

vapor from as little as 90 ng per Petri dish of compound 1 reduced egg hatch by 50%. Eggs were generally more sensitive than prepupae-pupae, although the ratios of prepupae-pupae ED<sub>50</sub>'s to the egg ED<sub>50</sub>'s varied from 0.36 to 78, a difference of 217-fold. However, five of the eight compounds had ratios in close agreement.

In summary, many similarities were found in the juvenile hormone activities of selected terpenoid ethers and esters on three unrelated species of Coleoptera; however, some striking differences were also observed. Thus, in making generalizations about structure-activity relations in regard to juvenile hormone activity, reliance upon one assay species as being representative of other insects in the same order is inadequate. The high ovicidal activity of compounds 1-3 and 5 on *E. varivestis* would not have been predicted by the results on either *T. molitor* or *L. decimlineata* pupae. Future studies are necessary to determine if the virtual inactivity of the polyalkoxy ethers (compounds 1-6) on *L. decimlineata* was caused by a sensitivity spectrum at the active site(s) distinct from the other species tested or whether other differences in the compound-organism interactions were of primary importance. Both the topical and vapor assays indicated a general correlation between the ovicidal and juvenile hormone activity on *E. varivestis*, again with some notable exceptions. There was an overall coefficient of +0.72 (significant at the 5% level) between the lowest dose causing a 90% or greater reduction in egg hatch and a 90% or greater reduction in adult emergence of *E. varivestis*. This finding supports the conclusion of Slama (1971) from results on the bug, *Pyrrhocoris apterus*, that a general corre-

lation exists between the ovicidal and morphogenetic potency of a given juvenile hormone compound. It remains to be determined to what extent the deviations from this generalization are caused by differences between the stages in penetrability or metabolic stability rather than by differences in potency at the site(s) of action.

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## Uptake and Metabolism of DDT by Six Species of Marine Algae

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The uptake and metabolism of DDT by six species of marine phytoplankton was studied. Uptake of DDT by the various species increased linearly with an increasing concentration of DDT but nonlinearly with an increasing concentration of cells. The species with the higher numbers of cells per unit of mass took up greater amounts of DDT per unit weight than species with lower numbers of cells. All species concentrated DDT to levels many times higher than the original concentration in the medium. DDT was accumu-

lated by the six species in the following order: *Skeletonema costatum* > *Cyclotella nana* > *Isochrysis galbana* > *Olisthodiscus luteus* > *Amphidinium carteri* > *Tetraselmis chuii*. All of the species converted small amounts of DDT to DDE. After 24 days of treatment, the amount of DDE produced by different species ranged from 0.03 to 12% of the total DDT in the cells. Maximum conversion of DDT to DDE was observed in cultures of *Tetraselmis*.

In recent years there has been a great deal of concern about the effects of organochlorine pesticides on the environment. Biological magnification of chlorinated hydrocarbon pesticides like 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane (DDT) has been well documented and is of great concern. Since algae represent the first link in the pelagic food chain, it becomes important to examine the interaction between DDT and algae. It has been shown that some species of algae possess a marked capacity to concentrate DDT from the surrounding medium (Gregory *et al.*, 1969; Kiel and Priester, 1969; Sodergren, 1968; Vance and Drummond, 1969; Ware *et al.*, 1968). The degree of DDT accumulation varied with the concentration

of the pesticide in the medium and with the algal species. Following an exposure of algae to a medium containing 0.1-5 ppm of DDT, the concentration of the pesticide in the organisms was 200-1000 times that in the medium. On the other hand, Cox (1970a) reported a concentration factor of 25,000-80,000 in three species of marine algae which were exposed to DDT concentrations ranging from 1 to 3 parts per trillion (ppt). Working in the laboratory with *Chlorella*, Sodergren (1968) found uptake to be rapid (15 sec) and permanent. He concluded that the uptake was passive since killed cells absorbed DDT as effectively as live cells. DeKoning and Mortimer (1971) found that uptake by *Euglena* was very rapid and that DDT was held without being desorbed.

