Pick-Up and Metabolism of DDT, Dieldrin and Photodieldrin by a Fresh Water Alga (*Ankistrodesmus Amalloides*) and a Microcrustacean (*Daphnia Pulex*)

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Introduction

The extensively used chlorinated hydrocarbon insecticides, DDT and dieldrin, are common microcontaminants of the environment (BROOKS, 1974; MATSUMURA, 1972; EDWARDS, 1970; WEAVER et al., 1965). The residues of these insecticides in aquatic environments are picked up by food-web and food-chain organisms and can become concentrated through successive trophic levels in organisms at top of the food-chain. This bioconcentration can result in ecological hazards to fish, birds and other wild life (RUDD, 1964; WURSTER et al., 1967).

The fate and effects of these persistent toxicants on food-chains have been extensively investigated in various laboratories. DDT is slowly converted to DDE by various marine algae (RICE and SIKKA, 1972); a marine diatom (MIYAZAKI and THORSTEINSON, 1972); two fresh water diatoms, certain bacteria and several fungi (MIYAZAKI and THORSTEINSON, 1972).

The aquatic invertebrates at lower trophic levels also convert DDT to DDE, DDD and other metabolites (JOHNSON et al., 1972; BROWN, 1971). Dieldrin is extremely resistant to biodegradation. It is slowly isomerized in environment by sunlight (ROSEN and SUTHERLAND, 1967) and by marine algae (MATSUMURA et al., 1970) to more toxic photodieldrin (KHAN et al., 1973). Photodieldrin, which is the "terminal residue" of dieldrin (EAGON, 1969), is also extremely persistent in the environment (REDDY and

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KHAN, 1974). Due to the lack of studies as to the fate of these insecticides in fresh water food chains, we investigated the absorption/adsorption and metabolism of DDT, dieldrin and photodieldrin in the fresh water alga, Ankistrodesmus amalloides and the fresh water microcrustacean Daphnia pulex.

Experimental

14-C-labeled DDT (specific activity 22.7 mCi/m-mole) and 14-C-dieldrin (sp. activity 79 mCi/m-mole) were pruchased from Amersham Searle (Arlington Heights, Illinois). 14-C-photodieldrin (sp. activity, 4.0 mCi/m-mole) was prepared from 14-C-dieldrin as described by Rosen and Cary (1968). The unicellular freshwater alga Ankistrodesmus amalloides, was obtained from the Indiana Algae Culture Collection (number 190) and was grown under the conditions cited by Kessler (1963). Cultures of Daphnia pulex were grown in the lab in 50% Chu-10 media (CHU, 1942).

Uptake in algae was carried out using 0.72 ppb (parts per billion) pesticide added in ethylene glycolmonomethyl ether to 100-ml algal medium containing algae. The 100-ml flasks were periodically shaken by hand to keep the cells in suspension. The experiments were carried out under continuous laboratory light. After the appropriate time, an 80-ml aliquot was taken and centrifuged at 2,000 rpm for 12 minutes on a Sorvall RC-2B. After discarding the supernatant the pellets were washed in distilled water and filtered on Whatman No. 3 filter paper (2.3 cm. disks in a 15 ml Millipore apparatus) and placed in a dessicator for 48 hrs. after which the filters were dissolved in 1 ml of Soluene-100 (Packard Instruments). Once the algae had dissolved, the filter paper and the algae were suspended in a toluene base scintillation fluid and counted on a Packard liquid scintillation spectrometer (model 3390) equipped with a model 544A Absolute Activity Analyzer.

This procedure works well for 100 and 1000 cell/ml concentrations (counted using a hemacytometer) but for larger concentrations of 10^4 and 2×10^4 cells/ml, the procedure was modified slightly. Due to the larger cell mass, 5 ml of Soluene 100 was used to digest the algae and a 1 ml aliquot of this was counted. By using this procedure over 80% of the initial pesticide can be accounted for. Possible sources of loss could be codistillation during incubation of the cells.

