Fate of Dieldrin in Selected Species of Marine Algae

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Certain chlorinated hydrocarbon pesticides, such as dieldrin, are known for their low biodegradability and high accumulation in living organisms. This accumulation has been shown to occur along various food chains including those in the marine environment (ROBINSON et al., 1967). Marine phytoplankton as primary producers occupy the first level of build-up in these pelagic food chains; therefore, it becomes important to study the interaction between algae and dieldrin. MENZEL et al. (1970) reported that photosynthesis of certain marine phytoplankton was affected by dieldrin to varying degrees. Concentrations of dieldrin as high as 1000 ppb showed no effect on photosynthesis in certain species, whereas other species were sensitive to dieldrin concentrations above 1 ppb. An increase in the concentration above 100 ppb, the solubility limit for dieldrin in water (EDWARDS, 1966), caused an increased inhibition of ^{14}C-uptake. In a study dealing with the absorption of dieldrin by Chlorella pyrenoidosa, WHEELER (1970) observed that 42% of the initially applied dieldrin, which represented maximum uptake, was absorbed by the cells within 6 to 24 hours. With time, dieldrin became progressively more difficult to extract, indicating movement, perhaps into subcellular organelles.

The dieldrin accumulated by the algae may undergo transformation by the organism. It is possible that the pesticide metabolites may be more persistent and almost as toxic as or more toxic than the parent compound. Metabolites of dieldrin, photodieldrin and metabolites G and F, were reported by BATTERTON et al. (1971) to be as toxic as dieldrin to cells of Anacystis nidulans. The purpose of this study was to investigate the accumulation and metabolism of dieldrin by species representing different taxonomic divisions of marine phytoplankton.

MATERIALS AND METHODS

The six species representing four algal divisions used in this study were Skeletonema costatum, Cyclotella nana (Bacillariophyta); Amphidinium carteri (Pyrrophyta); Olisthodiscus

Bulletin of Environmental Contamination & Toxicology,
Vol. 9, No. 2, © 1973 by Springer-Verlag New York Inc.

luteus, (Xanthophyta); Isochrysis galbana (Chrysophyta); and Tetraselmis chuii, (Euglenophyta). They were obtained from the University of Rhode Island, Narragansett Marine Laboratory. The algae were grown axenically in Guillard and Ryther's medium 'f' (GUILLARD and RYTHER, 1962) with the modification that glycyl-glycine (5mM) was added as a buffer (MCLACLAN, 1964), and the pH was adjusted to 7.5. The cultures were grown on a reciprocating shaker under controlled environmental conditions (14 hours of light per day at 500 foot-candles and a temperature of 23°C ± 1°).

A stock solution of ^{14}C-dieldrin (specific activity 168 μc/mg) in acetone was added to the cell suspension to give an acetone concentration not exceeding 0.4%. For the uptake experiments, the cultures were agitated on a New Brunswick rotary shaker at approximately 300 rpm to keep the cells in suspension. The flasks were stoppered during the experiment to minimize codistillation losses of dieldrin. After exposure to dieldrin, the cells were concentrated by centrifuging the cultures at 17,300 x g for ten minutes. The cells were then transferred to clean tubes and re-centrifuged as before. The washed cells were suspended in scintillation fluid and counted for ^{14}C in a nuclear Chicago liquid scintillation counter. Tubes containing medium plus dieldrin but no cells were subjected to the same washing procedure as the cells and the counts from the blank tubes were subtracted from the counts present in the cells. The amount of radioactivity remaining in the medium was determined by counting 1-ml aliquots of dieldrin solution in scintillation fluid containing Triton X (Packard Instrument Company, Downers Grove, Illinois).