Knowledge concerning the accumulation and metabolism of DDT by marine algae is limited. The DDT accu-

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mulated 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. Therefore, the identification and measurement of residue levels of DDT and its metabolites in the algae are necessary in order to fully understand the effects of this pesticide on the marine environment.

The present study was undertaken to investigate the accumulation and metabolism of DDT by species representing different taxonomic divisions of marine phytoplankton. The objective was to find out if these organisms differed in their capacity to accumulate and metabolize DDT.

#### MATERIALS AND METHODS

The six species representing four algal divisions used in this study were *Skeletonema costatum*, *Cyclotella nana* (Bacillariophyta), *Amphidinium carteri* (Pyrrophyta), *Olisthodiscus luteus* (Xanthophyta), *Isochrysis galbana* (Chrysophyta), and *Tetraselmis chuii* (Euglenophyta). They were obtained from the University of Rhode Island, Narragansett Marine Laboratory. The cultures were bacteria free and grown axenically on Guillard and Ryther's (1962) medium "f" with the modification that glycylglycine (5 mM) was added as a buffer (McLachlan, 1964), and the pH was adjusted to 7.5. The cultures were grown on a reciprocating shaker under controlled environmental conditions, 14 hr of light per day at 500 ft-candles and a temperature of  $23 \pm 1^\circ$ .

A stock solution of [ $^{12}\text{C}$ ]- or [ $^{14}\text{C}$ ]DDT (specific activity of 67  $\mu\text{Ci}/\text{mg}$ ) in acetone was added to the cell suspension to give an acetone concentration not exceeding 0.3%. Preliminary experiments indicated that this concentration of acetone had no adverse effect on the growth of the algae included in the study.

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 period of the experiment to minimize the loss of DDT due to codistillation. After exposure to DDT, duplicate 10-ml aliquots of cell suspension (0.1–1.1 mg cell dry weight) were removed and centrifuged at  $17,300 \times g$  for 10 min; the medium was decanted and the cells were transferred to clean tubes. The cells were washed once and suspended in scintillation fluid (dioxane base) for counting in a Nuclear Chicago liquid scintillation counter. Medium containing DDT but no cells was subjected to the same washing procedure and the counts from these 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 using a liquid scintillation fluid containing Triton-X (Packard Instrument Co., Downers Grove, Ill.).

To study the metabolism of [ $^{12}\text{C}$ ]- and [ $^{14}\text{C}$ ]DDT, gas-liquid chromatography and thin-layer chromatography were used, respectively. DDT was added at a concentration of 1 ppm to 100 ml of cell suspension. For glc analysis, 10-ml aliquots of cell suspension (0.1–7.0 mg cell dry weight) were removed after various periods of incubation. The cells were separated from the medium by centrifugation and the pellet was extracted twice with acetone. Using this procedure, more than 90% of the radioactivity was recovered from cells incubated with [ $^{14}\text{C}$ ]DDT. In order to minimize losses of DDT during removal of acetone from the cell extract, the acetone was allowed to evaporate at room temperature to a point just short of dryness, and then the crude extract containing the DDT and metabolites was quickly dissolved in petroleum ether. It was observed that the loss of DDT under these conditions averaged about 10%. Because of the small amount of cell material used for extraction, it was not necessary to subject the cell extract to cleanup procedures. The petro-

leum ether extract was adjusted to an appropriate volume, dried over anhydrous sodium sulfate, and injected into a Micro-Tek 220 gas chromatograph equipped with a nickel-63 high-temperature electron capture detector. A 6 ft  $\times$  0.25 in. i.d. borosilicate glass column packed with 4% SE-30 and 6% QF-1 coated on 80–100 mesh Supelcoport (Supelco, Inc., Bellefonte, Pa.) was used to separate the various components. Prior to use, the column was conditioned according to the method described by Thompson (1971) using Silyl 8 (Pierce Chemical Co., Rockford, Ill.) and a standard pesticide mixture. The conditions for separation of DDT and the petroleum ether-soluble metabolites of DDT were as follows: 72 ml/min of  $\text{N}_2$  carrier gas flow; column, detector, and injector temperatures of 207, 281, and 232 $^\circ$ , respectively; and sufficient detector sensitivity to give 10% full-scale recorder response with 10 pg of DDE. The respective amounts of DDT and DDE were quantitated using peak area measurements which were compared with the peak areas of standard DDT and DDE.

