ACCUMULATION AND METABOLISM OF DDT AND ITS METABOLITES BY *TETRAHYMENA*

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Abstract. *Tetrahymena pyriformis* readily accumulated DDT and its metabolites from the medium initially containing 1 ppm of each compound. These compounds were accumulated in a decreasing order of DDD, DDE, o,p'-DDT, DDMU, and p,p'-DDT. DDT was metabolized to DDE, o,p'-DDT, and DDMU. DDD was metabolized to DDMU whereas o,p'-DDT, DDE and DDMU were not metabolized. When the organisms were transferred to toxicant free medium excretion of p,p'-DDT and its metabolite occurred in two phases: (a) rapid phase of elimination which was completed during the first 3 h and (b) slow phase of elimination which continued for another 21 h. The implications of these results are discussed.

1. Introduction

Several bacteria and algae have been used to study the accumulation and metabolism of DDT, however, such studies are lacking for protozoans (Johnsen, 1976). It is well known that the aquatic environment generally contains both DDT and its metabolites, such as DDD and DDE which are quite abundant and more persistent than DDT, and may have different affinities towards microorganisms.

In view of the importance of ciliate protozoans in the food chain, studies on accumulation, metabolism and elimination of DDT and its metabolites are required to understand the role played by these compounds in the environment. Hence studies were undertaken in a common fresh water ciliate, *Tetrahymena pyriformis*.

2. Materials and Methods

T. pyriformis were grown in 1% proteose peptone (*w/v*) supplemented with 0.3% yeast extract and 0.5% sodium chloride. Stock cultures were maintained in 10 ml test tubes containing 2 ml of the culture medium. All inoculations were made under aseptic conditions in a UV chamber. Cultures were subcultured once in 15 days.

Stock solutions of p,p'-DDT and its metabolites (p,p'-DDD, p,p'-DDE, o,p'-DDT, and DDMU) were prepared in acetone. The purity of DDT and its metabolites was more than 99% as determined by gas liquid chromatography (GLC). DDT/metabolites were used at a concentration of 1 ppm which has no adverse effects on the ciliate (Rup Lal and Saxena, 1979). Two ml of 72 h old culture were added to 98 ml of sterilized proteose peptone medium (under aseptic conditions) in 250 ml conical flasks. Cultures were grown for 24 h before treatment with one ppm p,p'-DDT/metabolite. Appropriate controls were set up simultaneously. Treated cultures were periodically shaken to keep the cells in suspension. Cultures were harvested at regular intervals by centrifugation

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at 5000 rpm for 10 min and the resulting pellet was washed three times with toxicant free medium and analyzed by GLC. All treatments were replicated three times. For determination of dry weight, treated and control cultures were pelleted by centrifugation, washed and resuspended in distilled water and dried to a constant weight at 80 to 90 °C.

For elimination studies, ciliates were treated with one ppm p,p'-DDT/metabolite under standard experimental conditions for 4 days and then transferred to a toxicant free medium. Samples were collected for estimation of the levels of p,p'-DDT residues or its metabolites at 0, 3, 12, and 24 h after transfer to the toxicant free medium.

DDT residues were extracted from the pellet according to the method described by Rup Lal *et al.* (1981). The recovery of the standards from the spiked samples was p,p'-DDT 95%, p,p'-DDD 97%, o,p'-DDT 98%, p,p'-DDE 95% and DDMU 99%. The metabolic products of DDT were further confirmed by thin layer chromatography (TLC), dehydrochlorination and subsequent GLC. All residues were calculated on dry weight basis but not corrected for recovery.

3. Results and Discussion

The pattern of accumulation and metabolism of p,p'-DDT in *Tetrahymena* is shown in Table I. It is evident that total DDT concentration in *Tetrahymena* increased from 6.3 ppm after 1 h to a maximum of 23.8 ppm after 12 h. Subsequently the total DDT concentration decreased to 1.92 ppm after 6 days and again increased to 7 ppm on the 8th day. The accumulation factor varied from 2 to 24. Similarly, the accumulation of DDMU, p,p'-DDE, o,p'-DDT and p,p'-DDD when incubated separately was fairly rapid in the beginning and was in the order of p,p'-DDD, p,p'-DDE, o,p'-DDT, and DDMU (Figures 1, 2). It is apparent that the initial rapid buildup is mainly due to adsorption whereas slow and continuous accumulation after this period may be mainly due to absorption which continues until the equilibrium is reached. This assumption is also supported by Kenaga (1974).

