Metabolism of Thiabendazole in Laying Hen and Lactating Goats

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Thiabendazole (TBZ), an anthelminthic and fungicide of the benzimidazole class, was rapidly metabolized by lactating goats and laying hens. In lactating goats dosed orally with 120 mg of [^{14}C]TBZ daily for 7 consecutive days and sacrificed at about 24 h after the final dose, an average of 74% of the total administered dose was accounted for in the matrices analyzed. Nearly all of this recovered radioactivity was found in the excreta (urine, 69%; feces, 28%) with very little (≤3%) in milk and tissues (liver, kidney, fat, muscle). In laying hens dosed orally with 3.19 mg of [^{14}C]-TBZ daily for 10 consecutive days and sacrificed at about 24 h after the final dose, an average of about 97% of the total administered dose was accounted for in the matrices analyzed. Nearly all (>99%) of this recovered radioactivity was found in the excreta with very little in the eggs (~0.16%) and tissues (0.29%). Enzyme preparations (Glusulase, sulfatase, β-glucuronidase) and strong acid hydrolyses were used to release TBZ and its metabolites from hen and goat tissues and matrices. On the basis of these enzymic and acid hydrolyses, and subsequent HPLC radiochromatographic and GC/MS analyses of the extracts, the major metabolite of TBZ in the excreta, edible tissues, milk, and eggs was determined to be 5-hydroxythiabendazole or its *O*-sulfate conjugate. Minor amounts of benzimidazole and TBZ were also found in most of the goat and hen tissues examined. The results of this study show that the metabolic fates of TBZ in lactating goats and laying hens are similar, predominantly involving hydroxylation at the 5-position followed by sulfation.

Keywords: *Thiabendazole; 5-hydroxythiabendazole; 5-hydroxythiabendazole O-sulfate; benzimidazole; GC/MS*

INTRODUCTION

Thiabendazole [2-(4′-thiazolyl)benzimidazole, TBZ] (Figure 1) is the active ingredient in fungicidal preparations effective against several fungal genera (including *Aspergillus*, *Colletotrichum*, *Cytospora*, Fusarium, *Penicillium*, *Sclerotinia*, *Thielaviopsis*, and *Phoma*) that affect plants and is also an anthelminthic used in both animals and humans (Spencer, 1981). It is currently used for postharvest treatment of food products to prevent deterioration (Edgington et al., 1971; Logan et al., 1975; Meredith, 1977). Since livestock might consume TBZ residues on treated foodstuffs, it is important to determine its metabolic fate in animals.

The metabolism of TBZ in humans and farm animals has been previously studied; oral administration of TBZ to sheep (Tocco et al., 1964), cattle and goats (Tocco et al., 1965), and dogs and humans (Tocco et al., 1966) resulted in rapid absorption from the gastrointestinal tract. Peak plasma levels varied with species and ranged from 1 (dogs, humans) to 7 h (goat, cattle). In dogs, goats, and cattle, approximately 82% of the dose was excreted in urine and feces within the first 72 h following oral administration. The excretion rate in humans was more rapid, with approximately 80% being found in urine within the first 24 h. In all of the species studied, hydroxylation of the benzimidazole ring at the 5-position to form 5-hydroxythiabendazole (5HTBZ) and subsequent conjugation to the glucuronide and sulfate are the major metabolic steps, accounting for between 70 and 95% of the urinary metabolites in sheep, goats, and swine. In dogs and humans, these conjugates account for 23 and 38%, respectively, of the urinary metabolites.

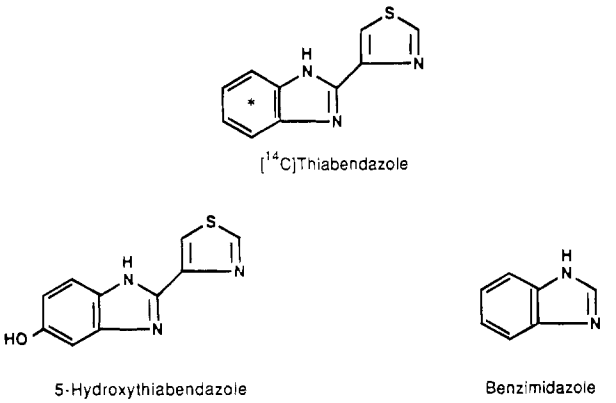

Figure 1. Structures of [^{14}C]thiabendazole (TBZ) and its two metabolites, benzimidazole (BNZ) and 5-hydroxythiabendazole (5HTBZ). The asterisk denotes the position of the ^{14}C label.