For tlc analysis, cells from 100-ml aliquots of culture (1 to 70 mg cell dry weight) were extracted with acetone as described before; the acetone extract was concentrated by evaporation under a stream of nitrogen and spotted on silica gel-coated glass plates. The plates were developed with *n*-pentane (Mendoza *et al.*, 1968). By developing each plate twice with pentane for 15 cm, it was possible to effectively separate DDE ( $R_f$  0.43) from DDT ( $R_f$  0.28). Authentic labeled compounds were cochromatographed for comparison with the unknowns. The plates were scanned for radioactivity on a Nuclear Chicago Actigraph.

#### RESULTS AND DISCUSSION

Preliminary experiments were performed to determine the ability of the algae to remove DDT from a solution containing 1 ppb of the pesticide. The cell density in each case was adjusted to give an absorption reading of approximately 0.1 at 750 nm. DDT was rapidly taken up from the solution by all the species studied. About 90% of the maximum DDT uptake by a given species occurred within 2 hr after exposure to DDT. Figure 1 shows the kinetics for DDT uptake by *Tetraselmis chuii*. A similar time course of DDT uptake was observed in the other species. The total amount of DDT accumulated by the

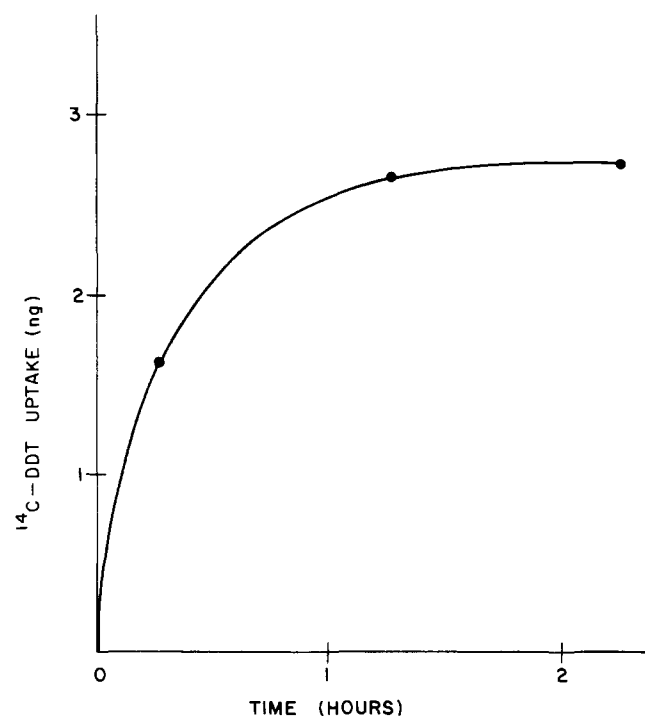


Figure 1. Time course for uptake of DDT by *Tetraselmis chuii*.

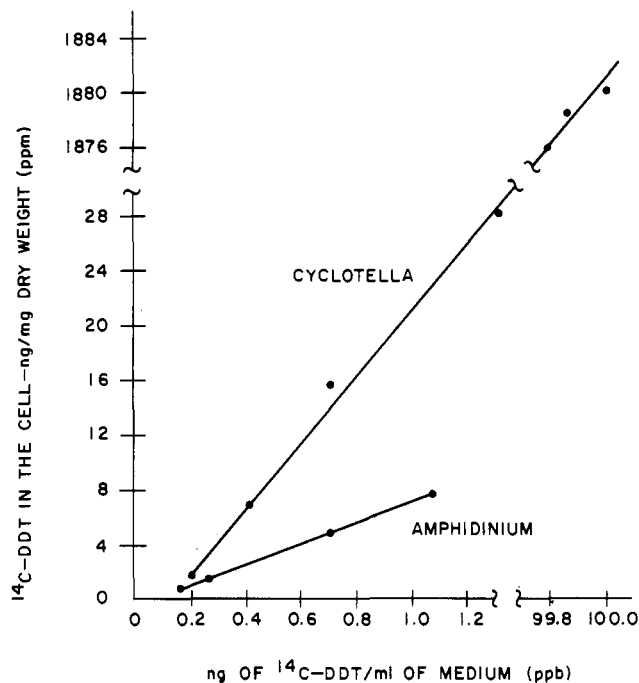


Figure 2. Uptake of DDT by *Cyclotella nana* and *Amphidinium carteri* as a function of DDT concentration.

