Excretion Balance, Metabolic Fate, and Tissue Residues following Treatment of Lactating Goats and Laying Hens with Thidiazuron Cotton Defoliant

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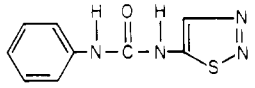
Thidiazuron (*N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea) cotton defoliant was administered for 10 consecutive days to lactating goats and laying hens. The vast majority of the radioactive material (>70%) was eliminated in goat urine and feces; less than 1.5% of the administered radioactivity was in the milk. Hens excreted 72% of the total consumed radioactive material during the 10-day feeding period. In addition to the parent compound, which was present in low levels in goat milk and in chicken excreta, eggs, liver, and kidney, *N*-4-hydroxyphenyl-*N'*-1,2,3-thiadiazol-5-ylurea or 4-hydroxyphenylthidiazuron and phenylurea were detected. 4-Hydroxyphenylthidiazuron was the major thidiazuron metabolite and was present in the free and/or conjugated form in goat and hen excreta, milk, eggs, and certain tissues. Phenylurea was detected only in goat urine. Other unidentified compounds also were present.

Thidiazuron (DROPP) or *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea is a new cotton defoliant with low acute mammalian toxicity (SN 49537 Experimental Cotton Defoliant; NOR-AM Agricultural Products, Inc., Technical Information Bulletin, 1976). Crecelius and Knowles (1978)



Thidiazuron

studied the fate of radiolabeled thidiazuron in rats following administration as a single oral dose and as a dietary supplement. The compound was rapidly metabolized, primarily by hydroxylation at the 4 position of the phenyl moiety, and eliminated in the urine and feces in the free form and as glucuronic acid and ethereal sulfate conjugates.

Since certain components of the cotton plant are used in the preparation of animal feed, it was necessary to study the excretion balance, metabolic fate, and tissue residues in lactating goats and laying hens following treatment with thidiazuron.

MATERIALS AND METHODS

Compounds. Two radioactive samples of thidiazuron were provided by NOR-AM Agricultural Products, Inc. (Woodstock, Ill.) for this study. Thidiazuron-aniline-^{14}C, or thidiazuron-A-^{14}C (sp act. 18.85 mCi/mmol), was uniformly labeled with radiocarbon in the phenyl moiety. Thidiazuron-thiadiazole-^{14}C, or thidiazuron-T-^{14}C (sp act. 13.1 mCi/mmol), was labeled with radiocarbon at the thiadiazole carbon adjacent to the urea nitrogen. Both samples were greater than 99% radiochemically pure as determined by TLC, autoradiography, and radioassay.

The following nonradioactive compounds also were provided by NOR-AM: thidiazuron; *N*-2-hydroxyphenyl-*N'*-1,2,3-thiadiazol-5-ylurea or 2-hydroxyphenylthidiazuron; *N*-3-hydroxyphenyl-*N'*-1,2,3-thiadiazol-5-ylurea or 3-hydroxyphenylthidiazuron; *N*-4-hydroxyphenyl-*N'*-1,2,3-thiadiazol-5-ylurea or 4-hydroxyphenylthidiazuron; and 5-amino-1,2,3-thiadiazole or thiadiazole amine. Samples of phenylurea, 2-hydroxyacetanilide, 3-hydroxyacetanilide, and 4-hydroxyacetanilide were obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis., and samples of aniline, 2-aminophenol, 3-aminophenol, 4-aminophenol, and acetanilide were obtained from Fisher Scientific Co., St. Louis, Mo.

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Treating and Handling of Animals. Two lactating goats weighing about 41 kg (Nubian) and 54 kg (Saanen), respectively, were housed individually in stainless steel restraining cages. They were provided grain, hay, and water free choice. Average daily food consumption measured over a period of 3 days prior to treatment was 2.6 kg (1.0 kg of grain, 1.6 kg of hay) for the Nubian goat and 1.7 kg (0.7 kg of grain, 1.0 kg of hay) for the Saanen goat. Goats were milked by hand twice daily, and the morning and evening milk were combined.

