THIABENDAZOLE

IDENTITY

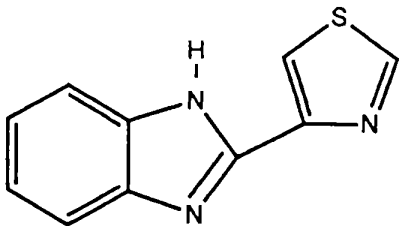
Chemical name: Thiabendazole

CAS Number: 148-79-8

CAS Nomenclature: 2-(4-thiazolyl)-1H-benzimidazole,
4-(2-benzimidazolyl)thiazole

Synonyms: Omnizole, Thiaben, Thibenzole, Bovizole, Eprofil, Equizole, Mintezol, Mertect, Lombristop, Minzolum, Nemapan, Polival, TBZ

Structural formula:



Molecular formula: $C_{10}H_7N_3S$

Molecular weight: 201.3

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Thiabendazole contains not less than 98.0% and not more than 101.0 % $C_{10}H_7N_3S$ calculated on the anhydrous basis. Thiabendazole is a chelating agent and forms stable complexes with a number of metals, including iron. It does not bind calcium.

Appearance: White, crystalline

Melting point: 304-305°C

Solubility: Slightly soluble in water, more soluble in dilute acid and alkali, maximum solubility is at pH 2.2. Soluble in DMF and DMSO.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Thiabendazole is a member of a well-known and widely used chemical class of compounds known as the benzimidazoles. It is related in chemical structure and pharmacological properties to other compounds such as fenbendazole, oxibendazole, oxfendazole, mebendazole, febantel and triclabendazole.

Thiabendazole is a potent, orally effective, broad spectrum anthelmintic with significant activity against gastrointestinal parasites in cattle, swine, sheep, goats, poultry, horses, dogs and many species of exotic animals. In addition, thiabendazole has been found to be effective in the control of a large number of fungal diseases affecting plants, animals and man.

Dosages

Thiabendazole is administered orally to cattle (3-5 g/100 lb BW), sheep (2 g/100 lb BW), and goats (2-3 g/100 lb BW) as a suspension, medicated feed, bolus, drench or as top dressing pellets. Additionally, it is available for cattle as an oral paste (3-5 g/100 lb BW) and in a medicated block (0.33 lb of block/100 lb BW). Preparations available for swine include an oral paste (30-40 mg/lb BW) and a premix (0.05-0.1% thiabendazole/ton feed).

METABOLISM

General

Metabolic studies have shown that thiabendazole is rapidly absorbed, metabolized, and excreted in man, cattle, rats, sheep, dogs, and goats. Excretion generally is completed within 3 to 8 days via urine and feces. In cows, approximately 0.1% of the dose was excreted in milk within 60 hours. The major metabolite found in all species studied was 5-hydroxythiabendazole, as a glucuronide or sulfate ester.

Rat

Eight rats were given single oral doses of ^{14}C or ^{35}S labeled thiabendazole and blood, urine, and feces samples were radiometrically analyzed (Tocco 1966). In 4 rats dosed with 100 mg/kg, maximum concentrations of drug and metabolites in whole blood were found 2 to 6 hours after dosing and ranged from 15 to 24 μg/ml. In 4 rats dosed with 25 mg/kg, 92% of the dose was excreted with 66% in urine and 26% in feces by 48 hours post treatment. After the 100 mg/kg dose, 79% of the dose was excreted by 48 hours with 49% in urine and 30% in feces. After 48 hours through day 8, excretion was close to the limit of detection at 0.1 μg/ml. Using paper chromatography, the composition of 24 hour urine from one rat dosed with 25 mg/kg was identified as unchanged thiabendazole (3%), free 5-hydroxythiabendazole (4%), the sulfate conjugate of 5-hydroxythiabendazole (39%), and the glucuronide conjugate of 5-hydroxythiabendazole (50%).

Radioactivity was not detected in the carcass of rats dosed with 25 mg/kg and sacrificed after 14 days.

Dog

Four dogs, given a single oral dose of 50 mg/kg body weight of thiabendazole-^{14}C, had urine, feces, and plasma analyzed radiometrically and spectrofluorometrically (Tocco 1966). Metabolites appeared in the plasma 30 minutes after dosing and reached maximum levels of 7 to 21 μg/ml within 2 hours. Three days after dosing, an average of 82% of thiabendazole was excreted, with 35% in urine and 47% in feces.

Cattle

Eighteen calves were administered single oral doses of 50 to 200 mg/kg thiabendazole (^{14}C, ^{3}H, or nonradioactive) in capsules or in a drench suspension. Urine, blood, feces samples were analyzed radiometrically and spectrofluorometrically. In results reported for 3 calves dosed with 50 and 200 mg/kg, plasma concentrations peaked from 4 to 7 hours (Tocco 1965; Anonymous 1981). In 2 calves dosed with 50 mg/kg, plasma concentrations were zero by 2 days. In one calf dosed with 200 mg/kg, the plasma concentration was 0.3 μg/ml on day 8, the last day of collection. In results reported for 4 calves (3 dosed with 50 mg/kg and one dosed with 200 mg/kg), 63% of the drug was excreted after 4 days with 55% in the urine and 8% (determined spectrofluorometrically) or 30% (determined radiometrically) in the feces (Tocco 1965; Anonymous 1981). After a 50 mg/kg dose, radioactivity was undetectable in urine by day 10 (Tocco 1965). By 40 days, radioactivity was undetectable in urine after a dose of 150 mg/kg. It took 45 to 50 days for radioactivity in urine to drop to undetectable levels after a dose of 200 mg/kg. In 3 animals, after the 50 mg/kg dose, tissue residues determined by spectrofluorometry were barely detectable (detection limit = 0.2 μg/g wet tissue), 3 days after treatment and in 1 calf radioactivity was not detected in tissue after 30 days (Tocco 1965). In 12 animals, after a 110 mg/kg dose, residues were not detected 3, 7, and 14 days after dosing (Anonymous 1981). In one calf, 57 days after 200 mg/kg dose, all tissue residues were at or below 0.6 ppm and 4 tissues had levels below the detection limit of the radiometric method (0.07 ppm) (Tocco 1965). In another calf, 34 days after the 110 mg/kg dose, radioactivity levels were highest in liver at 1.5 μg/g by radiometric analysis (Tocco 1965). Spectrofluorometric analysis did not agree with radiometric results in 2 cases. This discrepancy may be due to degradation of labeled thiabendazole.