different species varied from 33 to 93% of the pesticide present. *Skeletonema* removed 93% and *Cyclotella* removed 73%, whereas the other four species removed much lower amounts of DDT, as follows: *Isochrysis*, 57%; *Olisthodiscus*, 38%; *Amphidinium*, 44%; and *Tetraselmis*, 33%. In the subsequent studies, DDT uptake by various species was measured after 2 hr of incubation of the cells with the pesticide.

The effect of DDT concentration and cell density on the uptake of the pesticide was investigated. Figure 2 shows the uptake of DDT by *Cyclotella* and *Amphidinium* exposed to varying concentrations of the pesticide. The amount of the pesticide accumulated by *Cyclotella* increased linearly with an increase in the concentration of the pesticide up to 100 ppb. At concentrations above 100 ppb, the increase in uptake was not proportional to the increase in the amount of DDT in the medium. A non-linear relationship at concentrations higher than 100 ppb may be due to the fact that at these concentrations all of the DDT was not in solution and/or suspension. The pat-

Table I. Effect of Cell Density on the Ability of Various Species of Marine Algae to Concentrate DDT<sup>a</sup>

Species	Dry weight of cells/ml of culture, mg	[ <sup>14</sup> C]DDT uptake (% of DDT in the medium)	Concentration factor, cell concn of DDT/culture concn of DDT
<i>Cyclotella nana</i>	0.017	62.8	37,600
	0.008	48.8	58,100
<i>Isochrysis galbana</i>	0.039	50.0	11,300
	0.019	42.0	28,800
<i>Olisthodiscus luteus</i>	0.108	49.7	4,600
	0.054	38.0	7,000
<i>Amphidinium carteri</i>	0.066	28.4	4,300
	0.033	23.7	9,600
	0.106	55.4	5,200
<i>Tetraselmis chuii</i>	0.053	33.3	6,300
	0.029	92.8	31,900
<i>Skeletonema costatum</i>	0.015	55.9	38,400

<sup>a</sup>Initial DDT concentration in the medium was 0.7 ppb.

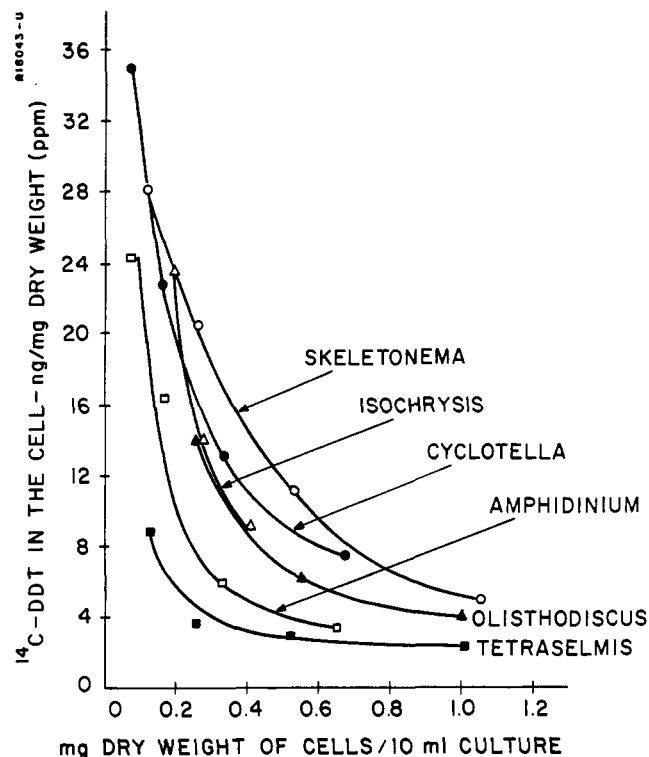


Figure 3. Uptake of DDT by different algae as a function of cell density.

tern of uptake was also linear for *Amphidinium* in the range of DDT concentrations tested (0.17 to 1.08 ppb).