The goats were treated for 10 consecutive days immediately following the morning milking with thidiazuron equivalent to a concentration of 1.5 ppm/day based on pretreatment food consumption values. The Nubian goat received daily 3.9 mg of nonradioactive thidiazuron plus 0.25 mg (15 μCi) of thidiazuron-A-^{14}C. The Saanen goat received daily 2.6 mg of nonradioactive thidiazuron plus 0.22 mg (15 μCi) of thidiazuron-T-^{14}C. The thidiazuron was formulated in gelatin capsules containing a starch carrier.

Two days prior to the initial treatment the goats were catheterized to facilitate collection of urine. Milk production and the amount of urine and feces voided were recorded daily during the test period, and samples were kept for analysis. Food consumption and milk production for the goat treated with thidiazuron-A-^{14}C generally increased during the experiment. However, there was a decrease in food consumption and milk production for the goat treated with thidiazuron-T-^{14}C during the course of the experiment. At the end of the 10-day experiment the goats were killed and tissues were removed for analysis.

Ten White Leghorn laying hens about 1.5-years old and weighing between 1.5 and 2.0 kg each were kept individually in poultry battery units. Water and a standard laying mash diet were provided ad libitum for 3 days after which time the hens were given thidiazuron-treated laying mash. Thidiazuron-treated laying mash was prepared to yield a final concentration of about 1.0 ppm of thidiazuron as follows. To 8 kg of standard laying mash were added an acetone solution containing 4.44 mg of thidiazuron-A-^{14}C (300 μCi) and 3.56 mg of nonradioactive thidiazuron. To another 8 kg of laying mash were added an acetone solution containing 5.06 mg of thidiazuron-T-^{14}C (300 μCi) and 2.94 mg of nonradioactive thidiazuron. Combustion analysis following thorough mixing (Hobart mixer) indicated that the thidiazuron concentration was 1.08 ppm for the mash containing thidiazuron-A-^{14}C and 1.1 ppm for the mash containing thidiazuron-T-^{14}C. Five hens were provided with thidiazuron-T-^{14}C treated laying mash and five were provided with thidiazuron-A-^{14}C treated laying mash ad libitum for 10 consecutive days. Records of food

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consumption, excreta voided, and egg production were kept.

Four hens from each experiment were killed after 10 days. The remaining hen from each experiment was returned to untreated laying mash after the 10-day period and killed 4 days later. Tissues were removed from all hens for analysis.

Chromatography and Radioisotopic Methodology. Separation of thidiazuron and its radiocarbon-containing metabolites was accomplished by TLC with 20 × 20 cm glass plates coated with a 500-μ layer of silica gel GF_{254}. The solvent systems used for two-dimensional TLC were ethyl acetate (first direction) and chloroform–ethyl acetate (1:1) (second direction). The chromatographic behavior of thidiazuron and each of the potential metabolites in these two solvent systems, both single-direction and two-dimensional, was reported by Crecelius and Knowles (1978). Further, an attempt was made to correlate the Roman numeral designations for thidiazuron metabolites in the present study with those in the rat study (Crecelius and Knowles, 1978). Following TLC the plate was placed in contact with no-screen x-ray film and exposed for a minimum of 21 days. For quantitative determination of thidiazuron and its metabolites from TLC, the silica gel corresponding to the darkened images on the film was scraped into a scintillation vial, the cocktail was added, and the mixture was radioassayed.

The radiocarbon content of each sample was measured with a Picker Liquimat 220 liquid scintillation spectrometer. All data were corrected for background, dilution, quenching, and counting efficiency. The scintillation cocktail for all samples, except for hen tissues and excreta analyzed by combustion, was a mixture of toluene (1.5 L), methyl Cellosolve (1.5 L), 2,5-diphenyloxazole (15 g), and 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (0.9 g). Permafluor V (Packard Instruments Co., Inc., Downers Grove, Ill.) counting solution was used for hen tissues and excreta.

Solid samples from goats were combusted in a Schöniger flask as described by Crecelius and Knowles (1978). Solid samples from hens were combusted in a Packard Model 306 Tri-Carb Sample Oxidizer.

