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Effects of p,p′-DDT, p,p′-DDD, and p,p′-DDE on Oxygen Uptake in the Freshwater Planarian (*Phagocata gracilis*)

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Increased oxygen uptake is a feature of DDT poisoning in insects. Oxygen uptake increases within either minutes or hours after poisoning depending upon the dosage and particularly upon the route of administration of DDT.

KOUYOUMJIAN & UGLOW (1974) found that with a freshwater triclad, Polycelis felina, 10 ppm of p,p'-DDT, p,p'-DDD, and p,p'-DDE in acetone affected the nervous system as revealed by the onset of uncoordinated, loop-like, lateral movements and the retardation of flipover times of these poisoned planarians. In view of this finding, it might be suspected that, with planarians as with insects, oxygen uptake increases significantly following DDT poisoning.

The present work reports the effects of DDT and two of its metabolites (DDD and DDE) on the oxygen uptake in another freshwater planarian, Phagocata gracilis.

MATERIALS AND METHODS

All planarians were collected from a spring-fed stream located in an abandoned limestone quarry alongside State Road 141 south of the dam at Center Hill Reservoir near Smithville in Dekalb County, Tennessee. Planarians were sustained in enamel pans containing pond water maintained at 20°C and fed beef liver weekly. After each feeding, planarians were transferred to enamel pans containing fresh water. Planarians were kept in the laboratory for at least one week prior to being used in a test series.

The first test series was to determine the effect of beef liver and beef liver perfused with corn oil on the oxygen uptake. For this test series 45 planarians were selected and separated into nine groups (three control groups and six test groups) of five worms each. Each of these nine groups was placed in separate glass culture dishes containing pond water and starved for seven days. On the eighth day, the test groups received about 1 g of beef liver that was either untreated or perfused with corn oil. Following 1 h of feeding, the six test groups together with the three control groups were transferred to Warburg vessels for measurement of oxygen uptake on a Warburg respirometer (UMBREIT et al. 1964). Oxygen uptake was measured for 3 h at 30-min intervals at 20°C without agitation. Each group was returned to its respective culture dish until assayed again at 6, 12, 24, 48, 72, and 96 h after treatment.

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The second test series was to determine the effect of DDT, DDD, and DDE on the oxygen uptake. For each test series 60 planarians were selected and separated into 12 groups (three control groups and nine test groups) of five worms each. Each of these 12 groups was placed in separate glass culture dishes containing pond water and starved for seven days. On the eighth day, each group received about 1 g of beef liver perfused with corn oil. The test groups received beef liver perfused with corn oil containing 10 ppm of p,p'-DDT, p,p'-DDD, or p,p'-DDE. After 1 h of feeding, the 12 groups of planarians were transferred to Warburg vessels for measurement of oxygen uptake. Oxygen uptake was measured for 3 h at 30-min intervals at 20°C without agitation. Each group was returned to its respective culture dish until assayed again at 6, 12, 24, 48, 72, and 96 h after treatment. Both test series were repeated four times.

Protein determinations were by the method of LOWRY et al. (1951). Statistical analysis was by Duncan's Multiple Range Test.

RESULTS

Data presented indicate that 60-min exposure of P. gracilis to beef liver perfused with 10 ppm of p,p'-DDD, or p,p'-DDE in corn oil did not produce symptoms of DDT poisoning as reported by KOUYOUMJIAN & UGLOW (1974). There was no mortality in any of the groups used in this study.

Oxygen uptake of planarians fed beef liver significantly increased to 23.5 ± 0.1 ul O_2/mg protein/h, 6 h after feedings, (Table 1). Peak uptake was 1.3 times the basal value 6 h after

Table 1. Oxygen uptake in P. gracilis following feeding of beef liver perfused with corn oil.

