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Distribution of Pendimethalin in an Aquatic Microecosystem

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The dinitroaniline herbicide, pendimethalin (N-(1-ethylpropyl) -3,4-dimethyl-2,6-dinitrobenzeamine) is used to control annual grasses in a wide range of crops. With normal agricultural usage, any pendimethalin entering the aquatic environment would most likely be associated with pesticide-treated soils transported during runoff or in soil after flooding for rice production. Dinitroaniline herbicides are known to degrade more rapidly in flooded anaerobic soil than under aerobic conditions (HELLING 1976). The anaerobic degradation of pendimethalin has been studied, (SMITH et al. 1979) but its fate in the aquatic environment is not known. This paper reports the distribution of pendimethalin between sediment, water, and aquatic organisms in an aquatic microecosystem.

METHODS AND MATERIALS

Model Ecosystem. The overall procedures and design of the recirculating static model ecosystem have been described (ISENSEE 1980). For the ecosystem study, 400-g quantities of Matapeake silt loam soil (pH 5.3; organic matter content 1.5%; sand, silt, and clay contents of 38.4, 49.4, and 12.2%, respectively), treated with ^{14}C-labeled pendimethalin (25.67 µCi/mg) at 1 and 10 ppm were placed in the bottom of 20-L glass tanks (41 x 20 x 24 cm). Three replicates of each rate plus one control (400 g untreated soil) were prepared and then flooded with 16 L of water. The next day, 16 mosquito fish (*Gambusia affinis*), 16 snails (*Helisoma* sp.), and 1 g of algae (*Oedogonium cardiacum*) were added to the main tank. About 200 daphnids (*Daphnia magna*) were placed in the daphnid chamber (1.5-L capacity glass tank with a stainless steel screen bottom to restrict daphnid passage) which was suspended in the 20-L tank. A percolator water pump continuously moves water into the daphnid chamber and ensured uniform mixing of the water and transport of food to the daphnids. Samples of water and organisms were taken at regular intervals and analyzed as described below.

Sampling and Analysis. Water samples (triplicate 1-mL) were taken at 2-day intervals and analyzed by standard liquid scintillation (LS) methods for total ^{14}C. 100-mL samples were taken 2, 9, 16, 23, and 30 days after flooding, and these were extracted

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using C18 Sep-Paks (Waters Associates, Inc.)[1]. The samples were passed through prewashed (acetone followed by distilled water) Sep-Paks at 2-4 mL/min. Radioactivity was extracted from the Sep-Pak with 10 mL of acetone. Pendimethalin extraction efficiency from water was 99+%. Acetone extracts were spotted on TLC plates (20 x 20 cm GF-254, E. Merck, Darmstadt) and developed for 10 cm with toluene. Each plate was autoradiographed for 2 weeks with Kodak No-Screen medical X-ray film, NS-54T. Tissue samples (two fish, two snails, 45-50 mg daphnids, and 80-100 mg algae) were taken 1, 3, 7, 15, and 30 days after the start of the experiment. After day 30 the experiment was terminated, and the remaining organisms were placed in untreated water and then harvested after 3 and 10 days "desorption". Fish and snails were homogenized whole in acetonitrile, and the homogenate was assayed directly by LS. Daphnids were weighed, placed in LS vials, then ruptured by the cocktail, and analyzed directly by LS. Algae samples were oxidized to determine total ^{14}C. Fish and snail extracts were spotted on each of two silica gel TLC plates which were developed 10 cm with toluene and ethyl acetate:toluene (1/9, v/v), respectively. Plates were autoradiographed as described above.

Soil samples taken after 10 days were vacuum filtered to remove excess water. Ten-g samples of the moist soil were shaken with 50 mL ethyl acetate:hexane (7/3, v/v) for 1 h. After decanting the solvent, 100 mL additional ethyl acetate:hexane was added, and the vessels were shaken for 24 h. After filtration the soil was shaken for an additional 24 h with 100 mL methanol and filtered. The ethyl acetate:hexane and methanol extracts were analyzed separately by LS and TLC. Soil samples taken before and after extraction were oxidized to determine total ^{14}C.

