Interactions of Pesticides and Phytoplankton

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INTERACTIONS OF PESTICIDES AND PHYTOPLANKTON

BY

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A Research Report Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science in Environmental Systems Management

FLORIDA TECHNOLOGICAL UNIVERSITY

June 1972
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I. INTRODUCTION

The balance among the natural processes which have been responsible for the earth's atmosphere is being severely disturbed by natural instabilities. Further complicating these instabilities, and to a far greater extent, is the burning of fossil fuels, excessive release of fertilizers into the hydrological cycle, and the large scale changes of the environment by pesticides.

The distribution and occurrence of organic pesticides has come under close scrutiny as evidence of persistence has increased. The widespread use of pesticides has resulted in the development of resistant insect populations, changes in status for pest species and resurgence of treated populations, adverse effects on nontarget organisms, and pollution of the environment with persisting residues (1).

Biological magnification of chlorinated hydrocarbons through food chains has been widely reported by Wurster (2) and others. In an aqueous environment the chlorinated hydrocarbons, for example, may contaminate virtually all organisms at all levels of the trophic structure. Each species eats many organisms from the next lower trophic level and is, in turn, ingested by organisms of the next higher trophic level. The organisms are ingested, metabolized, and excreted, but much of the pesticide is retained. Great pesticide concentrations may occur at the higher trophic levels.
Effects of these pesticides are not limited to organisms at the top of the food chain. It has been reported by Wurster (3) that even a few ppb of DDT in the water can decrease photosynthesis in some marine phytoplankton.

According to Johnson (4) the oxygen in the atmosphere results partly from photodissociation of water vapor but mainly from photosynthesis in excess of decay. Unlike land plants, phytoplankton are unable to utilize to maximum efficiency high light intensities; therefore, net oxygen production per unit area is lower in the marine environment (4). However, what the natural oceanic phytoplankton environment lacks in average net production per unit area is compensated by its total size. It is now apparent that the oceans in their entirety are at least 2-3 times more productive than all the land masses together (5).

Many herbicides such as acylanilides, phenylureas, and triazines are potent inhibitors of photosynthesis. Wessels and Van der Veen (6) first discovered that monuron and other substituted phenylureas were powerful inhibitors of the Hill reaction. The inhibition of photosynthesis by pyriclor has been studied by Meikle (7). The inhibitory effect of herbicides on photo reactions has been reviewed by Moreland (8). Work done by Lawler and Rogers (9) suggested that in certain varieties of barley, DDT affected the light reaction in photosynthesis. Bollen (10) has also documented interactions between pesticides and soil microorganisms.

Many of the same relationships that exist between soil microorganisms and pesticides may exist between aquatic microorganisms and
pesticides. It is the purpose of this paper to review the inter-
actions of pesticides and phytoplankton.
II. OCCURRENCE AND PERSISTENCE OF PESTICIDES IN AQUATIC ENVIRONMENTS

Environmental contamination may be classified as intentional (direct) or unintentional (indirect) contamination. Table I reproduced from Ware and Roan (11) shows a further classification of sources of pesticides in the aquatic environment.

TABLE I

SOURCES OF PESTICIDES IN THE AQUATIC ENVIRONMENT

<table>
<thead>
<tr>
<th>A. Intentional introductions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control of objectional flora and/or fauna</td>
</tr>
<tr>
<td>2. Industrial wastes</td>
</tr>
<tr>
<td>a. Pesticide manufacturers and formulators</td>
</tr>
<tr>
<td>b. Food industry</td>
</tr>
<tr>
<td>c. Moth-proofing industry</td>
</tr>
<tr>
<td>3. Disposal of unused materials</td>
</tr>
<tr>
<td>4. On-site field cleaning of application, mixing, and dipping equipment</td>
</tr>
<tr>
<td>5. Disposal of commodities with excessive residues</td>
</tr>
<tr>
<td>6. Decontamination procedures</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Unintentional introductions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Drift from pesticide applications to control objectional flora or fauna</td>
</tr>
<tr>
<td>2. Secondary relocation from target area via natural wind and water erosion</td>
</tr>
<tr>
<td>3. Irrigation soil water from target areas</td>
</tr>
<tr>
<td>4. Accidents involving water-borne cargo</td>
</tr>
<tr>
<td>5. Application accidents involving missed targets or improper chemicals</td>
</tr>
</tbody>
</table>