Metabolism studies in algae were carried out using a 3×10^4 cell/ml (143.4 mg) culture of A. amalloides grown on the previously described media. The algae were exposed to 0.72 parts per billion 14-C-DDT for 30 days under

continuous light after which the algae were isolated by centrifuging at 2,000 rpm, filtering on Whatman No. 3 filter paper and dessication for 48 hrs; after which the disks were ground up in 2-3 grams of sand and extracted with 75 ml acetone using a Soxhlet apparatus. After 48 hrs, the acetone was concentrated under a gentle stream of air and the metabolites were separated by thin layer chromatography on .25 mm Thick Silica gel (F-254) plates (E. Merck, (Dermsdadt, Germany) using n-heptane as a solvent (KOVACS, 1965).

Metabolism in Daphnia pulex was carried out by exposing approximately 100 organisms (25.3 mg) to 0.31 parts per trillion 14-C DDT for 24 hrs without feeding. After the exposure, the organisms were filtered on Whatman No. 3 filter paper, dessicated, ground up and extracted in 75 ml acetone using a Soxhlet apparatus. After concentrating the solvent, the metabolites were separated using TLC (n-heptane). The plates were then exposed to Kodak No-Screen Medical X-Ray film for 30 days. After developing the film, Rf values were calculated and were compared to Rf values of non-radioactive DDD and radioactive DDE.

TABLE 1

Recovery of ^{14}C-DDT in various steps involving its extraction from algae and daphnids.

Extraction Step [1]	% Radioactivity Recovered	
	algae	daphnids
1. Homogenization	72.8	97.0
2. Soxhlet Extraction of homogenate with acetone	58.7	78.3
3. Concentration of acetone extract	42.9	57.1

[1] The amount of activity recovered at the termination of the expt.) as a percent of the initially added radioactivity to water was: algae 36%; daphnids 81%; the radioactivity recovered from the water was 36% for the algal media and 16% for the media in which daphnids were exposed.

Results and Discussion

The amounts of DDT, dieldrin, and photodieldrin adsorbed/absorbed by the fresh water alga, Ankistrodesmus

amalloides are shown in Fig. 1A and 1B. The total pick-up of DDT during 1-3 hours (100 algal cells/ml) was 2.5 times higher than that of dieldrin and 10 times higher than that of photodieldrin (Fig. 1A). These differences in the uptake of the insecticides seem to be related with their water solubilites. For example, their solubilites in water (ppb) are: DDT, 3.4 (GUNTHER et al., 1968); dieldrin 12.5, photodieldrin 55.5 (KHAN and KHAN, 1974; KHAN, 1974). Increasing the number of cells, and thus their concentration in the medium by 10-fold (1,000 cells/ml) under same conditions showed similar ratios in the amounts of absorption of the three insecticides (Fig. 1B). Although the total amount of each insecticide in algae at 1,000 cells/ml was about 5 times higher than that at 100 cells/ml, the efficiency of each cell to absorb/adsorb these insecticides decreased on increasing the cell density (Fig. 2). The ratios of biological magnification of these insecticides by A. amalloides at the two cell concentration is shown in Table 2. Lower values are seen with higher cell concentration. This, again, indicates that higher densities lower the efficiency of insecticide absorption on unit cell or unit weight basis. Rice and Sikka (1973), studying the absorption of DDT by marine algae, reported a similar inverse-type of relationship of insecticide absorption and magnification (single cell basis) with the cell concentration. They emphasized that the surface area exposed to the insecticide was higher in dilute cell culture as compared with the more concentrated cultures and also that smaller cells, yielding more cells/unit wt. basis, should absorb more insecticide than a similar culture of a species with larger cells. However, their data does not support the latter hypothesis. For example (using 0.03 mg dry algal wt/ml) Isochrysis galbane (4 x 4 μ cell size) at 1.4 x 10^6 cells/ml absorbed 12.5 ng DDT/mg dry wt giving a magnification ratio of 17,900, while Skeletonema costatum (7 x 14 μ cell size) at 1.19 x 10^6 cells/ml absorbed 18.4 mg DDT/mg dry wt giving a magnification ratio of 26,300. Also, Amphidinium cacteri (cell size 15 x 15 μ) at .178 x 10^6 cells/ml absorbed 6.6 ng DDT/mg dry wt giving a magnification of 9,400, while Tetraselmis chuii (9 x 14 μ cell size) at .134 x 10^6 cells/ml absorbed only 4.0 ng DDT/mg dry wt and gave a magnification of only 5,700 (RICE and SIKKA, 1972). However, the surface area-to-volume ratios are higher for smaller cellular and multicellular spherical organisms and facilitate the rate of diffusion (KROGH, 1945; PROSSER and BROWN, 1961). The smaller cells or organisms should show higher biological magnification as compared with larger ones (KENAGA, 1972; KHAN and KHAN, 1974); although a number of other factors including the chemical composition of the cell membrane and cell constituents are equally important. More than one factor may be responsible for species differences in the absorption/adsorption of insecticides.

