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# Effect of DDT Metabolites on Soil Respiration and on an Aquatic Alga

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1,1,1-Trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), which is still widely used in the tropics, is subject to biomagnification and, as a result, is toxic to some nontarget species. Mortality in fish (WURSTER 1972), egg-shell thinning and the consequent decline in bird populations (FISHBEIN 1974), and pulmonary carcinoma in experimental mice (DURHAM & WILLIAMS 1972) are among the adverse effects reported for DDT. However, no association has been found between levels of DDT plus 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) in fatty tissues and the occurrence of cancer in humans (DURHAM & WILLIAMS 1972). On the contrary, the use of 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (DDD) has been suggested for the chemotherapy of adrenal cortex tumors (DURHAM & WILLIAMS 1972).

Numerous reports exist on the effects of DDT on microbial communities and microbial processes in soil (MARTIN 1963, MARTH 1965, MENZEL et al. 1970). However, less is known about the possible adverse effects of DDT and its metabolites on freshwater and marine algae. WURSTER (1968) reported reduced rates of photosynthesis by representatives of coastal and oceanic phytoplankton even at a concentration of several nanograms per milliliter of DDT, but a paucity of data exists on the influence of DDT metabolites on microbial communities and algal fora. These compounds might even be more hazardous than the parent molecule to the communities involved in nutrient regeneration and biotic interactions. In the present study, the effect of DDT metabolites on microbial communities of soil and on development of one alga is assessed in order to establish the possible influence of these compounds.

## MATERIALS AND METHODS

DDT and its metabolites were from Aldrich Chemical Co., Milwaukee, Wis. Bis(p-chlorophenyl)methane (DDM) obtained from Eastman Organic Chemicals, Rochester, N.Y., was recrystallized prior to use. Tetraphenyldimethyl ethers were synthesized as previously described (SUBBA-RAO & ALEXANDER 1977).

Studies of the effect of DDT, DDD, DDE, 2,2-bis(p-chlorophenyl)acetic acid (DDA), DDM, p,p'-dichlorobenzhydrol (DBH), p,p'-dichlorobenzophenone (DBP), and p-chlorophenylacetic acid (PCPA) on the respiration of the microbial community of soil

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were conducted. 1,1,1',1'-Tetraphenyldimethyl ether (BHE) was also tested because of the possibility of the formation in nature of its chlorinated analogue from DDT (SUBBA-RAO & ALEXANDER 1977). The method of WHITESIDE and ALEXANDER (1960) was used to assess the effects of DDT metabolites on respiration of the soil. Air-dried Lima loam (pH 7.0, 3.6% organic matter) was passed through a 1.0-mm sieve before use. The chemicals were added as acetone solutions to 0.5 g of soil, and the acetone was allowed to evaporate. This treated soil was then mixed with 3.5 g of untreated soil, and the mixture was transferred to the main compartment of Warburg flasks. This procedure was used to minimize possible effects of residual acetone on the microorganisms. Each flask also received 0.2 ml of 20% KOH in the center well, and 1.0 ml of distilled water was added to the soil in each flask prior to placement of the manometers in the 30°C water bath.

Standard manometric procedures were used for measuring oxygen uptake (UMBREIT et al. 1972). The treatments were replicated three times, except that four flasks were used for each concentration in experiments with DDM, DBH, and DBP. The manometers were arranged in a completely randomized block design. The initial manometric readings were taken 1 h after the addition of water to the soil, and oxygen uptake was measured for 2 to 3 days at 2 to 4 h intervals.

To measure the influence of the test compounds as well as 1,1,1',1'-tetra(p-chlorophenyl)dimethyl ether (DCBHE) on *Chlorella vulgaris*, the growth medium consisted of 250 ml of Bold's inorganic salts broth (BOLD 1942) in 500-ml Erlenmeyer flasks. Two replicates were used for each treatment, except that three replicates were used in tests of PCPA, DDD, DDE, and DDT. The flasks were inoculated with a 10-day-old *C. vulgaris* culture and were incubated at 22°C on a rotary shaker (250 rpm) under constant illumination of 5,380 lux. A 10-ml portion was taken for analysis at regular intervals from each flask. Algal productivity was measured as total chlorophyll *a* and organic carbon.

