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THIDIAZURON UPTAKE, DISTRIBUTION AND METABOLISM IN BLUEGILLS AND CHANNEL CATFISH[1]

Key Words: Thidiazuron, DROPP®, Cotton Defoliant, Uptake, Distribution, Metabolism, Bluegills, Channel Catfish

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ABSTRACT

Bluegills (Lepomis macrochirus) exposed to 0.1 ppm of thidiazuron-^{14}C cotton defoliant for 28 days under continuous flow conditions accumulated relatively low levels of radiocarbon. The maximum detected was 5.4 ppm in fillet tissue after 1 day. During a 14 day depuration period, radioactivity declined to 1.0 ppm

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or less. Fractionation of offal and fillet tissues from bluegills collected at 28 days indicated that most of the radioactive material was water soluble, although appreciable amounts of organosoluble radioactive material also were present. When bluegills were injected intraperitoneally with thidiazuron-^{14}C, metabolism and elimination were relatively rapid. Organosoluble radioactive material isolated from fish tissue included thidiazuron, its 2-hydroxyphenyl derivative, phenylurea, and several unknowns. Channel catfish (Ictalurus punctatus) exposed under static conditions to a system containing 0.15 ppm of thidiazuron-^{14}C incorporated into soil also accumulated only low concentrations of radiocarbon. The maximum detected was 2.5 ppb in offal tissue at 7 days. In fillet tissue, radioactivity did not exceed 0.5 ppb. There was no evidence from these studies to indicate that thidiazuron would pose a hazard to the aquatic ecosystem.

INTRODUCTION

Thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea) is a new cotton defoliant that promotes the formation of the leaf abscission layer and the early shedding of green leaves. Its excretion balance, metabolic fate, and tissue residues after treatment of rats, goats, and hens have been previously investigated[1,2]. This paper describes thidiazuron uptake, distribution, and metabolism by bluegills (Lepomis machrochirus) and channel catfish (Ictalurus punctatus).

MATERIALS AND METHODS

Two radioactive samples of thidiazuron were provided by NOR-AM Agricultural Products, Inc. (Woodstock, Ill.). Thidiazuron-aniline-^{14}C or thidiazuron-A-^{14}C (specific activity 18.85 mCi/mmol) was uniformly labeled with radiocarbon in the phenyl moiety. Thidiazuron-thiadiazole-^{14}C or thidiazuron-T-^{14}C (specific activity 13.1 mCi/mmol) was labeled with radiocarbon at the thiadiazole carbon adjacent to the urea nitrogen. Radiochemical purity of both samples exceeded 99%.

A flow-through diluter system, modified from that of Mount and Brungs[3], was used to determine the uptake, distribution, and metabolism of thidiazuron under continuous flow conditions. A Micromedic automatic pipette was used to deliver the solvent control and exposure concentrations. Dimethylformamide, which was used as the carrier solvent, did not exceed 50 µL/L in control or exposure aquaria. Three glass aquaria (60 cm long x 30 cm wide x 30 cm deep) each containing 42 L of aerated well water were set in a constant temperature water bath (22 ±1°C). Each aquarium received 250 ml/min of well water containing enough thidiazuron-A-^{14}C or thidiazuron-T-^{14}C to maintain a final concentration of 0.1 ppm. The control received well water containing carrier solvent. After a 2-day equilibration period, 120 bluegills weighing about 1 g each were added to each aquarium. Five fish were removed for analysis for total radiocarbon from the aquaria containing thidiazuron after each of the exposure intervals of 1,

3, 7, 10, 14, 21, and 28 days. On day 28, an additional 20 fish were removed for analysis of distribution and metabolism. Water samples (500 ml) were taken for analysis at 0, 7, 14, and 28 days. After the 28-day exposure period, the thidiazuron exposure was terminated, and a 14-day depuration period was begun for the remaining fish. Five fish were analyzed for total radiocarbon at each sampling interval (1, 3, 7, and 14 days).

