Experimental Absorption of Dieldrin by *Chlorella*

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The absorption of ^{14}C-dieldrin from aqueous medium by actively growing *Chlorella* has been demonstrated. Dieldrin penetrated algal cells rapidly and a maximum per-cell level was reached within 6 to 24 hr after introduction of the insecticide. The changes in distribution of radioactivity in various extracts of the experimental system with time are presented. The cellular portion contained increasing amounts of labeled insecticide with time, and more exhaustive extraction was required for its removal from these tissues; no metabolites were detected.

As a part of the overall pollution problem, the effects of residual pesticides upon the environment are being carefully studied. It would seem appropriate, therefore, to examine the interactions occurring between insecticides and algae.

The more persistent compounds, even as their use diminishes, will be present in the environment for many years. The role played by algae in maintaining the "balance of nature" is substantial. Food production, carbon dioxide fixation, and oxygen production are but a few of the many functions these microfloral populations perform. Thus, any interactions between these organisms and insecticides are important.

Raghu and MacRae (1967) discussed the effect of lindane on the micro-flora of submerged rice soils after the application of 5, 6, and 50 kg of lindane per hectare. The insecticide caused no detrimental effect on total algal populations and, in fact, resulted in a marked stimulation of their growth. Woodwell *et al.* (1967) demonstrated that DDT in the soil of a Long Island salt marsh caused the contamination of the lowest forms of plant and animal life, resulting in the eventual contamination of higher animals.

Recently, a physiological effect of DDT on algae has been reported. Wurster (1968) showed that aqueous solutions containing DDT substantially reduced the photosynthetic rate of four algal species as measured by the uptake of $^{14}CO_2$. The cells were incubated in a solution containing DDT for 20–24 hr before exposure to ^{14}C.

Södergren (1968) demonstrated the uptake of DDT by *Chlorella* sp. Cells grown in medium containing ^{14}C-DDT absorbed the radio-labeled insecticide within 15 sec. Furthermore, cells exposed for 1 min contained at least as much DDT as cells exposed for 25 hr. The quantity of insecticide absorbed increased with increasing DDT concentration in the medium. Södergren proposed that the mechanism of uptake was passive since the rate of uptake of mercuric-chloride-killed cells was equal to that of living cells.

The purpose of this report is to demonstrate the absorption of dieldrin by *Chlorella pyrenoidosa*.

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MATERIALS AND METHODS

Chlorella pyrenoidosa Chick, Indiana Algae Culture Collection Number 251, were maintained in Bristol's Growth Medium (Starr, 1964). The cells in 150 ml of medium were grown in 500-ml Erlenmeyer flasks which were fitted with two-hole rubber stoppers. A mixture of 5% CO_2–95% air was piped through a glass tube into the culture flasks at a rate ranging from 10–20 cc per min and a trap consisting of gas chromatographic column packing (5% DC 200) was attached to the exit port (Wheeler, 1969). The surface of the rubber stopper exposed to the interior of the culture flask was coated with a 1 to 2 mm thickness of paraffin. The culture flasks were maintained on an Eberback Corp. reciprocating shaker (95 excursions per min).

The photoperiod was 16 hr light and 8 hr dark, using 40 watt Gro-Lux tubes; the temperature was maintained at 25° C ± 1°.

^{14}C-dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,-6,7,8,8a - octahydro - 1,4 - endo - exo - 5,8 - dimethanonaphthalene) was supplied by the Shell Development Corp., Modesto, Calif., and was purified by preparative thin-layer chromatography prior to use. The specific activity of the purified insecticide was 51.1 μc per mg. Dieldrin was introduced to the algae in 1.0 ml of 95% ethanol.

Culture flasks were set up to contain 150 ml of sterile Bristol's medium. The flasks were inoculated with *Chlorella* from an actively growing stock culture four days prior to the start of the uptake study.

The number of cells per ml was determined by observing the absorbance (at 670 nm) of the suspension each time a sample was taken. A graph relating the absorbance to numbers of cells had been prepared previously.

Duplicate 10-ml samples were taken at 0, 6, 12, 24, 36, 48, and 72 hr after the introduction of the radiolabeled dieldrin.

When each sample was taken, a standardized procedure was followed:

The cells were centrifuged for 10 min at 15,000 rpm (30,000 × G at tube bottom) at 4° C in an IEC Model B-20 refrigerated centrifuge and the medium was poured off.