Several solvent systems were tried in attempts to extract the chlorophyll completely. Algal pellets, which were obtained by centrifuging 10-ml portions of the broth at 4,100 X *g* for 5 min, were extracted with 5-ml portions of various solvents for 10 min at room temperature. The pellet was mixed with the solvent with a glass rod, the solvent portions were then centrifuged at 4,100 X *g* for 5 min to remove the cell debris, and the absorbance of the chlorophyll extract was measured at 665 nm using a Beckman DB-G spectrophotometer (Beckman Instruments, Fullerton, Calif.). The solvent systems tested were 90% acetone in water, 90% acetone in methanol, 95% ethanol in water, 90% methanol in water, 99% methanol in ether, and methanol; of these, 99% methanol in ether was found to remove the greatest amount of chlorophyll *a*, and hence it was used. Most of the pigment was extracted in 10 min based on tests involving longer time periods for extraction. The procedure finally adopted was to extract the algal pellet from 5.0 ml of broth with 5.0 ml of 99% metha-

nol in ether for 10 min at room temperature. The amount of chlorophyll per unit volume was calculated as described elsewhere (AMERICAN PUBLIC HEALTH ASSOCIATION 1975).

To determine the biomass of C. vulgaris by total organic carbon analysis, 1.0 to 5.0 ml of algal suspension was used either directly or after centrifugation at 4,100 X g for 5 min. When centrifuged, the algal pellet was resuspended in 1.0 to 2.0 ml of distilled water. Organic carbon was determined by the method of LU et al. (1959) using 1.0-ml samples mixed with 2.0 ml of dichromate-sulfuric acid reagent, and sucrose was the standard.

## RESULTS AND DISCUSSION

Studies of the influence of different concentrations of DDT metabolites on the rate of oxygen consumption by the microbial community of soil revealed no statistically significant effect (1% confidence level) by 1, 10, 100, and 1000 µg of: DDM and DBH; 1, 10, and 100 µg of DDE, DDD, DDT, and BHE; and 1 and 10 µg of DBP per gram of soil. The respiration rate ranged from 204 to 275 nmol $O_2$ consumed/g of soil per hour. PCPA and DDA were toxic but only at concentrations of 100 µg/g of soil (Table 1). The

TABLE 1

Effect of DDT and its metabolites on soil community respiration

| Test chemical | µg metabolite/g soil | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 10 | 100 | 1000 |
| | nmol $O_2$ consumed/g soil per h | | | | |
| PCPA | 297a[a] | 285a | 283a | 241b | ND[b] |
| DDA | 263a | 270a | 266a | 184b | ND |
| DDM + glucose | 490a | 522a | 495a | 498a | 486a |

[a]Values in the same line that are followed by the same letter do not differ significantly at the 1% level.

[b]Not determined.

inhibition by DDA and PCPA may be attributable to their solubility because the nonpolar metabolites of DDT and the parent molecule did not have any effect; nevertheless, no evidence exists that such high concentrations are encountered in nature. ALBONE et al. (1972) observed a decrease in the bacterial counts of estuary sediment at a DDT concentration of 100 µg/g of sediment. PATHAK et al. (1961) reported no adverse effects on microbial populations and no inhibition of nitrogen-fixing or nitrifying bacteria when DDT was applied to soils at concentrations of 50 and 100 kg/ha. Because the respiratory activity of soil was low, glucose (6.0 µmol per flask) was added in one study, but even under these conditions, DDM was not toxic.

The effect of DDT metabolites on the growth of C. vulgaris was assessed by measuring the rate of synthesis of chlorophyll a. The trichromatic method (AMERICAN PUBLIC HEALTH ASSOCIATION 1975) entails an elaborate extraction of centrifuged algal pellets with 90% acetone in water and involves maceration and extraction at 4°C for 24 h. The optical density at 665 nm of 5.0 ml of the chlorophyll extract thus obtained was 0.06. However, much larger amounts of chlorophyll were extracted with other solvents. The optical densities of different solvent extracts were: 0.12, 0.33, 0.38, 0.91, and 1.35 for 90% acetone in methanol, 95% ethanol in water, 90% methanol in water, methanol, and 99% methanol in ether, respectively. The extraction with 99% methanol in ether for 10 min at room temperature was thus found to be faster, much simpler, and gave higher chlorophyll yields than the trichromatic method.