Fish tissue was dissected into two fractions, the offal, which consisted of the head, tail, and viscera, and the fillet. Thus there were five offal and fillet fractions for each radiolabel at each sampling interval. To determine the uptake of radiocarbon by the fish, each tissue fraction was combusted (Packard Model 306 Sample Oxidizer), and the $^{14}CO_2$ was measured by liquid scintillation spectrometry[2]. Data are expressed as means (±SD) of five replicates for each sampling interval.

For determination of the distribution of radiocarbon, the 20 fish collected at 28 days were dissected, and the respective offal and fillet portions were combined. The total offal or fillet tissue was homogenized in a mixture of acetone:water (2:1) (3 x 15 ml) to yield an acetone-water fraction and a tissue residue. The acetone was evaporated, and the water was extracted with ethyl acetate (2 x 15 ml). The ethyl acetate was evaporated, and the residue was partitioned between n-hexane (5 ml) and acetonitrile (5 ml). The total radioactivity in the water, acetonitrile, n-hexane, and tissue residue was measured[2].

To monitor the concentration of thidiazuron in the water during the course of the experiment, samples (500 ml) were extracted with ethyl acetate (3 x 50 ml). The ethyl acetate extract was reduced in volume on a rotary evaporator, and an aliquot was radioassayed. The remainder was subjected to two-dimensional thin-layer chromatography (TLC), autoradiography, and radioassay to determine the nature and concentration of the radioactive material[2].

To determine the extent of thidiazuron metabolism by bluegills, 6 fish (weight 12.8 ±2.8 g) were injected intraperitoneally with 2.75 µCi each of thidiazuron-A-^{14}C or thidiazuron-T-^{14}C. Treated fish were kept in 40-L glass aquaria containing 15 L of well water under static conditions. Three fish were removed for analysis 6 and 24 hr after injection. The fish were dissected, and offal and fillet fractions, respectively, from the three fish were combined. The tissue was fractionated into water, ethyl acetate, and residue as described above, and the total radioactivity in each fraction was measured. In addition, the nature and concentration of the radioactive material in the ethyl acetate fraction was determined by two-dimensional TLC, autoradiography, and radioassay[2].

A static study with channel catfish also was conducted. In this case, thidiazuron-A-^{14}C or thidiazuron-T-^{14}C was thoroughly incorporated into sandy loam soil to give a concentration of 0.15 ppm. The soil (5.0 kg) was spread on the bottom of a stainless

steel tank (1.8 m long x 0.9 m wide), and aged aerobically for 14 days. Well water (400 L) was slowly added to the tank, and 50 channel catfish (weight 11.9 ±5.1 g) were introduced. The system was aerated continuously. Three fish were removed for analysis for total radiocarbon after 1, 3, 7, 10, 14, 21 and 28 days. After the 28 day exposure period, 20 of the channel catfish were placed in a stainless steel tank (0.7 m long x 0.36 m wide) containing well water (100 L) and untreated soil (1.25 kg) for a 14-day depuration period. Three catfish were taken for analysis on each of days 3, 7, 10 and 14. Samples of water and hydrosoil also were taken at each sampling interval during exposure and depuration periods.

Channel catfish were dissected into offal and fillet portions for analysis. The offal or fillet from individual fish was placed in a mortar, frozen with liquid nitrogen, and ground until homogeneous. The total radioactivity in a 0.5- to 0.8-g sample was determined by combustion. Data are means (±SD) from three fish. Soil samples (0.25 g) also were combusted. The total radioactivity in water samples was determined by direct counting of 0.5-ml aliquots.

RESULTS AND DISCUSSION

The average recoveries of thidiazuron from water during the continuous flow bluegill exposure were 93% and 97% of theory for thidiazuron-A-^{14}C and thidiazuron-T-^{14}C (Table 1). For both

TABLE 1

Recovery of Thidiazuron-^{14}C from Water During a Continuous-Flow Bluegill Exposure[a]

Time (days)	Recovered radioactivity (%)	
	Thidiazuron-A-^{14}C	Thidiazuron-T-^{14}C
0	81.0	89.0
7	94.8	96.7
14	97.1	99.3
21	91.8	91.6
28	98.4	108.0

[a] 100% recovery = 0.1 ppm.

radiolabels, about 90% of the radioactive material partitioned into ethyl acetate, and greater than 90% of the material co-chromatographed with thidiazuron.