The cells were thoroughly mixed with 5 ml of acetone for approximately 30 sec, and centrifuged; the acetone wash was decanted.

The cells were then extracted with 3 ml of boiling 95%

ethanol and centrifuged. The alcohol was poured off, and the extraction was repeated. The ethanol extracts were combined.

The cells were then extracted with 3 ml of boiling chloroform-methanol (1 to 1) and centrifuged. The solvents were decanted, and the extraction was repeated. The chloroform-methanol extracts were combined.

The cellular tissue remaining after extraction was digested in 1 *M* hyamine hydroxide [*p*-(diisobutyl-cresoxy-ethoxy-ethyl) dimethylbenzylammonium hydroxide] in methanol (Packard Instrument Co.).

The trap on the effluent port of the culture flask was replaced with a fresh one. Accumulated dieldrin was eluted with 5 ml of acetone.

An aliquot of each sample (*i.e.*, medium, acetone, ethanol extract, chloroform-methanol extract, hyamine hydroxide, trap eluant) was counted in a Packard Tricarb liquid scintillation spectrometer Model 3375. The samples were corrected for quenching through the use of the Automatic External Standardization feature of the instrument. Two scintillation fluids were used: Seven grams per liter of PPO (2,5-diphenyloxazole) (Packard Instrument Co., Inc.), 0.3 g per liter dimethyl POPOP [1,4 bis-2(4methyl-5-phenyloxazolyl) benzene] (Packard Instrument Co., Inc.), 100 g per liter of naphthalene (Eastman Organic Chemicals), *p*-dioxane (Spetroquality, Matheson, Coleman and Bell) to make 1.0 liter. Five grams per liter of PPO, 0.10 g per liter of dimethyl POPOP, redistilled toluene to make 1.0 liter.

The dioxane-base fluid was used for counting aqueous samples and hyamine hydroxide solutions of cellular residues. The toluene-base solution was used for all other samples.

All extracts were scrutinized by thin-layer chromatography and autoradiography. Aluminum oxide plates were spotted with aliquots from each extract and developed in 5% acetone–95% *n*-heptane. The migration of radioactivity was compared with the migration of authentic dieldrin.

RESULTS

Figure 1 shows cellular growth, radioactivity associated with cells, and radioactivity in the growth medium. The results from two experiments which contained substantially different initial levels of ^{14}C-dieldrin per cell are presented (Figure 1A and 1B).

In Experiment A (Figure 1A), the dieldrin concentration in the medium initially was 0.3 ppm and the quantity of pesticide per cell was 12.8×10^{-12} g.

The zero-hour sample did show some radioactivity, caused at least in part by a 15 min lag between the time the dieldrin was added and the time the medium was removed from the cells after centrifugation.

Figure 1B graphically presents the data from Experiment B. The significant variable introduced here was that the dieldrin level was 7×10^{-12} g per cell initially, approximately one-half that of Experiment A. Again, 0.3 ppm was added to the culture.

One major difference between the two experiments is that it took substantially longer for the level of radioactivity associated with the cells in "B" to reach a stage of equilibrium (*i.e.*, relatively constant ratio of cell numbers and cellular radioactivity) than it did for "A." A second difference may be seen at the 72-hr sampling. Although the number of cells continued to increase, the associated radioactivity did not.

Figure 1 C shows the quantity of radioactivity in the medium (on a per-ml basis) from the two experiments at each sampling period.

Table I. Dieldrin in Algae at Each Time Period[a]

Time (hr)	Dieldrin Per Cell ($g \times 10^{14}$) (Expt. A)[b]	(Expt. B)[c]
0	0.20	0.097
6	0.98	0.49
12	1.14	1.02
24	1.11	1.30
36	1.20	1.26
48	1.34	1.44
72	1.50	1.08

[a] Calculated from total radioactivity found in the ethanol, chloroform-methanol, and hyamine hydroxide extracts. [b] 12.8×10^{-12} g dieldrin per cell initially. [c] 7×10^{-12} g dieldrin per cell initially.

Table I gives the actual quantities of dieldrin on a per-cell basis as calculated from the specific activity of the radioactive dieldrin, the total dpm in a 10-ml sample of cells (ethanol, chloroform-methanol, and hyamine hydroxide extracts), and the number of cells in a 10-ml sample. The difference in time required to reach a state of "equilibrium" between cells and associated radioactivity is illustrated here by the fact that the per-cell level is higher, earlier, in Experiment A than in Experiment B. In both experiments, the maximum level of dieldrin on a per-cell basis was in the range of 1.3 to 1.5×10^{-14} g.