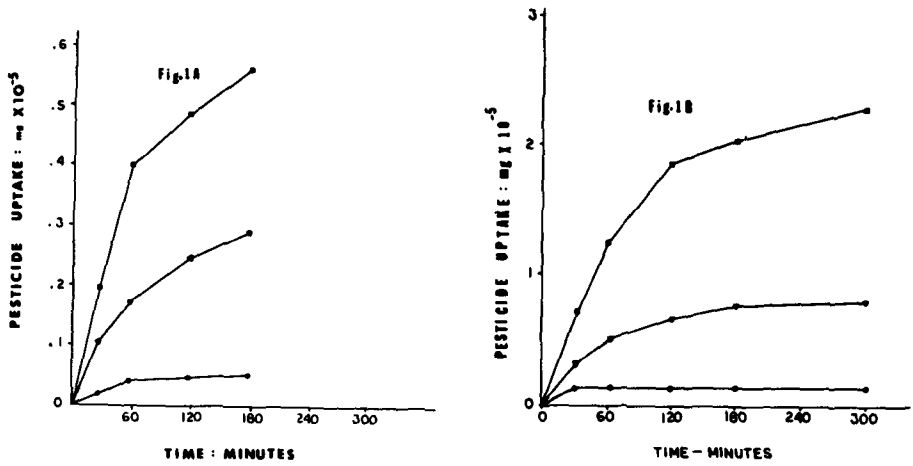

Fig. 1. Absorption of ^{14}C-labelled DDT (■—■), dieldrin (▲—▲) and photodieldrin (●—●) by freshwater alga, A. amalloides at 100 cells/ml (Fig. 1A) and 1,000 cells/ml (Fig. 1B) concentrations.

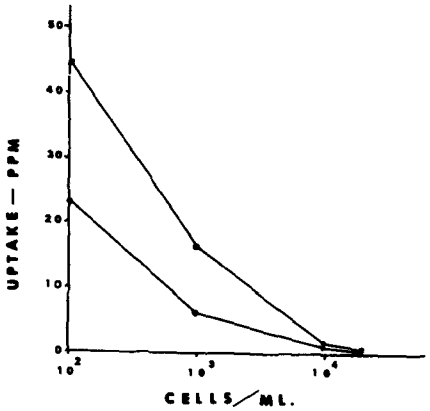

Fig. 2. Effect of cell concentration in the medium on the uptake of DDT (■—■) and dieldrin (▼—▼) by cells of A. amalloides.

For Ankistrodesmus amalloides the time for maximum absorption is 120 min for DDT, 30-60 min for dieldrin and 30 min for photodieldrin (Fig. 1A, 1B). While cells still continue to absorb/adsorb DDT and dieldrin, though slowly, after this period, photodieldrin concentration in cells does not seem to increase up to 3 hrs. The cells of marine algae, e.g. Tetraselmis chuii, absorb maximum amount of DDT within one hour, while the maximum amount of dieldrin, e.g. by Cyclotella nana, is absorbed in less than one hour (RICE and SIKKA, 1972, 1973). However, the fresh water

TABLE 2

Biological Magnification of DDT, Dieldrin and Photodieldrin by A. amalloides.

