Growth of Pure Cultures of Marine Phytoplankton in the Presence of Toxicants

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ABSTRACT

UKELES, RAVENNA (U.S. Bureau of Commercial Fisheries, Milford, Conn.). Growth of pure cultures of marine phytoplankton in the presence of toxicants. Appl. Microbiol. **10**:532–537. 1962.—The effects of 17 toxicants on the growth of five species of algae in pure culture were studied. The two species displaying the greatest sensitivity to the action of each of the compounds tested were *Monochrysis lutheri* and *Phaeodactylum tricornutum*, and the most resistant species was *Protococcus*. Of eight different classes of toxicants tested, substituted urea compounds and a mercuric compound were most effective in inhibiting growth of all algal species at the lowest concentrations.

The widespread use of toxic chemicals, as a means of pest control, aroused interest in the effects of these agents on all plants and animals. Since the introduction of 1,1,1,-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) as an insecticide, other highly toxic organic compounds have been synthesized and used in large quantities over land and aquatic areas. The broad effects of these practices on plants, wildlife, livestock, and public health are now recognized (Tarzwell, 1960).

Contamination of estuaries can occur through runoff from treated lands or from the application of herbicides and insecticides to marsh areas adjoining water basins. In addition to this accidental introduction of toxicants into estuarine waters, chemical methods are being developed for the control of predators, competitors, and fouling organisms on commercial shellfish beds. These methods include spreading of chemically treated sand over beds, chemical treatment of cultch, and use of chemically treated baits (Loosanoff, 1960). Data are needed on the effects on phytoplankton of the introduction of these compounds, since the fertility of natural waters and their ability to support fish or shellfish populations depend upon phytoplankton which constitute the base of the marine food chain (Provasoli and Pinter, 1953).

Shellfish hatchery practices being developed at Milford Laboratory include chemical methods for the control of bacterial and fungous infections of lamellibranch larvae (Davis et al., 1954; Guillard, 1959). To evaluate the efficacy of these methods, the tolerance to these chemicals of algal species utilized as foods by larvae should be investigated. Finally, there is a demand for information on chemicals that can destroy nuisance algae in water supplies (Palmer and Maloney, 1955) and aquatic weeds in estuarine and fresh-water environments (Maloney, 1958).

This study was undertaken to provide information on the tolerance of marine phytoplankton that are important foods for oyster and clam larvae (Davis and Guillard, 1958) to compounds classified as "toxicants" (DeOng, 1953). These compounds include bactericides, fungicides, herbicides, and insecticides.

MATERIALS AND METHODS

The test species included two motile flagellates, *Monochrysis lutheri* Droop [*Chrysophyceae* (Droop, 1953)] and *Dunaliella euchlora* Lerche [*Chlorophyceae* (Lerche, 1937)], and two nonmotile unicells, *Chlorella* sp. and *Protococcus* sp. [*Chlorophyceae* (isolated at Milford)]. The fifth species, *Phaeodactylum tricornutum* Bohlin [*Phaeodactylaceae* (Lewin, 1958)], has also been classified as a diatom under the name *Nitzschia closterium* Ehrenberg Wm. Smith forma *minutissima* (Allen and Nelson, 1910).

The basal medium (autoclaved at a pressure of 15 psi for 20 min) used for the culture of algae had the following composition: KH_2PO_4, 20 mg; thiamine hydrochloride, 0.2 mg; biotin, 2 μg; vitamin B_{12}, 2 μg; $NaNO_3$, 150 mg; ferric sequestrene (13% Fe), 10 mg; NH_4Cl, 50 mg; $CuSO_4 \cdot 5H_2O$, 0.019 mg; $ZnSO_4 \cdot 7H_2O$, 0.044 mg; $CoCl_2 \cdot 6H_2O$, 0.022 mg; $MnCl_2 \cdot 4H_2O$, 0.360 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.0126 mg; and sea water (salinity 22 to 28 parts per thousand) to make 1 liter of solution.

Toxicants were prepared as concentrated or saturated stock solutions in sterile sea water. At least 24 hr later, serial dilutions in sterile sea water were made, and 1-ml samples of the appropriate dilution were added to 9 ml of sterile basal medium. Final concentrations of toxicants were calculated on the basis of the per cent of active ingredient in aqueous solution and given as parts per million of the pure compound. The compounds tested, with commercial names, chemical composition, manufacturers, and common usages, are given in Table 1. The formulations and solubility in water, as given by the manufacturer or indicated in the literature, are also listed.