Table II shows a typical distribution of the radioactivity at each sampling time. Extracts of the cells possessed increasing percentages of the labeled material with time. The zero-hour ethanol extract contained only 2% of the radioactivity, whereas by 6 and 12 hr it had increased to 11 and 25%, respectively. The ethanol-fraction radioactivity continued to increase, finally reaching 45% by the end of the three-day period.

Prior to the 24-hr sample, no radioactivity was detected in either the chloroform-methanol extracts or the hyamine hydroxide moiety. Between the 24 and 48 hr samples, the chloroform-methanol extracts always possessed more than the hyamine hydroxide portions, but during these time periods these fractions accounted individually for less than 0.5% of the total radioactivity. At 72 hr, however, these two extracts totaled 3.4%.

The paraffin covering on the rubber stopper possessed 224,000 dpm, approximately 5% of the total initial radioactive material introduced to the system.

Ninety-five percent of the labeled material was recovered in the various fractions examined. Recovery of radioactivity was excellent, since a small excess of cells and medium remained after the 72-hr sample had been taken.

Thin-layer chromatography and autoradiography confirmed that all of the detectable radioactivity in each experimental fraction was dieldrin.

DISCUSSION

The demonstration of dieldrin absorption by *Chlorella* is based upon the following reasoning. First, the quantity of radiolabeled dieldrin per cell increased, for varying periods of time, after the insecticide had been introduced to the culture. Secondly, the label became more difficult to extract with time, indicating movement, perhaps into subcellular organelles. These two facts strongly support that dieldrin is absorbed by *Chlorella*.

The rate of dieldrin absorption is several orders of magnitude slower than that of DDT (Södergren, 1968). An explanation for this is probably related to the water solu-