However, these early studies were not conducted with doses based on anticipated total daily feed intake of TBZ from treated foodstuffs. In addition, the nature and amounts of the residues in milk, eggs, and edible tissues were not determined. The objective of the present studies was to determine, using dosing rates expected from feed intake, the nature and amounts of residues of orally administered TBZ present in edible tissues of

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lactating goats and laying hens and in milk and eggs. Residues in urine and feces were also determined. With an understanding of the nature, levels, and distribution of TBZ residues, especially in edible tissues, following oral administration, the safety of these residues with respect to human exposure can be better assessed.

MATERIALS AND METHODS

Chemicals. Two separate batches of [U-^{14}C-*phenyl*]-2-(4'-thiazolyl)benzimidazole ([^{14}C]TBZ) with radiochemical purities of >99.3% were prepared by the Labeled Compound Synthesis Group at Merck Research Laboratories. Prior to oral administration, these [^{14}C]TBZ batches were diluted to specific activities of 0.0047 and 0.0079 mCi/mg for the goat and hen studies, respectively. The hydroxylated [^{14}C]TBZ derivative, 5-hydroxy[^{14}C]thiabendazole ([^{14}C]5HTBZ), was isolated from urine of a goat treated with [^{14}C]TBZ and purified to >99% by flash and thin layer chromatography. Unlabeled analytical grade benzimidazole (BNZ) was purchased from Sigma Chemical Co., St. Louis, MO, while unlabeled 5HTBZ was synthesized at Merck Research Laboratories in Rahway, NJ. The structures of [^{14}C]TBZ and its two metabolites are shown in Figure 1. All of the solvents used in this study were of analytical grade (J. T. Baker, Phillipsburg, NJ).

Preparation of Capsules. For treatment of hens, a solution of [^{14}C]TBZ was prepared daily by dissolving the labeled compound in DMSO at 50 °C. Aliquots, each equivalent to 3.19 mg (~25.2 μCi) of [^{14}C]TBZ, were added to individual cellulose capsules. Capsules containing only cellulose were also prepared for administration to control hens. For treatment of goats, aliquots, each equivalent to 120 mg (564 μCi) of [^{14}C]TBZ, were pipetted into the cellulose capsules. Capsules containing only cellulose were also prepared for administration to control goats.

Animal Handling and Dosing. All hens used in this study were housed in cages and given water and food. Twenty-five hens (*Gallus domesticus*, Schnuck's Agri-Foods, Hawkpoint, MO, ~26 weeks old, 1–2 kg each, 20 treated and 5 controls) separated into five groups (1 control and 4 treated per group) were acclimated for 7 days prior to dosing. Following acclimation, [^{14}C]TBZ was administered to the test hens orally by manual insertion of the capsule into the esophagus. Each control and treated hen received a single capsule on each morning during the 10 consecutive day dosing period. The hens were sacrificed humanely by use of cervical dislocation and necropsy performed on the 11th day (about 24 h following the final dosing). For the lactating goats (*Capra hircus*, Leslie Findling Farm, Atlanta, MO, ~1 year old, 45–60 kg each), three treated and two controls were acclimated for 14 days prior to dosing. All goats were given water, hay, and goat chow and housed in individual stalls throughout the study. Following acclimation, the capsules containing [^{14}C]TBZ were administered to the test goats orally using a balling gun. Each goat (control and treated) received a single capsule on each morning during the 7 consecutive day dosing period. The goats were humanely sacrificed by use of electrocution and necropsy performed on the eight day (about 24 h following the final dosing).