Although, to avoid alteration of the compound, stock solutions of toxicants were not sterilized, the high concen-

tration of the solutions and the subsequent aseptic handling of media and cultures presumably kept all experimental cultures essentially free of contaminants. Lindane was an exception to this method of preparation. To obtain high concentrations, this toxicant was added directly to the basal media and sterilized for 10 min.

Cultures were incubated in Pyrex screw-cap test tubes with the plastic cap liners removed. Each tube was inoculated with 0.5 ml of a bacteria-free unialgal stock culture maintained on the enriched sea-water basal medium. The initial number of cells in the test medium was about 150,000 per ml for four species and about

TABLE 1. *Toxicants used in study of marine phytoplankton*

Common name	Chemical name, formulation, and solubility in water	Manufacturer	Common use
	Organic phosphates		
TEPP	Tetraethylpyrophosphate; 40% + 60% related organic esters; very soluble in water	Niagara Chemical Division, Middleport, N.Y.	Insecticide
Dipterex	*o,o*-Dimethyl-1-hydroxy-2,2,2-trichloroethyl phosphonate; 50% soluble powder; 130,000 ppm	Chemagro, New York, N.Y.	Insecticide
	Phenolics		
Carbolic acid	Phenol; very soluble in water	Mallinckrodt Chemical Works, St. Louis, Mo.	Bactericide
Dowacide A	Sodium orthophenyl phenate·$4H_2O$; 97%; 1,220,000 ppm	Dow Chemical Co., Midland, Mich.	Bactericide
	Oils		
o-Dichlorobenzene	Orthodichlorobenzene; 130 ppm	Commercial Solvents Corp., Terra Haute, Ind.	Organic solvent
Niagara compound 3514	2-Chloro-1-nitropropane; 8,000 ppm	Niagara Chemical Division, Middleport, N.Y.	Experimental compound
	Iodophor		
PVP	Polyvinylpyrrolidone-iodine	Antara Chemical Co., New York, N.Y.	Bactericide-fungicide
	Chlorinated hydrocarbons		
DDT	1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)-ethane, wettable powder; 0.0002 ppm (0.2 ppm colloidal solution)	Naugatuck Chemical Division, Naugatuck, Conn.	Insecticide
Lindane	γ-1,2,3,4,5,6-Hexachlorocyclohexane; 10 ppm	California Spray-Chemical Corp., Richmond, Calif.	Insecticide
Toxaphene	Mixture of polychloro dicyclic terpenes with chlorinated camphene predominate, 60% emulsion concentrate; 1.5 ppm	Hercules Powder Co., Wilmington, Del.	Insecticide
	Carbamates		
Nabam	Disodium ethylene bis-dithiocarbamate hexahydrate; very soluble in water	Niagara Chemical Division, Middleport, N.Y.	Fungicide
Sevin	1-Naphthyl-*N*-methylcarbamate; 95%; 1,000 ppm	Union Carbide Chemicals Co., New York, N.Y.	Insecticide
	Organic mercury		
Lignasan	Ethyl mercury phosphate; 6.25%; very soluble in water	E. I. du Pont de Nemours and Co., Inc., Wilmington, Del.	Bactericide-fungicide
	Urea derivatives		
Fenuron	3-Phenyl-1,1-dimethyl urea; 2,900 ppm	E. I. du Pont de Nemours and Co., Inc., Wilmington, Del.	Herbicide
Monuron	3-(*p*-Chlorophenyl)-1,1-dimethyl urea; 230 ppm	E. I. du Pont de Nemours and Co., Inc., Wilmington, Del.	Herbicide
Diuron	3-(3,4-Dichlorophenyl)-1,1-dimethyl urea; 42 ppm	E. I. du Pont de Nemours and Co., Inc., Wilmington, Del.	Herbicide
Neburon	1-*n*-Butyl-3-(3,4-dichlorophenyl)-1-methyl urea; 4.8 ppm	E. I. du Pont de Nemours and Co., Inc., Wilmington, Del.	Herbicide

250,000 per ml for *P. tricornutum*. Loosely capped culture tubes were incubated in a culture room with a continuous light intensity of about 500 ft-c and a temperature of 20.5 ± 1 C. After an incubation period of 10 days, during which tubes were frequently rotated, growth in experimental cultures was visually compared to that of controls and recorded on a scale of 0 to +. After finding the range of concentrations that had no effect and those that appeared to be lethal, sublethal toxicant concentrations were investigated. In these experiments, optical density measurements of growth were made with a Beckman DU spectrophotometer at a wavelength of 530 mμ. To test for the presence of viable organisms where there was no visible growth, a 1-ml sample was transferred to the basal medium with no added toxicant and observed for growth after 10 to 14 days.