A study of the persistence of pesticides in river waters by Eichelberger and Lichtenberg (12) was conducted to investigate the
stability of some common pesticides in raw river water. Also an
effort was made to identify the chemical or biological degradation
products. The following pesticides were studied at concentrations
of 10 µg/liter:

<table>
<thead>
<tr>
<th>Organochlorine</th>
<th>Organophosphorus</th>
<th>Carbamates</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHC</td>
<td>Parathion</td>
<td>Sevin</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>Azodrin</td>
<td>Zectran</td>
</tr>
<tr>
<td>Aldrin</td>
<td>Methyl parathion</td>
<td>Baygon</td>
</tr>
<tr>
<td>Heptachlor epoxide</td>
<td>Malathion</td>
<td>Mesurol</td>
</tr>
<tr>
<td>Telodrin</td>
<td>Ethion</td>
<td>Matalal</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>Trithion</td>
<td>Monuron</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>Fenthion</td>
<td>Fenuron</td>
</tr>
<tr>
<td>DDE</td>
<td>Dimethoate</td>
<td></td>
</tr>
<tr>
<td>DDT</td>
<td>Merphos</td>
<td></td>
</tr>
<tr>
<td>DDD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlordane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endrin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Of the organochlorine compounds tested no measurable degradation
or chemical change was observed for BHC, heptachlor epoxide, dieldrin,
DDE, DDT, DDD, and endrin. Aldrin underwent epoxidation to form dieldrin.
The only organophosphorus compound that remained stable was
azodrin. All the carbamate compounds were greatly changed after one
week, and all but Baygon were completely lost after eight weeks. At
eight weeks, only 5 percent of the Baygon remained, the rest having
been hydrolyzed to its phenol.

The results of a synoptic survey by the Public Health Service
of pesticide pollution in various U.S. rivers in 1964 reported by
Weaver, Gunnerson, Breidenbach, and Lichtenberg (13). The surveillance
was for nine chlorinated hydrocarbon pesticides: dieldrin, endrin,
DDT, DDE, DDD, aldrin, heptachlor, heptachlor epoxide and BHC.
In order of decreasing frequency of occurrence, dieldrin, endrin, DDT, and DDE were found in all major river basins. Heptachlor and aldrin were less abundant. DDD was detected at only one sampling station; presumptive evidence of BHC was observed at only one station, and there was no indication of heptachlor epoxide seen at any station. It was thought that the lack of detection of the latter three compounds may have been due to low sensitivity of the analytical procedures.

Research conducted by Woodwell, Wurster, and Isaacson (14) established that DDT residues (including DDT + DDE + DDD) in the soil of a salt marsh on the south shore of Long Island averaged more than 13 pounds per acre.

The annual use of DDT in the United States has declined in the past decade. However, research performed by Cox (15) revealed total concentrations of DDT residues in phytoplankton were approximately three times greater in later samples from the period of 1955 to 1969.

Karinen, Lamberton, Stewart and Terriere (16) studied the persistence of carbaryl in the marine estuarine environment. They concluded that at low temperatures and under conditions which prevented adsorption by mud, carbaryl was degraded slowly, persisting only for several weeks. One of the products of decomposition under these conditions was 1-naphthol which is as toxic as the carbaryl to some aquatic species.

It has been reported by Risebrough (17) that polychlorinated biphenyls, chlorinated hydrocarbons which are extensively used in industry and agriculture are now widely distributed in marine ecosystems.
Results indicated that wind transport can account for the observed distribution of DDT compounds in California waters and that the amount of chlorinated hydrocarbons entering the tropical Atlantic as fallout from the northeast Trades is comparable to that entering the sea from a major river system.

Further studies of the occurrence and persistence of pesticides have been done by Faust and Suffet (18) and Cope (19).
III. EFFECTS OF PESTICIDES ON PHOTOSYNTHESIS AND GROWTH

The effects of pesticides on phytoplankton may be varied and numerous. However, the only direct effects that are included in this review concern changes in growth rate and changes in specific metabolic rates caused by various pesticides.

Copper in the ionic form has been used in pest control for many years. The influence of copper on the rate of photosynthesis and growth of planktonic algae has been studied by Steemann Nielsen, Kamp-Nielsen, and Wium-Andersen (20), Steemann Nielsen and Kamp-Nielsen (21), and Steemann Nielsen and Wium-Andersen (22).