Sample Collection. In the hen metabolism study, excreta were collected daily, while eggs were collected twice daily, in the morning and evening. Tissue samples were collected from each of the animals upon necropsy, and each tissue type was pooled by treatment group. Hen tissues collected were liver, kidney, gizzard, heart, abdominal fat, breast muscle, and thigh muscle. In the goat metabolism study, feces, urine, and milk were collected twice daily, while tissue samples were collected upon necropsy. Goat tissues collected and composited by weight were fat (perirenal and omental fat, 1/1 w/w), liver, kidney, heart, blood, gall bladder contents, and muscle (semimembranosus, triceps, and longissimus dorsi muscles, 1/1/1 w/w/w).

Equipment. Radioactivity contained in liquid samples and fat tissues, and in sample extracts, was quantified by direct liquid scintillation counting (LSC) on either a Searle Model 300 or a TM Analytic Delta 300 liquid scintillation counting system. Radioactivity contained in solid samples was quantified by LSC following oxidative combustion with either a Packard 306B Tricarb sample oxidizer or a Harvey OX-500 biological material oxidizer. In both cases, Carbosorb was used as the [^{14}C]CO_2 trap. HPLC was typically conducted using a Shimadzu gradient HPLC system and a Gilson Model 202C fraction collector connected in series with an Alltech Econosphere, 4.6 mm i.d. × 250 mm C_{18} column. A flow rate of about 2.0 mL/min was used in conjunction with a gradient solvent system consisting of water [buffered with 0.01 M potassium hydrogen phosphate (K_2HPO_4) and 0.01 M triethylamine (Et_3N)] and methanol. Gas chromatographic/mass spectrometric (GC/MS) analyses of sample extracts and of the corresponding authentic standards were accomplished using a Finnigan MAT 5100 quadrupole mass spectrometer interfaced with a Finnigan Model 9611 gas chromatograph, controlled by a Super Incos data system. Chromatographic separation was achieved using a DB-5 (J&W Scientific) phenylmethyl silicone, bonded-phase fused-silica capillary column (30 m × 0.25 mm film) with carrier gas (helium) flow at about 2 mL/min. Gas chromatographic separation involved a splitless injection, with an injector temperature of about 250 °C and a column oven temperature program of 50–150 °C at 10 °C/min and then 150–220 °C at 60 °C/min. The mass spectrometer was run in EI mode using full scans. The presence of 5HTBZ was investigated by GC/MS following either methylation with trimethylanilinium hydroxide in methanol (MethElute, Pierce, Rockford, IL) or trimethylsilylation with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, Alltech, Deerfield, IL) in acetonitrile. The presence of BNZ was investigated using the selective ion monitoring (SIM) method described by VandenHeuvel and Liesch (1986).

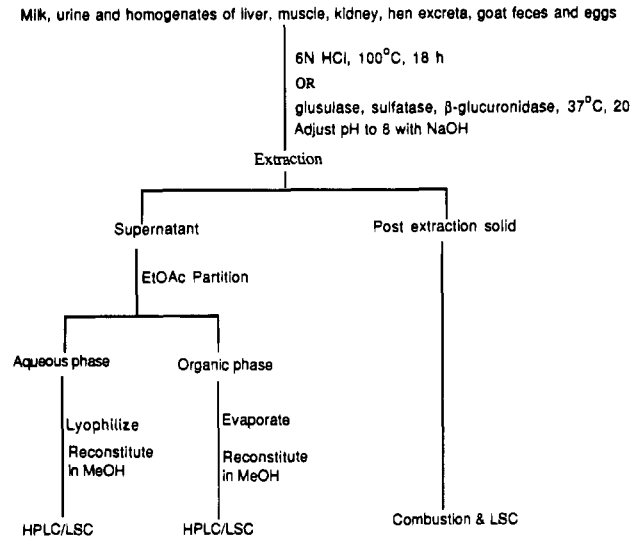

Figure 2. Flow sheet for the extraction and characterization of TBZ residues in hen and goat matrices.

Determination of Total Radioactive Residues (TRR). Aliquots of milk, fat, and urine samples from the goat were mixed directly with the appropriate scintillation cocktail and analyzed by LSC. Composited and processed goat feces, muscles, liver, kidney, heart, and blood were combusted, and the resulting $^{14}CO_2$ was analyzed by LSC for the determination of TRR. These procedures were also repeated in the hen for composited excreta, egg, fat, liver, kidney, gizzard, heart, breast muscle, and thigh muscle.