RESULTS

The growth response in the presence of toxicants is given for each species in Table 2. The results are expressed as

TABLE 2. *Effect of toxicants on growth of phytoplankton*[a]

Toxicant	Concn	*Protococcus* sp.	*Chlorella* sp.	*Dunaliella euchlora*	*Phaeodactylum tricornutum*	*Monochrysis lutheri*	Toxicant	Concn	*Protococcus* sp.	*Chlorella* sp.	*Dunaliella euchlora*	*Phaeodactylum tricornutum*	*Monochrysis lutheri*
	ppm							*ppm*					
Dipterex	10	0.75	0.84	0.59	1.00	1.00	Nabam	0.1	0.93	0.80	0.27	0.82	0.48
	50	0.65	0.70	0.54	0.85	0.55		1	0.53	0.63	0.00	0.00[b]	0.00
	100	0.54	0.68	0.42	0.39	0.00		10	0.00	0.00	0.00	0.00	0.00
	500	0.29	0.00	0.00	0.00	0.00							
	1,000	0.00	0.00	0.00	0.00	0.00	Lindane	1	1.40	0.88	1.00	1.00	0.86
								2.5	1.40	0.73	1.00	0.82	1.00
TEPP	100	0.62	0.65	0.90	0.58	0.83		5	0.75	0.57	1.00	0.30	1.00
	300	0.17	0.27	0.49	0.25	0.38		7.5	0.75	0.36	0.73	0.00[b]	0.00[b]
	500	0.00[b]	0.00[b]	0.00	0.00	0.00		9	1.00	0.33	0.60	0.00[b]	0.00[b]
	1,000	0.00[b]	0.00	0.00	0.00	0.00							
							Toxa-	0.01	1.00	0.70	0.90	0.54	0.00[c]
Phenol	10	0.77	0.83	0.86	1.20	0.78	phene	0.04	0.77	0.70	0.55	0.00[b]	0.00
	100	0.71	0.84	0.51	0.00[b]	0.00[b]		0.07	0.80	0.00[b]	0.53	0.00[b]	0.00
	300	0.59	0.63	0.47	0.00[b]	0.00[b]		0.15	0.00	0.00	0.00	0.00	0.00
	500	0.00	0.00	0.00	0.00	0.00							
							DDT	0.02	1.00	1.00	0.83	1.00	0.57
Dowacide A	10	0.90	0.90	0.70	0.82	0.68		0.04	1.00	1.00	0.85	0.91	0.57
	25	0.74	0.90	0.81	0.48	0.22		0.60[d]	0.50	1.00	0.74	0.91	0.28
	50	0.76	0.74	0.52	0.22	0.00							
	100	0.00[b]	0.00[b]	0.00[b]	0.00	0.00	Lignasan	0.0006	0.86	0.78	0.64	0.55	1.00
	1,000	0.00	0.00	0.00	0.00	0.00		0.006	0.00	0.00	0.31	0.17	0.00
								0.06	0.00	0.00	0.00	0.00	0.00
Orthodichloro-	1.3	0.71	0.82	0.71	0.74	1.00							
benzene	7.6	0.80	0.95	0.90	0.80	0.65	Fenuron	0.03	0.93	0.95	1.00	1.00	1.00
	13	0.00[b]	0.00[b]	0.00[b]	0.00[b]	0.00[b]		0.29	0.72	0.82	0.46	0.82	0.67
	130	0.00	0.00	0.00	0.00	0.00		2.90	0.33	0.00[b]	0.00[b]	0.00[b]	0.00
								29	0.00	0.00	0.00	0.00	0.00
Chloronitro-	0.8	0.94	0.90	0.81	1.00	1.00							
propane	8	0.72	0.25	0.83	0.72	0.69	Neburon	0.04	0.41	0.31	0.47	0.10	0.00[e]
	80	0.00	0.00	0.00	0.00	0.00		0.20	0.00[b]	0.00	0.00[b]	0.00[b]	0.00
								0.40	0.00	0.00	0.00	0.00	0.00
PVP-iodine	10	0.87	0.90	1.00	1.00	0.96							
	20	0.88	0.65	1.00	0.90	0.71	Monuron	0.001	0.90	0.30	1.00	0.65	0.83
	50	0.80	0.30	0.00[b]	0.00[b]	0.61		0.02	0.00[b]	0.00[b]	0.00[b]	0.00[b]	0.00
	100	0.59	0.27	0.00	0.00[b]	0.00		0.20	0.00	0.00	0.00	0.00	0.00
Sevin	0.1	0.80	0.90	0.90	0.00[b]	0.87	Diuron	0.00002	0.52	1.00	0.80	0.83	0.00
	1	0.74	0.80	0.65	0.00[b]	0.00[b]		0.0004	0.22	0.62	0.44	0.79	0.00
	10	0.00[b]	0.00	0.00[b]	0.00	0.00		0.004	0.00	0.34	0.00[b]	0.00	0.00
	100	0.00	0.00	0.00	0.00	0.00		0.04	0.00	0.00[b]	0.00[b]	0.00	0.00
								0.40	0.00	0.00	0.00	0.00	0.00