The time for maximum adsorption/absorption of a particular compound varies from one organism to another and depends on the chemical nature of the compound. *Tetrahymena* took 12, 24, and 6 h to accumulate maximum concentrations of total DDT, DDMU and p,p'-DDE, respectively, whereas accumulation of DDD continued until the end of the experiment when organisms accumulated about 777 ppm of DDD. Protozoans such as *Crithedia fasciculata* (French, 1976) and *Stylonychia notophora* (Rup Lal *et al.*, 1981) took only 6 h to accumulate maximum concentration of DDT.

Tetrahymena pyriformis biomagnified DDT by 24 times from the medium initially containing 1 ppm DDT. Interestingly enough the accumulation factor was maximum for DDD (777) followed by p,p'-DDE (486), o,p'-DDT (418) and DDMU (147). Thus it is seen that certain metabolites of DDT are accumulated by *Tetrahymena* several times more than DDT itself. The protozoans, *Paramecium multimicronucleatum*, *P. bursaria* and *Euglena gracilis* exposed to 1 ppm DDT for 7 days accumulated 99 to 964 ppm DDT (Gregory *et al.*, 1969). It is quite possible that the presence of large proportions of some of these metabolites in higher animals may partly be due to such a preferred

TABLE I

Concentration of DDT and its metabolites (ppm) at different time intervals in *Tetrahymena pyriformis* exposed to one ppm of DDT for 10 days[a].)

Exposure (time in hr)	DDMU	p,p'-DDE	o,p'-DDT	p,p'-DDT	Total DDT AF[b]
1	1.20 ±0.087 (0.19)	2.3 ±0.35 (0.36)	1.16 ±0.13 (0.18)	1.64 ±0.08 (0.26)	6.30
6	3.12 ±0.18 (0.26)	3.84 ±0.24 (0.32)	1.56 ±0.07 (0.13)	3.37 ±0.12 (0.28)	11.89
12	2.34 ±0.12 (0.098)	16.38 ±0.081 (0.68)	1.83 ±0.08 (0.07)	3.25 ±0.2 (0.13)	23.80
24	1.93 ±0.25 (0.25)	1.97 ±0.14 (0.25)	0.77 ±0.03 (0.10)	3.018 ±0.20 (0.39)	7.70
48	1.29 ±0.06 (0.27)	1.72 ±0.33 (0.36)	0.38 ±0.03 (0.08)	1.28 ±0.29 (0.27)	4.67
72	2.48 ±0.32 (0.32)	2.48 ±0.32 (0.32)	0.92 ±0.14 (0.12)	1.83 ±0.28 (0.23)	7.71
96	0.69 ±0.02 (0.16)	1.00 ±0.04 (0.47)	0.61 ±0.02 (0.14)	0.91 ±0.05 (0.22)	4.20
144	0.26 ±0.03 (0.13)	0.46 ±0.02 (0.23)	0.28 ±0.02 (0.14)	0.92 ±0.03 (0.47)	1.92
192	1.11 ±0.02 (0.15)	1.64 ±0.04 (0.23)	0.49 ±0.02 (0.06)	3.82 ±0.13 (0.54)	7.06
240	1.52 ±0.03 (0.22)	3.89 ±0.10 (0.57)	0.24 ±0.02 (0.04)	1.07 ±0.04 (0.15)	6.72

[a] Values in parenthesis indicate the quotient for each compound with respect to total DDT.
[b] AF = Accumulation factor.

accumulation rather than metabolism by the organism itself. It has been shown that the catfish, *Heteropneustes fossilis* can metabolize DDT to only DDE but also contains other metabolites (Agarwal and Gupta, 1974).

Tetrahymena pyriformis metabolized p,p'-DDT to p,p'-DDE, o,p'-DDT and DDMU (Table I). When p,p'-DDD, p,p'-DDE, o,p'-DDT and DDMU were incubated separately none of these compounds were metabolized further except p,p'-DDD which was