The first test objects were the freshwater green alga *Chlorella pyrenoidosa* and the marine diatom *Skeletonema costatus* Gr. After initial tests, it was realized that the two algae did not react to excess concentrations of copper in exactly the same way, and it was decided to first study the *Chlorella*. First studies concerned only the influence of copper on photosynthesis during the first 24 hours (20). They concurred with McBriam and Hassall as cited in (20) that it was extremely important whether the copper treatment was made under aerobic or anaerobic conditions.

The growth medium was a modification of Österlind's Medium B, and the carbon-14 technique (23) was used for measuring the rate of photosynthesis.
They found that when studying the influence of excess concentrations of copper on the rate of photosynthesis, many other factors were important. Factors cited were time, the light intensity at which the algae had grown previously, the light intensity during the experiments, the variation of the copper concentration, the cell concentration, and the composition of the experimental medium. As cited in (20), Greenfield had shown that the maximum deleterious effect of copper on the photosynthetic rate was obtained after 20 minutes. Greenfield had used a solution containing only CuSO₄ for treatment. When CuSO₄ was administered with all the other ions in the medium, a different time dependence was observed. For the first three hours no effect was observed; then the rate of photosynthesis gradually decreased. The maximum decrease was normally found after 24 hours if the concentration of copper was not too high. They also found that the decisive factor was not directly the duration of the copper treatment. It was necessary that photosynthesis occur after the copper treatment before the influence of copper could be observed. In the first two hours after copper treatment, no influence of copper on the rate of photosynthesis was found, but during the following two hours, the rate had decreased to one-third. However, if during the first two hours the algae had been in the dark, during the next two hours in the light no influence of copper was observed.

The rate of photosynthesis of algae which had been growing at a high light intensity was more affected by the addition of copper than was the rate of photosynthesis of algae which had been growing at a lower light intensity.
Experiments made in media with ordinary concentrations of other ions in addition to copper showed that apparently only the dark reactions were affected during the first 4-8 hours. However, the rate of photosynthesis was influenced both at a high and a low rate of illumination.

The factors of copper concentration, cell concentration, and composition of the medium were found to be highly interdependent. It was determined that the concentrations of cells was very important in regulating the influence of the copper. At pH 8.0 the influence of copper was much more pronounced than at pH 5.0.

It was surmised that the effect of deleterious concentrations of copper in balanced solutions was not due to a marked penetration of this ion into the plasma but to a binding to the cytoplasmic membrane whereby the cells became more or less unable to divide. The cells became saturated with assimilation products which had a depressant effect on the rate of photosynthesis. Also, other cations competed with copper for the active sites of the cell membrane.

Studies were then undertaken to determine the influence of deleterious concentrations of copper on the growth of Chlorella pyrenoidosa (21). Cell concentrations were determined.

It was determined that the direct influence of copper depended on the division stage of the algae. If the initial stages of cell division had taken place before addition of copper, the cell continued to divide.
The cell concentration was found to be very important in the influence of copper on growth. The effect of a certain copper concentration stopped at a certain concentration of the algae regardless of whether the experiment was started at this cell concentration or this concentration was attained during the experiment. This was thought to be due to the binding of copper by the organic matter of cell walls and slime envelopes.

Hydrogen ions competed with copper both when combining with the organic matter in the cell walls and when occupying the active sites of the cell membrane. This explained the fact that the influence of copper was only slight at pH 5.0 as compared with that at pH 8.0.

If the concentration of copper added to the medium was not too high, it was possible for the algae to re-establish a normal growth rate in the same medium. However, even if higher concentrations of copper were added, the algae were not killed. After being transferred to an ordinary medium the algae started to grow again.

Next, the influence of copper on photosynthesis and growth in the diatom Nitzschia palea was studied (22). The carbon-14 technique was used for measuring the rate of photosynthesis, and growth was measured by means of counting the cells in a haemocytometer.

The studies showed that the rate of photosynthesis decreased at all illumination intensities even in short-term experiments. This was in contrast with the observations with Chlorella where only the rate of photosynthesis at a high illumination rate was influenced. This indicated that copper most likely penetrated the cells of Nitzschia
palea. Simultaneously organic matter was lost by the cells. This implied that copper, even in balanced solutions, loosened the cell membranes in diatoms.

Although pretreatment with copper in the dark had no influence on the subsequent rate of photosynthesis in the light, organic matter was excreted in the dark immediately after the addition of the copper. This did not take place in the Chlorella.