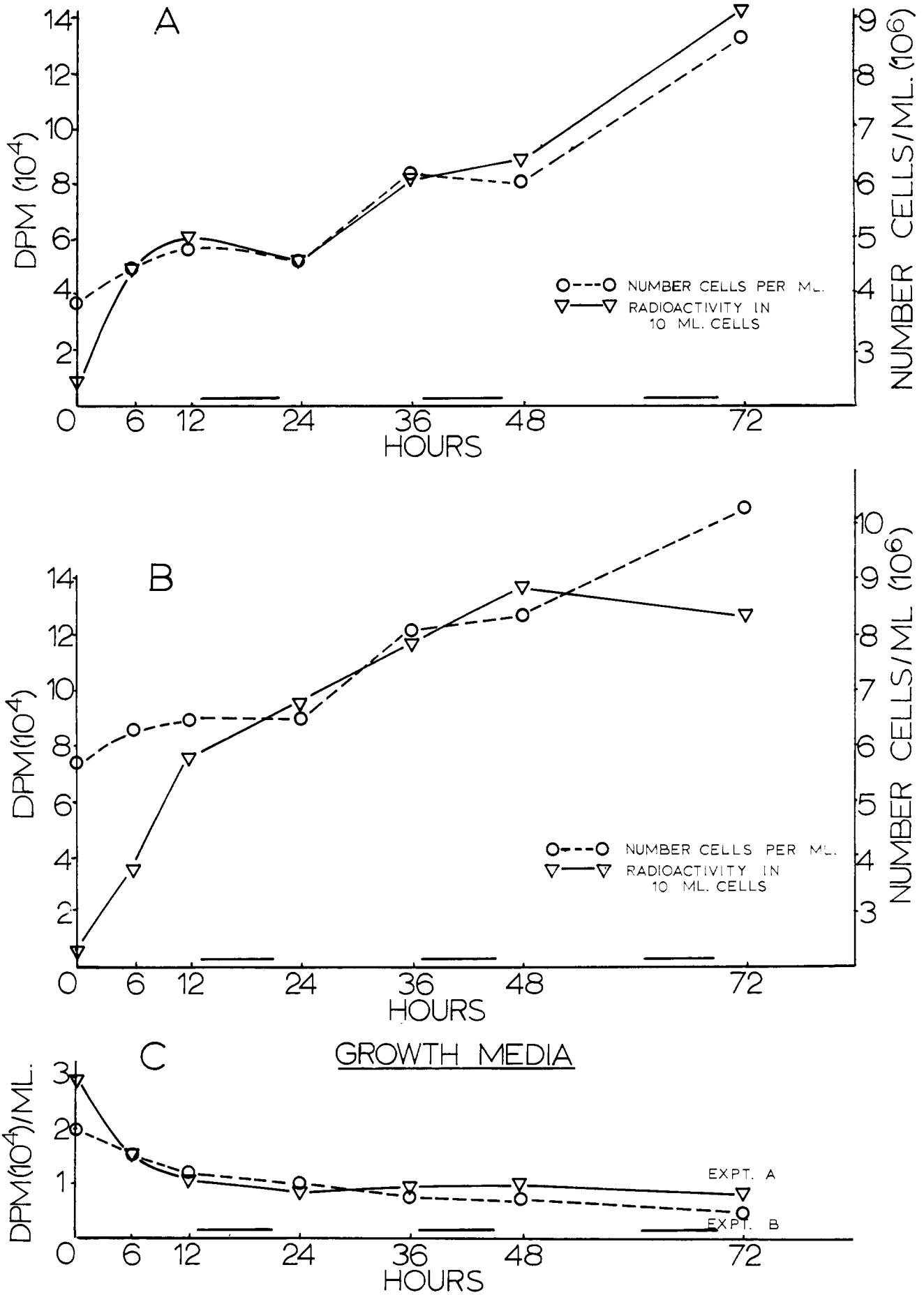

Figure 1. Cellular growth, radioactivity associated with cells (A, B), and media radioactivity (C) *vs.* time exposed to ^{14}C-dieldrin (periods of darkness are designated by a horizontal line above the abscissa)

Table II. Distribution of Radioactivity of Each Sampling Period[a] (expressed as a percentage of the total dpm)

Time (Hr)	Medium[b]	Cells[b]				Traps	Total Radioactivity[e] (dpm)
		Acetone Rinse	Ethanol Extract	C—M[c]	H—H[d]		
0	64.5	33.5	2.0	0	0	...	313,300
6	44.8	44.1	10.9	0	0	0.51	330,700
12	40.5	30.6	25.2	0	0	3.56	301,000
24	33.0	33.6	29.8	tr[f]	tr	3.29	315,400
36	28.1	26.1	39.5	tr	tr	6.07	291,300
48	23.9	25.6	45.1	tr	tr	4.71	296,900
72	18.0	22.7	45.4	2.6	0.8	10.5	225,500

[a] These illustrative data are from experiment B. Averages of duplicate samples. [c] C—M Chloroform-methanol extract. [d] H—H Tissue residue solubilized in hyamine hydroxide. [e] Total radioactivity in each 10-ml sample. [f] Tr measurable radioactivity but less than 0.5%.

bilities of the two compounds and their affinities for cellular lipid materials. Dieldrin is approximately 100 times more soluble in water (250 ppb; Richardson and Miller, 1960) than is DDT (3.4 ppb) (Gunther *et al.* 1968). DDT would have, therefore, a far greater affinity for cell-water interfaces and subsequent cellular absorption than would dieldrin.

This lesser affinity of dieldrin for nonpolar materials could be significant in relation to the environmental exposure of higher animals to insecticides through biological concentration.

It would be of interest to determine the absorptive capabilities of *Chlorella* for the two compounds. Södergren (1968) exposed cells to a maximum concentration of 0.6 ppb DDT and reported the absorption of 0.38 μg by 1.9×10^5 cells. This is 0.20×10^{-14} g of DDT per cell. The data in Table I (above) show levels of 1.3 to 1.5×10^{-14} g of dieldrin per cell after exposure to 300 ppb. The quantity absorbed is certainly related, to some degree, to the level of exposure. Further work is in progress on per-cell insecticide levels, resulting from various chemical concentrations.

The per-cell level of 1.3 to 1.5×10^{-14} g dieldrin appears to represent a maximum under these experimental conditions. This is based on the assumption that a twofold increase in dieldrin per-cell should have caused a substantially higher cellular level unless a maximum level were reached. Since no significant per-cell differences were detected, it is assumed such a maximum was reached.

The potential utilization of algae as pesticide scavengers might be investigated. It one assumes there were 1.4×10^{-14} g dieldrin per cell and 9×10^6 cells per ml, then 150 ml of cells absorbed 42% of the original 45×10^{-6} g insecticide added to the culture.

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