Eight dairy cows were given 3, 5, or 10 g/100 lbs thiabendazole drench formulation or 5 g/100 lbs thiabendazole bolus formulation (Tocco 1965). Recovery of thiabendazole and metabolites ranged from 72 to 120% for compounds with concentrations between 0.05 to 5.0 ppm. Approximately 0.1% of the dose was excreted in milk with over 99% of the drug present as metabolites. Highest concentrations of thiabendazole and metabolites appeared within 24 hours after dosing. Residues were not detected 60 hours after dosing.

The absorption and pharmacokinetics of fenbendazole and thiabendazole in cattle were compared (Prichard *et al.* 1981). Three cattle were dosed intraruminally with

5 mg fenbendazole/kg, 100 mg thiabendazole/kg and the marker, chromium ethylenediaminetetraacetate (Cr-EDTA). Samples of digesta, blood, urine and feces were analyzed by spectrofluorometry. Drug movement through the gastrointestinal tract was derived by compartmental analysis of Cr-EDTA concentrations and integration of benzimidazole concentrations. Approximately 12% of thiabendazole left the rumen in digesta, indicating a net absorption from the rumen of 88%. No metabolites of thiabendazole were found in rumen contents. Elimination of thiabendazole from the rumen was virtually complete within 48 hours of administration. Approximately 10% and 8% of the thiabendazole dose appeared at the pylorus and terminal ileum, respectively. Of these amounts, 9% in the abomasum and practically 100% in the ileum was present as 5-hydroxythiabendazole, indicating that metabolites of absorbed thiabendazole were recycled to the GI tract. Thiabendazole reached maximum levels (about 3 mg/L) in plasma approximately 4 hours after treatment. Thiabendazole plasma levels dropped to about 0.3 mg/L at 24 hours. At 0.5 hours, 5-hydroxythiabendazole was present and contributed about half of the total thiabendazole in plasma throughout sampling. Between 17 and 36% of thiabendazole dose was excreted in the first 24 hour urine with urinary thiabendazole excretion ceasing 40 hours after dosing. Approximately 5% of the total urinary excretion occurred as unchanged drug.

The plasma concentrations of anthelmintics and their metabolites were compared (Prichard *et al. 1985*). Ten cattle were treated with fenbendazole, oxfendazole, febantel, albendazole, and thiabendazole using seven treatment procedures. Three cattle were treated with 176 g/L (from 88 mg/kg concentration) by oral drench. Plasma samples were analyzed by HPLC with a limit of detection of 70 ng/ml. The parent compound was not detected. The levels of 5-hydroxythiabendazole peaked at approximately 2 μg/ml by 4 hours post treatment. By 30 hours post dosing, the levels of 5-hydroxythiabendazole dropped to approximately 0.1 μg/ml.

Swine

Eleven young pigs were dosed with thiabendazole (nonradioactive, ^{14}C, ^{3}H, or ^{35}S) by feed or by capsule. During the medicated feeding of 0.02% feed for 4 days, plasma levels of thiabendazole in 2 pigs ranged from 1.2 to 2.0 μg/ml and were undetectable in 30 days (Anonymous 1981). The limit of detection was 0.02 ppm for thiabendazole and 0.05 ppm for total 5-hydroxythiabendazole. In 4 pigs dosed 50 mg/kg, an average of 76% of the drug was excreted with 66% in urine and 10% in feces in 3 days (Tocco 1965). Only 2 pigs out of 11 had significant drug radioactivity in tissues. One pig treated with a single dose of 50 mg/kg had the highest residue value in large intestine (0.36 μg/g) at 10 days withdrawal (Tocco 1965). The second pig after being treated with 0.02% feed for 4 days had the highest residue value in liver (8.9 μg/ml) at 1 day withdrawal (Anonymous 1981). Questionable or invalid data was reported for some plasma and tissue samples. It was suggested that accidental contamination of samples occurred during analysis. Eight pigs dosed with 0.1% nonradioactive thiabendazole feed for 17 days, and then fed 0.02% for 4 weeks with 30 day withdrawal had an average residue of 0.08 ppm in liver and 0.40 ppm in kidney determined by spectrofluorometric method; no residues were found in muscle (Anonymous 1981).

Sheep

Twenty-two sheep orally received 50, 100, or 200 mg/kg thiabendazole (nonradioactive, ^{14}C, or ^{35}S). Plasma, tissue, urine and feces samples were analyzed radiometrically and spectrofluorometrically. After 50 mg/kg dose, plasma concentrations of thiabendazole in 5 sheep peaked between 4 and 8 hours after dosing with values ranging from 7.7 to 10.4 μg/ml (Anonymous 1981). In 4 days after a 50 mg/kg dose, 8 sheep excreted an average of 89% of the drug, 75% in urine and 14% in feces (Tocco *et al.* 1964). Tissue residues of radioactivity in one sheep which received 50 mg/kg thiabendazole were highest in cecum (34.4 mg/g), small intestine (33.6 mg/g) and kidney (13.9 μg/g) at 6 hours after dosing (Tocco *et al.* 1964). At five days post treatment, residues were low and by 24 days, residues were below detection (0.06 μg/g). Tissue residues of radioactivity in 2 sheep that received 100 mg/kg were highest in bladder (average 0.26), spleen (average 0.26), skin (0.38 and 0.44), and pancreas (0.66, 0.08) (Anonymous 1981). With 1 day withdrawal, twelve sheep dosed orally with 100 and 200 mg nonradioactive thiabendazole/kg had the following residues: muscle - 0.36 to 3.87, liver - 2.05 to 3.69, kidney - 1.11 to 3.80. At 7 and 28 days after treatment, residues were below the detection limit of 0.2 μg/g (Anonymous 1981).