In fillet and offal tissues of bluegills exposed to a concentration of 0.1 ppm of the cotton defoliant, levels of thidiazuron-A-^{14}C equivalents remained nearly constant during both exposure and depuration periods (Figure 1). Radioactivity in these tissues did not exceed 1.0 ppm and often was less than 0.5 ppm. However, with thidiazuron-T-^{14}C, the radioactivity was higher in both fillet and offal tissues. The maxima detected were 5.4 ppm in fillet tissue and 3.8 ppm in offal tissue at 1 day. During the depuration period, radioactivity decreased to 1.0 ppm or less.

In fractionated tissue samples from bluegills collected at 28 days, the relative distribution of radioactivity in the water

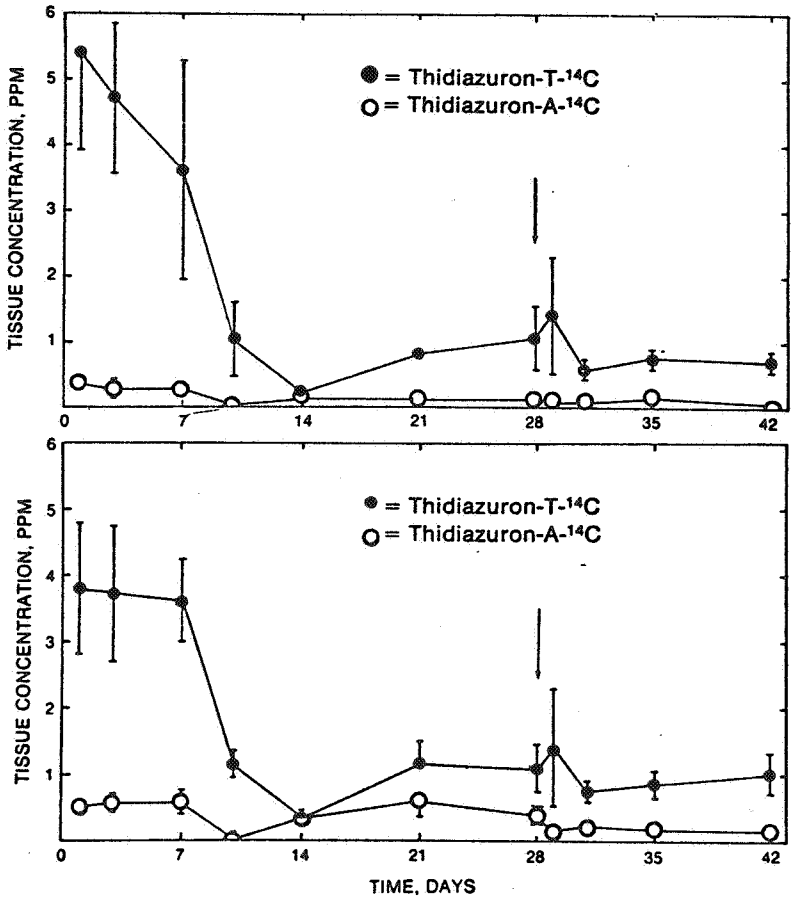

FIGURE 1

Concentration of thidiazuron-^{14}C equivalents in fillet (upper) and offal (lower) tissues of bluegills during a 28-day continuous exposure to a concentration of 0.1 ppm. Vertical lines denote standard deviation about the mean. Arrow indicates initiation of depuration period.

and acetonitrile fractions was similar for the two radiolabels in both fillet and offal tissue (Table 2). However, a different relationship existed for the n-hexane and residue fractions. With thidiazuron-A-^{14}C, radioactivity in both fillet and offal

TABLE 2

Fractionation of Tissue Samples from Bluegills Following Exposure to Thidiazuron-^{14}C for 28 Days at a Concentration of 0.1 ppm in a Continuous-Flow Exposure

Fraction	Radioactivity (%)			
	Thidiazuron-A-^{14}C		Thidiazuron-T-^{14}C	
	Fillet	Offal	Fillet	Offal
Water	49.5	33.6	51.3	32.5
Acetonitrile	19.6	28.2	19.5	27.7
n-Hexane	20.2	23.6	8.1	9.0
Residue	10.7	14.6	21.1	30.8

tissues was higher in the n-hexane fraction than in the residue, while the converse was true for the corresponding tissue fractions from the thidiazuron-T-^{14}C exposure (Table 2).