The uptake and concentration of DDT by different algae were studied in the presence of various cell densities. The cells were exposed to a medium containing 0.7 ppb of DDT. Increasing the cell density resulted in an increase in the total amount of pesticide removed, although the increased uptake was not directly proportional to the increase in cell density (Table I). Our findings demonstrate that the degree to which the cells of different species concentrated DDT was greatly influenced by the cell density. Figure 3 shows that as the cell density increased, the degree to which the algal cells concentrated DDT decreased. These results are in agreement with those of Cox (1970b), who reported that the partition coefficient (concentration factor) of DDT residues for phytoplankton collected off the California coast diminished as the density of the phytoplankton increased. The logical explanation is that decreasing the cell density caused an increase in DDT localized around each cell, thereby making larger amounts of DDT available for uptake by the cell.

Table I gives the DDT concentration factors for different species at various cell densities. Several workers (Gregory *et al.*, 1969; Kiel and Priester, 1969; Sodergren, 1968; Vance and Drummond, 1969) have reported that the concentration of DDT in different species of algae was 200-1000 times greater than that in the medium. Though the species used by us were different from those of other workers, the DDT concentration factors far exceeded the values reported by the above investigators. The observed differences are probably due to the fact that in this study different cell densities and DDT concentrations were employed. Kiel and Priester (1969), Gregory *et al.* (1969), and Vance and Drummond (1969) did not examine the effect of varying cell densities on uptake of DDT. Moreover, they used DDT concentrations which exceed the solubility limit of DDT in water (1.2 ppb) as reported by Bowman *et al.* (1960). In our studies the cells were exposed to DDT concentrations well within the water solubility limit for DDT, and the accumulation of pesticide was studied as a function of cell concentration.

Table II. Influence of Cell Number and Cell Size on the Uptake of DDT by Different Species of Algae<sup>a</sup>

Species	Number of cells/ ml of culture	Cell size, $\mu$	Uptake of DDT 2 hr after treatment, ng of DDT/mg of dry wt	Concentration factor, cell concn of DDT/ culture concn of DDT
<i>Skeletonema costatum</i>	$1.19 \times 10^6$	$7 \times 14$	18.4	26,300
<i>Cyclotella nana</i>	$0.83 \times 10^6$	$8 \times 8$	14.5	20,700
<i>Isochrysis galbana</i>	$1.40 \times 10^6$	$4 \times 4$	12.5	17,900
<i>Olisthodiscus luteus</i>	$0.115 \times 10^6$	$11 \times 11$	12.0	17,100
<i>Amphidinium carteri</i>	$0.178 \times 10^6$	$15 \times 15$	6.6	9,400
<i>Tetraselmis chuii</i>	$0.134 \times 10^6$	$9 \times 14$	4.0	5,700

<sup>a</sup>One milliliter of cell suspension in each treatment contained 0.03 mg (dry weight) of cells and 0.7 ppb of DDT.

Our findings indicate that the various species differ significantly in their capacity to accumulate DDT from the medium (Figure 3). The algae accumulated DDT in the following order: *Skeletonema* > *Cyclotella* > *Isochrysis* > *Olisthodiscus* > *Amphidinium* > *Tetraselmis*. These differences could be due to the taxonomic characteristics of the cells or they may result from variations in the number of cells/unit of weight or cell size. In general, species having a greater number of cells per milliliter accumulated greater amounts of DDT than species having lesser numbers of cells (Table II). This suggests that the species with a smaller cell size will concentrate more DDT; this was confirmed in our findings, with the exception of *Skeletonema*. The two species belonging to the division Bacillariophyta were found to be the most efficient concentrators of DDT. A possible explanation for the greater accumulation of DDT by these diatoms compared to the other algae included in this study may be the unique cellular constituents of diatoms, such as storage fats and cell wall components. The differential accumulations of DDT by these marine phytoplankton indicate that organisms feeding on them will receive varying amounts of DDT, depending on the relative abundance of these species in the surrounding waters.