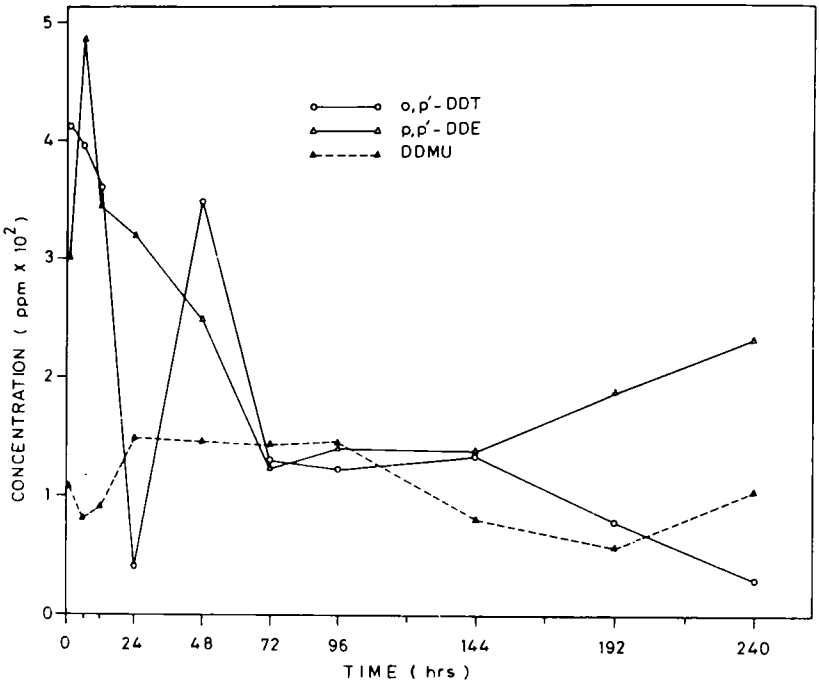

Fig. 1. Concentration of DDMU, p,p'-DDE, and o,p'-DDT (ppm) at different time intervals in *Tetrahymena pyriformis* exposed to one ppm of each compound separately for 10 days.

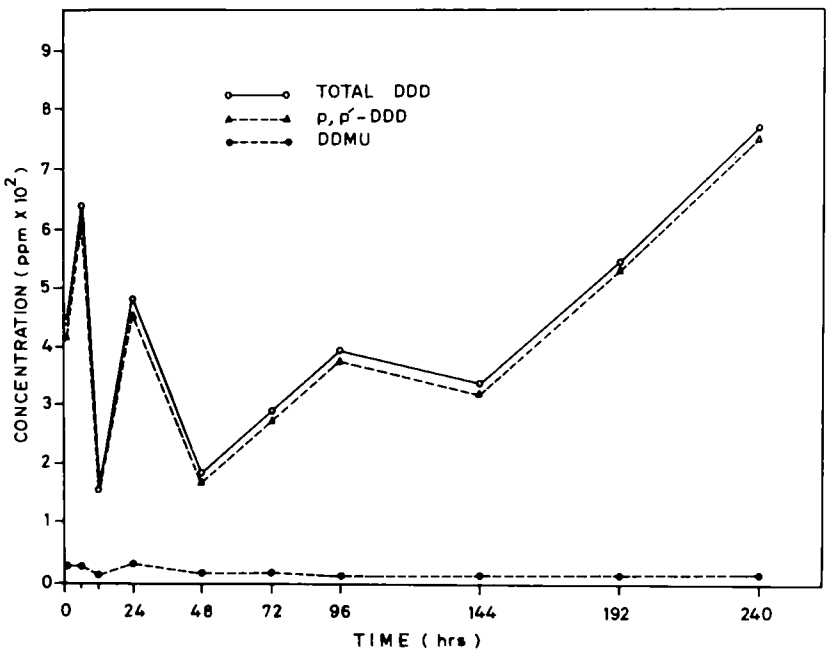

Fig. 2. Concentration of p.p'-DDD and its metabolite, DDMU (ppm) at different time intervals in *T. pyriformis* exposed to one ppm of p,p'-DDD for 10 days.