To study the metabolism of ^{14}C-dieldrin by the algae, the cells were incubated with ^{14}C-dieldrin for two weeks. At the end of this period, the cells were separated from the medium by centrifugation and extracted with acetone. The extracts were analyzed by both gas-liquid chromatography and thin-layer chromatography. For TLC analysis, the extracts were concentrated by evaporation under a stream of nitrogen and spotted on silica gel-coated glass plates. The plates were developed 15 cm with a solvent mixture consisting of diethyl ether and n-hexane (9:1) (MATSUMURA and BOUSH, 1967). The plates were scanned for radioactivity on a Nuclear Chicago Actigraph. Samples for GLC analysis were dried over sodium sulfate, and the acetone was removed by evaporation. They were made up to appropriate volumes with petroleum ether and then analyzed on a Microtek Model MT-220 gas chromatograph equipped with a ^{63}Ni electron capture detector. Operating temperatures for the various components were as follows: inlet 225°C; oven 186°C; detector 283°C. The chromatographic column, an 800 cm x 4 mm (i.d.) glass U-tube, was packed with an equal weight mixture of 1.5%

OV-17 and 1.95% QF-1 on 80 - 100 mesh size Supelcoport (Supelco, Inc., Bellefonte, Pennsylvania). The carrier gas was nitrogen with a flow rate of 50 ml/min.

RESULTS AND DISCUSSION

Preliminary studies were done to study the kinetics of dieldrin uptake by various algae. Figure 1 shows the uptake of ^{14}C-dieldrin by Cyclotella (0.06 mg/ml, dry weight) from a solution containing 1 ppb dieldrin. Maximum uptake of the dieldrin occurred within the first hour of its addition to the culture, and no significant changes in the level of absorbed dieldrin were observed after this time. A similar time course for dieldrin absorption was noticed in the other species. In subsequent studies, dieldrin uptake was measured after two hours of incubation to permit its maximum absorption by the cells.

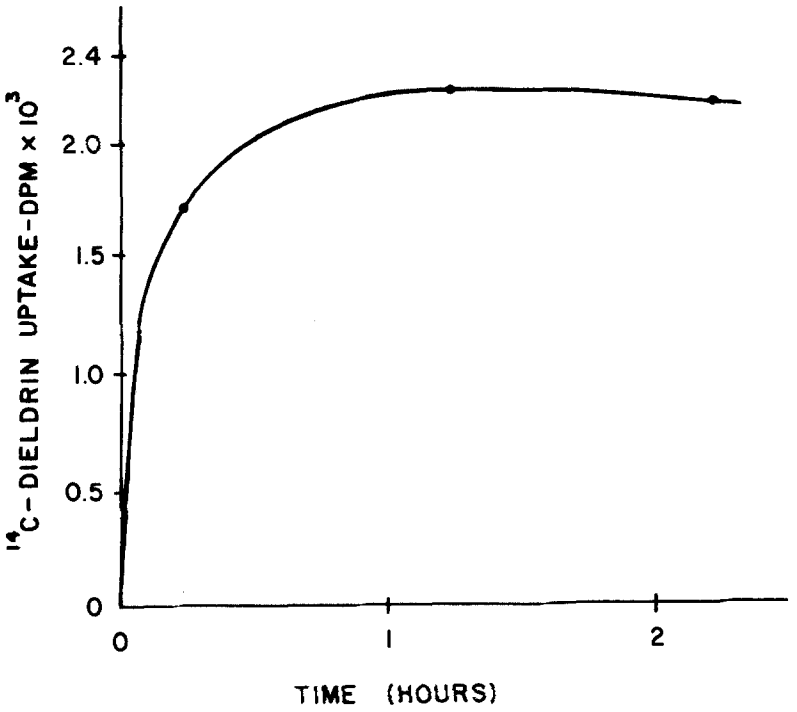

Figure 1. Time course for uptake of dieldrin by Cyclotella nana

Some of the factors that may affect the accumulation of dieldrin by algae were studied. When cells of Tetraselmis and Amphidinium were incubated with various concentrations of dieldrin, the uptake of dieldrin increased linearly with an increase in dieldrin concentration up to 1000 ppb (Figure 2). With an increasing concentration of dieldrin, the linear uptake continued above its maximum solubility limit in water, 100 ppb (EDWARDS, 1966), which indicated, as suggested by MENZEL et al., (1970) that the algae were incorporating dieldrin as small particles or that saturation of dieldrin was maintained while the algae concentrated the dieldrin from solution.