The influence of low concentrations of copper on photosynthesis was about three times as high in Nitzschia palea as in Chlorella pyrenoidosa; however, growth seemed to be less influenced in the diatom. This was thought to be due to the excretion of organic matter by the diatom. Part of this matter was able to bind the copper and thus remove the toxic effect.

The above mentioned studies showed that copper in the ionic form is very poisonous for photosynthesis and growth of unicellular algae at concentrations of copper usually found in natural waters. According to Steemann Nielsen and Wium-Andersen (24), this indicates that copper is not ordinarily present in the ionic form but is complexed by organic matter. Complexed copper is not poisonous to algae.

Addition of CuSO₄ to lakes to suppress algae growth has been a common practice for many years. This treatment has been used especially to destroy water blooms of certain blue-green algae. As cited in (24), Rodhe described such a case from the Swedish lake Norrviken. By adding CuSO₄ to the lake, he increased the copper concentration from 9 to 24-28 µg/l. This prevented a water bloom
but permitted the more or less normal development of green algae. This indicated that the copper was not immediately complexed upon addition to the water and that some ionic copper was present immediately after the addition. It was concluded that in strongly contaminated waters, the possibility of copper acting as a poison must not be disregarded.

This paper (24) also included a warning that copper is a complication in the carbon-14 technique. Some manufacturers of carbon-14 ampoules have used ordinary distilled water, which often has a content of about 250 μg/l of copper. It was thought likely that some productivity measurements have been influenced.

Kamp-Nielsen (25) studied the effect of mercury ions on the photosynthesis and growth of Chlorella pyrenoidosa in Österlind's Medium B and compared these effects with those of copper. The carbon-14 technique was used for measuring the rate of photosynthesis (23). Growth experiments and determinations of cell numbers were made according to Steemann Nielsen and Kamp-Nielsen (21).

The aim of the investigation was to compare the influence of mercury with that of copper on photosynthesis. Factors considered were time, light intensity, concentration of mercury, chemical composition of the medium, cell concentration, and excretion of potassium.

A varying time dependence was observed when mercury was administered together with the other ions. It was necessary that photosynthesis occur after the mercury treatment before the influence of
mercury could be observed. A light duration of one hour gave the same reduction in the rate of photosynthesis as one hour in the dark followed by one hour in the light.

In short-term experiments, there was a marked reduction in photosynthesis at light saturation. In the longer term experiments there was also a marked decrease of photosynthesis at the low light intensities.

As in experiments with copper, the factors of mercury concentration, cell concentration and composition of the medium were highly interdependent. It was found that the maximum effect was smaller with mercury than with copper in experiments lasting four hours, but in 21-hour experiments the maximal effect of mercury was approaching that of copper. It was thought possible that a smaller number of "active sites" on the membrane were available for binding mercury than for binding copper. As time passed, mercury may have penetrated into the interior of the cells affecting both the light and the dark reactions of the photosynthesis.

In contrast to experiments with copper poisoning, potassium and sodium showed no counteracting effect on mercury toxicity.

Cell concentration affected the toxicity of mercury in a manner similar to that described for copper; that is, the influence of the mercury was regulated by the concentration of cells.

Preliminary investigations pointed to an excretion of potassium ions as the primary effect of both copper and mercury poisoning in a balanced medium.
In the growth experiments, it was found that mercury caused a delay in the normal exponential growth of Chlorella; however, the duration of this lag phase was only about half that noted caused by copper. In the mercury experiments, the length of the lag phase was highly dependent on the ratio of the mercury concentration and the initial cell concentration.

It was concluded that the physiological response of Chlorella to poisoning with mercury was similar to that of copper. However, there were important differences. Mercury acted at a lower and in a more narrow range of concentrations than did copper. The depressing effect of mercury was not counteracted by other cations such as potassium and sodium, and iron had only a slight effect. Cell division was stopped after addition of mercury and there was no accumulation of assimilation products. It was thought that there was a light-independent leakage in the cytoplasmic membrane leading to an outflow of potassium ions. In the growth experiments, the effect of mercury was more quickly counteracted than that of copper.

Harriss, White, and Macfarlane (26) studied the effects of several organomercurial fungicides on photosynthesis and growth of plankton. The species studied were a marine diatom, Nitzschia delicatissima Cleve isolated from waters near Puerto Rico and a naturally occurring phytoplankton population taken from a shallow freshwater lake in Florida. The dominant genera in the freshwater phytoplankton population included Merismopedia sp. (Agmenellum), Navicula sp., Crucigenia sp., Staurastrum sp., and Ankistrodesmus sp. These phyto-
plankton were exposed to concentrations of from 0 to 50 ppb of the following organomercurial compounds: phenylmercuric acetate (PMA or Phix), methylmercury dicyandiamide (Panogen), N-methyl-mercuric-1,2,3,6-tetrahydro-3,6-methano-3,4,5,6,7,7-hexachlorophthalimide (MEMMI), and diphenylmercury.

