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Metabolism of Aldrin by the Freshwater Planarian *Phagocata gracilis*

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Metabolism of the organocnlorine pesticide DDT by planarians is well documented (Phillips et al. 1974; Kouyoumjian and Villeneuve 1979; Onwumere and Wells 1983). Baldwin and Wells (1978) demonstrated the presence of a microsomal enzyme system in the planarian Phagocata velata. The purpose of the present study was to examine the metabolism of the organochlorine pesticide aldrin by the planarian Phagocata gracilis.

METHODS AND MATERIALS

All planarians (Phagocata gracilis) were collected from a spring-fed stream located across State Road 141 from the Center Hill Dam in DeKalb County, Tennessee. The worms were maintained in water collected from the spring in DeKalb County or from a spring located on the grounds of Oaklands Mansion in Murfreesboro, Tennessee.

The planarians were maintained in 5 liter glass aquaria containing approximately 1 liter of spring water at room temperature (18°-20°C) and fed beef liver weekly. After feeding for one hour, the worms were transferred to clean water and aerated for 2 to 3 minutes. Each aquarium was aerated for several minutes every other day. All planarians were maintained in the laboratory for one week prior to being used in any test series.

Planarians were separated into 10 groups of 20 worms each and placed in glass fingerbowls containing fresh spring water. Three groups were used as controls and were fed 0.25 g of beef liver perfused with corn oil only. The remaining test groups were fed 0.25 g of beef liver perfused with corn oil containing 125 ppm aldrin. Three test groups and three control groups were allowed to feed for one hour then sacrificed immediately after feeding. The remaining test groups were allowed to feed for one hour then transferred to 25 ml of clean water. Three test groups were sacrificed at 20, 40, 60, 120, and 180 minutes after feeding.

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After worms were removed from their bowls, the water samples were collected for extraction and analysis by gas chromatography.

Each group of planarians was rinsed in 1% acetone and weighed. The worms were then homogenized in 2.5 ml of acetonitrile with a Ten Broek tissue grinder. The homogenate was transferred to a conical centrifuge tube and shaken vigorously. All extraneous material was allowed to settle and the liquid fraction decanted into a 125 ml separatory funnel. Extraction of extraneous material with acetonitrile was repeated twice. The combined acetonitrile fraction received 7.5 ml of 2% NaCl and was shaken again. Each sample then received 3 ml of ice cold hexane and was shaken vigorously. The hexane layer was collected and extraction with hexane repeated twice. The combined hexane fractions were transferred to large test tubes and allowed to evaporate to dryness.

Extracts from both the planarians and water samples were cleaned up on florisil columns prior to analysis by gas chromatography. Each column was prepared by plugging a 10 mm (I.D.) glass column with a small piece of glass wool and adding 2.2 g of activated florisil and 1.6 g of $NaSO_4$. Each column was used only once. Samples were dissolved in 0.5 ml of hexane and transferred to the florisil columns. Residues were eluted with 12 ml of hexane followed by 37 ml of 1% methanol/hexane. The eluate was collected in a large test tube and evaporated to dryness under the exhaust hood. Residues were re-dissolved in 0.25 ml of hexane and transferred to a 1 ml reaction vessel. A 1 ul aliquot of each sample was analyzed by gas chromatography. Recovery of residues with this method was 92.6%.

Aldrin epoxidase assays were carried out using a method similar to that of Carlson (1974). Worms (1 g wet weight) were homogenized in 1 ml of ice cold phosphate buffer (0.1 M, pH 7.6). The homogenate was centrifuged at 9000 x g for 20 minutes at 0°C in a Beckman model L3-50 preparative ultracentrifuge. A 0.4 ml aliquot of the supernatant resulting from the 9000 x g centrifugation was added to a reaction medium consisting of 0.1 ml of NADPH (48 mg/ml), 0.1 ml of glucose-6-phosphate (48 mg/ml), 1.39 ml of phosphate buffer, and 0.01 ml of aldrin (18.25 mg/5 ml isopropyl alcohol) as a substrate. Each reaction proceeded at 25°C with constant shaking for thirty minutes. The reaction was terminated by the addition of 2 ml of hexane. After thorough mixing, the two layers were separated and 5 ul of the hexane layer was removed for analysis by gas chromatography.

The remainder of the 9000 x g supernatant was centrifuged at 105,000 x g for one hour at 0°C. The microsomal pellet obtained was dissolved in 5 ml of buffer. Both the microsomal and the soluble fractions were then assayed for aldrin epoxidase activity. Aliquots of all subcellular fractions were removed for protein assays.

All chromatographic analyses were performed in a Varian model 3700 gas chromatograph equipped with an electron capture detector. The gas chromatograph was equipped with a 6' x 2 mm I.D. glass column packed with 11% OV-17+QF, by wt. (80/100) on Gas Chrom Q. The operating temperatures were as follows: column 210°C, detector 270°C. The carrier gas was ultrapure nitrogen maintained at a flow rate of 30 cc/min and head pressure of 20 psig. All chromatographs were recorded on a Beckman linear recorder.