Extraction and Characterization of TRR. Techniques used for the release of [^{14}C]TBZ residues in tissues consisted of enzyme and acid hydrolyses and Raney nickel reduction. Although all three techniques were not used in each individual sample, the most quantitative residue-releasing technique was utilized for each matrix analyzed. A flow sheet for the enzymic and acid hydrolytic extraction and characterization of the TRR in hen and goat matrices is presented in Figure 2. For Glusulase treatment, the pH of the medium was buffered at 5 with a 0.1 M sodium acetate buffer. In the case of sulfatase,

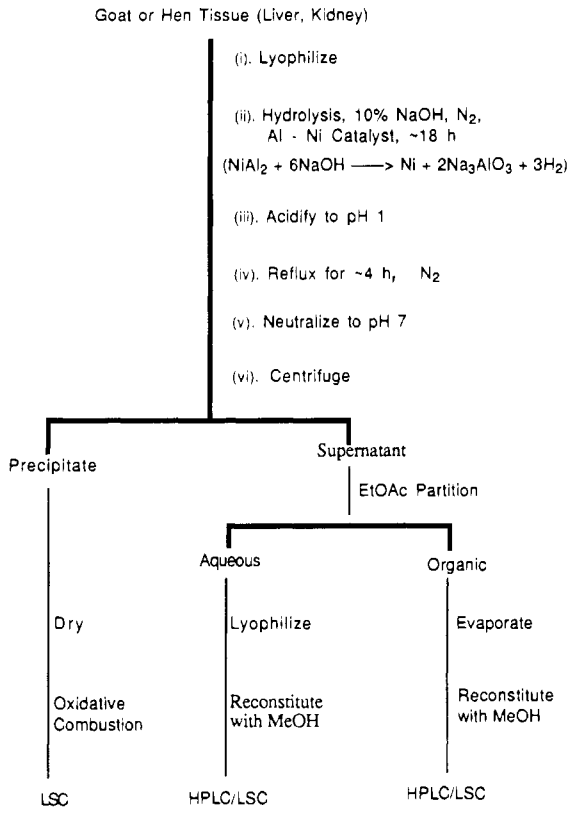

Figure 3. Flow sheet for the release and characterization of unextracted TBZ residues in goat and hen tissues using Raney nickel treatment.

the pH was buffered at 7 with 0.05 M Tris buffer. For β-glucuronidase hydrolysis, the pH was buffered at 7 with 0.5 M potassium phosphate buffer. Following enzyme or acid hydrolytic release, the supernatants were partitioned against ethyl acetate into organic and aqueous phases. Respective aliquots from these phases, following solvent evaporation or lyophilization, were then reconstituted in methanol for HPLC analysis. Postextraction solids, when present, were dried using a gentle stream of air and then taken up for oxidative combustion and LSC.

Raney Nickel Reduction. Activated metal catalysts are known to desulfurize thiazoles, producing α-methylamines (Badger and Kowanko, 1957). The method used to release potential thiolate-bound residues from animal tissues using Raney nickel reduction has been previously described by Monson (1991) and is summarized in Figure 3. Tissue sample (liver, kidney) aliquots were lyophilized and then reductively hydrolyzed for about 18 h by treatment with 10% NaOH followed by nickel–aluminum alloy under a blanket of nitrogen. Following reaction of the tissue samples with the generated Raney nickel (according to the reaction $NiAl_2$ + $6NaOH \rightarrow Ni + 2Na_3AlO_3 + 3H_2$), the resulting hydrolysate was acidified and eventually partitioned with ethyl acetate into aqueous and organic phases. These phases, following lyophilization or solvent evaporation, were then reconstituted with MeOH for HPLC analysis.

RESULTS AND DISCUSSION

Distribution of TBZ Residues. *Goat.* Total radioactive residue levels in goat tissues, milk, urine, and feces are shown in Tables 1 and 2. The majority of these residues, per day, were found in the urine (~51%) and feces (~21%). The residue levels in urine, feces, and milk reached steady state by 2 days of dosing. These steady state levels were ~42 ppm in urine, ~26 ppm in feces, and ~1 ppm in milk (Table 2). Following the cessation of dosing and subsequent necropsy, the average residue levels in the composited tissues were determined (Table 1). The majority of the recovered radioactivity, 97.6%, was found in the excreta. About 1% of dose was excreted in the milk. For edible tissues the TBZ residue levels followed the order liver (~4.8 ppm) > kidney (~1.4 ppm) > muscle (0.1 ppm) > fat (0.03 ppm) (Table 1). These residue levels were relatively low, cumulatively accounting for about 1% of the recovered radioactivity.