[a] The optical density of the control cultures was as follows: *Protococcus* sp., 0.407, *Chlorella* sp., 0.570, *Dunaliella euchlora*, 0.630, *Phaeodactylum tricornutum*, 0.600, *Monochrysis lutheri*, 0.314. Growth expressed as the ratio of optical density with toxicant/optical density with no toxicant.

[b] No growth but organisms were viable.

[c] Lower concentrations gave the following results: 0.0015 ppm, 0.00; 0.00015 ppm, 0.00; 0.000015 ppm, 0.78.

[d] A concentration of 1.00 ppm as an acetone solution did not inhibit growth of any organism.

[e] Lower concentrations gave the following results: 0.004 ppm, 0.00; 0.0004 ppm, 0.62.

the ratio of optical density of growth in the presence of toxicants to optical density in the basal medium with no added toxicants. Results are presented for both resistant and sensitive species, illustrating the range of concentrations (i) that did not significantly inhibit growth; (ii) that appreciably decreased the rate of growth or inhibited cell division entirely, although viable organisms were present; and (iii) that appeared to be lethal since there was no growth, and organisms did not appear to be viable under the conditions of the test.

The organic phosphates, *o,o*-1-hydroxy-2,2,2-trichloroethyl phosphonate (Dipterex) and tetraethylpyrophosphate (TEPP), were the toxicants tolerated in the highest concentrations by all species. Dipterex at 50 ppm caused less than 50% inhibition of each species, and the concentration of 100 ppm was lethal to only one species. There was also no appreciable inhibition of growth with TEPP at 100 ppm. Growth of all species was inhibited by 500 ppm of either compound, but the most resistant species, *Protococcus*, was still viable in 500 ppm of each compound, and *Chlorella* was viable in 500 ppm of TEPP.

The resistance to phenolic compounds was also high. Although phenol at 300 ppm, and Dowacide A at 50 ppm, caused some reduction in the rate of growth of three chlorophytes, only growth of *P. tricornutum* and *M. lutheri* was inhibited at these concentrations. Phenol was lethal at 500 ppm to all species, and Dowacide A caused inhibition of growth of the three chlorophytes and death of the two less resistant species at 100 ppm.

Orthodichlorobenzene and chloronitropropane had no significant effect on growth at 8 ppm, except that *Chlorella* seemed sensitive to this concentration of chloronitropropane. Lethal or inhibitory concentrations were reached at about ten times this amount. The iodophor compound polyvinylpyrrolidone-iodine also seemed to have little effect at a concentration of 10 ppm, but became toxic as concentrations approached 100 ppm.

The carbamate compounds 1-naphthyl-*N*-methylcarbamate (Sevin) and nabam may be considered next in a general order of increasing toxicity. Although 1 ppm of Sevin did not seriously influence the growth of the three most resistant species, growth of *P. tricornutum* and *M. lutheri* was completely suppressed. Concentrations of 10 ppm were lethal to three species. Nabam allowed growth of two of the resistant species at 1 ppm, but 10 ppm appeared lethal to all organisms.