Hours after feedings	ul O_2/mg protein/h		
	Basal[a]	Liver[a]	Liver + oil[a]
3	18.4 ± 0.4	23.4 ± 0.1**	25.5 ± 0.1**
6	18.5 ± 0.6	23.5 ± 0.2**	24.6 ± 0.1**
12	18.1 ± 0.6	22.8 ± 0.3**	23.4 ± 0.3**
24	19.3 ± 0.6	22.8 ± 0.4**	22.6 ± 0.2**
48	17.9 ± 0.4	21.6 ± 0.3**	22.0 ± 0.2**
72	17.6 ± 0.3	20.2 ± 0.2**	20.9 ± 0.2**
96	17.2 ± 0.3	20.2 ± 0.2**	19.2 ± 0.2**

Significant difference at p=0.05 (*) and p=0.01 (**).

[a]Sample size is nine groups of five worms each.

feedings, and then declined, falling to 1.2 and 1.1 times the basal values 24 and 72 h after feedings, respectively. Mean values of total oxygen uptake were 22.1 ± 0.2 and 18.2 ± 0.5 ul O_2/mg protein/h for liver-treated and control groups, respectively. Planarians fed beef liver perfused with corn oil significantly increased their oxygen uptake above the basal level to 25.5 ± 0.1 ul O_2/mg protein/h, 3 h after feedings. Oxygen uptake peaked at 1.4 times the basal value 3 h after feedings, and then declined, falling to 1.3, 1.2, and 1.1 times the basal values 6, 24, and 96 h after feedings. Mean values of total oxygen uptake in oil-treated groups were 22.6 ± 0.2 ul O_2/mg protein/h and in control groups 18.1 ± 0.5 ul O_2/mg protein/h.

There was no significant difference over a 96-h period between the oxygen uptake in planarians fed beef liver perfused with p,p'-DDT in corn oil and planarians fed beef liver perfused with corn oil (Table 2). Mean values of total oxygen uptake in DDT-

Table 2. Oxygen uptake in P. gracilis following feeding of beef liver perfused with 10 ppm p,p'-DDT in corn oil.

Hours after feedings	ul O_2/mg protein/h Liver + oil[a]	Liver + oil + DDT[a]
3	23.5 ± 0.1	23.3 ± 0.1
6	24.6 ± 0.1	24.3 ± 0.2
12	23.4 ± 0.3	23.5 ± 0.2
24	22.6 ± 0.2	22.5 ± 0.2
48	22.0 ± 0.2	22.1 ± 0.1
72	20.9 ± 0.2	20.3 ± 0.2
96	19.9 ± 0.2	19.5 ± 0.2

Significant difference at p=0.05 (*) and p=0.01 (**).

[a]Sample size is nine groups of five worms each.

treated and control groups were 22.2 ± 0.2 and 22.42 ± 0.2 ul O_2/mg protein/h, respectively.

Oxygen uptake in planarians fed beef liver perfused with p,p'-DDD in corn oil was not significantly different over a 96-h oil (Table 3). Mean values of total oxygen uptake in DDD-treated groups were 22.2 ± 0.1 ul O_2/mg protein/h and in control groups 22.5 ± 0.2 ul O_2/mg protein/h.

Planarians fed beef liver perfused with p,p'-DDE in corn oil did not increase their oxygen uptake significantly above that in

Table 3. Oxygen uptake in P. gracilis following feeding of beef liver perfused with 10 ppm of p,p'-DDD in corn oil.

Hours after feedings	ul O_2/mg protein/h Liver + oil[a]	Liver + Oil + DDD[a]
3	23.5 ± 0.1	23.5 ± 0.1
6	24.6 ± 0.2	24.3 ± 0.1
12	23.6 ± 0.2	23.3 ± 0.1
24	23.2 ± 0.3	22.6 ± 0.2
48	22.0 ± 0.2	21.8 ± 0.1
72	20.9 ± 0.2	20.8 ± 0.2
96	19.7 ± 0.2	19.4 ± 0.1

Significant difference at p=0.05 (*) and p=0.01 (**).