Hen. Total radioactive residue levels in hen tissues, egg, and excreta are shown in Tables 3 and 4. An average of 96.6% of the total administered dose was recovered following 10 days of oral administration of [^{14}C]TBZ. The large majority of this recovered dose (99.6%, 26 ppm) was found in the excreta. The total

Table 1. Total Radioactive Residues (TRR) in Tissues of Goats Dosed with [^{14}C]TBZ[a]

	TRR (ppm)			
tissue	goat 1	goat 2	goat 3	av
liver	3.70	4.36	6.24	4.77
kidney	1.37	1.32	1.47	1.39
gall bladder contents	0.68	0.37	1.49	0.85
heart	0.23	0.19	0.24	0.22
composited fat	0.01	0.02	0.05	0.03
composited muscle	0.08	0.11	0.12	0.10
blood	0.17	0.21	0.19	0.19
% of dosed radiocarbon recovered in urine, feces, and milk	63.1	77.6	79.3	73.3
% of dosed radiocarbon recovered in tissues	0.80	1.00	1.10	0.97
% of dosed radiocarbon recovered in urine, feces, milk, and tissues	63.9	78.6	80.4	74.3

[a] Total administered dose is based on a nominal dose of 840 mg (~3.948 mCi) for each animal (120 mg/day × 7 days).

Table 2. Average Daily Distribution of [^{14}C]TBZ Residues in Goat Urine, Milk, and Feces

	TRR					
	urine		feces		milk	
study day	ppm	%[a]	ppm	%[a]	ppm	%[a]
1	40.25	52.54	13.83	12.92	0.49	0.49
2	41.91	37.46	26.44	20.08	0.96	0.85
3	42.53	50.85	27.09	22.68	1.13	0.91
4	37.98	52.01	28.55	25.87	1.09	0.84
5	44.29	53.16	26.58	19.42	1.24	0.94
6	43.24	59.74	24.85	20.74	1.16	0.96
7	30.99	54.15	22.60	25.95	1.02	0.80
av per day	40.17	51.42	24.28	21.09	1.01	0.83

[a] Represents the percent of daily administered dose (120 mg/day). ppm is μg/g.

Table 3. Total Radioactive Residues in Hens Dosed with [^{14}C]TBZ[a]

	TRR (ppm)				
matrix	group 1	group 2	group 3	group 4	av
excreta	25.95	23.88	27.46	27.07	26.09
egg	0.12	0.10	0.09	0.11	0.10
liver	1.60	1.39	1.45	1.45	1.47
kidney	1.22	1.17	1.25	1.18	1.20
gizzard	0.31	0.28	0.34	0.25	0.29
heart	0.31	0.31	0.34	0.32	0.32
composited fat	0.02	0.02	0.02	0.01	0.02
breast muscle	0.08	0.06	0.06	0.07	0.07
thigh muscle	0.11	0.09	0.08	0.08	0.09

[a] Total administered dose is based on a nominal dose of 31.9 mg (~0.252 mCi) for each hen (3.19 mg/day × 10 days). ppm is μg/g.

