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Behavior of Trifluralin in Aquatic Model Ecosystems

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The preemergence herbicide, trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) is widely used throughout the United States. With normal agricultural usage, any trifluralin entering the aquatic environment would most likely be associated with pesticide-treated sediments during runoff. PARKA and WORTH (1965) have shown the LC_{50} of trifluralin in treated fish ponds was 0.58 ppm for bluegills and 0.94 ppm for fathead minnows. They also noted that when trifluralin was sprayed on soil and then added to a static pond, the trifluralin was not as toxic, with a bluegill LC_{50} of 28 ppm with Princeton fine sand and 13.2 ppm with Brookston silty clay. PROBST et al. (1969) reported that trifluralin has a low water solubility (1 ppm) and is tenaciously adsorbed to soil, particularly organic matter and clay. Both of these properties would be expected to limit trifluralin availability in the aquatic ecosystem. Considering these physical and toxicological properties, we were interested in studying trifluralin to assess its impact on the aquatic ecosystem.

MATERIALS AND METHODS

In this study two different model ecosystems were used. In the first experiment, the recirculating static model ecosystem, previously described by AMBROSI et al. (1978) was used. Briefly, this ecosystem consists of a 16-liter aquarium divided into two chambers by a glass partition, a screen mounted on top of the partition and pump for circulation between the chambers. This ecosystem design, introduces the pesticide by incorporating it with soil at field application rates. We treated 400 g of Matapeake (Typic Hapludult) 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) with [u-ring-^{14}C-]trifluralin (45.25 μCi/mg) mixed with unlabeled trifluralin of chemical purity greater than 97%, at the rates of 1, 10, and 100 ppm. Trifluralin in ethanol was applied to the soil, the soil was air dried and then thoroughly mixed. Treated soils (in triplicate) were placed in the large chamber of the ecosystem tanks, then flooded with 16 liters of water. Two tanks with untreated soil were used as a control. One day after we flooded the soils, we placed ca. 100 water fleas (*Daphina magna*), 24 snails (*Helosoma* sp.) and 1.0 g of algae (*Oedogonium cardiacum*) in the large chamber and 24 mosquito fish (*Gambusia affinis*) in the small chamber.

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In the second experiment, a continuous dosing system (MOUNT and BRUNGS, 1967) was designed to show the effect of direct application of trifluralin into the ecosystem. The ecosystem tanks (75-liter aquariums) were designed like the recirculating static systems of the first experiment. Three replicates were used at each concentration of 1, 10, and 100 ppb plus three control tanks. Trifluralin in ethanol was injected into the dosing system. The maximum ethanol concentration was 6 ppm, a level used in previous experiments with no adverse effects. Snails, algae, and mosquito fish were used in this experiment.

In both experiments, organisms were sampled 1, 3, 7, 15, and 30 days after the start of the experiment. Water samples (50 ml) were extracted with ethyl acetate:hexane (70:30 V/V), concentrated and analyzed by liquid scintillation counting. This extraction procedure was found to be 95% efficient in extracting trifluralin from the water. In addition, 1-ml water samples were collected and counted directly on days 15 and 30. Fish, snails, and water fleas were analyzed by homogenizing in methanol and then scintillation counting of the homogenates. Algae samples were analyzed for $^{14}CO_2$ using a combustion furnace and trap, followed by scintillation counting. Thin layer chromatograms (TLC) using silica-gel-coated plates, developed with ethyl acetate:cyclohexane (1:1), were used to determine the identity of the ^{14}C found in the snail and fish tissues.

During both experiments, the ^{14}C levels were treated as though all the radioactivity was trifluralin. When TLC analyses were conducted, the percentage of the radioactivity that was trifluralin was determined. Bioaccumulation ratios (BR = ^{14}C conc. in tissue/ conc. in water) are based on total ^{14}C, thus including trifluralin and all of its degradation products.

RESULTS AND DISCUSSION

During the first experiment, the amounts of trifluralin available for accumulation by the organisms depended on the rate of desorption from the soil. The ^{14}C levels in water are an indication of the rate at which this process was occurring (Table 1). By day 15, the hexane-extractable fraction of the ^{14}C present at the 100-ppm treatment rate represented 66% of the radioactivity, and by day 30, this had decreased to 47%, indicating the degree of degradation in the static system. The fish by this time had accumulated 3 ppm of ^{14}C at the 10-ppm treatment rate and 11 ppm at the 100-ppm treatment rate (Table 2). Of these values, 38% was in the form of trifluralin and another 36% was present as a major metabolite; the remainder was unidentified. The ^{14}C rate after 30 days averaged 0.1 ppm at the 10-ppm rate and 3.7 ppm at the 100-ppm rate (Table 4). The TLC analysis of the snail tissue showed 70% of this material was trifluralin and 26% was the same metabolite found in the fish. The BR values of the species tested (after 30 days) were water fleas, 33; snails, 19; fish, 68; and algae, 147 for the 100 ppm treatment rate. No toxic effects were detected in this experiment with any of the life forms.