[a]Sample size is nine groups of five worms each.

planarians fed beef liver perfused with corn oil (Table 4). Mean values of total oxygen uptake were 22.1 ± 0.2 and 21.9 ± 0.2 ul O_2/mg protein/h for DDE-treated and control groups, respectively.

Table 4. Oxygen uptake in P. gracilis following feeding of beef liver perfused with 10 ppm p,p'-DDE in corn oil.

Hours after feedings	ul O_2/mg protein/h Liver + oil[a]	Liver + oil + DDE[a]
3	23.2 ± 0.1	23.1 ± 0.1
6	23.9 ± 0.2	24.0 ± 0.2
12	23.2 ± 0.2	23.3 ± 0.2
24	22.0 ± 0.2	22.4 ± 0.1
48	21.3 ± 0.3	22.1 ± 0.1
72	20.1 ± 0.3	20.3 ± 0.2
96	19.7 ± 0.3	19.4 ± 0.2

Significant difference at p=0.05 (*) and p=0.10 (**).

[a]Sample size is nine groups of five worms each.

DISCUSSION

The three most common transformations of DDT are dehydrochlorination to DDE, oxidation to dicofol and other compounds, and

reductive dechlorination to DDD. Not all of these transformations are, however, detoxifying. KOUYOUMJIAN & UGLOW (1974) found, for example, that p,p'-DDD was more toxic to the freshwater planarian, P. felina, than p,p'-DDT; the LC50 (mean lethal concentration) values approximated a 4:3 ratio of toxicity for p,p'-DDT and p,p'-DDD, respectively. PHILLIPS et al. (1974) reported, however, that with another freshwater planarian, Phagocata velata, p,p'-DDT was detoxified into p,p'-DDD and p,p'-DDE with no adverse effects on this species; reductive dechlorination of DDT to DDD was the major metabolite. With the exception of the reductive dechlorination product DDD, all of the metabolites of DDT so far identified in lower organisms such as planarians are less acutely toxic than DDT.

The data presented indicate that 60-min exposure of P. gracilis to beef liver perfused with 10 ppm of p,p'-DDT, p,p'-DDD, or p,p'-DDE in corn oil was not toxic to this species. It is possible, therefore, that P. gracilis possesses a microsomal enzyme system capable of degrading DDT into DDD and DDE by reductive dechlorination and dehydrochlorination, respectively. Involvement of such a system in the transformation of DDT into DDE in P. velata was suggested by PHILLIPS et al. (1974) and confirmed by BALDWIN & WELLS (1978), who reported that 60-min exposure of P. velata to 10 ppm of p,p'-DDT in corn oil activated NADH-cytochrome b_5 reductase; the activity of NADH-cytochrome b_5 reductase was inversely related to DDT concentration.

It is also conceivable that P. gracilis failed to accumulate within 60 min sufficient amounts of DDT, DDD, or DDE to produce toxic effects. Longer exposure of P. gracilis to DDT and its metabolites could perhaps result in the buildup of dangerously toxic levels in its adipose tissue. Storage of these liposoluble compounds in this tissue would prevent their immediate release and diffusion into surrounding tissues, their excretion, and their metabolism. Under conditions of stress such as starvation, low temperatures, etc., P. gracilis would, however, mobilize fat reserves for energy, resulting in the release and diffusion of these compounds from their storage sites into various tissues, and in the subsequent risk of toxicity. This hypothesis could perhaps explain the findings of KOUYOUMJIAN & UGLOW (1974), who found that, despite a relatively high resistance of P. felina to organochlorine insecticides, prolonged starvation combined with 96-h exposure of this planarian to 10 ppm of p,p'-DDE, p,p'-DDT, and p,p'-DDD in 1% acetone resulted in 92, 96, and 100% mortalities, respectively. In a preliminary study, we observed that exposure of P. gracilis to 1% acetone resulted in 100% mortalities. The death of these planarians appeared to follow the same general pattern as exhibited by P. felina, consisting of a short period in the contracted state, followed by a series of uncoordinated, loop-like, lateral movements (KOUYOUMJIAN & UGLOW 1974).

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