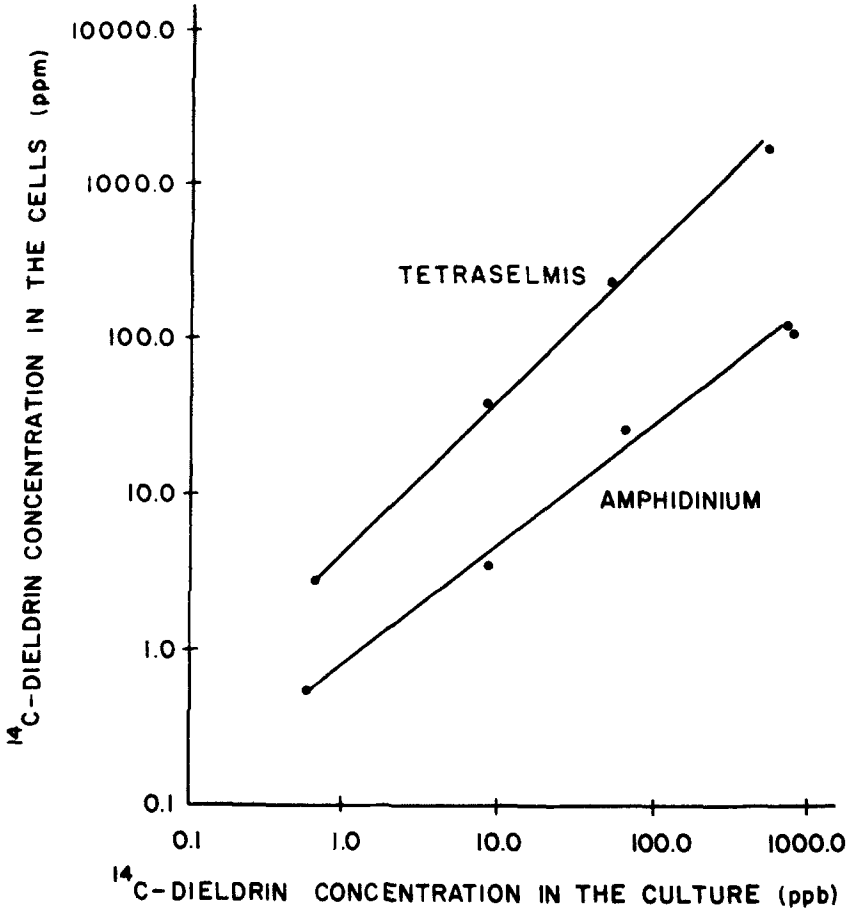

Figure 2. Uptake of dieldrin by Tetraselmis chuii and Amphidinium carteri as a function of dieldrin concentration.

When dieldrin uptake was measured at cell densities ranging from 0.1 to 1.3 mg/10 ml (dry weight) using a constant initial dieldrin concentration of 1.7 ppb, it was observed that the uptake of dieldrin by the algae increased with an increase in the amount of cells in the medium (Figure 3). The relationship was found to be linear in the cultures of Amphidinium, but non-linear in Skeletonema, Tetraselmis, Cyclotella, Isochrysis, and Olisthodiscus.