TABLE 1
Trifluralin Water Concentrations (ppb) in Aquatic Model Ecosystems.

Treatment	rates[1]	Days after start of experiment 1	3	7	15	30
Static	1	0.2	0.7	0.4	0.8	0.9
	10	3.4	2.5	2.9	8.4	9.1
	100	36.9	31.3	55.1	148.8 (223.9)[2]	160.1 (337.8)
Flowing	1	0.1	0.2	0.2	0.5	0.8
	10	0.5	0.9	1.4	2.6	2.5
	100	9.3	16.2	19.6	29.8 (25.3)	21.6 (21.7)

[1] Treatment rates: static - ppm, flowing - ppb.
[2] Values in parenthesis are 1-ml direct counts, others are 50-ml extraction values.

In the second experiment where a continuous dose of trifluralin was introduced into the ecosystem, the actual water levels obtained in the experiment were 0.8, 2.5, and 21.6 ppb in the 1-, 10-, and 100-ppb treatments, respectively, by day 30 (Table 1). With this system, virtually all of the radioactivity present in the water was present in the hexane-extractable form. In this experiment, all organisms had an initial ^{14}C uptake reaching a high in the 100-ppb treatments of 120, 112, and 32 ppm in fish, algae, and snails respectively, by day 15. The ^{14}C uptake decreased through day 30, at which time the levels were 82, 102, and 19 ppm for fish, algae, and snails, respectively. The concentration in water also decreased during this time. By day 30, the ^{14}C in the fish was 62% (46 ppm) trifluralin and 26% (19 ppm) was an unidentified metabolite (the same metabolite as in the first experiment) with the remainder unidentifiable. In the snails, the radioactivity was 38% (12 ppm) trifluralin and 36% (11 ppm) metabolite. The BR values by day 30 were fish, 3919; snails, 878; algae 3961. Except for the BR values for snails, these values are in the range generally regarded as environmentally hazardous.

With this second experiment, we observed several toxic effects in the fish and algae. The algae growth in the 100-ppm treatments was greatly inhibited; by day 30 the mat formed was roughly 2 cm in surface diameter, as compared with 10 cm diameter at start of experiment and a mat diameter of ca. 30 cm in controls and remaining tanks. The fish and snails reproduced in this experiment; however, the young fish were abnormal. By day 16, toxicity was noticeable at the highest concentrations. The young fish were swimming upside down, lying upside down on the bottom, and jerking. We observed darkening of the tail region and unusual curvatures of the back. Adults had unusual curvatures of the back, problems in maintaining proper swimming orientation, yellow bellies and erratic movements. No fish died during this period. The young all lived for another 67 days after trifluralin inputs were stopped on day 30. By the end of this 67-day desorption period, the fish had a

TABLE 2

Tissue Concentrations (ppm ± Standard Deviation) and Bioaccumulation Ratios of ^{14}C-Trifluralin in Mosquito fish *(Gambusia affinis)* in Aquatic Model Ecosystems.[1]

Treatment rates[2]	Days after start of experiment						
	1	3	7	15	30	35[3]	50
Static 1	0.2±0.3 (1000)[4]	2.2±2.2 (3140)	0.3±0.4 (750)	4.6±4.5 (5750)	2.1±1.5 (2630)	0.7±0.7	0.2±0.0
10	1.1±0.9 (320)	2.7±0.8 (1080)	1.1±0.9 (380)	4.2±5.3 (500)	2.7±3.2 (300)	0.4±0.3	0.5±0.5
100	25.4±3.8 (690)	36.0±5.3 (1150)	19.2±5.6 (350)	11.6±1.5 (80)	10.7±2.4 (70)	4.1±1.3	1.8±1.7
Flow. 1	1.1±1.0 (1100)	0.6±0.5 (3000)	1.2±1.6 (6000)	0.9±1.0 (1800)	2.6±2.6 (3250)	1.9±1.4	0.7±0.7
10	1.1±1.2 (2200)	2.4±1.9 (2670)	8.0±1.1 (5710)	5.4±2.0 (2080)	12.7±15.2 (5080)	2.1±1.0	1.0±0.6
100	11.1±1.6 (1190)	30.9±7.7 (1910)	77.7±25.4 (3960)	120.6±24.0 (4050)	82.3±19.5 (3810)	31.3±9.0	-- --

[1] Recirculating static and continuous dosing ecosystems.
[2] Static - ppm, flowing -ppb.
[3] After day 30 the organisms were placed in untreated water.
[4] Bioaccumulation ratio = tissue concentration/water concentration (in parenthesis).