The response to the chlorinated hydrocarbons varied within the group. Lindane was inhibitory to the two most sensitive species at 7.5 ppm, but other species were little affected, and even the two most sensitive species were viable.

1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)-ethane (DDT) in colloidal solution at 0.6 ppm did not affect growth, with the exception of *M. lutheri*. There was some inhibition of growth of *M. lutheri*, but this may not be of significance since a solution of DDT in acetone at 1 ppm was not lethal. Toxaphene, a mixture of chlorinated camphenes, was the most toxic of this group of chlorinated hydrocarbons. A concentration of 0.01 ppm of toxaphene was tolerated by four species but 0.15 ppm was lethal to all organisms. *M. lutheri* showed a striking sensitivity to this compound and as little as 0.001 ppm was lethal.

The compounds with the greatest degree of toxicity were Lignasan, and the substituted ureas. Lignasan was lethal to all species at 0.06 ppm, and 0.0006 ppm was the only concentration tested that did not cause drastic inhibition of growth. The least toxic of the substituted urea compounds, fenuron, was tolerated at 0.29 ppm, but 1-*n*-butyl-3-(3,4-dichlorophenyl)-1-methylurea (neburon) at 0.04 ppm caused more than 50% reduction in growth of three species and had a still more drastic effect on the two most sensitive species. 3-(3,4-Dichlorophenyl)-1,1-diphenylurea (diuron) and 3-(*p*-chlorophenyl)-1,1-dimethylurea (monuron) were the most toxic compounds in this group. Monuron had little effect at 0.001 ppm, and diuron at 0.0004 ppm, although even the latter was lethal to *M. lutheri*.

There were some fairly consistent differences in the sensitivity of the five species of phytoplankton to toxicants. The naked, free-swimming chrysomonad, *M. lutheri*, was in every case most susceptible to the action of the toxicants and, in the case of toxaphene, the difference was very marked. The other brown pigmented species, *P. tricornutum*, often showed tolerances similar to *M. lutheri*. In contrast, the green pigmented chlorophytes, both motile and nonmotile, were always the most resistant and, of this group, *Protococcus* was usually the most resistant.

Even the most sensitive species, *M. lutheri*, could withstand exposure to sublethal doses of a toxicant for long periods of time. Cultures of *M. lutheri* that were incubated for as long as 6 months in 1 ppm Sevin, 1 ppm lindane, and 0.0006 ppm Lignasan were still capable of reproducing when transferred to the basal media without toxicant.

Microscopic observations showed concentrations of toxicants that were lethal frequently caused cell lysis and, in some cases, resulted in morphological abnormalities of the cell.

Discussion

For practical purposes, bio-assays of toxicants are valuable in indicating concentrations that are harmful and concentrations that appear to have no effect and, therefore, may be considered "safe." Experiments with pure cultures under defined conditions yield specific information important in the understanding and managing of ecological situations. However, field studies should be conducted before laboratory-determined "safe" concentrations can be extrapolated for use in natural environments. It seems reasonable to assume that, under certain ecological conditions particularly favorable to the growth of algae, or where the ambient concentrations of toxicant fluctuate widely, some phytoplankton species may survive in an otherwise inhibitory or lethal concentration of toxicant. For example, copper ore, at concentrations that ap-

peared promising in laboratory experiments (Marvin, Lanford, and Wheeler, 1961), was not useful in the treatment of natural waters to suppress harmful flagellate blooms. On the other hand, even sublethal concentrations of toxic chemicals in isolated pools may drastically alter natural populations.

Some toxicants used for predator control in shellfish hatcheries may be "safe" at higher concentrations than under natural conditions. In hatchery procedures, plankton food obtained from another source is being continuously supplied to lamellibranch cultures. In this case, where massive concentrations are being utilized as foods, compounds used for the control of predators may be "safe" for feeding at concentrations that cause inhibition of algal growth. Polyvinylpyrrolidone-iodine, for example, has been found useful in hatchery trays to control predators at 100 and 250 ppm, yet these concentrations are lethal to some algal species and inhibit growth of others. In nature, however, blooms are important as a source of food and because of this an inhibition of phytoplankton blooms and long-term effects on the natural flora and fauna must be considered. Although many toxicants have a low water solubility, the introduction of even traces of certain metals, toxic ions, and organic molecules may influence the basic ecology of a particular environmental niche and result in a change in the complex relationship between endogenous phytoplankton populations, phytoplankton blooms, and phytoplankton feeders (Provasoli, 1958).