Analysis of Urine and Feces. The total radioactivity in goat urine was determined by counting duplicate 0.5-mL aliquots. For fractionation 20 mL of urine was extracted twice with 20-mL aliquots of ethyl acetate. The ethyl acetate extracts were combined, dried over anhydrous sodium sulfate, and concentrated to a volume of about 0.5 mL. The radioactivity in the ethyl acetate fraction (organosoluble) and that remaining in the urine after ethyl acetate extraction (water soluble) was measured. An aliquot of the organosoluble fraction was subjected to TLC, autoradiography, and radioassay to determine its nature and concentration. The nature and concentration of the water-soluble radioactive material were determined by incubating aliquots of the concentrated water fraction with enzymes (β-glucuronidase and aryl sulfatase) and hydrochloric acid for 18 h followed by ethyl acetate extraction, TLC, autoradiography, and radioassay (Crecelius and Knowles, 1978).

The total radioactivity in goat feces and hen excreta was determined by combustion of triplicate 100–300-mg aliquots. Five grams of goat feces or 10 g of hen excreta were extracted twice with a mixture of acetone and water (2:1); the extracts were combined, and the acetone was evaporated. The remaining water fraction was extracted twice with ethyl acetate, and the ethyl acetate extracts were combined and concentrated to about 1 mL. The total radioactivity in the residue, water fraction, and ethyl acetate fraction was measured. An aliquot of the ethyl acetate fraction was subjected to TLC, autoradiography, and radioassay. The water fraction from hen excreta was analyzed in a similar manner to that described above for goat urine.

Analysis of Milk and Eggs. The total radioactivity in goat milk was determined by radioassay of triplicate 0.2-mL aliquots. Goat milk samples (100 mL) were analyzed further by the procedure of Timmerman et al. (1961) to yield acetonitrile, *n*-hexane, water, and residue fractions. The total radioactivity in each fraction was measured, and aliquots of the acetonitile fraction were subjected to TLC.

Hen eggs were homogenized to mix the white and yolk, and triplicate 100– 200-mg aliquots were combusted to determine total radioactivity. Aliquots (5 g) of homogenized hen eggs were extracted three times with a mixture of acetone and water (2:1); the extracts were combined, and the acetone was evaporated. The remaining water fraction was extracted twice with ethyl acetate, and the ethyl acetate extracts were combined and concentrated to an oily residue which was partitioned between *n*-hexane and acetonitrile. The radioactivity in the acetonitrile, *n*-hexane, and water fractions, and residue was measured. The acetonitrile fraction also was analyzed by TLC.

Analysis of Tissues. Tissue samples from goats and hens were analyzed for total radioactivity by combustion of triplicate 100-mg aliquots, except for fat where 50-mg aliquots were analyzed. Certain tissues were fractionated as described previously for hen eggs to determine the distribution of the radioactive material. TLC analysis also was used.

To determine the feasibility of using the Bleidner distillation procedure for analysis of thidiazuron residues, samples of goat liver, kidney, and milk from the thidiazuron-A-^{14}C study were analyzed before and after acid hydrolysis (1 N HCl, 1 h) essentially as described by Lowen et al. (1964).

RESULTS AND DISCUSSION

Excretion Balance. Table I gives the cumulative percentage elimination of radiocarbon in the urine, feces, and milk of goats and in the excreta of hens treated with thidiazuron-^{14}C for 10 days. By the end of the 10-day experimental period goats had eliminated 74.2% (thidiazuron-T-^{14}C) and 86.4% (thidiazuron-A-^{14}C) of the total administered dose. Urine was the major route for elimination of thidiazuron-^{14}C equivalents accounting for slightly more than 50% of the total dose. It seems likely that this figure should be even higher as problems were encountered with the catheters and some of the urine was not collected. Rats treated with thidiazuron-^{14}C as a single oral dose or as a dietary supplement generally eliminated the majority (>50%) of the dose in the feces (Crecelius and Knowles, 1978). An appreciable amount of radioactive material, 20.5% (thidiazuron-T-^{14}C) and 32.0% (thidiazuron-A-^{14}C), was present in goat feces at 10 days. The cumulative percentage of the dose eliminated in goat milk was low comprising only 0.4% (thidiazuron-T-^{14}C) and 1.3% (thidiazuron-A-^{14}C) (Table I).