Table 4. Daily Distribution of TBZ Residues in Hen Eggs and Excreta

	TRR (ppm)									
	group 1		group 2		group 3		group 4		av	
day	egg	excreta	egg	excreta	egg	excreta	egg	excreta	egg	excreta
1	ND[a]	27.2	ND	22.1	ND	26.3	ND	21.3	ND	24.2
2	0.1	25.5	0.1	20.6	0.1	26.2	0.1	26.4	0.1	24.7
3	0.1	25.5	0.1	23.4	0.1	28.4	0.1	27.5	0.1	26.2
4	0.1	24.4	0.1	24.1	0.1	29.2	0.1	32.3	0.1	27.5
5	0.1	24.6	0.1	25.5	0.1	25.9	0.1	25.9	0.1	25.5
6	0.1	28.5	0.1	22.3	0.1	27.3	0.1	28.3	0.1	26.6
7	0.2	26.6	0.1	26.1	0.1	28.9	0.1	24.4	0.1	26.5
8	0.2	25.7	0.1	23.1	0.1	27.4	0.2	27.0	0.2	25.8
9	0.2	25.1	0.1	25.6	0.1	25.6	0.1	28.3	0.1	26.1
10	0.2	26.6	0.2	26.1	0.1	29.4	0.1	29.5	0.1	27.9
daily av	0.1	26.0	0.1	23.9	0.1	27.5	0.1	27.1	0.1	26.1

[a] ND, <0.005 ppm.

Table 5. Composition of Extracted TBZ Residues in the Matrices of [^{14}C]TBZ-Treated Goats

	residues (ppm)[a]				
matrix	TBZ	5HTBZ	*O*-sulfate of 5HTBZ	BNZ	%[b]
urine	ND[c]	7.87	9.49	ND	29.9
feces	0.34	2.10	ND	0.41	4.91
milk	0.02	ND	0.40	ND	0.72
liver	0.20	0.12	ND	0.08	0.69
kidney	0.07	0.10	ND	0.05	0.38
muscle	ND	0.01	ND	0.01	0.02
fat	ND	ND	ND	ND	ND

[a] ppm stands for μg/g. [b] Expressed as a function of the total radioactive residues found in the treated goats. [c] ND, <0.005 ppm.

Table 6. Composition of Extracted TBZ Residues in the Matrices of [^{14}C]TBZ-Treated Hens

	residues (ppm)[a]				
matrix	TBZ	5HTBZ	*O*-sulfate of 5HTBZ	BNZ	%[b]
excreta	ND[c]	4.40	3.40	ND	29.9
egg	0.01	0.03	ND	0.02	0.23
liver	0.05	0.07	ND	0.03	0.57
kidney	0.11	0.40	ND	0.12	2.41
muscle	ND	ND	ND	ND	ND
fat	ND	ND	ND	ND	ND

[a] ppm stands for μg/g. [b] Expressed as a function of the total radioactive residues found in the treated hens [c] ND, <0.005 ppm.

residue levels reached steady state by day 2 in excreta and in egg. These steady state levels were ~25 ppm in excreta and ~0.1 ppm in egg (Table 4). The total TBZ residue levels in edible tissues followed the order liver (~1.5 ppm) > kidney (1.2 ppm) > muscle (~0.1 ppm) > fat (0.02 ppm) (Table 3). Cumulatively, these residues account for about 0.4% of the recovered radioactivity. Similarly, the total TBZ residues in egg (0.1 ppm) accounted for about 0.01% of recovered radioactivity.

Nature of the TBZ Residues. HPLC radiochromatographic assays of urine, excreta, feces, milk, eggs, and tissue extracts following a combination of 6 N HCl, β-glucuronidase, sulfatase, and Glusulase hydrolytic extractions indicate that the metabolites of TBZ are BNZ and 5HTBZ, the latter present either unconjugated or as the *O*-sulfate. The presence of the *O*-sulfate conjugate of 5HTBZ was based on its successful hydrolysis to 5HTBZ with both Glusulase and sulfatase and the lack of success with β-glucuronidase.

Goat. The composition of the TBZ residues in the matrices of treated goats is summarized in Table 5. In urine, the metabolites were unconjugated 5HTBZ (~7.9 ppm, 13.6%) and 5HTBZ *O*-sulfate conjugate (~9.5 ppm, 16.3%). These percentages are expressed relative to the total recovered radioactivity. The feces contained unconjugated 5HTBZ (2.1 ppm, 2.4%) together with low levels of BNZ (~0.4 ppm, 0.5%) and unmetabolized TBZ (~0.3 ppm, 0.4%). In milk, unmetabolized TBZ (0.02 ppm, 0.02%) and the *O*-sulfate conjugate of 5HTBZ were found (0.4 ppm, 0.4%). Qualitatively, the residues in tissues (i.e., liver, kidney, muscle, fat) were similar to those of feces and were comprised mostly of unmetabolized TBZ, unconjugated 5HTBZ, and BNZ. Unextracted residues remained in the liver and kidney following the hydrolytic extraction process, at concentrations of about 0.3 (0.05%) and 0.1 ppm (0.005%), respectively. Attempts made to release these residues using Raney nickel reduction/hydrolysis for characterization by HPLC, ^{1}H NMR, and mass spectrometry were inconclusive, due in part to the low levels involved.

