and several weeks after hand-applied formulations of DDT, Baytex and endrin. Actual concentrations in the water were not determined but the

Bulletin of Environmental Contamination & Toxicology,
Vol. 5, No. 6, 1971, published by Springer-Verlag New York Inc.

formulations were at levels adequate for mosquito control on the Spartina patens marshes tested. There was no effect from Baytex and a slight but statistically significant gain in net primary productivity in the DDT and endrin plots. The cause for this phenomenon was never determined but it was suggested that a more rapid recycling of a limiting nutrient due to insecticide caused mortalities of copepods and aquatic insects might be involved.

More recently Wurster (4) using twenty-four hour exposures and ^{14}C demonstrated inhibition of several species of phytoplankters by concentrations of DDT as low as 0.001 ppm.

Menzel et al. (5) have extended these studies including two more chlorinated hydrocarbons, dieldrin and endrin and evaluating by ^{14}C and by growth rates.

Present trends seem to be towards a reduction in use of chlorinated hydrocarbon insecticides with a substitution of organophosphates which generally have shorter residual times. As a consequence, the present study deals with the effects of some of the newer insecticides on four species of marine phytoplankton: Dunaliella euchlora, Phaeodactylum tricornutum, Skeletonema costatum, and Cyclotella nana. The insecticides used were Abate and Baytex, both organophosphates; Baygon, a carbamate; and DDT which was included as a reference-point (6). Abate, Baytex, and Baygon all have shorter residual times than DDT. They have each had some application in insect control and the first is considered to be of such low mammalian toxicity as to have been used in human drinking water as an Aedes aegypti control measure (7).

Methods

The change in oxygen production of phytoplankton was used as an indicator of the effect of the insecticide investigated. The amount of oxygen produced is related to the amount of carbon fixed by the phytoplankton. Approximately one molecule of oxygen is produced for each atom of carbon fixed. For the exact conversion value, variables such as nitrogen source, illumination pattern and photosynthetic quotient must be taken into account (8). Even excluding consideration of these factors, oxygen evolved is a valid index of net primary production which is our best measure of the available energy at the base of any given trophic system.

The phytoplankton cultures were maintained in "f/2" medium (9), with constant aeration and at an illumination of 250 footcandles supplemented by daylight. Insecticides were prepared in acetone at concentrations of one percent by weight; these solutions were diluted to the required strength in distilled water just prior to use.

TABLE I

Summary of percentage reductions in oxygen production and tests of significance.

Pesticides (ppm)	Dunaliella euchlora %	Dunaliella euchlora t	Phaeodactylum tricornutum %	Phaeodactylum tricornutum t	Skeletonema costatum %	Skeletonema costatum t	Cyclotella nana %	Cyclotella nana t
DDT								
1.0	42	8.4	35	2.9	39	5.5	33	2.9
0.1	32	5.3	10	0.8*	32	2.5	33	2.3
0.01	30	5.3	24	1.9*	36	5.2	1	0.1*
Abate								
1.0	36	6.1	38	3.8	55	4.8	80	6.8
0.1	23	4.1	28	2.5	23	2.2	13	1.0*
0.01	30	5.1	12	1.0*	16	1.2*	1	0.2*
Baygon								
1.0	25	4.5	23	1.9	30	4.3	53	11.0
0.1	32	4.6	28	2.5	23	2.1	13	0.9*
0.01	27	6.8	40	3.8	29	2.9	13	0.4*
Baytex								
1.0	27	5.4	29	2.5	19	2.3	50	3.8
0.1	27	6.7	29	2.5	51	5.9	48	2.7
0.01	16	2.6	35	3.5	26	3.2	1	1.2*

% Percent reduction in oxygen production as compared with acetone controls.
t Value calculated by t-test from actual oxygen production values.
* Probability not significant, all other values are significant at least at the .05 level.

For each test one thousand ml of stock culture was pumped into 1,500 ml erlenmeyer flasks in replicate. The appropriate amount and concentration of insecticide was added to each pair of flasks so that the final insecticide concentrations were 1.0, 0.1, and 0.01 ppm. The flasks were illuminated at 250 footcandles for 20 hours. At the end of this period the material from each flask was transferred to 3 D.O. (dissolved oxygen) bottles; one of these 3 bottles was fixed immediately and the other 2 received either light or dark for the remaining 4 hours of the test. The light bottles were stoppered and illuminated at 500 footcandles for 4 hours. The dark bottles were stoppered and covered with aluminum foil to prevent light penetration. At the end of the four hour period the bottles were fixed and titrated by the Winkler Method (10). The bottles fixed at the start of the four hour period, when titrated, gave the initial concentration of oxygen which was used as a reference point. Each series of tests was run twice.

Results and Discussion

Results are reported as a percent reduction in dissolved oxygen level of the various test cultures as compared with the controls. Results were analyzed using a one tailed-t-test (Table 1). While percentage reductions are reported here, actual differences in oxygen evolved expressed as parts per million were t-tested.