The uptake of DDT by living and dead algal cells was compared. The cells were killed by treatment with a 0.03% solution of mercuric chloride or by boiling the cell suspension. With the exception of *Amphidinium*, similar amounts of DDT were removed from the medium by the living or dead cells of the various species. These findings

do not indicate whether the uptake of DDT by these algae occurs by adsorption and/or absorption. The fact that the living and dead cells accumulated equal amounts of the pesticide indicates that uptake of DDT is a passive process. *Amphidinium* cells which were boiled or treated with mercuric chloride took up 1.9 and 2.5 times more DDT, respectively, than untreated cells. On the basis of the available data, it is not possible to explain the increased uptake of DDT by dead cells of *Amphidinium*.

Glc analysis of the cell extracts indicated that the various species converted DDT to DDE, although the degree of conversion was not extensive (Table III). A typical gas chromatogram of an extract of an algal culture treated with DDT is shown in Figure 4. It can be seen that the low amount of cellular material present in the extract did not interfere with the chromatography of DDT and its metabolites. It was observed that the amount of DDE produced by the algae increased with an increase in the time of incubation. The algae included in the study differed in their ability to produce DDE. The percentage conversion of DDT associated with algal cells ranged from 0.03 to 11.84% in the various species. Maximum conversion of DDT to DDE was observed in cultures of *Tetraselmis*. In this species, 11.84% of the total DDT in the cells was

Table III. Conversion of DDT to DDE by Various Species of Marine Algae

Species	Days after addition of DDT <sup>a</sup>	Percent conversion to DDE, <sup>b</sup> cell fraction
<i>Olisthodiscus luteus</i>	5	< Control
	24	0.94
	24	0.71
<i>Isochrysis galbana</i>	5	0.03
	24	0.31
<i>Cyclotella nana</i>	5	1.19
	10	0.09
	24	0.33
<i>Amphidinium carteri</i>	5	2.81
	10	0.46
	24	1.84
<i>Tetraselmis chuii</i>	5	11.51
	10	0.18
	24	3.29
<i>Skeletonema costatum</i>	5	4.90
	10	
	24	

<sup>a</sup>DDT added at an initial concentration of 1 ppm in the medium.

<sup>b</sup>Calculated by dividing the weight of DDE by the total weight of DDT + DDE, after first multiplying the DDE by 1.12 to convert it to its original DDT weight.

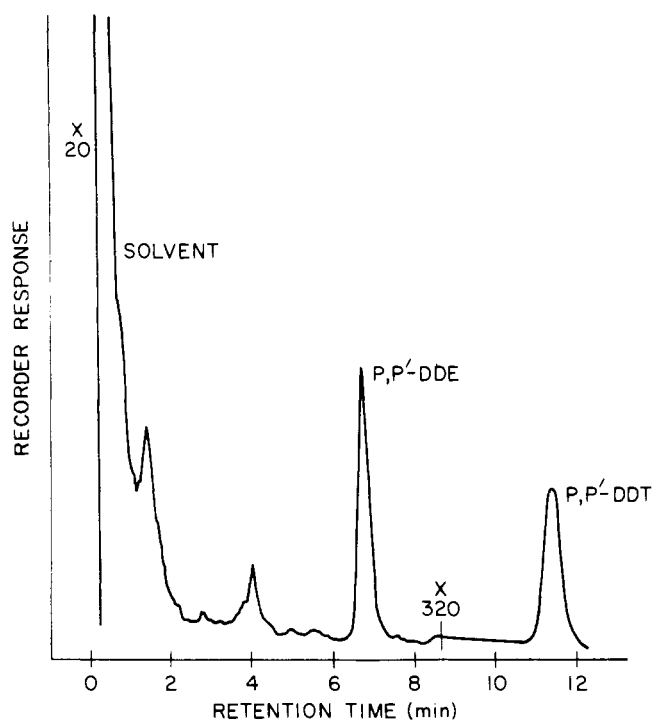


Figure 4. Typical gas chromatogram of an extract of an algal culture treated with DDT for 21 days.

present as DDE 24 days after treatment. Our findings agree with those of Bowes (1972), who recently reported that *Skeletonema costatum* and *Cyclotella nana* produced low amounts of DDE. He did not observe any conversion of DDT to DDE by *Amphidinium carteri*, whereas our studies indicate that this organism was able to convert small amounts of DDT to DDE.