Goat

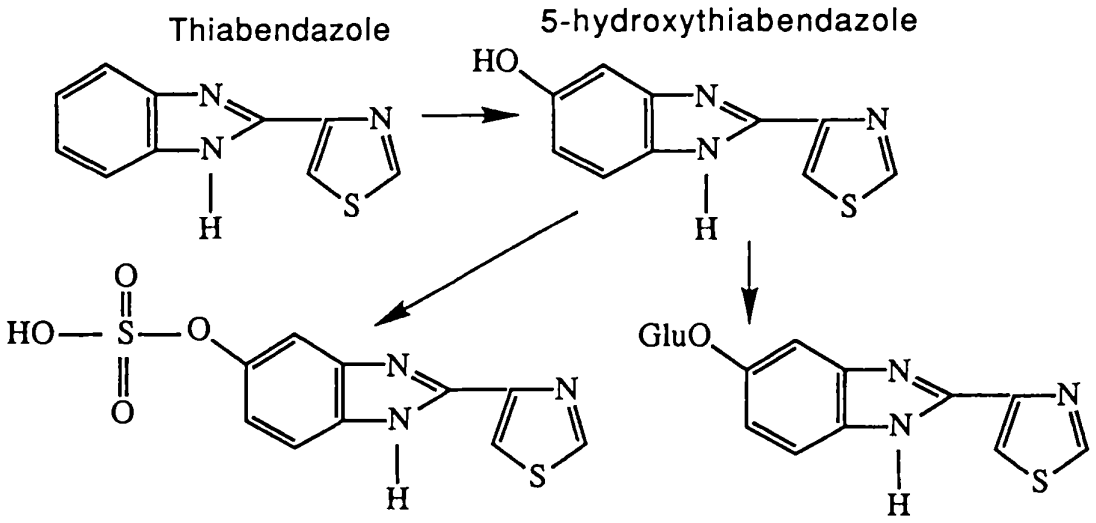
Seven goats were orally treated with 50 or 150 mg/kg thiabendazole (^{3}H, or ^{35}S and ^{14}C) (Tocco *et al.* 1965). Plasma, tissue, urine and feces samples were analyzed radiometrically and spectrofluorometrically. After 50 or 150 mg/kg dose, plasma concentrations of thiabendazole from 7 goats peaked between 2 and 8 hours with values ranging from 1.9 to 10.9 μg/ml (Tocco *et al.* 1965; Anonymous 1981). After 24 hours, plasma levels dropped substantially. At time of sacrifice (from 1 to 30 days), 6 goats dosed with 50 or 150 mg/kg excreted an average of 85% of the drug with 59% in urine and 26% in feces (Tocco *et al.* 1965). In tissue of goats that received thiabendazole, highest levels of residues appeared in abomasum (2.1 μg/g), kidney (2.7 μg/g) and large intestine (2.3 μg/g) after 24 hours. By 17 days post dosing, residue levels were zero except in abomasum (0.25 μg/g), heart (0.2 μg/g), large intestine (0.35 μg/g), liver (0.2 μg/g), and lung (0.1 μg/g). By 30 days post dosing, all residues were zero. In six goats given a thiabendazole drench formulation in dosages of 50, 150, or 225 mg/kg, approximately 1% of the dose was secreted in milk with the highest levels of thiabendazole and metabolites appeared within 24 hours (Tocco *et al.* 1965). Ninety percent of the drug was present as metabolites. No drug or metabolites were detectable in the milk 4 days later.

Man

Four male subjects were dosed with a suspension of 1.0 g ^{14}C-thiabendazole (Tocco *et al.* 1966). Plasma drug concentrations were measured radiometrically (detection level 0.2 ppm) and spectrofluorometrically (detection level 0.1 ppm for thiabendazole and 0.2 ppm for its metabolites). Plasma concentrations of thiabendazole peaked from 1-2 hours after treatment with values ranging from 13 to 18 μg/ml and then dropping to the detection level between 24 and 48 hours.

By 48 hours, 87 to 100% of the dose was excreted in the urine (81-91%) and feces (2-7%). Twenty-five percent of the dose detected in the urine was glucuronide and 13% was sulfate esters of 5-hydroxythiabendazole. Less than 1% of the dose was excreted as unchanged thiabendazole or unconjugated 5-hydroxythiabendazole. After 5 days, 4 to 9% of the dose was excreted in feces.

Twelve male subjects were dosed with 2 g thiabendazole in the form of tablets, wafers, capsules, and suspension in a cross-over experiment (Anonymous 1981). Plasma concentrations of thiabendazole peaked about 3 hours after treatment with values ranging from 11 to 18 μg/ml. Thiabendazole in the wafer was more rapidly absorbed with peak values of 30 to 50% higher than the other forms. For all 4 forms, sixteen to 21% of the drug was excreted in the urine after 24 hours.