Utilizing $^{14}$C NaHCO$_3$, photosynthesis was measured by uptake of carbon-14. Cell counts were used to measure growth.

Diphenyl mercury was the least toxic. One ppb of the other three mercurials caused a significant reduction in photosynthesis and growth in cultures of N. delicatissima and the freshwater phytoplankton. At 50 ppb essentially all uptake of inorganic carbon was stopped, and the cell counts indicated complete inhibition of growth. With all the mercurial compounds, toxicity decreased with increasing cell concentration in lake samples. This was in the same manner as noted by Wurster (3) in tests of the chlorinated hydrocarbons.

Tests were made by Menzel, Anderson, and Randtke (27) to determine whether organisms isolated from different oceanic environments varied in response to three chlorinated insecticides, DDT, endrin, and dieldrin. The species tested were Skeletonema costatum, a coastal centric diatom isolated from Long Island Sound; the naked green flagellate Dunaliella tertiolecta, typical of tide pools and estuaries; the coccolithophorid Coccolithus Huxleyi and the centric diatom, Cyclotella nana, both from the Sargasso Sea.

The cultures were grown in half-strength medium "f" (28), an enriched sea water. Cell carbon concentrations were adjusted to 100,
250, and 500 μg of carbon per liter since this was considered to be within the range of naturally occurring carbon concentrations in surface oceanic waters. The insecticides were added in ethanol or acetone solvent. To each sample one μc of \(^{14}\)C Na\(_2\)CO\(_3\) was added. After 24 hours exposure to light, the samples were filtered and counted in a Geiger-Muller end-window counter. Long term effects of DDT and endrin on cell division were studied by counting cells each day for seven days.

Dunaliella was insensitive to all the insecticides tested and there was no effect on the rate of cell division measured over the seven-day period. The rate of carbon-14 uptake in Skeletonema and Coccolithus was reduced significantly at concentrations above 10 ppb by all three insecticides. The DDT at 100 ppb blocked cell division after two or three divisions in Skeletonema but had no apparent effect on Coccolithus. Endrin had little effect on the final concentration of Skeletonema although there was a reduced growth rate over the first five days. Reduced growth rates occurred throughout the experiment in Coccolithus. Cyclotella was inhibited by all three insecticides at concentrations above one ppb. Cell division was completely inhibited by dieldrin and endrin. Cells exposed to DDT divided but more slowly than the controls.

Solubilities of the three insecticides are all very low (29). Since some of the species responded to concentrations above solubility limits, it was thought that this might have been accommodated by the precipitation or adsorption to surfaces.
Wurster (3) studied the effects of DDT on photosynthesis by marine phytoplankton. Species representing four classes of phytoplankton were chosen. They were *Skeletonema costatum*, *Coccolithus huxleyi*, *Pyramimonas*, and *Peridinium trochoideum*. Also, water containing a typical neritic phytoplankton community dominated by various diatoms was studied. The cultures were grown in half-strength medium "f" (28). DDT was added to the cultures in an ethanol solution in the desired concentrations. Photosynthesis at various concentrations of DDT (one to 500 ppb) was measured by uptake of $^{14}C$ relative to uptake by controls. Cell concentrations were chosen to approximate those in nature. Carbon-14 uptake was inhibited in all the samples to which DDT had been added.

It was thought that the effect on phytoplankton occurred after absorption of the DDT by the cells because of the very low solubility of DDT in water and the higher solubility in the lipid of the cells. Therefore, an increased effect with decreased cell concentration caused by the additional amount of DDT per cell might be expected. One experiment was conducted in which the concentration of DDT was held constant at various cell concentrations. *Skeletonema costatum* was tested using 10 ppb DDT. Photosynthesis decreased to half that of the controls as the cell concentration was reduced by two orders of magnitude.