Hens treated with thidiazuron-^{14}C as a dietary supplement eliminated about 72% (both labels) of the total dose in the excreta by the end of the 10-day treatment period (Table I). An additional 5 to 8% of the dose was eliminated by those hens held for 4 days on untreated food.

Metabolic Fate. The nature and concentration of radioactive material isolated from the urine of goats treated with thidiazuron-^{14}C are given in Table II. The major

Table I. Elimination of Radiocarbon in Urine, Feces, and Milk of Goats and in the Excreta of Hens Treated with Thidiazuron-^{14}C for 10 Days

	Cumulative % of dose eliminated[a]									
	Goat								Hen excreta	
	Thidiazuron-T-^{14}C				Thidiazuron-A-^{14}C					
Day	Urine	Feces	Milk	Total	Urine	Feces	Milk	Total	Thidiazuron-T-^{14}C	Thidiazuron-A-^{14}C
On treated food										
1	37.7	4.6	0.3	42.6	63.7	8.7	0.7	73.1	31.9	50.1
2	39.2	15.0	0.3	54.5	59.8	23.5	1.0	84.3	49.6	64.1
3	47.6	19.5	0.3	67.4	60.4	28.5	1.1	90.0	60.1	68.7
4	55.3	22.5	0.3	78.1	53.8	33.1	1.1	88.0	66.6	70.1
5	55.9	20.4	0.4	76.7	55.3	31.9	1.2	88.4	68.6	70.2
6	57.0	19.5	0.4	76.9	53.9	35.1	1.3	90.3	71.2	71.6
7	56.8	21.3	0.4	78.5	53.2	36.1	1.3	90.6	70.3	70.4
8	56.5	21.4	0.4	78.3	53.9	35.3	1.3	90.5	68.4	71.7
9	58.4	22.8	0.4	81.6	55.6	35.2	1.3	92.1	69.6	72.5
10	53.3	20.5	0.4	74.2	53.1	32.0	1.3	86.4	72.0	72.3
Off treated food										
11									79.0	75.1
12									79.5	75.9
13									79.9	76.5
14									80.3	77.0

[a] Cumulative thidiazuron-^{14}C equivalents eliminated/cumulative thidiazuron-^{14}C administered × 100 = % elimination. Thidiazuron-T-^{14}C "on treated food" data from five hens; "off treated food" data from two hens. Thidiazuron-A-^{14}C "on treated food" data from four hens; "off treated food" data from one hen.

Table II. Nature and Concentration of Radioactive Material Isolated from the Urine of Goats Treated Orally for 10 Days with Thidiazuron-^{14}C

	% radioactive material at indicated days following initial treatment									
	Thidiazuron-T-^{14}C					Thidiazuron-A-^{14}C				
Metabolite or fraction	1	3	5	7	9	1	3	5	7	9
Organosoluble										
I	2.2	5.1	6.7	6.7	5.8	2.1	1.2	0.8	4.1	1.3
III	1.9	3.6	0.6	0.6	0.8	0.1	0.2	0.1	0.9	0.2
V	0.1	0.5	0.2	0.3	0.4	0.2	0.5	0.3	1.0	0.3
VI	14.0	11.8	5.3	7.6	13.2	1.6	2.4	1.8	4.1	3.7
VII	0.1	0.5	0.1	0.2	0.1	0.4	1.4	0.8	1.4	1.3
VIII	0.4	0.3	0.2	0.2	0.5	0.1	<0.1	<0.1	<0.1	0.2
XIV						2.7	6.2	2.4	5.7	5.3
Water soluble	81.3	78.2	86.9	84.4	79.2	92.8	88.1	93.8	82.8	87.7

Table III. Cleavage of Water-Soluble Radioactive Material Isolated from Urine of Goats Treated Orally for 10 Days with Thidiazuron-^{14}C when Incubated for 12 h with Enzymes and Acid