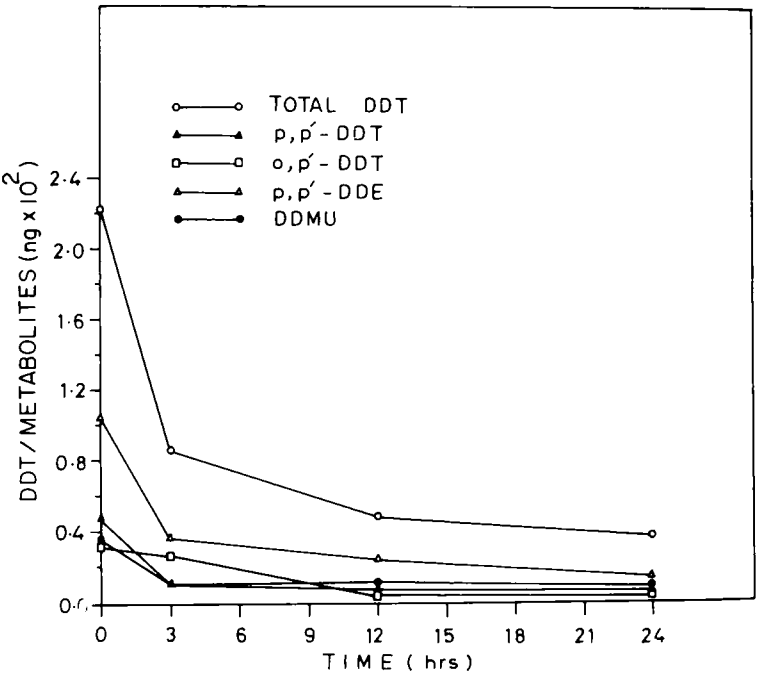

Fig. 3. DDT and its metabolites (ng × 10^2) retained at different time intervals in *T. pyriformis* when organisms (53 mg.-dry wt.) were transferred to toxicant free medium after an initial exposure to 1 ppm DDT for 4 days. Three replicates were used in each case.

converted to DDMU(Figure 2). The products most widely reported from DDT metabolism by microorganisms are DDD, DDE and DDMU (Johnsen, 1976; Williams, 1977). Wedemeyer (1967a,b) has proposed the pathway for DDT metabolism as DDT → DDD → DDMU and DDT → DDE in *Aerobacter aerogenes*. The conversion of DDD to DDMU in *Tetrahymena* may also follow a similar pathway for DDT metabolism. The absence of DDD formation for DDT in *Tetrahymena* may be due to rapid conversion of DDD to DDMU as suggested in *Stylonychia notophora* (Rup Lal *et al.*, 1981). o,p'-DDT, an isomeric product of p,p'-DDT was observed during the present investigation in *Tetrahymena*. Conversion of photodieldrin to photoaldrin and epoxy rings to ketones has been reported in microbes (Matsumura, 1972). *Tetrahymena* did not metabolize o,p'-DDT suggesting that o,p'-DDT is not acted upon by the ciliate as compared to p,p'-DDT.

Tetrahymena pyriformis containing DDT/its metabolites when transferred to toxicant free medium eliminated the accumulated compounds into the medium in two phases: (1) initial rapid phase and (2) slow phase. During the initial rapid phase more than 50% of DDT residues were eliminated from the organisms and during the slow phase about 35% of residues were lost (Figure 3). Elimination of DDT metabolites followed a similar pattern (Figure 4). In *Aerobacter aerogenes* and *Bacillus subtilis* exposed to DDT and later washed with the DDT-free medium, the DDT residues decreased by 55 and 70%,

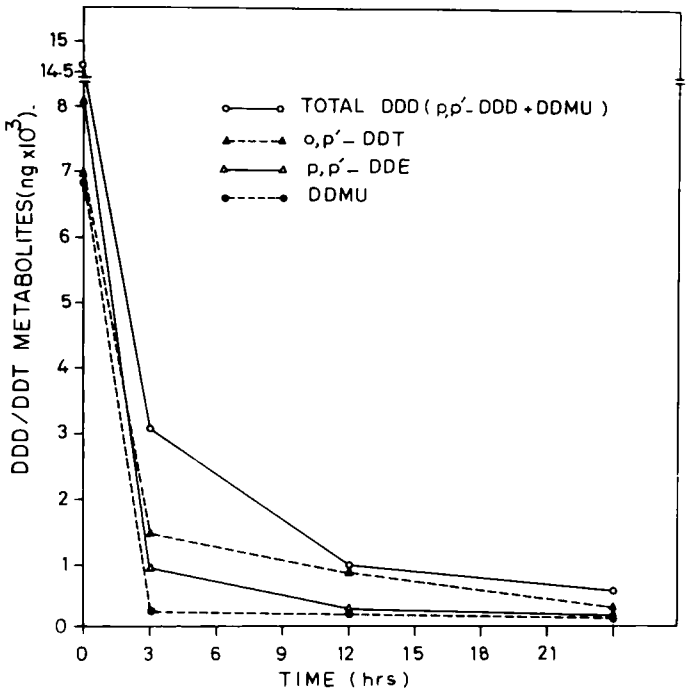

Fig. 4. Amounts of DDMU, p,p'-DDE, o,p'-DDT and total DDD (ng × 10^3) retained at different time intervals in *T. pyriformis* when the organisms (49.3 mg.-dry wt.) were transferred to toxicant free medium after an initial exposure to 1 ppm of each compound separately for 4 days. Three replicates were used in each case.

respectively (Johnson and Kennedy, 1973). *Chlamydomonas* also eliminated α-HCH within 30 min (Canton *et al.*, 1977). However, DDT residues were retained and not released into the medium by *Chlorella* (Sodergren, 1968) and *Euglena* (DeKoning and Mortimer, 1971).

Acknowledgments

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