Hen. Analysis of the tissues and excreta from the treated hens indicates that their composition of [^{14}C]-TBZ residues is similar to that in goat and the farm animals studied earlier (Tocco et al., 1964, 1965). The results of these analyses are summarized in Table 6. Unconjugated and conjugated 5HTBZ are the major metabolites (total of 7.8 ppm, 30% of the total recovered radioactivity in excreta). Measurable levels of TBZ were not found in the excreta. In egg, the residues were unmetabolized TBZ (0.01 ppm, 0.01%), unconjugated 5HTBZ (0.03 ppm, 0.05%), and BNZ (0.02 ppm, 0.02%). The residue profiles in the liver and kidney were similar to that found in egg, consisting of unmetabolized TBZ, unconjugated 5HTBZ, and BNZ. Neither parent TBZ, 5HTBZ, nor BNZ was individually present, in detectable amounts (≥0.005 ppm), in the muscle and fat.

GC/MS Confirmations. Isolates corresponding to the metabolites 5HTBZ and BNZ were obtained from goat urine and liver, respectively, by preparative HPLC. Total ion chromatograms and mass spectra from the goat urine isolate were the same as for the authentic 5HTBZ standard. The presence of BNZ in the goat liver isolate was also confirmed by GC/MS. The similarities in retention time and of characteristic ions (*m/z* 118, parent; *m/z* 91, loss of HCN; *m/z* 63) between authentic BNZ standard and the purified liver isolate demonstrate the presence of BNZ in the liver extract.

Similarly, a hen excreta isolate corresponding in RP-HPLC retention time to 5HTBZ was analyzed by GC/MS. The results confirm the presence of 5HTBZ in the excreta, based on the same GC retention time and similar mass spectrum as authentic 5HTBZ. The major fragment ions were the molecular ion (M^+ 217) and the M − 27 fragment ion (*m/z* 190) resulting from the