Sulfate ester of 5-hydroxythiabendazole

Glucuronide of 5-hydroxythiabendazole

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Rat

In a series of studies, rats were divided into four treatment groups. Rats were treated with ^{14}C-thiabendazole, alone or with sodium formate, with $NaH^{14}CO_3$ and with $H^{14}COONa$. Total excretion of thiabendazole was 69-99% in rats receiving ^{14}C-thiabendazole alone and 96-97% for rats receiving ^{14}C-thiabendazole with sodium formate. Terminal excretion rates and tissue retention of radioactivity were similar for all three compounds. Table 1 shows the residues in tissues of rats treated with ^{14}C-thiabendazole, $NaH^{14}CO_3$ and $H^{14}COONa$ (Anonymous 1981).

Table 1. Tissue residues (μg/g) in the Rat

Compound	Dose (mg/kg)	Kidney	Liver	Carcass	Terminal Excretion (Days $^{-1}$)
^{14}C-Thiabendazole	96	0.04	DL	0.19	-0.032
	173	0.07	0.03	0.46	-0.031
^{14}C-Thiabendazole + Formate	110	0.02	DL	0.10	-0.040
	202	0.04	0.02	0.13	-0.042
$NaH^{14}CO_3$	1.7	0.00047	0.00034	0.00013	-0.017
	2280	0.53	0.62	1.5	-0.016
$H^{14}COONa$	20.3	0.096	0.053	0.34	-0.033
	4570	3.26	2.74	8.5	-0.028

DL = Detection Limits

The study suggests that all three compounds contribute to a common carbon pool of small molecules that are subsequently incorporated into other molecules. Radioactive residues resulting from administration of ^{14}C-thiabendazole would, by extension, be the products of reincorporation from this common carbon pool and not a measure of thiabendazole residues.

Cattle

A single calf was treated orally with a capsule containing ^{3}H-thiabendazole at a dose of 50 mg/kg. Ninety percent of the administered dose was recovered in the urine and feces (65% and 25%, respectively). Plasma drug concentrations are shown in Table 2. At a detection limit of 0.08 ppm there were no detectable tissue residues 30 days following treatment.

Table 2. Concentration (μg/ml) of thiabendazole in the plasma of a calf treated with 50 mg/kg ^{3}H-Thiabendazole

Time after Dosing (h)	Concentration (μg/ml)
1	0.5
2	0.8
4	1.8
6	2.0
7	NA
24	≤1

Three calves were treated orally via capsule with ^{14}C-thiabendazole at doses of 110, 150 and 200 mg/kg. Calves were slaughtered 34, 59 and 57 days postdosing. At the 200 mg/kg dose, 81% of the administered dose was excreted in the urine and feces (47 % and 34 %, respectively). Plasma drug concentrations are shown in Table 3. Tissue residues at slaughter are shown in Table 4 (Anonymous 1981).

Table 3. Concentration (μg/ml) of thiabendazole in the plasma a calf treated orally with 200 mg/kg ^{14}C-Thiabendazole

Time after Dosing	Concentration (μg/ml)
1 h	4.1
2	5.0
4	7.5
6	NA
7	9.6
24	3.8
2 days	1.3
3	1.1
4	0.4
5	0.5
6	0.5
7	0.4
8	0.3

Table 4. Tissue residues (ppm) in calves treated orally with ^{14}C-Thiabendazole

Tissue	Dose (mg/kg) [Sacrifice, days]		
	110 [34]	150 [59]	200 [57]
Fat	0.1*	0.0**	0.18*
Kidney	0.15	0.11	0.13
Liver	1.5	0.39	0.59
Muscle	0	0.13	0.16

* Estimated detection limit ≈ 0.08
**Estimated detection limit ≈ 0.07

As simultaneous determination of thiabendazole residues using spectrofluorometric analysis showed only very low residues, a series of fractionation studies were conducted to demonstrate that the radioactivity measured in the tissues of treated calves was the result of the degradation of labeled thiabendazole and subsequent incorporation of small molecules containing ^{14}C into several metabolic cycles (Anonymous 1981).

Fractionation of liver from a calf treated with 200 mg/kg ^{14}C-Thiabendazole:

Procedure 1 utilizes a methylene chloride extraction of the acid hydrolyzate of ^{14}C-thiabendazole-containing liver. At a detection limit of 0.16 μg thiabendazole (of the ≈ 12 μg/20 g tissue sample), negligible residues, indistinguishable from the control, were detected. The method accounts for approximately 1% of the anticipated residue.

Procedure 2 also employs an initial methylene chloride extraction. The aqueous phase that results is extracted with ethyl acetate and back extracted with 0.1 N HCl. The majority of the radiolabel is detected in the solid residue following combustion analysis. The method accounts for approximately 60% of the anticipated residue.

Procedure 3 utilizes an acid hydrolysis followed by chromatography on resin. The acid eluate accounts for approximately 4% of the expected residue. The major fractions are the basic eluate (49%), containing the bulk of the amino acids, and the solid residue (39%, after combustion analysis). No residue is associated with the spent resin. Accountability is approximately 90%.

Procedure 4 uses salt extraction at pH 5.5 and pH 10.0. Approximately 60% of the activity is in the acidic fraction. An additional 18% is found in the basic fraction. Solid residues contain approximately 20% of the residues.

Procedures 6 and 7 produce purified nucleic acid and protein fractions. Recoveries range from 65% for nucleic acids to 90% for proteins. Radioactivity in the nucleic acid fraction is negligible. The protein fraction contains approximately 25% of the anticipated thiabendazole.

Fractionation of liver from a calf treated with 110 mg/kg ^{14}C-Thiabendazole:

Procedure 4 demonstrated an accountability of approximately 90%. Radioactivity is extracted at both pH 5.5 (29%) and pH 10.0 (36%). Approximately 25 % remains in the solid material.