It must be emphasized that the effects of environmental factors on the stability and potency of toxicants present a problem in the evaluation and correlation of results. For example, DDT is virtually insoluble in water, and its effectiveness varies with the type of solvent used. Although, in general, the toxicity to crops of DDT in aqueous solution is low, an oil solution may be phytotoxic (Brown, 1951). DDT is also affected by the type of experimental container, being more toxic in a glass than an enamel, aluminum, or plastic vessel (Cole, 1961). Dipterex and TEPP hydrolyze in solution within a few days (Albert, 1960), and lindane decays 70% in sea water after 30 days (Werner and Waldichuk, 1961). In our experiments, it has been found that a freshly prepared solution of nabam is more toxic than an aged solution. The instability of toxicants may be advantageous in certain applications and may account for the resistance to the action of the organic phosphates. Resistance to the action of a toxicant may also be due to permeability barriers, and, therefore, modifications of environmental factors influencing permeability could produce different results.

The commercial use of a toxicant is based on selective toxicity, i.e., the chemical must be more toxic to pest species than to the valuable species. This selectivity of toxicants may be judiciously employed in the commercial application of toxicants to remove predators so that the desirable forms of phytoplankton are still maintained in aquatic environments; or, conversely, it would seem possible to use certain toxicants to remove undesirable algal species and leave other populations unharmed. For example, organic phosphorus and chlorinated hydrocarbon compounds have been used to control an arthropod invasion of an open-tank culture of *Chlorella* and *Chlamydomonas* (Loosanoff, Hanks, and Ganaros, 1957). It was found that 1 ppm of Dipterex, lindane, DDT, or TEPP did not affect the culture, although copepod invaders were sucessfully eliminated. Similarly, compounds of Lignasan or monuron may be used in very low concentrations to rid areas of troublesome algal species. In fact, it has been reported that Lignasan was used to clear fish ponds of the toxic flagellate *Prymnesium parvum* (Sarig and Lahav, 1961). Monuron has also been used successfully to eradicate filamentous algae from a fresh-water pond, with no adverse effects on fish (Maloney, 1958). These two classes of compounds may also be useful as algicides in marine environments.

It is important to examine the basis of the selective action of various toxicants so that a logical use of these chemicals may be pursued with a minimal amount of damaging side effects. The effect to TEPP has been traced to an anticholinesterase action (Brown, 1951), and, as a nerve poison, it follows that its phytotoxicity should be low. Even 100 ppm of TEPP had no effect on the phytoplankton tested. Dipterex, another organic phosphorus compound, at 100 ppm was lethal to only the most sensitive organism.

The chlorinated hydrocarbons, lindane and DDT, are also reported to have a nerve action and stimulate nerve fibers to convulsions (Albert, 1960). Although 0.05 ppm of lindane is a 100% lethal dose for *Anopheles* larvae (Spector, 1956) and 0.2 ppm killed crabs (Loosanoff et al., *unpublished data*), 5 ppm had no effect on any of the five species of phytoplankton tested. It has previously been reported that protozoa are resistant to the action of DDT (Richards and Cutkomp, 1946), and DDT has also been found nontoxic to six species of fresh-water algae at 2 ppm (Palmer and Maloney, 1955).

Certain toxicants act through chromosomal effects, and it is to be expected that these compounds would be more general protoplasmic poisons. Lignasan induces multipolar spindles in some plants (Spector, 1956), and carbamate compounds have also been reported to suppress cytoplasmic division (Spector, 1956), which may account for the low "safe" concentrations of these compounds.

The asymmetric substituted urea compounds are important herbicides and there is recent evidence that these compounds act by interfering with the utilization of light and CO_2 in the photosynthetic process (Craft, 1961). With the group of substituted urea compounds, the parent derivative, fenuron, a phenyl urea, was the least toxic of the series, and the chlorinated phenyl ureas were most toxic.

Determination of the capacity of algae to develop strains that are resistant to the action of some of these toxicant compounds would be of interest. Because of their rapid

growth and ease of handling under controlled conditions, protozoan organisms have been a valuable assay tool. The algal species used here may be suitable for bio-assays of certain toxicants, particularly herbicides.

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