TABLE 3

Tissue Concentrations (ppm ± Standard Deviation) and Bioaccumulation Ratios of ^{14}C-Trifluralin in algae (*Oedogonium cardeacum*) in Aquatic Model Ecosystems.[1]

Treatment rates[2]	Days after start of experiment						
	1	3	7	15	30	35[3]	50
Static 1	0.2±0.3 (1000)	0.2±0.2 (290)	--	0.2±0.2 (250)	<0.1	0.2±0.1	
10	1.7±1.1 (500)	0.6±0.2 (240)	0.7±0.4 (240)	1.8±1.0 (210)	2.1±0.8 (230)	1.2±0.9	0.9±0.4
100	38.1±15.1 (1030)	5.0±0.9 (160)	15.2±2.9 (280)	31.6±6.1 (210)	35.4±4.1 (220)	25.4±7.9	16.6±5.5
Flow. 1	<0.1	0.9±1.5 (4500)	4.0±8.4 (20000)	1.3±1.3 (2600)	1.5±1.3 (1880)	0.6±0.4	12.5±22.8
10	0.1±0.1 (1000)	1.4±1.7 (1560)	33.1±22.9 (23640)	11.1±6.9 (4270)	3.1±2.1 (1240)	2.7±0.8	38.4±65.3
100	2.6±0.6 (280)	41.5±5.2 (2560)	90.8±32.4 (4630)	112.3±88.3 (3770)	102.2±78.8 (4730)	26.7±12.2	7.8±3.2

[1] Recirculating static and continuous dosing ecosystems.
[2] Static - ppm, flowing -ppb.
[3] After day 30 the organisms were placed in untreated water.
[4] Bioaccumulation ratio = tissue concentration/water concentration (in parenthesis).

TABLE 4

Tissue Concentrations (ppm ± Standard Deviation) and Bioaccumulation Ratios of ^{14}C-Trifluralin in Snails (*Hellisoma sp.*) in Aquatic Model Edosystems.[1]

Treatment rates[2]	Days after start of experiment						
	1	3	7	15	30	35[3]	50
Static 1	0.2±0.1 (1000)[4]	0.1±0.1 (140)	0	< 0.1	< 0.1	0.1±0.1	< 0.1
10	0.5±0.3 (150)	0.5±0.5 (200)	< 0.1	0.3±0.2 (40)	0.1±0.1 (10)	0.1±0.1	< 0.1
100	5.6±2.1 (150)	4.4±2.6 (140)	2.4±0.8 (40)	3.5±0.8 (20)	3.7±1.8 (20)	2.1±1.0	0.9±0.3
Flow. 1	0.2±0.1 (2000)	0.1±0.3 (500)	0.2±0.4 (1000)	0.3±0.3 (600)	0.1±0.3 (130)	0.3±0.2	0.2±0.1
10	0.4±0.2 (800)	0.7±0.5 (780)	3.3±5.3 (2360)	4.9±5.3 (1880)	1.5±2.3 (600)	0.6±0.3	0.3±0.1
100	2.5±2.4 (270)	18.0±17.1 (1110)	31.1±21.7 (1590)	32.5±16.7 (1090)	18.7±12.4 (870)	4.9±3.5	4.3±2.0

[1] Recirculating static and continuous dosing ecosystems.
[2] Static - ppm, flowing -ppb.
[3] After day 30 the organisms were placed in untreated water.
[4] Bioaccumulation ratio = tissue concentration/water concentration (in parenthesis).

TABLE 5

Tissue Concentrations (ppm ± Standard Deviation) and Bioaccumulation Ratios of ^{14}C-Trifluralin in Water Fleas (*Daphnia magna*) in Recirculating Static Aquatic Model Ecosystem.

Treatment rates[1]	Days after start of experiment				
	1	3	7	15	30
1	0.2±0.1 (1000)	0.1±0.1 (140)	0.5±0.3 (1250)	0	0.1±0.1 (110)
10	1.9±0.3 (560)	2.7±0.2 (1080)	0.8±0.1 (280)	0.2±0.1 (20)	0.4±0.1 (40)
100	19.6±0.8 (530)	19.6±1.9 (630)	14.0±2.4 (250)	6.4±0.9 (40)	5.2±0.2 (30)

[1] Theoretical water concentration of trifluralin (ppb).
[2] Bioaccumulation ratios = tissue concentration/water concentration (in parenthesis).

body level of 4.5-ppm trifluralin. Trifluralin represented 90% of all the ^{14}C present in the fish at this time.

CONCLUSIONS

These data illustrate the variation that can be obtained when using different model ecosystem designs in studying the behavior of pesticides in the aquatic ecosystem. These differences were probably related to the rapid metabolism and degradation of soil-incorporated trifluralin in the first experiment and to the continuous input of trifluralin in the second experiment. A very important difference between the two ecosystems was the toxic effect of trifluralin on the algae and fish during the second experiment. We believe that the risks of trifluralin in the aquatic ecosystem are greatest when there is a continuous input of tribluralin. With an occasional soil-incorporated input, the degradation processes would minimize this risk.

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