Procedure 5 results in the separation and identification of lipid and polysaccharide fractions from the liver tissue. Radioactivity is essentially absent from those fractions anticipated to contain thiabendazole or its metabolite. The lipid fraction contains approximately 14% of the initial radioactivity and the polysaccharide fraction contains ≈ 1%. Upon combustion, the solid residue contains 34% of the expected radioactivity. The polysaccharide filtrate, containing protein hydrolysate and other water soluble constituents, contains ≈ 29% of the radioactivity. Accountability is approximately 80%.

Procedure 7 results in isolation of protein from the liver tissue. Protein contains approximately 60% of the initial radioactivity. Nonprecipitating material accounts for approximately 14% of the total and 12% is attributed to fatty material. Total accountability is approximately 85%.

From procedures 5 and 7 the company concludes that the radioactivity is not restricted to a single component or fraction. The "residues" derived from calves treated with radiolabeled thiabendazole are apparent rather than real.

Sheep

Two lambs were treated orally with 50 mg/kg ^{14}C-thiabendazole. Total urine and feces were analyzed. Table 5 shows the percent of the dose excreted in 96 hours (Tocco 1960).

Table 5. Urinary and fecal excretion (% of total) of ^{14}C-thiabendazole following an oral dose of 50 mg/kg in lambs

	Time After Dose (h)	Lamb No 981	Lamb No 979
Urine	96	77	74
Feces	96	14	16

OTHER RESIDUE DEPLETION STUDIES

Cattle

Fifteen calves were treated with thiabendazole via capsule (950 mg/kg) or drench (50 or 110 mg/kg). Plasma levels of drug were determined in one calf and tissue residues were analyzed in three animals treated at 50 mg/kg and slaughtered at one and three days postdosing. A fluorometric assay was used (detection limit of 0.2 μg/g). Tissue residues were low at one day postdosing, negligible to absent after three days, and absent in samples collected more than three days after drug treatment (Anonymous 1981)

Two Friesian cows in midlactation were given thiabendazole orally, at a dose of 66 mg/kg. Cows were milked twice daily at approximately 12 hour intervals. Milk samples were collected at 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144 hours after treatment. Milk was analyzed for thiabendazole residues as whole milk and, following fractionation into aqueous (whey) and lipid (curds) phases, the aqueous phase was analyzed. With an assay limit of detection of 0.5 μg/ml, neither thiabendazole nor its 5-OH metabolite were detected in any of the samples (Weir 1987).

Table 6. Residues (μg/ml) of thiabendazole and its 5-OH metabolite in milk

Sample	Dose (g/100 lbs)	Formulation	TBZ	5-OH-TBZ
0-12	3	drench	0.06	2.39
12-24			ND	1.10
24-36			ND	0.26
36-48			ND	0.07
48-60			ND	0.02
60-72			ND	ND
0-12	5	drench	0.03	3.59
12-24			0.03	2.08
24-36			ND	0.79
36-48			ND	0.15
48-60			ND	0.05
60-72			ND	ND
0-12	10	drench	0.22	4.14
12-24			0.05	3.82
24-36			ND	0.83
36-48			ND	0.29
48-60			ND	0.09
60-72			ND	ND
0-12	5	bolus	0.16	3.27
12-24			ND	2.15
24-36			ND	0.53
36-48			ND	0.17
48-60			ND	0.03
60-72			ND	ND

Six dairy cows were treated orally via drench with thiabendazole at doses of 3, 5 or 10 g/100 lbs. Two other cows were given thiabendazole in a bolus formulation at a dose of 5 g/100 lbs. The animals were milked at 12 hour intervals for six days. Milk was analyzed for thiabendazole and its 5-OH metabolite (including 5-glucuronide and 5-OSO_3). Samples were analyzed spectrofluorometrically. The assay has a limit of quantitation of 0.05 μg/ml. Residues of thiabendazole and its 5-OH metabolites are shown in Table 6 (Robinson et al. 1963).

Seven Friesian cows were divided into control and treatment groups. Control animals received an oral drench with water equivalent in volume to the largest volume given to cows in the treatment group. Animals in the treatment group received a 17.6% preformed suspension of thiabendazole at a dose of 66 mg/kg. Milk samples were collected prior to treatment and at 8, 24, 32, 48, 72 and 108 hours after dosing. High pressure liquid chromatography with fluorescence detection was used to measure the concentration of thiabendazole residues in the milk. Results of the study are shown in Table 7 (Batty et al. 1985).

Table 7. Mean residues (ppm) of thiabendazole and 5-OH thiabendazole in the milk of treated cows

Sample	TBZ	5-OH-TBZ	Total
0	0	0	0
8	<0.1*	0.93	0.97
24	<0.1	1.05	1.07
32	<0.1	<0.1	<0.1
48	<0.1	<0.1	<0.1

* Limit of detection = 0.1 ppm

Nine lactating Holstein cows were treated orally with encapsulated thiabendazole at doses of 0.5, 1.5 and 5.0 g daily for a period of 28 days. Two control animals received cellulose-filled gelatin capsules. Two cows from each treatment group were slaughtered at the end of the 28-day treatment period. One control cow was euthanized on day 14 of the treatment period due to poor health. At the end of the 28 day dosing period (study day 29), all cows but one from each treatment group were humanly killed to obtain tissues for residues analysis. The remaining cows were slaughtered after a 28-day washout period. Milk samples were collected on days -1, 1, 2, 4, 7, 14, 21, 28, 29, 35, 42 and 56. Subsamples of 500 ml were composited on each sample day and retained for analysis. Tissues were collected for residue analysis at the time of slaughter. Residues were determined spectrofluorometrically. Summaries of milk residues are shown in Tables 8 and 9. A summary of tissue residues are shown in Table 10 (Predmore and Justin 1987).