Of the insecticides used, Baytex had the greatest effect in depressing oxygen production of the algae tested. This insecticide lowered the oxygen production a statistically significant amount in all cases tested except for Cyclotella nana at a concentration of 0.01 ppm. Baygon had the second greatest effect on the oxygen production: In all experiments using D. euchlora, S. costatum, and P. tricornutum oxygen production was significantly lowered while with C. nana the decrease was significant only at a concentration of 1.0 ppm. Baygon was followed by DDT which significantly depressed the oxygen production of D. euchlora, and S. costatum at all concentrations tested while P. tricornutum was depressed at 1.0 and 0.1 ppm. Abate had the least effect on the oxygen production: It lowered the oxygen production of D. euchlora at all concentrations tested, of Skeletonema costatum and Phaeodactylum tricornutum at concentrations of 1.0 and 0.1 ppm, and of Cyclotella nana only by a concentration of 1.0 ppm. In all cases the results obtained from the dark bottles were not significantly different from the controls, hence, all changes refer only to the light bottles, e.g. to net primary production.

Menzel et al. (5) have demonstrated a DDT suppression of ^{14}C uptake at about .001 ppm for Cyclotella nana and .005 ppm for Skeletonema costatum at 24 hours. Our concentrations did not go below 0.01 ppm at which level the latter was affected. The former however,

was not affected below 0.1 ppm DDT in our system. There seem to be two probable explanations for the difference: (1) The ^{14}C technique is far more sensitive than the Winkler D.O. method and (2) we measured the productivity of the algae during the last 4 hours of a twenty-four exposure. The fact that we are able to detect effects of organophosphate insecticides with this method suggests that effects at even lower concentrations will be detected when measured for a full twenty-four hours by the ^{14}C technique.

Each alga reacted differently to the various concentrations of the several insecticides used. The results indicated that all of the test insecticides lowered the oxygen production of the selected algae in at least some of the concentrations used. Care must be taken in using the results to predict what will happen in the environment. In the laboratory more control was provided than is found in nature; only one insecticide, at set concentrations, was used for any one set of tests and only uni-algal cultures were used. Factors such as light intensity and long-term effects of low dosages need to be considered. In the environment many insecticides, in varying concentrations, are acting not upon a single species of algae, but upon several species at once. The consequences of differential effects of pesticides on competition, diversity and total productivity of a mixed community are unpredictable.

The tests showed that under the conditions imposed, the insecticides used did significantly lower the oxygen production of the algae, in at least some concentrations. This in itself accents again that caution is necessary in the widespread introduction of insecticides into the environment. Further, it may be pointed out that an attack on DDT or even on chlorinated hydrocarbons will not solve all of our environmental problems.

Summary

Photosynthesis of four estuarine phytoplankters was inhibited by Baytex and Abate (organophosphates), Baygon (carbamate) and DDT (chlorinated hydrocarbon). Responses varied with the algal species and with the insecticide. The order from most to least toxic insecticide was Baytex, Baygon, DDT, Abate and the order from least to most sensitive alga was *C. nana*, *P. cornutum* = *S. costatum*, *D. euchlora*.

Acknowledgement

We thank Mrs. Barbara E. Hawkes for valuable technical assistance. The senior author was supported, in part, by an institutional N.S.F. fellowship.

References

1. UKELES, R., Appl. Micro. 10, 532 (1962)
2. BUTLER, P.A., Fish and Wildlife Circ. No. 167, 10 (1963)
3. RUBER, E. and FERRIGNO, F., Proc. 51st Ann. Mtg. N.J. Mosquito Extermination Ass'n. 84 (1964)
4. WURSTER, C.F. Jr., Science 159, 1474 (1968)
5. MENZEL, D.W., ANDERSON, J., and RANDTKE, A., Science 167, 1724 (1970)
6. Technical grades of Baytex (fenthion; 0,0-dimethyl 0 - 4-(methylthio)-m-tolyl phosphorothioate) and Baygon (0-isopropoxyphenyl methylcarbamate) were supplied by Chemagro Corp. Technical grade Abate (0, 0, 0', 0' - tetramethyl 0, 0' -thiodi-p-phenylene phosphorothioate) was provided by the American Cyanamid Company. The DDT used was purchased from Nutritional Biochemicals Corp.
7. LAWS, E.R. Jr., SEDLAK, V.A., MILES, J.W., JOSEPH, C.R., LACOMBA, J.R., and RIVERA, A.D., Bull. Wld. Hlth. Org. 38, 439 (1968)
8. RYTHER, J.H., Limnol. Oceanog. 1, 72 (1956)
9. GUILLARD, R.L., Woods Hole Oceanog. Inst. - mimeo. (1968)
10. _____ Standard Methods for the examination of water, sewage, and industrial wastes. Amer. Pub. Health Assoc. 11th ed. (1960)