RESULTS AND DISCUSSION

Distribution of radioactivity among various components in the ecosystems and the percent of radioactivity in each component (based on the total ^{14}C added to each tank) are shown in Table 1. Total recovery ranged from 95 to 97% for the two application rates. Apparently very little of the ^{14}C-pendimethalin was converted to $^{14}CO_2$ which would rapidly be lost from the open tanks. However, the amount of ^{14}C pendimethalin available for metabolism was relatively low since only 15 to 18% of the total ^{14}C applied to soi desorbed after 30 days.

Of the ^{14}C remaining in soil, an average of 68% was unextractable and assumed bound, while 32% was extractable (27% in the ethyl acetate:hexane fraction and 5% in the methanol fraction). About 45 and 12% of the ^{14}C in the ethyl acetate:hexand and methanol extracts, respectively, was chromatographically identical to pendimethanlin, while polar metabolite recovery (^{14}C remaining at the origin) was 16 and 44%, respectively.

[1]Mention of a trade name or proprietary product does not constitute a guarantee or warranty of product by the U.S. Dept. Agric. and does not imply its approval to the exclusion of other products that may be suitable.

Table 1. Distribution of ^{14}C-Pendimethalin in an Aquatic Model Ecosystem[1]

Component	1.0 ppm Sum of CPM X 10^5	1.0 ppm %	10.0 ppm Sum of CPM X 10^5	10.0 ppm %
Total CPM initially added to each tank	183.632	100.00	420.420	100.00
- - - - - - - - - - - - - - - - - - Soil[2] - - - - - - - - - - - - -				
Ethyl acetate:hexane (7:3) extractable	40.867	22.25	95.336	22.68
Methanol extractable	7.216	3.93	17.334	4.12
Nonextractable	108.770	59.23	229.906	54.68
- - - - - - - - - - - - - - - - - - Water[3] - - - - - - - - - - - - -				
Total in tanks	19.755	10.76	52.821	12.56
- - - - - - - - - - - - - - - - - - Organisms[4] - - - - - - - - - - -				
Snails	0.914	0.50	3.116	0.74
Fish	0.384	0.21	1.046	0.25
Algae	0.57	0.03	0.145	0.03
Daphnids	0.020	0.01	0.045	0.01
Total recovery	177.983	96.92	399.749	95.08

[1]Average for three replications.
[2]Amounts recovered at the end of the experiment.
[3]Calculated on the day 13 (maximum) water concentration.
[4]Total ^{14}C recovered in all samples taken.

High-performance LC (HPLC) also confirmed the presence, of pendimethalin. An additional spot on the TLC plates, which accounted for 9% and 14% of the ^{14}C in the ethyl acetate:hexane and methanol extracts, respectively, was chromatographically identical to 1-(1-ethylpropyl)-5,6-dimethyl-7-nitrobenzimidazole. However, HPLC failed to confirm its presence. We were interested in looking for this benzimidazole since it was the metabolite reported by SMITH et al. (1979) as forming when Pendimethalin-treated soil was incubated under anaerobic conditions. The remaining ^{14}C on the TLC plates was not identified.

The radioactivity in water reached a plateau concentration after 13 days and did not decline until day-30 (Table 2). The concentration at day-13 represented between 11-13% of the total ^{14}C-labeled pendimethalin initially added to the tanks (Table 1). The amount of extractable radioactivity (Table 2) declined from about 92% (day-2) to 60% (day-30) which indicates a moderate rate of polar metabolite formation. However, according to TLC analysis, pendimethalin accounted for only 30% of the total

Table 2. Concentration of ^{14}C-Pendimethalin Found in Water as a Function of Treatment Rate, Time and Analytical Procedure.

	DC,[1] ppb[2]		% extracted[3]	
Days	1.0[4]	10.0[4]	1.0	10.0
1	1.2	13.5		
2	1.2	13.1	92.0	92.8
3	1.3	12.7		
7	1.8	19.8		
9	2.6	28.5	82.4	86.5
13	2.7	31.4		
16	2.2	25.3	70.8	75.7
20	2.2	25.4		
23	2.6	30.9	57.9	70.3
27	2.6	30.7		
30	1.6	23.6	59.9	61.8

[1]DC = Direct count of water samples.