	% radioactivity recovered at indicated days following initial treatment					
	Organic phase			Aqueous phase		
Treatment	1	3	9	1	3	9
	Thidiazuron-T-^{14}C					
β-Glucuronidase	66.9	68.9	68.5	33.1	31.1	31.5
Aryl sulfatase	17.8	12.0	15.9	82.2	88.0	84.1
HCl	47.2	43.6	40.1	52.8	56.4	59.9
Control	18.2	9.5	11.6	81.8	90.5	88.4
	Thidiazuron-A-^{14}C					
β-Glucuronidase	76.3	71.5	72.1	23.7	28.5	27.9
Aryl sulfatase	19.2	15.9	15.3	80.8	84.1	84.7
HCl	37.2	43.5	38.9	62.8	56.5	61.1
Control	17.4	15.6	15.3	82.6	84.4	84.7

organosoluble metabolite in urine from goats treated with thidiazuron-T-^{14}C was metabolite VI; it also was present in the thidiazuron-A-^{14}C study. Metabolite VI was a major metabolite in the rat study, and it was identified as 4-hydroxyphenylthidiazuron (Crecelius and Knowles, 1978). Metabolite XIV was the other major organosoluble radioactive urinary component, and it occurred only with thidiazuron-A-^{14}C. It was identified as phenylurea on the basis of its cochromatographic behavior with authentic phenylurea and on the basis of its mass spectrum. Low-resolution mass spectra of metabolite XIV and of authentic phenylurea were similar. Measurement of metabolite XIV parent ion yielded a mass of 136.06601 (calculated for $C_7H_8OH_2$: 136.06365). Phenylurea also was present in urine of rats treated with thidiazuron-^{14}C (Crecelius and Knowles, 1978). Metabolite I, ostensibly a major metabolite, chromatographed at the TLC origin and was probably a mixture.

The vast majority (>78%) of the urinary radioactive material was water soluble (Table II), and the influence of treatment with enzymes and acid on this material is given in Table III. When compared to the control treatment with β-glucuronidase released an average of 56% (range 48.7 to 59.4%) of the water-soluble radioactive

Table IV. Results of Fractionation of Feces from Goats Treated Orally for 10 Days with Thidiazuron-^{14}C

Days following initial treatment	% radioactivity in		
	Organic phase	Aqueous phase	Residue
	Thidiazuron-T-^{14}C		
1	77.4	5.6	17.0
5	78.8	7.5	13.7
9	74.0	7.2	18.8
	Thidiazuron-A-^{14}C		
1	59.0	4.0	37.0
5	67.2	16.3	16.5
9	69.6	7.5	22.9

material to the organic phase, suggesting the formation of glucuronides. TLC analysis revealed that of the radioactive aglycons released by β-glucuronidase treatment >88% was metabolite VI (4-hydroxyphenylthidiazuron). Results of the aryl sulfatase treatments were erratic, and there appeared to be little, if any, radioactive material in goat urine in the form of ethereal sulfates (Table III). When the urinary water-soluble radioactive material was incubated with HCl the amount of radioactivity that partitioned into the organic phase was less than that observed with β-glucuronidase treatment (Table III). Thidiazuron and metabolite VI (4-hydroxyphenylthidiazuron) form salts in the presence of acid. Thus it was probable that the HCl effected cleavage of the glucuronides; however, certain of the aglycons subsequently formed salts and did not partition into the organic solvent. The stability of thidiazuron and metabolite VI (4-hydroxyphenylthidiazuron) in acid would preclude actual degradation of these compounds following their release as aglycons.

The results of fractionation of feces from goats treated with thidiazuron-^{14}C are given in Table IV. The majority of the radioactive material was in the organic phase, and TLC analysis revealed that from 81 to 100% of this material was metabolite VI (4-hydroxyphenylthidiazuron).

Table V gives the distribution and concentration of radioactive material in the excreta of hens treated with thidiazuron-^{14}C. An average of 41.4% (range 37.9 and 47.5%) of the radioactive material in the excreta was organosoluble. The majority of this material was metabolite VI (4-hydroxyphenylthidiazuron). Metabolite XV cochromatographed with the parent compound. Metabolite XIV (phenylurea) was not detected in hen excreta. The water-soluble radioactive material in hen excreta averaged 34.7% (range 29 to 41.4%). Treatment of this material with β-glucuronidase or aryl sulfatase did not result in any appreciable increase in organosoluble aglycons.