The rate of chlorophyll increase in the C. vulgaris culture was linear for 8 days in one experiment and for 13 days in another experiment; hence, the rates calculated were based on the data from 8- or 13-day incubation periods. The rate of chlorophyll a increase was unaffected by either of the two test concentrations of DDD, DDA, DBH and DBP; i.e., the differences were not statistically significant at the 5% level (Table 2). On the other

TABLE 2

Effect of DDT metabolites on increase of chlorophyll a content of Chlorella vulgaris

| Expt | Chemical | Conc of metabolite, µg/ml | | |
|---|---|---|---|---|
| | | 0[a] | 1 | 10 |
| | | ng chlorophyll a increase/ml per day[b] | | |
| 1 | DDT | 461ab | 638de | 629de |
| | DDE | 461ab | 614cde | 651e |
| | DDD | 461ab | 536bcd | 543bcd |
| | DDA | 461ab | 486ab | 410a |
| | PCPA | 461ab | 601cde | 521bc |
| | BHE | 461ab | 604cde | 698e |
| | DCBHE | 461ab | 671e | 659e |
| 2 | DDM | 562ab | 873c | 1090d |
| | DBH | 562ab | 613ab | 501a |
| | DBP | 562ab | 630ab | 609ab |

[a] Values in soil with no added chemical are the same because only one replicated series was run.

[b] Values followed by the same letter are not significantly different at the 5% level for any combination of chemical and concentration within the same experiment.

hand, significant increases (at the 5% level of probability) in the rate of chlorophyll accumulation were observed at a concentration of 1 µg of DDT, DDE, PCPA, BHE, or DCBHE/ml, and the 10 µg/ml concentrations of these compounds did not further enhance the rate. With DDM, moreover, not only was the lower concentration stimulatory, but the higher concentration enhanced the effect even more. The differences in the rate of chlorophyll accumulation at the two concentrations of DDM were significant at the 1% level. WURSTER (1968) reported a 70% decrease in the rate of $^{14}C$ uptake by Skeletonema costatum in solutions containing 0.1 µg of DDT/ml and a 90% decrease in the rate of $^{14}C$ uptake by phytoplankton communities. On the contrary, MENZEL et al. (1970) observed that Dunaliella tertiolecta was not affected by DDT up to a concentration of 1.0 µg/ml. The present data show a stimulation in autotrophic development of an alga by DDT metabolites at much higher concentrations.

The rate of biomass accumulation by C. vulgaris was also affected by low concentrations of some of these compounds. A significant stimulation (1% level) in rate of biomass increase was observed with 1.0 µg DDE, DDM, and DBH/ml, but a statistical reduction in the rate of biomass increase was observed with 1.0 µg of DCBHE/ml (Table 3). The higher concentration of the three

TABLE 3

Effect of DDT metabolites on biomass of Chlorella vulgaris

| Expt | Chemical | Concn of metabolite, µg/ml | | |
|---|---|---|---|---|
| | | 0 | 1 | 10 |
| 1 | DDT | 13.0bc[a] | 9.83ab | 9.95ab |
| | DDE | 13.0bc | 18.5 d | 10.5 ab |
| | DDD | 13.0bc | 11.9 ab | 16.2 cd |
| | DDA | 13.0bc | 10.1 ab | 11.3 ab |
| | PCPA | 13.0bc | 10.4 ab | 11.1 ab |
| | BHE | 13.0bc | 10.8 ab | 11.9 ab |
| | DCBHE | 13.0bc | 8.85a | 9.55ab |
| 2 | DDM | 6.60b | 11.2 e | 10.2 d |
| | DBH | 6.60b | 7.60c | 2.60a |
| | DBP | 6.60b | 6.15b | 6.85b |

[a]Values followed by the same letter are not significantly different at the 1% level for any combination of chemical and concentration within the same experiment.

stimulatory chemicals reversed their beneficial effect. On the other hand, DDT, DDA, PCPA, and BHE had no detectable influence at either concentration.

These data show that, at the lower levels tested, none of the test compounds except DCBHE adversely affected either the microbial communities involved in soil respiration or a widespread aquatic alga, and sometimes these compounds promoted microbial activity.

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