Although it was possible to gain insight into the partitioning behavior of the radioactive material in bluegill tissue from the previous experiment, radioactivity was insufficient for chromatographic analysis. Consequently, bluegills were injected intraperitoneally with thidiazuron-^{14}C to study its metabolic fate. At 6 hr, 34.9% of the thidiazuron-A-^{14}C dose and 55.4% of the thidiazuron-T-^{14}C dose remained in the fish. At 24 hr, these respective percentages were 29.7 and 32.3. The total recovery of radiocarbon at 24 hr was 82.7% for the thidiazuron-A-^{14}C study and 69.8% for the thidiazuron-T-^{14}C study. Fish excretions that settled to the bottom of the aquarium, which were not sampled, may have contained the rest of the radiocarbon.

Analysis of the aquarium water at 24 hr indicated that 75% of the radioactive material partitioned into ethyl acetate.

Table 3 gives the distribution of radioactivity following intraperitoneal injection of bluegills with thidiazuron-^{14}C. Most of the radioactive material was water soluble after 6 and 24 hr. However, appreciable radioactive material was present in the ethyl acetate fraction. In the ethyl acetate fraction, thidiazuron was the main component (Table 4). Other components were 2-hydroxyphenylthidiazuron or N-2-hydroxyphenyl-N'-1,2,3-thiadiazol-5-ylurea and phenylurea; phenylurea was detectable only in bluegills injected with thidiazuron-A-^{14}C. At least three unknowns and radioactive material at the TLC origin were present (Table 4).

With regard to the radioactivity in the water fractions (Table 3), neither β-glucuronidase nor aryl sulfatase treatment[2] released appreciable radioactive material soluble in ethyl acetate. However, treatment with acid[2] released a considerable amount of radioactive material soluble in ethyl acetate from both radiolabels at 6 and 24 hr. The average amount released was 45% for thidiazuron-A-^{14}C and 37% for thidiazuron-T-^{14}C.

The concentration of thidiazuron-^{14}C equivalents was generally higher in offal than in fillet tissues of channel catfish during a 28-day exposure to thidiazuron-^{14}C incorporated into soil at a concentration of 0.15 ppm; the pattern was similar for both radiolabels (Figure 2). The maxima were about 2.5 ppb in

TABLE 3

Distribution of Radioactivity at 6 and 24 Hours Following Intraperitoneal Injection of Bluegills with Thidiazuron-^{14}C

Fraction	Relative % radioactivity			
	Thidiazuron-A-^{14}C		Thidiazuron-T-^{14}C	
	6 hr	24 hr	6 hr	24 hr
Fillet				
Water	36.5	66.0	16.2	69.0
Ethyl Acetate	46.4	14.0	72.1	10.3
Residue	17.1	20.0	11.7	20.7
Offal				
Water	69.2	86.3	67.3	87.2
Ethyl Acetate	25.0	6.0	26.6	5.7
Residue	5.8	7.7	6.1	7.1

TABLE 4

Nature and Relative Concentration of Radioactive Material Soluble in Ethyl Acetate 24 Hours After Intraperitoneal Injection of Bluegills with Thidiazuron[a]

Compound	Radioactivity (%)			
	Thidiazuron-A-^{14}C		Thidiazuron-T-^{14}C	
	Fillet	Offal	Fillet	Offal
Thidiazuron	43.9	46.3	66.4	66.9
2-Hydroxyphenyl-thidiazuron	8.4	28.8	<0.1	4.9
Phenylurea	25.0	11.1		
Unknowns (3)	12.1	4.7	7.8	2.2
Origin	10.6	9.1	25.8	26.0

[a] TLC was used to separate the radioactive material. The adsorbent was silica gel GF_{254} and the solvent system was ethyl acetate (first direction) and chloroform-ethyl acetate (1:1) (second direction).