The concentration of radioactive material in milk from goats treated with thidiazuron-^{14}C is given in Table VI. The highest concentration was 83.5 ppb after 10 days in the goat treated with thidiazuron-T-^{14}C. Levels of radioactivity generally were higher in the goat treated with

Table V. Distribution and Concentration of Radioactive Material in the Excreta of Hens Treated with Thidiazuron-^{14}C as a Dietary Supplement

Metabolite or fraction	% radioactivity at indicated days							
	Thidiazuron-T-^{14}C				Thidiazuron-A-^{14}C			
	2	5	7	10	2	5	7	10
Organosoluble								
I	1.3	1.2	1.0	1.6	1.8	1.7	1.2	2.1
II	1.2	0.5	0.3	0.5				
III	0.8	0.7	1.0	0.6	2.5	2.3	2.0	3.7
IV					0.6	0.6	0.4	<0.1
VI	30.8	33.2	35.5	30.9	31.2	26.9	28.9	30.0
VII	4.3	1.1	1.1	1.3				
VIII	0.2	<0.1	<0.1	<0.1	2.5	3.6	2.9	6.7
XIII	<0.1	0.5	<0.1	0.4	1.0	<0.1	<0.1	<0.1
XV	3.2	2.0	3.1	2.6	1.1	2.5	1.5	2.2
XVI					2.5	3.4	1.8	2.8
Water soluble	29.0	31.2	31.1	35.3	37.1	39.0	41.4	33.2
Residue	29.2	29.6	26.9	26.8	19.7	20.0	19.9	19.3

Table VI. Concentration of Radioactive Material in Milk from Goats and Eggs from Hens Treated with Thidiazuron-^{14}C for 10 Days

Day	Ppb[a]			
	Goat milk		Hen eggs	
	Thidiazuron-T-^{14}C	Thidiazuron-A-^{14}C	Thidiazuron-T-^{14}C	Thidiazuron-A-^{14}C
On treated food				
1	10.3 (1.3)	27.8 (4.0)	<1.0	<1.0
2	33.7 (3.1)	32.4 (2.3)	2.4 (0.2)	1.5 (0.2)
3	50.9 (5.3)	36.7 (3.2)	3.3 (0.2)	3.6 (0.8)
4	53.6 (2.0)	33.2 (3.9)	9.9 (0.5)	6.8 (0.9)
5	66.6 (4.9)	33.2 (3.9)	15.3 (0.7)	10.9 (0.9)
6	68.8 (0.9)	33.4 (2.8)	23.5 (0.6)	17.1 (1.1)
7	70.0 (0.6)	22.1 (6.3)	30.0 (0.7)	21.8 (0.6)
8	76.8 (0.4)	18.5 (2.6)	34.1 (1.4)	21.8 (1.2)
9	81.5 (9.9)	24.4 (0.7)	34.7 (0.8)	19.7 (2.3)
10	83.5 (2.3)	19.7 (1.2)	37.2 (1.4)	23.5 (1.0)
Off treated food				
11			38.9 (1.8)	
12			34.2 (0.7)	26.1 (0.3)
13				22.3 (0.3)
14			29.0 (1.4)	16.6 (0.5)

[a] Data are means with standard deviation given in parentheses.

Table VII. Results of Fractionation of Milk from Goats Treated Orally for 10 Days with Thidiazuron-^{14}C

Days after initial treatment	% radioactivity			
	Acetonitrile phase	Hexane phase	Aqueous phase	Residue
	Thidiazuron-T-^{14}C			
1	29.1	2.7	44.1	24.1
3	37.9	0.1	22.2	39.8
5	58.8	0.7	14.9	25.6
7	48.7	0.9	16.1	34.3
9	47.2	0.4	14.9	37.5
	Thidiazuron-A-^{14}C			
1	52.6	<0.1	32.8	14.6
3	44.3	0.6	23.0	32.1
5	56.1	<0.1	21.8	22.1
7	43.1	<0.1	20.2	36.7
9	40.0	<0.1	17.6	42.4