Table 8. Summary of milk residues (Days 2 - 28)[ppm]

		TBZ		5-OH-TBZ		Total†	
Dose	Cow No	Range	Mean	Range	Mean	Range	Mean
Control*	8	0.010-0.019	0.014	0.001-0.005	0.003	0.013-0.024	0.017
0.5	14	0.010-0.018	0.014	0.010-0.012	0.010	0.020-0.028	0.024
	22	0.014-0.017	0.016	0.016-0.018	0.017	0.030-0.036	0.033
	9	0.010-0.014	0.012	0.010-0.013	0.010	0.020-0.025	0.022
1.5	7	0.010-0.014	0.013	0.116-0.148	0.135	0.130-0.162	0.148
	10	0.014-0.018	0.016	0.016-0.096	0.042	0.033-0.112	0.058
	12	0.012-0.016	0.014	0.082-0.091	0.087	0.098-0.105	0.101
5.0	3	0.011-0.022	0.016	0.143-0.2446	0.176	0.159-0.262	0.192
	6	0.014-0.018	0.016	0.064-0.092	0.081	0.082-0.106	0.097
	15	0.014-0.016	0.015	0.090-0.103	0.096	0.105-0.123	0.112

* Data from the entire study period days -1 - day 57
† Total = TBZ + 5-OH-TBZ

Table 9. Summary of milk residues (Days 35 - 56)[ppm]

		TBZ		5-OH-TBZ		Total†	
Dose	Cow No	Range	Mean	Range	Mean	Range	Mean
Control	8	0.014-0.016	0.015	0.002-0.004	0.003	0.017-0.019	0.018
0.5	22	0.010-0.015	0.013	0.002-0.004	0.003	0.012-0.019	0.016
1.5	7	0.013-0.014	0.014	0.004-0.006	0.005	0.017-0.020	0.018
5.0	15	0.012-0.018	0.016	0.002-0.004	0.003	0.016-0.020	0.018

† Total = TBZ + 5-OH-TBZ

Table 10. Summary of mean tissue residues (ppm)

Tissue	Dose	TBZ		5-OH-TBZ		Total	
		Day 29	Day 57	Day 29	Day 57	Day 29	Day 57
Blood	Control	0.014	0.013	0.002	0.004	0.016	0.017
	0.5	0.015	0.013	0.004	0.004	0.019	0.017
	1.5	0.013	0.012	0.015	0.003	0.028	0.015
	5.0	0.016	0.013	0.019	0.004	0.035	0.017
Fat	Control	0.017	0.016	0.002	0.003	0.019	0.019
	0.5	0.017	0.016	0.003	0.003	0.020	0.019
	1.5	0.015	0.016	0.010	0.002	0.025	0.018
	5.0	0.015	0.017	0.009	0.002	0.024	0.019
Kidney	Control	0.018	0.021	0.009	0.010	0.027	0.031
	0.5	0.012	0.020	0.044	0.010	0.056	0.030
	1.5	0.016	0.020	0.25*	0.008	0.27*	0.028
	5.0	0.027	0.022	0.439	0.014	0.466	0.036
Liver	Control	0.016	0.021	0.012	0.020	0.028	0.041
	0.5	0.022	0.018	0.027	0.016	0.049	0.034
	1.5	0.048	0.018	0.086	0.015	0.134	0.033
	5.0	0.068	0.020	0.138	0.017	0.206	0.037
Muscle	Control	0.015	0.013	0.003	0.002	0.018	0.015
	0.5	0.013	0.014	0.002	0.000	0.015	0.014
	1.5	0.014	0.012	0.005	0.002	0.019	0.014
	5.0	0.016	0.016	0.005	0.002	0.021	0.016

* Report offers no explanation for these anomolously high residue values.

Sheep

Three sheep were dosed orally with thiabendazole. One animal was treated at a rate of 60 mg/kg and slaughtered four hours after dosing. Two animals were slaughtered seven days after being treated with either 82 mg/kg or 100 mg/kg. Concentrations of thiabendazole in the plasma and erythrocytes were determined in the sheep receiving the 82 mg/kg dose and are shown in Table 11 (Tocco 1960). Tissue residues resulting from this study are shown in Table 12.

Table 11. Concentration of thiabendazole in the plasma and erythrocytes of a sheep treated orally with 82 mg thiabendazole/kg

Time after dose (h)	Conc. in Plasma (μg/ml)	Conc. in Erythrocytes (μg/ml)
1	0.06	0.08
2	0.28	0.29
3	0.53	0.54
4	1.51	1.54
5	1.00	1.39
6	1.06	1.22
24	0.01	0.01

Thiabendazole concentration in the blood at 24 hours postdosing are consistent with control levels in the blood of untreated sheep.

Table 12. Tissue residues (μg/g) of thiabendazole in sheep

	Dose [Slaughter]		
Tissue	60 mg/kg [4 h]	82 mg/kg [7 days]	100 mg/kg [7 days]
Muscle	0.38	0.02	0.02
Kidney	0.68	0.03	0.03
Liver	4.12	0.04	0.09
Heart Fat	4.40	NA	0.04
Omental Fat	NA	NA	0.03

Tissue residues of thiabendazole were low, even at four hours postdosing when blood levels appeared to be maximal. Recoveries based on internal standards were greater than 90%.

Only 4% of the administered dose was recovered in urine and feces. Two metabolites were detected and were under investigation (Tocco 1960).

Fourteen sheep were maintained on a continuous diet containing various amounts of thiabendazole (TBZ). Blood levels were determined in the treated sheep and in four untreated controls. Results are shown in Table 13 (Tocco 1960).