Time: Min.	Magnification[1] (ppb in algae / ppb in water) x10[2]		
	DDT	Dieldrin	Photodieldrin
30	213 (78)	117 (30)	47 (17)
60	436 (139)	191 (69)	38 (19)
120	531 (192)	270 (71)	49 (19)
180	616 (229)	320 (87)	47 (17)
300	(248)	(88)	(17)

[1] values in parenthesis for 1,000 cells/ml

alga, Chlorella pyrenoidosa showed maximum absorption of dieldrin after 48 hr (WHEELER, 1970) and another species of Chlorella absorbed maximum amount of DDT in one minute (SODERGREN, 1968). More work is needed on the kinetics of absorption/adsorption of insecticides, using standard methods by these microorganisms before valid comparisons can be made.

The biological magnification ratios (Table 2) for 1,000 cells/ml are comparable with those reported for marine algae at higher cell concentrations (RICE and SIKKA, 1972). Dieldrin is magnified 2-3 times less and photodieldrin about 6 times less than DDT in Ankistrodesmus amalloides. These differences are due to the factors discussed earlier, solubilites of these insecticides in water. This alga has very low affinity for photodieldrin which is most polar (least lipophilic) of the three insecticides.

The fresh water alga metabolizes DDT to both DDE (3.5%) and DDD (0.8%) (Table 3). DDE formation by marine phytoplanktons has also been reported. Marine algae convert 0.94% (Olisthodiscus luteus) to 11.51 % of DDT to DDE (Tetrasalmis chuii) (RICE and SIKKA, 1972). Miyazaki and Thorsteinson (1972) reported several microorganisms (Baker's yeast; several actinomycetes, bacteria; a marine and two fresh water diatoms) to convert DDT to DDE at less than 1%. However, DDD formation has not been observed in these organisms. In aqueous media, especially in the presence of reduced porphyrins, DDT has been shown to be converted to DDD (MISKUS et al., 1965). But porphyrins are present in marine algae, which do not convert DDT to DDD, as well as in the fresh water alga, A. amalloides which dechlorinates DDT. Therefore DDD in this case is most likely a metabolic product. The microorganisms in

ruminants as well as in soils (KHAN et al., 1974) convert DDT to DDD. Since our incubations were 1-month long, this possibility can not be ruled out. The fresh water flea, Daphnia pulex shows about 13.6% conversion of DDT to DDE (Table 3). No other metabolites of DDT were detected using these methods. Other fresh water crustaceans, Daphnia magna, Gammarus fasciatus (Amphipoda) and Palaemonetes kadiakensis (Decapoda) metabolize DDT to DDE (JOHNSON et al., 1972); the DDE formation being (% of total body radioactivity); 19.7, 20.9, and 13.2, respectively. Daphnia and Palaemonetes also produced about 7% DDD (JOHNSON et al., 1972) which we were unable to detect in D. pulex kept unfed before and during the experimentation.

TABLE 3

Recovery of DDT and its metabolites [2] from algae and daphnids exposed to ^{14}C-DDT for 30 days and 24 hours, respectively.

Organism	% radioactivity [1]		
	DDT	DDE	DDD
algae	95.6	3.5	0.8
daphnids	86.0	13.9	Nil

[1] percent of total radioactivity extracted from the organisms.

[2] The Rf values were: DDT, .225; DDE, .326; DDD, .117 on silica gel F-254 plates using n-heptane as a solvent.

^{14}C-dieldrin and ^{14}C-photodieldrin (4 ppb) were recovered unchanged from Daphnia pulex after a 4-day exposure (KHAN, 1974; RIO, unpublished data, this lab) and from Ankistrodesmus spiralis after a 30-day exposure (REDDY and KHAN, 1974a).

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