thidiazuron-T-^{14}C than in the goat treated with thidiazuron-A-^{14}C. The thidiazuron-T-^{14}C treated goat did not adjust to the restraining cage as well as the thidiazuron-A-^{14}C treated goat, and this difficulty was manifested primarily by a decrease in milk production throughout the experimental period. Thus the higher levels of radioactivity in milk from the thidiazuron-T-^{14}C treated goat were not unexpected. Table VII gives the results of fractionation of goat milk. An appreciable amount of radioactivity was present in the acetonitrile phase (average 45.8%, range 29.1 to 58.8%). TLC of pooled acetonitrile fractions from days 1, 3, and 5 revealed that metabolite VI (4-hydroxyphenylthidiazuron) comprised an average of 36.4%, metabolite XV (thidiazuron) 30.2%, metabolite I (origin) 26.8%, and two other compounds 6.6%. Analysis of pooled acetonitrile fractions for days 7 and 9 indicated that the major component was metabolite VI (4-hydroxyphenylthidiazuron); several other metabolites were present, but the parent compound (metabolite XV) was not detected. There also was an appreciable amount of radioactive material in the aqueous phase and residue (Table VII). Treatment of the water-soluble radioactive material with β-glucuronidase or aryl sulfatase did not result in any appreciable increase in organosoluble radioactive material.

The concentration of radioactive material in eggs from hens treated with thidiazuron-^{14}C is presented in Table VI. Generally, the concentration of radioactivity increased with time on treated food. The maximum level detected was 38.9 ppb. Table VIII gives the distribution of radioactive material in eggs collected at days 6 and 10. There was apparently a difference in distribution relative to the two radiolabels, and this difference was in the ratio of radioactive material in the acetonitrile fraction as compared to the residue. TLC analysis of pooled day 6 and 10 acetonitrile fractions showed that metabolite VI (4-hydroxyphenylthidiazuron), metabolite VII (unknown), and metabolite XV (thidiazuron) were present; however, the low levels of radioactivity precluded quantitation and an explanation for the observed difference.

Tissue Residues. The concentration of radioactive material in tissues of goats and hens treated with thidiazuron-^{14}C is given in Table IX. Generally, tissue levels of radioactivity were somewhat higher with thidiazuron-T-^{14}C than with thidiazuron-A-^{14}C. A similar phe-

Table VIII. Distribution of Radioactive Material in Eggs and Tissues of Hens Treated with Thidiazuron-^{14}C as a Dietary Supplement

Tissue	% radioactivity in indicated fraction							
	Thidiazuron-T-^{14}C				Thidiazuron-A-^{14}C			
	Water	Acetonitrile	Hexane	Residue	Water	Acetonitrile	Hexane	Residue
Egg, day 6	27.0	24.0	<0.1	49.0	27.3	42.2	2.1	28.4
Egg, day 10	23.7	26.4	0.5	49.4	27.5	40.6	1.1	30.8
Liver, day 10	10.4	19.6	0.4	69.7	7.5	14.2	0.4	77.9
Kidney, day 10	14.7	14.0	1.4	69.9	15.4	15.0	1.1	68.5

Table IX. Concentration of Radioactive Material in Tissues of Goats and Hens Treated with Thidiazuron-^{14}C for 10 Days

Tissue	Ppb thidiazuron equivalents after indicated days[a]					
	Goat, 10 days[b]		Hen[c]			
			Thidiazuron-T-^{14}C		Thidiazuron-A-^{14}C	
	Thidiazuron-T-^{14}C	Thidiazuron-A-^{14}C	10 Days	14 Days	10 Days	14 Days
Abomasum	40.4 (10.0)	22.8 (15.3)				
Blood	18.8 (13.3)	16.8 (14.9)	69.1 (9.6)	23.0	55.6 (7.6)	16.3
Brain	<10.0	<10.0				
Fat, subcutaneous			3.2 (0.4)	1.4	2.2 (0.5)	2.0
Fat, visceral	<10.0	<10.0	3.1 (1.2)	1.2	2.6 (0.8)	4.8
Gizzard			22.6 (0.8)	9.1	18.2 (3.0)	6.4
Heart	15.0 (10.0)	<10.0	30.6 (5.8)	9.2	23.6 (4.5)	7.9
Intestine	207.7 (49.1)	15.7 (8.9)	53.8 (6.5)	12.9	43.5 (4.4)	15.0
Kidney	113.3 (9.2)	42.2 (11.4)	119.8 (16.9)	23.5	112.4 (28.3)	27.8
Liver	117.1 (17.3)	86.2 (4.4)	114.1 (35.3)	32.7	93.0 (16.8)	15.7
Lung	51.6 (22.2)	<10.0	62.4 (7.7)	17.1	51.7 (18.4)	20.1
Muscle, hind leg	10.9 (5.1)	<10.0				
Muscle, fore leg	<10.0	<10.0				
Muscle, neck	13.4 (8.2)	<10.0				
Muscle, leg			13.4 (1.4)	5.8	13.7 (2.2)	1.8
Muscle, breast			12.8 (0.7)	4.9	10.4 (2.2)	3.5
Oviduct			23.8 (3.0)	5.4	13.5 (3.0)	4.3
Pancreas	21.0 (15.6)	10.2 (2.7)	30.9 (2.5)	9.5	18.9 (7.0)	7.2
Spleen	27.3 (10.1)	11.8 (6.2)	34.2 (3.0)	12.1	26.8 (9.1)	9.7
Uterus	18.9 (3.2)	<10.0				