Table 13. Concentration of thiabendazole in the blood of sheep maintained on a continuous diet of TBZ in feed

TBZ in Feed (%)	Wethers		Females	
	No. of Sheep	Conc. (μg/ml)	No. of Sheep	Conc. (μg/ml)
None	2	0.01*	2	0.03*
0.01	1	0.00	2	0.00
0.032	1	0.01	2	0.01
0.1	2	0.04	2	0.03
0.32	1	0.09	2	0.52
1.0	1	0.47	NA	NA

* Control values have been subtracted from experimental blood sample values

Four sheep were treated orally with thiabendazole at a dose of 44 mg/kg. Animals were slaughtered 1, 2, 3 and 4 days postdosing. Both thiabendazole and its 5-OH metabolite were measured in the tissues. The results are shown in Table 14 (Weir 1987).

Table 14. Residues (μg/g) of thiabendazole and its 5-OH metabolite in the tissues of sheep treated orally with 44 mg thiabendazole/kg

Tissue	Withdrawal (days)	Animal No.	TBZ	5-OH-TBZ
Liver	1	91	2.24	2.44
	2	92	0.31	ND
	3	93	0.80	ND
	4	94	ND	ND
Muscle	1	91	0.34	0.24
	2	92	ND	ND
	3	93	ND	ND
	4	94	ND	ND

ND = Not detectable (limit of detection = 0.05 μg/g)

Six Finn-Dorset crossbred lambs were given thiabendazole orally at a dose of 44 mg/kg. Blood samples were collected before drug administration and at 2, 4, 6, 8, 12, 24, 36, 48 and 72 hours after treatment. Samples were assayed for thiabendazole and its 5-OH metabolite. Maximum blood concentrations of thiabendazole were detected between two and four hours postdosing. Measurable levels of thiabendazole were still present at 24 hours postadministration but were absent in the 36 hour collections. The 5-OH metabolite was present in the plasma, reaching a maximum concentration at six hours postdosing. Detectable levels of metabolite were present in the 12 hour samples but concentrations were below the limit of detection by 24 hours. Mean concentrations of thiabendazole and 5-OH-thiabendazole are shown in Table 15 (Weir 1987).

Table 15. Mean plasma concentrations of thiabendazole and 5-OH-thiabendazole in sheep given thiabendazole orally at a dose of 44 mg/kg

Time (h)	Thiabendazole (μg/ml)	5-OH-Thiabendazole (μg/ml)
0	0	0
2	2.01 ± 0.87	0.13 ± 0.04
4	2.16 ± 0.88	0.16 ± 0.05
6	1.66 ± 0.77	0.18 ± 0.07
8	1.38 ± 0.70	0.16 ± 0.04
12	0.27 ± 0.23	0.13 ± 0.08
24	0.02 ± 0.01	ND
36	ND	ND
48	ND	ND
72	ND	ND

Concentrations of thiabendazole are higher and persist longer in liver than residues in muscle or plasma (Weir and Bogan 1985).

Swine

Eight crossbred swine were given a single oral dose of 100 mg thiabendazole/kg. The dose was administered as a drench suspension. Pigs were given free access to water and were fed a nonmedicated hog feed. Four pigs were slaughtered seven days after dosing and four pigs were slaughtered 28 days after dosing. Four untreated controls were also slaughtered at the later time. Tissues were assayed for thiabendazole and metabolites using a spectrofluorometric method. The detection sensitivity for the method is 0.05 ppm thiabendazole + metabolites. Assay values for the control swine were at or below the detection limit. The assay values for the treated swine at both 7 and 28 days postdosing were also below the limit of detection (Anonymous 1981).

Three pigs were maintained for two weeks on a diet containing 0.1% thiabendazole. The pigs were slaughtered at zero, 2 and 7 days withdrawal from medicated feed. Tissues were analyzed for thiabendazole and the 5-glucuronide and 5-OSO_3 metabolites. Results for selected tissues are shown in Table 16 (Anonymous 1981).

Table 16. Tissue residues (μg/g) of thiabendazole and its 5-glucuronide and 5-OSO_3 metabolites in pigs receiving 0.1% TBZ in feed for 2 weeks

	Liver			Kidney			Muscle			Fat		
	TBZ	5-OH	Total	TBZ	5-OH	Total	TBZ	5-OH	Total	TBZ	5-OH	Total
Control*	0.02	0.09	0.11	0.03	0.09	0.12	0.04	0.04	0.08	0.05	0.04	0.09
0	3.9	1.8	5.7	2.7	5.3	8.0	2.1	0.16	2.3	3.5	0.22	3.7
2	0.12	0	0.12	0.19	0	0.19	0	0	0	0.17	0	0.17
7	0.05	0	0.05	0	0	0	0	0	0	0.02	0	0.02

*Control values have been subtracted from experimental tissue sample values

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

A radiometric method was used for thiabendazole, labeled with ^{3}H, or ^{35}S and ^{14}C, in urine, plasma and tissue samples and analyzed by liquid scintillation and low beta Geiger counting (Tocco *et al.* 1965). Feces samples were analyzed by gas proportional counting. The detection limit for tissue was 0.08 μg/g.

A chemical method first extracted thiabendazole and 5-hydroxythiabendazole into ethyl acetate and then into hydrochloric acid for urine, tissue, plasma, and milk samples (Tocco *et al.* 1965). In liver and kidney samples, the glucuronide

and sulfate esters of 5-hydroxythiabendazole were first converted to 5-hydroxythiabendazole using β-glucuronidase and sulfatase (glusulase - commercial enzyme mixture), and then extracted. Feces were first extracted with methylene chloride and then hydrochloric acid. Fluorescence was measured by a spectrofluorometer. Thiabendazole and 5-hydroxythiabendazole were recovered with adequate precision (95 ± 6%) for values from 0.1 to 5 μg/g for plasma and tissue and from 5 to 10 μg/g for urine and feces. The detection limit for tissue was 0.1 μg/g for thiabendazole and 0.2 μg/g for its metabolites. The detection limit for milk was 0.05 μg/g.