[2]Based on total ^{14}C activity, expressed as parent compound.

[3]% of direct count analysis.

[4]One and 10 ppm soil treatment.

^{14}C in water on day-2, then decreased to 3% by day-9 and to <1% than for days-16 to 30. At the same time polar metabolites (^{14}C remaining at the origin) increased from 54% (day-2) to 90% or more (day 9 to 30). Total ^{14}C in water was directly related to treatment concentration.

The total biomass of all aquatic organisms contained about 1% of the total ^{14}C in the system (Table 1). The large biomass of snails, compared to the other organisms, accounted for most of the ^{14}C. Distribution of radioactivity among the four species of organisms over time is shown in Table 3. Snails reached their peak ^{14}C level between day-7 and-15. TLC analysis of the snail extracts indicated that 50% of the radioactivity was pendimethalin on day-1 which then decreased to < 1% by day-15. Polar metabolites, R_f of 0.1 or less, ranged from 25 to 35% of the ^{14}C over all sampling times while an unidentified metabolite more polar then pendimethalin (lower R_f values) increased from 12% (day-1 to 45% (day-40) of the recovered ^{14}C. Attempts to identify this metabolite were unsuccessful. Fish reached the highest concentration of radioactivity on day-1, which then decreased steadily for the rest of the experiment. Pendimethalin accounted for 76% of the ^{14}C (TLC analysis) on day-1 which decreased slowly to 22% by day-30. The steady loss of ^{14}C in fish paralleled the rapid loss of pendimethalin from water, even

Table 3. Distribution of ^{14}C-Pendimethalin Among Four Species of Aquatic Organisms in an aquatic Model Ecosystem.

Organisms	Treatment rate[2] ppm	Tissue content ppb[1]						
		Treatment days[3]					Desorption days[4]	
		1	3	7	15	30	4	10
Algae	1.0	58±12[5]	44±7	117±3	269±20	376±32	268±41	151±20
	10.0	535±34	644±51	998±62	2312±114	4479±220	2620±120	1736±201
Snails	1.0	199±9	217±15	419±65	320±20	219±10	188±25	166±9
	10.0	1640±224	2504±75	3971±216	5170±574	3616±281	3470±343	2888±326
Daphnids	1.0	223±31	332±15	476±61	551±5	76±4	51±4	7±1
	10.0	2918±243	3319±96	3030±141	1202±83	525±52	513±32	64±3
Fish	1.0	370±32	355±28	255±18	68±4	26±2	56±22	16±1
	10.0	4218±296	3612±322	2632±226	946±56	567±86	106±17	53±10

[1]Based on total ^{14}C-activity, expressed as parent compound.

[2]Concentration of pendimethalin in 400 g of soil each tank.

[3]Days after start of experiment.

[4]Organisms harvested after 4 and 10 days in untreated water.

[5]Mean tissue content ± standard error for three replications.

though the concentration of ^{14}C in water increased with time. These data suggest that fish preferentially accumulated pendimethalin over other more polar metabolites and degraded it more slowly than snails.

Algae accumulated ^{14}C steadily, throughout the 30-day experiment. Algae growth was apparently not affected since no difference was observed between control and treatment tanks. Daphnids reached peak concentrations between day-7 and-15, organisms placed in untreated water for 10 days all lost ^{14}C, but at different rates. Daphnids, with their high surface area to mass ratio, lost ^{14}C much more rapidly than the other organisms. Maximum concentration of ^{14}C in organisms ranged from 190 (algae) to 312 (fish) times the water concentration.

Conclusions. This study has shown that if pendimethalin enters water adsorbed to soil or sediment, little will desorb, and that which does reach water will rapidly degrade. Pendimethalin accumulated by snails was degraded (or lost) much more rapidly than that accumula- ted by fish. Accumulation patterns indicate that a complex relationship exists between the metabolites found in water and organisms. The rapid degradation of pendimethalin suggests that it poses little danger to the aquatic environment.

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