The effect of DDT on the growth rate of *Euglena gracilis* and the rate of DDT uptake by actively growing *Euglena* cultures was studied by deKoning and Mortimer (30).
Euglena gracilis were grown in a synthetic medium at room temperature. The DDT was added to the cultures as a solution in ethanol. When 10 μg of DDT was added to cultures in either 1.0 ml or 0.1 ml of ethanol, the effect was apparently related to the ethanol concentration. DDT suppressed growth of Euglena when added in 1.0 ml of ethanol, but when added with 0.1 ml of ethanol or no ethanol, DDT had no effect. After four days, the inhibited cultures recovered. The possibility of other environmental factors influencing the apparent toxic effects of DDT was mentioned.

Rate of uptake of DDT was measured by extracting aliquots of a growing culture with hexane, discarding the aqueous layer, and analyzing both cells and hexane. Uptake of DDT at zero time was very rapid. During exponential growth of the culture, cell division seemed to dilute the DDT concentrations in the cells. In the later stages when cell growth slowed, DDT uptake paralleled cell growth. DDT accumulation by Euglena did not have an observable effect on cell division.

Wheeler (31) demonstrated the absorption of 14C-dieldrin from an aqueous medium by Chlorella pyrenoidosa. The dieldrin was introduced into the algae samples in 1.0 ml of 95 percent ethanol. The algae cultures were maintained in Bristol's growth medium.

After the radiolabeled dieldrin had been introduced into the culture, the quantity of insecticide per cell increased for varying periods of time. A maximum per-cell level was reached within six to 24 hours. The insecticide became increasingly difficult to remove with time. Wheeler suggested that this might have indicated movement
of the dieldrin into the subcellular organelles. The rate of absorption of dieldrin was several orders of magnitude slower than that of DDT. This was probably due to the differing water solubilities of the two compounds and differing affinities for cellular lipid materials. Dieldrin is approximately 100 times more soluble in water than DDT (29). No attempt was made to relate absorption of the radio-labeled dieldrin to the rate of photosynthesis by the Chlorella.

The effects of lindane, dieldrin, and DDT on algal populations were investigated by Lazaroff and Moore (32). The algae were obtained from the surface waters of New York State. Samples were examined, counted and used to inoculate enrichment media. These consisted of controls with basal medium alone or basal medium with various levels of the insecticides. Algal growth was inhibited by lindane in the majority of samples. Similar effects were observed with dieldrin and DDT in a few cases. Comparison of the types of algae developing in the controls with those which eventually developed at the intermediate and high levels of pesticide suggested that selection for resistant types had occurred. Sensitive and resistant clones were isolated from the appropriate cultures, and quantitative experiments to determine the effects of lindane and DDT on growth and developmental cycles of selected unialgal cultures were performed. Results indicated that chlorinated insecticides, at levels as low as one ppm, may have selected for resistant cells within populations of unialgal cultures. The composition of algal populations in natural bodies of water could be influenced by these selective effects.
Vickers and Boyd (33) studied the effects of ten organic insecticides on photosynthesis of a naturally occurring, freshwater phytoplankton community. The community was shown to be composed almost exclusively of a single unknown species of a blue-green alga. They utilized the scintillation method for measuring carbon-14 uptake in photosynthesis as reported by Schindler (34). The insecticides tested were dieldrin, endrin, DDT, toxaphene, malathion, methyl paration, mirex, sevin, matacil, and furadan.

Acetone was used as a carrier of the pesticide. Known amounts of carbon-14 Na₂CO₃ and pesticides were added to a constant volume of sample, and the sample was incubated in situ in the pool from which the water was originally taken. The culture flasks were floated beneath the water surface in culture cribs similar to those described by Goldman and Wetzel (35).

At concentrations of 10 ppm, only sevin, matacil, furadan, and toxaphene consistently inhibited carbon-14 uptake under the test conditions. Further tests showed that concentrations of three to five ppm of sevin and matacil caused about a 50 percent reduction of carbon-14 uptake. Concentrations of 15 ppm of sevin and matacil almost completely inhibited carbon-14 uptake. Furadan at 15 ppm reduced carbon-14 uptake 60 percent. At the higher concentrations, chlorophyll bleaching was caused by the sevin, matacil, and furadan.

The chlorinated hydrocarbons DDT, aldrin, dieldrin, and mirex were tested at concentrations up to eight ppm. Only a slight reduc-
tion, about 10 percent in carbon-14 uptake was observed with DDT. Results of tests with mirex, aldrin, and dieldrin were similar.

Initial tests with malathion indicated that it is unlikely that it inhibits carbon-14 uptake to any substantial degree.