A high-performance liquid chromatographic method for measuring eight benzimidazoles in liver, kidney, and muscle was reported (Marti *et al.* 1990). Tissue samples were homogenized with acetonitrile and defatted with hexane. Sodium chloride was added and three phases formed. The hexane layer was discarded, and the remaining two layers were mixed with dichloromethane. The acetonitrile layer was dried over sodium sulfate and evaporated first in a rotovap, then in an N-Evap. The extract was cleaned by passage through a Sep-Pak C_{18} cartridge, evaporated to 0.2 ml and cleaned by a Sep-Pak Florisil cartridge conditioned with chloroform-methanol-triethylamine. A final evaporation step under nitrogen took place before HPLC analysis. An octadecylsilane column with mobile phases of acetonitrile-water with an ion-pair reagent and UV detection was used. Positive results were verified by gas chromatograph-mass spectrometry in the electron-impact or positive or negative chemical-ionization mode. The authors claimed that the limit of detection ranged from 20 to 50 ng/g with a recovery of 66 to 87% (standard deviations from 2.2 to 11.6) except for oxfendazole which had recoveries between 40-45% in kidney and liver. The sensitivity could not be verified since control tissue chromatograms were not provided. Disadvantages of this procedure were the time consuming extractions and drying steps, use of highly toxic halogenated solvents, the additional expense of two SPE cartridges, and the inability to resolve all eight benzimidazoles in a single LC system. The major advantage was high precision.

A liquid chromatographic method using UV detection for measuring eight benzimidazoles in bovine, ovine, and swine muscle and liver was developed (Wilson *et al.* 1991). After extraction with ethyl acetate, evaporation, and final extraction with hexane, ethanol, and hydrochloric acid, samples were separated by reverse-phase liquid chromatography, using methanol and aqueous buffer as the mobile phase. Presumptive positive samples were confirmed by selected ion monitoring electron-impact gas chromatography/mass spectrometry. At 100 ng/g, overall recovery averaged 92% in liver tissue and 88% in muscle tissue for calves, swine, and sheep. Standard deviations for mean % recovery for all benzimidazoles at 50, 100, and 200 ng/g ranged from 15.3 to 23.0. The limit of detection was 50 ng/g. The major disadvantage of this method was lack of precision.

APPRAISAL

Information was provided on the metabolism of thiabendazole and the depletion of residues of thiabendazole from the edible tissues of cattle, sheep, goats and swine.

Residue depletion studies have shown thiabendazole is rapidly metabolized and excreted in all animal species and concentrations of the drug and its metabolites in tissue and excreta decrease rapidly to control levels. The major metabolite is 5-hydroxythiabendazole, generally found as its glucuronide or sulfate ester.

Cattle - No detectable residues of thiabendazole were found in liver, kidney, and muscle of cattle 3 days following oral doses of 50 or 110 mg/kg body weight thiabendazole. Residue levels in fat tissue approximate those found in muscle tissue.

In lactating animals, approximately 0.1 per cent of an oral dose was detectable in milk within 60 hours. Thiabendazole and its 5-hydroxythiabendazole were not detectable in the milk 60 hours after oral doses of 66, 110 or 220 mg/kg body weight.

Sheep - Seven days after oral administration of 82 and 100 mg/kg body weight doses of thiabendazole for sheep, no residues of the drug were found in muscle, liver and kidney.

Goats - Radioactive residue levels in goats which received labeled thiabendazole showed no drug residues 30 days after oral administration of 50 or 150 mg/kg body weight. Twenty-four hours after dosing with 50 mg/kg body weight only liver and kidney had detectable residues of 1.1 and 2.7 mg/kg, respectively. At 17 days post dose, no residue was found in the kidney while a residue of 0.2 mg/kg remained in the liver.

Swine - No residues of thiabendazole or related metabolites were found in swine tissues 7 days following a single oral dose of 100 mg thiabendazole per kg body weight.

When thiabendazole was administered to swine in the feed at 40 mg/kg body weight for two weeks, no residues of the drug or its metabolites were found in muscle, liver, kidney or fat after 7 days withdrawal, and only minute amounts (0, 0.12, 0.19, 0.17 mg/kg respectively) at a two day withdrawal period.

The following information was utilized in setting MRLs for thiabendazole:

An ADI of 0-100 μg/kg of body weight was established. This would result in a maximum ADI of 6 mg for a 60 kg human.

The total residues of thiabendazole can be approximated by the sum of thiabendazole and 5-hydroxythiabendazole and its conjugates.

The sum of the residue levels of thiabendazole and 5-hydroxythiabendazole depletes to below 0.1 mg/kg in all tissues and milk of animals within a few days of withdrawal. Assuming a total daily food intake from edible tissues and milk containing 0.1 mg/kg of thiabendazole residues, the theoretical maximum daily intake would be 0.2 mg.

$$\{(0.1 \text{ mg/kg} \times 0.5 \text{ kg tissue}) + (0.1 \text{ mg/kg} \times 1.5 \text{ kg of milk}) = 0.2 \text{ mg}$$

The theoretical maximum daily intake of 0.2 mg of thiabendazole of veterinary origin is much less than the value of 1.412 mg obtained from other agricultural uses of thiabendazole. The calculation of the latter figure was based on the Codex Maximum Residue Limits of thiabendazole in major foods and the estimated average daily global intake of these commodities. The theoretical maximum daily intake from both uses of thiabendazole utilizes 27% of the ADI.

The JMPR has set MRLs for the edible tissues of cattle, goats, horses, pigs and sheep at 0.1 mg/kg on the sum of the thiabendazole and 5-hydroxythiabendazole.

The Committee recommends MRLs for thiabendazole of 100 μg/kg for all edible tissues of cattle, goats, pigs and sheep and milk of cattle and goats. The marker residue is the sum of thiabendazole and 5-hydroxythiabendazole.

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