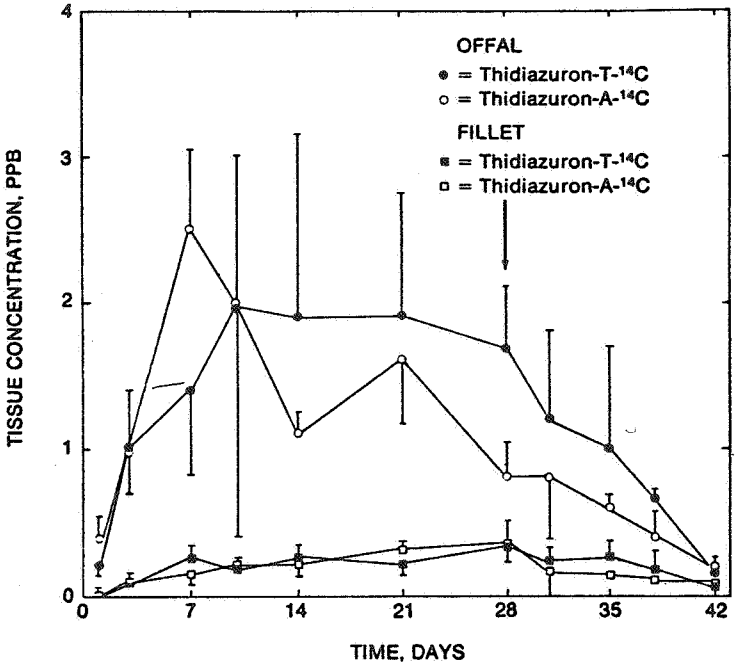

FIGURE 2

Concentration of thidiazuron-^{14}C equivalents in fillet and offal tissues of channel catfish during a 28-day exposure under static conditions. The thidiazuron was incorporated into the soil at a concentration of 0.15 ppm. Vertical lines denote standard deviation about the mean. Arrow indicates initiation of depuration period.

the thidiazuron-A-^{14}C offal tissue at 7 days and about 2.0 ppb in the thidiazuron-T-^{14}C offal tissue at 10 days. The radioactivity decreased to less than 0.5 ppb by the end of the depuration period. Radioactivity in fillet tissue did not exceed 0.5 ppb during either the exposure or depuration periods.

The concentration of thidiazuron-^{14}C equivalents in the water during the exposure and depuration periods never exceeded

TABLE 5

Thidiazuron-^{14}C-Equivalents in Water and Hydrosoil During and After Exposure of Channel Catfish

Days	Concentration (ppb)			
	Thidiazuron-A-^{14}C		Thidiazuron-T-^{14}C	
	Water	Hydrosoil	Water	Hydrosoil
Exposure				
1	0.53	140.0	0.89	170.0
3	0.48	150.0	0.39	130.0
7	0.37	100.0	0.38	150.0
10	0.30	80.0	0.36	110.0
14	0.11	100.0	0.06	100.0
21	0.09	70.0	0.07	70.0
28	0.12	110.0	0.13	100.0
Depuration				
31	<0.05	<0.05	<0.05	<0.05
35	<0.05	<0.05	<0.05	<0.05
38	<0.05	<0.05	<0.05	<0.05
42	<0.05	<0.05	<0.05	<0.05

1.0 ppb, and usually was less than 0.5 ppb. Thus most of the thidiazuron-^{14}C remained in the hydrosoil (Table 5).

In both bluegills and channel catfish, tissue levels of radiocarbon, although relatively low overall, generally were higher for thidiazuron-T-^{14}C than for thidiazuron-A-^{14}C. A similar phenomenon has been observed in other organisms[1,2]. It seems probable that metabolites from the radiolabeled aniline moiety do not form bound residues in fish to a significant extent, possibly because they are water soluble or are converted to water soluble compounds that are subsequently excreted.

Judging from these studies with bluegills and channel catfish, it seems unlikely that thidiazuron will pose a hazard if it gains access to the aquatic ecosystem as a consequence of its use as a cotton defoliant.

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