The effects of pesticides on low density populations of Scenedesmus quadricula data, a fresh water green alga, in terms of changes in growth and metabolism was studied by Stadnyk, Campbell, and Johnson (36).

The unialgal cultures were established in a culture mixture with a nutrient level approximating that of eutrophic lakes. The pesticides diuron, carbaryl, 2,4-D, DDT, dieldrin, toxaphene and diazinon were investigated. After treatment of the cultures with the pesticides, determinations were made of the cell number, biomass and carbon-14 uptake over a period of up to 10 days. Concentrations of 0.1 mg/l and 1.0 mg/l of pesticide in acetone were used.

Diuron was the most toxic to Scenedesmus. There was a drastic reduction in cell numbers, which was also reflected in a conspicuous reduction in biomass and a significant suppression of carbon assimilation. Carbaryl, in contrast to diuron, stimulated cell growth, and there was a consequent increase in carbon assimilation and an increase in cell biomass. Less severe changes were noted with 2,4-D. However, there was a decrease in cell density days four through eight. There was some indication of carbon fixation stimulation beginning on day six, and there was a small reduction in biomass by day 10.
DDT, dieldrin, and toxaphene all decreased cell numbers at all levels of treatment. Cell biomass was reduced 25 and 51 percent with DDT, 22 and 32 percent with dieldrin, but only three and four percent with toxaphene. In two-day cultures at both concentrations there was a 75 percent inhibition in carbon assimilation with DDT, while with toxaphene there was a 450 percent increase in carbon fixation. With dieldrin, an initial stimulation in carbon assimilation was observed at the two-day interval, followed by a significant inhibition at the six-day interval, with a final stimulation at day 10. Diazinon produced no effect on cell number, photosynthesis, or biomass over the 10-day study.

The effects of pesticides on the growth and survival of *Euglena gracilis* Z were studied by Moore (37). In the growth experiments, varying concentrations of malathion, parathion, nabam and vapam were added to the culture medium as aqueous solutions and thoroughly mixed before addition of the cells. The cultures were then grown under constant illumination from fluorescent lamps for five days. A second set of cultures was grown in the dark for nine days. Cell numbers were determined. Results were reported as percent inhibition, which was determined by comparing growth in the test cultures with growth in the control cultures.

Vapam appeared to inhibit growth more than the other pesticides tested. There was almost complete inhibition at 5 and 10 ppm. Nabam was also toxic at all concentrations tested. Nabam is hydrolyzed rapidly in aqueous solutions and was not detectable after 24 hours. At
the highest concentrations, malathion produced some inhibition, but there seemed to be little, if any, inhibition by parathion.

In the survival experiments, cells were plated on the growth medium solidified with 1.0 percent agar. The plates were wrapped in plastic film and incubated in the light for 14 days. Cells exposed to parathion and nabam were killed after one and two hours. Nabam appeared to be the most toxic. An attempt to offer protection against the killing action of nabam by chelation of divalent metal ions was not successful.

The gamma-isomer of hexachlorocyclohexane (gamma-BHC) can effectively control the rice stem borer, one of the major insect pests of rice. Raghu and MacRae (38) found that submerged field plots treated with BHC showed more abundant growth of algae than untreated plots. The marked stimulation of algal growth after addition of the insecticide was attributed to the elimination by the insecticide of small animals (Ostracoda) which feed on the algae. It was found that the most significant effect was upon the blue-green algae, which were much more abundant in the treated floodwaters. Populations of green algae and diatoms were apparently suppressed by an application of the insecticide.