Tlc analysis of the cells incubated with [ $^{14}\text{C}$ ]DDT indicated the presence of only one metabolite having an  $R_f$  value of 0.43, which corresponded to authentic DDE.

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## Fate of Aldrin- $^{14}\text{C}$ in Potatoes and Soil Under Outdoor Conditions

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Aldrin- $^{14}\text{C}$  has been applied to soils under outdoor conditions in Germany (2.9 kg/ha) and England (3.2 kg/ha) and potatoes have been sown. At harvest, more than 60% of the total radioactivity recovered from the soil and plants was due to metabolites, mainly dieldrin and a group of hydrophilic products, of which the main compound was identified as dihydrochlordene- $^{14}\text{C}$  dicarboxylic acid (1,2,3,4,8,8-hexachloro-1,4,4a,6,7,7a - hexahydro-1,4-endo-methyleneindene - 5,7-dicarboxylic acid). Photodieldrin- $^{14}\text{C}$  was also detected in small amounts in the

potato haulm from England, as were traces of photoaldrin- $^{14}\text{C}$  in both soils. The conversion of aldrin- $^{14}\text{C}$  was least in the upper soil layer and greatest in deeper soil layers (10-60 cm from surface) and in the plants. Only very low residues were detected in the deeper soil layers in England, whereas more radioactivity was found in the deeper soil samples in Germany. The leaching water of the experiment in Germany contained only dihydrochlordene- $^{14}\text{C}$  dicarboxylic acid (0.02 ppm).

The chlorinated insecticide aldrin (Figure 1) is widely used for the control of a range of soil pests. It may be applied to the soil before planting at dosage rates between 2 and 4 kg/ha or it may be used to dress seeds before sowing.

It is well established that aldrin is readily converted to its epoxide, dieldrin, in soil (Gannon and Bigger, 1958; Lichtenstein and Schulz, 1959, 1965) and in plants (Gannon and Decker, 1958; Glasser, 1955; Lichtenstein, 1959; Lichtenstein *et al.*, 1965, 1967). The conversion of aldrin to dieldrin by plant enzymes has also been demonstrated *in vitro*; e.g., by root homogenates (Yu *et al.*, 1971). Numerous studies have been reported on aldrin and dieldrin residues in crops and in soil (Decker *et al.*, 1965; Elgar, 1966; Harrison *et al.*, 1967; Lichtenstein, 1965; Onsager *et al.*, 1970), in food moving in commerce (Duggan and Weatherwax, 1967), and in total diets (Duggan and Lipscomb, 1969). With the exception of dieldrin and its photoisomer

photodieldrin (Lichtenstein *et al.*, 1970; Weisgerber *et al.*, 1970), no further conversion products of aldrin in plants or soil have been reported. Recently we reported (Weisgerber *et al.*, 1970) that under glasshouse conditions, 4 weeks after foliar application of aldrin- $^{14}\text{C}$  to cabbage, nearly 80% of the radioactivity detected in the plants consisted of metabolites which were more hydrophilic than metabolites of aldrin that had been identified previously. Application of aldrin- $^{14}\text{C}$  to spinach, carrot, and cabbage soil resulted in hydrophilic metabolites amounting to 62, 55, and 12%, respectively, of the total residues.

It is difficult to predict from glasshouse experiments the behavior of a compound under practical conditions. It is frequently found that the concentration of a metabolite that is found in indoor radiochemical studies is far greater than is subsequently found when the work is undertaken under practical field conditions. We have therefore studied the metabolism of aldrin- $^{14}\text{C}$  under outdoor conditions to complement the previous indoor studies. Different crops have been grown out-of-doors and have been treated in reasonable accordance with the recommended agricultural practice. However, plot sizes were necessarily limited since radioisotopes were being used. Experiments took place in Germany and in England to permit a comparison of the overall effect of different climatic conditions, soil

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