Insecticide residues were identified by comparison of retention times with those of known standards. Residue levels were quantitated by comparison to standards of known concentration. Results were expressed as ng insecticide/mg wet wt.

RESULTS AND DISCUSSION

Aldrin was detected in all tissue samples and was also the only insecticide recovered (Table 1). An unidentified hydrophilic compound was recovered from several water samples. There was no mortality in any group and no signs of toxicity were observed.

Table 1. Levels of aldrin and dieldrin detected in planarians following treatment with 125 ppm aldrin for one hour. Values expressed as ng insecticide/mg wet wt. (ppm).

Time (in minutes)	Aldrin ppm	Dieldrin ppm
0	0.27 ± 0.08	ND
20	0.14 ± 0.05	ND
40	1.12 ± 0.99	ND
60	0.26 ± 0.04	ND
120	0.16 ± 0.04	ND
180	0.16 ± 0.04	ND

Mean values expressed as ppm ± SE for a triplicate sample.
ND = none detected.

Analysis of water samples collected after the planarians were removed showed an unidentified compound eluted from the column after 5.35 minutes. This compound was recovered from water samples collected from 2 groups sacrificed at 120 minutes and from all groups sacrificed at 180 minutes.

No aldrin epoxidase activity was detected in any subcellular fractions assayed.

Phagocata gracilis (Turbellaria:Tricladida) is a free-living freshwater flatworm. P. gracilis is normally found in springs and the headwaters of creeks but members of the species have been found in cool stagnant pools and subterranean waters. The primary factor in determining the distribution of P. gracilis is thought to be temperature. These worms can survive at temperatures as high as 25°C but prefer a range of 0°C to 9.5°C (Kenk 1970). Speight and Chandler (1980), however, have shown that planarians found in the spring in DeKalb County prefer temperatures of 12.6°C and 14.8°C.

On several occasions, usually during the first three days in the laboratory, some of the planarians fragmented. These fragments were placed in separate dishes and kept for observation. Of the 20 pieces that were removed, 7 disintegrated and the rest regenerated new heads. When first observed, the new tissue was colorless but developed the natural coloration 3 to 6 weeks later. P. gracilis has been known to "encyst" as a response to environmental stress (Kenk 1970). It is possible that the fragmentation and regeneration observed in this study was a response to temperature changes that occurred when the worms were removed from the spring and placed in the laboratory.

Metabolism of organochlorine pesticide is known to occur in several species of planarians. Phillips et al. (1974) showed that P. velata converted DDT to DDE and DDD. Results presented by Kouyoumjian and Villeneuve (1979) revealed that both Polycelis felina and Crenobia alpina have the ability to convert DDT to DDE and DDD. Dugesia was able to metabolize aldrin but only 1.03% of the residues recovered were in the form of dieldrin (Khan et al. 1971). Based on the data presented in this study, P. gracilis does not convert aldrin to dieldrin. A hydrophilic compound was detected in water samples from animals treated with aldrin. This suggests an alternate route of detoxification. By using this mechanism, these invertebrates avoid the formation of another toxic compound by converting aldrin directly into a polar metabolite that is readily excreted into their aquatic environment.

REFERENCES

Baldwin RL, Wells MR (1978) Effect of DDT on NADH-cytochrome b_5 reductase activity in the freshwater planarian, Phagocata velata. Bull Environ Contam Toxicol 19:428-340

Carlson GP (1974) Epoxidation of aldrin to dieldrin by lobsters. Bull Environ Contam Toxicol 11:577-582

Kenk R (1970) Freshwater triclads (Turbellaria) of North America. IV. The polypharyngeal species of Phagocata. Smithson Contrib Zool 80:1-17

Khan MAQ, Kamal A, Wolin RJ, Runnels J (1971) In vivo and vitro epoxidation of aldrin by aquatic food chain organisms. Bull Environ Contam Toxicol 8:219-228

Kouyoumjian HH, Villeneuve JP (1979) Further studies on the toxicity of DDT to planaria. Bull Environ Contam Toxicol 22:109-112
Onwumere EA, Wells MR (1983) Metabolism of p, p'-DDT by the freshwater planarian Phagocata gracilis. Bull Environ Contam Toxicol 31:18-20
Phillips J, Wells MR, Chandler CW (1974) Metabolism of DDT by the freshwater planarian, Phagocata velata. Bull Environ Contam Toxicol 12:355-358
Speight DC, Chandler CW (1980) A laboratory study of substrate and temperature preferences of three species of freshwater planarians (Turbellaria: tricladida). J Tenn Acad Sci 55:117-120
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