The effects of pesticides at concentrations of one ppm on estuarine phytoplankton as tabulated by Ware and Roan (11) are shown in Table II.
<table>
<thead>
<tr>
<th>Herbicides</th>
<th>Decrease of carbon fixation during 4 hours exposure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monuron (urea)</td>
<td>94</td>
</tr>
<tr>
<td>Neburon (urea)</td>
<td>90</td>
</tr>
<tr>
<td>2,4,5-T, polyglycol butyl ether ester (phenoxy)</td>
<td>89</td>
</tr>
<tr>
<td>Diuron (urea)</td>
<td>87</td>
</tr>
<tr>
<td>Silvex (phenoxy)</td>
<td>78</td>
</tr>
<tr>
<td>DEF (defoliant)</td>
<td>75</td>
</tr>
<tr>
<td>Zytron (phosphate)</td>
<td>59</td>
</tr>
<tr>
<td>Paraquat</td>
<td>53</td>
</tr>
<tr>
<td>2,4-D, 2-ethylhexyl ester</td>
<td>49</td>
</tr>
<tr>
<td>Diquat</td>
<td>45</td>
</tr>
<tr>
<td>2,4-D, propyleneglycol butyl ether ester</td>
<td>44</td>
</tr>
<tr>
<td>Fenuron</td>
<td>41</td>
</tr>
<tr>
<td>Dacthal</td>
<td>37</td>
</tr>
<tr>
<td>Tillam</td>
<td>24</td>
</tr>
<tr>
<td>2,4-D, butoxyethanol ester</td>
<td>16</td>
</tr>
<tr>
<td>N-Serve</td>
<td>15</td>
</tr>
<tr>
<td>Hydram</td>
<td>9</td>
</tr>
<tr>
<td>Dalapon Na salt</td>
<td>0</td>
</tr>
<tr>
<td>Kurosal S1 (60 percent Silvex)</td>
<td>0</td>
</tr>
<tr>
<td>Tordon</td>
<td>0</td>
</tr>
<tr>
<td>2,4-D acid</td>
<td>0</td>
</tr>
<tr>
<td>2,4-D dimethylamine salt</td>
<td>0</td>
</tr>
<tr>
<td>2,4,5-T acid</td>
<td>0</td>
</tr>
<tr>
<td>MCP amine</td>
<td>0</td>
</tr>
<tr>
<td>Eptam</td>
<td>0</td>
</tr>
<tr>
<td>Vernam</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insecticides</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Kepone</td>
<td>95</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>94</td>
</tr>
<tr>
<td>Chlordane</td>
<td>94</td>
</tr>
<tr>
<td>Toxaphene</td>
<td>91</td>
</tr>
<tr>
<td>Ronnel</td>
<td>89</td>
</tr>
<tr>
<td>Thiodan</td>
<td>87</td>
</tr>
<tr>
<td>Methyl Trithion</td>
<td>86</td>
</tr>
</tbody>
</table>
It was thought that this, and the subsequent inhibition of respiration, could be due to the production of a toxic substance which damaged the dibromide added to Chlorella vulgaris in the light, caused a complete and irreversible inhibition of photosynthesis in less than 60 minutes. Turner, Stokes, and Gilmore (39) showed that 10⁻³ M diquat and irreversible inhibition of photosynthesis in less than 60 minutes.
functional membranes of the organelles. This substance could be hydrogen peroxide, produced by the oxidation of the free radical formed from diquat in the light in plastids.

Further studies on the effects of diquat in the light on chlorophyll bleaching and plastid structure by Stokes, Turner, and Markus (40) utilized electron micrographs to study the effects of diquat on the fine structure of the plastid and the cell. There was no visible damage to the plastid or to mitochondria after one hour of treatment in the light by $10^{-3}$ M diquat. Damage to both types of organelle was visible after 10 hours of diquat treatment in the light. They concluded that if the inhibition of photosynthesis and respiration was due to structural damage to the organelles, this must be during the first hour, at a level not detectable by electron microscopy.

Stokes and Turner (41) later studied the effects of diquat in the dark on the respiration rate of Chlorella vulgaris. They found that when the Chlorella samples were darkened for some hours and then treated with diquat in complete darkness, the rate of respiration was raised to higher levels. The effects of the stimulation was positively correlated with the concentration of diquat and the effects were measurable within the first 10 minutes of treatment.

Chemical designations and properties of pesticides may be found in the Pesticide Manual (42).
IV. DISCUSSION

It can be generalized that most of the pesticides studied are toxic to some of the microorganisms studied at some level. There was much variation in type and extent of response.

It is important to consider the rates of degradation of the pesticides as well as the end products. For example, some of the carbamate compounds, while very toxic to microorganisms, do not persist in the aqueous environment for very long. Some others such as DDT are known to be very persistent. Some of the degradation products may be toxic as is 1-naphthol, the degradation product of carbaryl.

There is great diversity in the species of both freshwater and marine environments. There is also great diversity in kinds and amounts of pesticides introduced into these environments. To extrapolate the adverse effects of certain pesticides or classes of pesticides on a relatively few species to include the whole ecosystem would not be valid. However, it is indicated that continued high usages of pesticides, worldwide, could conceivably affect primary production of oxygen at some future time. Therefore, it is very important that research be expanded in this area so that these interactions between pesticides and phytoplankton can be more precisely defined.
LIST OF REFERENCES