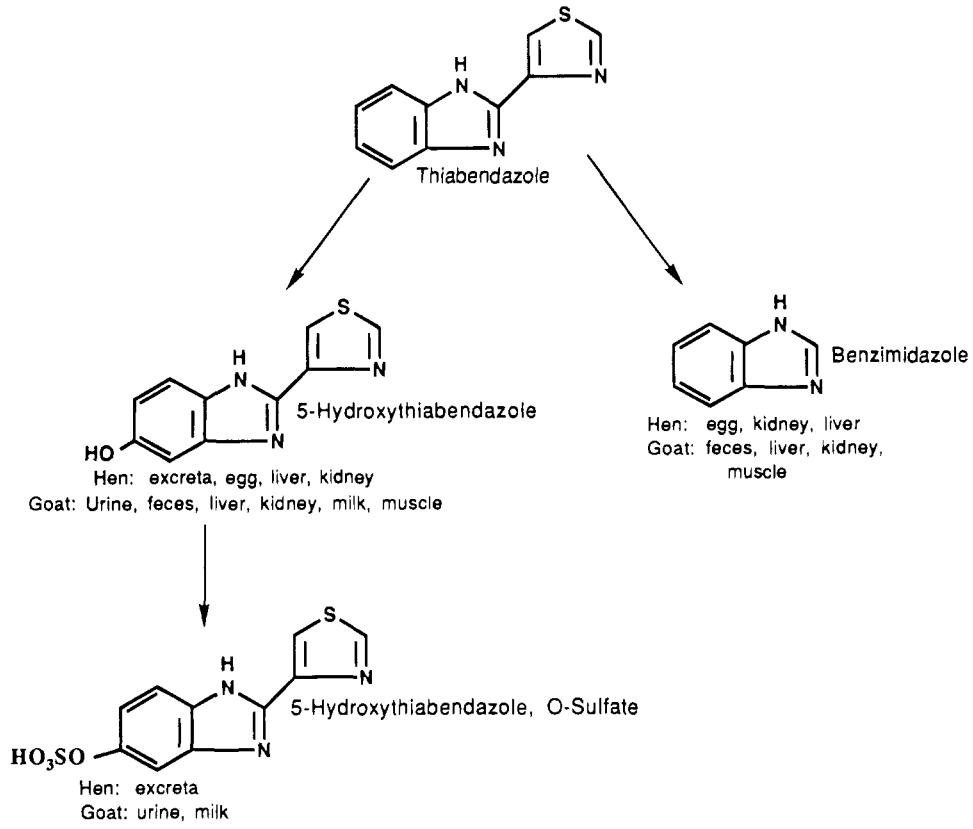

Figure 4. Proposed metabolic pathway of TBZ in dairy goat and laying hen.

elimination of HCN from the thiazole ring, a well-documented mode of fragmentation for TBZ-related benzimidazoles (Ellsworth et al., 1976; VandenHeuvel et al., 1977).

Proposed Metabolic Pathway. The proposed metabolic pathway of TBZ in the lactating goat and laying hen is presented in Figure 4. Metabolism occurs predominantly via hydroxylation at the 5-phenyl position to form 5HTBZ. Some or most of this 5HTBZ is subsequently converted to the *O*-sulfate conjugate. A minor metabolic pathway found in feces, liver, kidney, and eggs involves loss of the thiazolyl group of TBZ to form BNZ.

Conclusions. In lactating dairy goats, orally administered TBZ was metabolized to BNZ, 5HTBZ, and the sulfate conjugate of 5HTBZ. The majority of the residues were then excreted in the urine and, to a lesser extent, in feces. The average daily levels of total TBZ residues in urine and feces were approximately 40 and 24 ppm, representing 51 and 21%, respectively, of the daily administered dose. About 72.5% of the daily administered dose (or 97% of the recovered radioactivity) was found in the excreta collected up until 24 h following the termination of dosing. The lowest levels of incurred TBZ residues were found in the muscle (~0.1 ppm) and fat (~0.03 ppm), while higher levels were present in the kidney and liver (~1 and 5 ppm, respectively). However, due to the rapid excretion of TBZ and the rapid achievement of steady state levels in the urine and feces observed in this study, the half-life of the TBZ residues in goat is expected to be short. At a dose equivalent to 60 ppm in the diet, TBZ was absorbed rapidly by lactating dairy goats following oral administration and residues reached steady state levels in urine, feces, and milk by 2 days. These residues consist mainly of 5HTBZ and its sulfate conjugate. Lower levels of BNZ were found in edible tissues (liver, kidney, muscle). Neither parent TBZ nor its metabolites (5HTBZ, BNZ) were found in fat tissue in detectable concentration (≥0.005 ppm).

With laying hens, orally administered TBZ was also metabolized to BNZ, 5HTBZ, and the sulfate conjugate of 5HTBZ. The majority of the residues (~26 ppm/day) were then eliminated via the excreta. Greater than 96% of the total administered dose, or more than 99% of the recovered radioactivity, was excreted within 24 h following the final administration of TBZ. The muscle (breast, ~0.07 ppm; thigh, ~0.09 ppm), fat (~0.02 ppm), and egg (~0.10 ppm) had the lowest levels of TBZ edible tissue residues, while the kidney (~1.2 ppm) and liver (1.5 ppm) had higher levels. On the basis of the rapid excretion of TBZ residues in the excreta and the rapid achievement of steady state residue levels in hen matrices (by 2 days), the half-life of these residues in hen is expected to be short. At a dose equivalent to 29 ppm in feed, TBZ was absorbed rapidly by laying hens following oral administration, and residues reached steady state levels in excreta and egg by 2 days. These residues represent >99 and ~0.2%, respectively, of the recovered radioactivity and consist mainly of BNZ, 5HTBZ, and the sulfate conjugate of 5HTBZ.

The results of this study demonstrate that TBZ is metabolized to unconjugated 5HTBZ, conjugated 5HTBZ, and BNZ in lactating goats and laying hens. These residues are not likely to persist in edible tissues on the basis of the relatively small concentrations present and their rapid elimination.

LITERATURE CITED

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