[a] Standard deviation given in parentheses. [b] Lower limit of detectability was 10 ppb. [c] Data at 10 days are means of tissues from three hens; data at 14 days are from tissues of one hen. Lower limit of detectability was 1 ppb.

Table X. Recovery of Radioactive Material by Bleidner Distillation and Subsequent Radioassay from Milk, Liver, and Kidney Samples of a Goat Treated Orally for 10 Days with Thidiazuron-Aniline-^{14}C

Tissue	Dpm[a]	Recovery, %
Milk (50 mL)	2778 (135)	18.4
(302 dpm/mL; day 6)	2922 (51)	19.4[b]
	2652 (80)	17.6[b]
Liver (10 g)	2387 (172)	32.6
(732 dpm/g)	1767 (210)	24.1
	2335 (95)	31.9[b]
	4143 (81)	56.6[b]
Kidney (10 g)	1747 (54)	48.8
(358 dpm/g)	1613 (108)	45.1
	1931 (28)	54.0[b]

[a] Mean with standard deviation given in parentheses. [b] Recovery upon subjecting tissue sample to hydrolysis with HCl prior to distillation.

nomenon was observed with rats (Crecelius and Knowles, 1978). Kidney and liver contained highest concentrations of radioactivity with the exception of the goat intestine (thidiazuron-T-^{14}C). The distribution of radioactive material in hen liver and kidney is given in Table VIII. Most of the radioactivity was in the residue and was not analyzed further. However, TLC analysis was carried out on the acetonitrile fractions and indicated that metabolite VI (4-hydroxyphenylthidiazuron), metabolite XV (thidiazuron), and metabolite VII (unknown) were present. Fractionation of goat liver samples indicated that most of the radioactivity remained with the residue. TLC analysis was not conducted because of the low levels of organosoluble radioactive material.

The recovery of radioactive material by Bleidner distillation and subsequent radioassay from liver, kidney, and milk samples obtained from the goat treated with thidiazuron-A-^{14}C is given in Table X. Values generally were highest for kidney, intermediate for liver, and lowest for milk. However, the highest recovery value observed was 56.6%. Thus Bleidner distillation would not be expected to give extremely high recovery values if used to extract thidiazuron metabolites from these tissues even though essentially quantitative recovery of the parent compound can be achieved.

Residues of thidiazuron-^{14}C equivalents were high in goat and hen excreta and low in meat, milk, and eggs. Thidiazuron, 4-hydroxyphenylthidiazuron, and phenylurea were detected in those instances where levels of radioactivity were sufficient for characterization of the radioactive material. Further, these same compounds were detected when thidiazuron metabolism was studied in rats (Crecelius and Knowles, 1978). Thidiazuron itself possesses low toxicity to mammals, the acute oral LD_{50} to mice and rats being >4000 mg/kg (SN 49537 Experimental Cotton Defoliant; NOR-AM Agricultural Products, Inc., Technical Information Bulletin, 1976). We determined that the 24-h LD_{50} to mice (Swiss Webster, 20 g each) following intraperitoneal injection (dimethyl sulfoxide, 0.1 mL/mouse) was >500 mg/kg for 4-hydroxyphenylthidiazuron and phenylurea. Thus based on these studies thidiazuron would not be expected to pose a health hazard to animals.

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