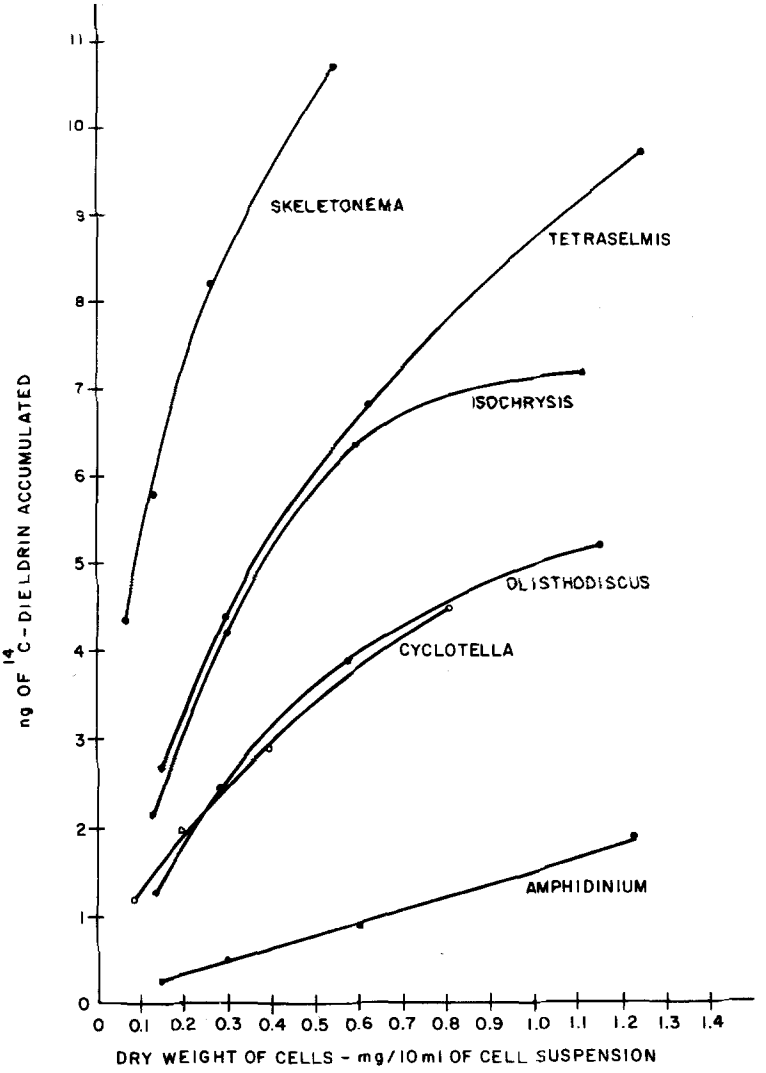

Figure 3. Uptake of ^{14}C-dieldrin by different algae as a function of cell concentration.

The various species differed significantly in their ability to remove dieldrin from the medium. When the algae were incubated each at a cell density of 0.2 mg/10 ml (dry weight) with 1.7 ppb of dieldrin, the percentage of the initial amount of the pesticide removed by the algae two hours after treatment was: Skeletonema 42.0%, Tetraselmis 16.0%, Isochrysis 15.5%, Olisthodiscus 13.0%, Cyclotella 13.0%, and Amphidinium 2.3%. An attempt was made to determine if the differential uptake of dieldrin by the different species could be explained by differences in the number of cells per unit mass of cells. Table 1 shows that there was no correlation between dieldrin accumulation and the number of cells per unit mass.

Our findings showed that various species of algae accumulated dieldrin to levels many times higher than the original concentration in the medium (Table 1). However, the degree of concentration of dieldrin by these algae was considerably less than that observed with DDT (RICE and SIKKA, 1972). Differences in the water solubility of the two pesticides and the differences in their affinities for cellular lipids may explain the differential accumulation of DDT and dieldrin by the algae. Because of the greater water solubility of dieldrin than of DDT, the former would be expected to have a lesser affinity for cell-water interfaces, thereby resulting in a lower uptake by the cells.

TLC analysis of the cell extracts and culture media two weeks after incubation with ^{14}C-dieldrin indicated the presence of one ^{14}C-compound having an R_f of 0.37. This compound co-chromatographed with authentic dieldrin, suggesting that all the radioactivity in the cells and media was present in the form of unchanged dieldrin. Gas-liquid chromatographic analysis of the cell extracts and media confirmed the results of the TLC analysis.

ACKNOWLEDGMENT

This research was supported by contract No. N00014-72-C-0010 from the Office of Naval Research. The algae were kindly supplied by Dr. Paul Hargraves, University of Rhode Island.

TABLE 1

UPTAKE OF DIELDRIN BY SIX SPECIES OF ALGAE

Species	Number of Cells/ml Culture	Uptake of Dieldrin After Two Hours[a]	
		ng dieldrin/ mg cell dry wt.	Concentration Factor Cell Conc. of Dieldrin/ Culture Conc. of Dieldrin
Skeletonema costatum	1.19×10^6	27.00	15,882
Tetraselmis chuii	0.134×10^6	14.60	8,588
Isochrysis galbana	1.40×10^6	14.00	8,238
Olisthodiscus luteus	0.115×10^6	8.33	4,900
Cyclotella nana	0.83×10^6	8.17	4,810
Amphidinium carteri	0.178×10^6	1.67	982

[a] One ml of cell suspension in each treatment contained 0.03 mg dry wt. of cells and 1.7 ppb dieldrin.

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