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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: ID # 60101-618. Thiabendazole. Phase V Review. Goat and Poultry Metabolism Studies. MRID # 420579-01 & 420117-01. CBRS # 8930 & 8719. DP Barcode: D170818 & D169697.

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Merck Sharp & Dohme has submitted animal metabolism studies to fulfill residue chemistry data requirement guideline # 171-4(b) for reregistration of thiabendazole. The 2 studies are:

1. Metabolism of ^{14}C-Thiabendazole (TBZ) in Lactating Dairy Goats, Project ID # 37729 - MRID # 420579-01.

2. Metabolism of ^{14}C-Thiabendazole (TBZ) in Poultry, Project ID # 37728 - MRID # 420117-01.

TBZ is on list B for reregistration. The Phase IV review stated that the registrant had committed to conduct animal metabolism studies (C. Olinger, 2/20/91). In other words, the submitted studies or their summaries have not previously been reviewed.

The in-life portion and the analytical work of both animal studies were carried out at ABC Labs, Columbia, MO. Sacrifice and necropsy were performed at the University of Missouri's Veterinary Medical Diagnostic Lab. ^{14}C-Thiabendazole (uniformly labeled in the phenyl ring) was employed in these experiments.

Samples were labeled and stored frozen, and processed frozen. Total radioactivity in tissues was determined by combustion followed by liquid scintillation counting.

Activity was analyzed on reverse phase HPLC using a gradient system of methanol and buffered water (0.01M K_2HPO_4 and 0.01M Et_3N). For unlabeled compounds, a UV detector set at 279 nm was coupled to the HPLC. TLC plates were developed with a solvent system of 90/10 v/v EtOAc/CH_3OH, with or without 0.05% of phosphoric acid.

A general extraction scheme was followed for tissues, milk, eggs, and excreta. Tissue samples were extracted with an organic solvent following or followed by hydrolysis (6N HCl, sulfatase, ß-glucuronidase or glusulase), or they were lyophilized prior to Raney nickel reduction. The unextractable residues were quantified by LSC.

Analytical standards employed in the chromatographic analysis included: thiabendazole, 5-hydroxythiabendazole (5-OH-TBZ), benzimidazole (BNZ), and benzimidazole carboxamide (BNZ-$CONH_2$).

In general, tissue residues were found to be relatively unextractable with organic solvents and hydrolytic methods were used directly for extraction of the tissue residues.

Metabolism of ^{14}C-Thiabendazole (TBZ) in Lactating Dairy Goats

Lactating goats (Capra hircus), weighing 45-60 Kg each, were housed in individual stalls. ^{14}C-Thiabendazole was administered orally in capsules containing cellulose at 120 mg per animal (3 goats total) per day for 7 days. Goats were expected to ingest 2 Kg of hay and goat chow per day (which would translate to a 60 ppm in the diet). Two goats were given plain cellulose capsules and served as control.

Milk was collected twice daily, once in the morning and once in the afternoon. Tissue samples were collected from each goat during necropsy which included fat (perirenal and omental), liver, kidney, heart, blood, gall bladder contents, and muscle.

Milk (1.02 ppm)

The daily samples were combined for activity counting. Data indicate that residues plateaued on day 3-5. Residues in milk represented ca 1.0% of the administered dose and peaked at 1.24 ppm. Initial extraction with an organic solvent released no radioactivity. The aqueous phase by HPLC analysis contained a major component (≈54%) which was suspected to be a sulfate conjugate of 5-OH-TBZ (see below); about 37% of the activity was not recovered on the column.

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Glusulase (a mixture of ß-glucuronidase and sulfatase) treatment followed by ethyl acetate extraction yielded "essentially one component" on HPLC (column recovery and chromatogram not provided) which matched the retention time of 5-OH-TBZ; the aqueous fraction was not addressed. Extraction of ß-glucuronidase-treated milk with ethyl acetate resulted in a fraction that did not contain any radioactivity; and the aqueous fraction's HPLC profile was similar to that of the aqueous-soluble fraction of milk without enzyme treatment. After sulfatase treatment, the organic extract contained 5-OH-TBZ as the only radioactive component (0.40 ppm, 39% of milk activity) by HPLC. The resulting aqueous phase contained 29% milk residue (0.29 ppm) and 22% (0.23 ppm) as unextractable (see Table IX in the submission; HPLC analysis was not provided). Treatment of goat milk with 6N HCl released 5-OH-TBZ as the primary component in the organic extractable fraction (distribution of activity in various fractions and HPLC chromatograms not provided).

Milk (1.02 ppm)

Treatment

		organic phase	aqueous phase
1)	None	no activity	5-OH-TBZ conj (54%) 37% activity unaccounted for
2)	glusulase	5-OH-TBZ	not addressed
3)	glucuronidase	no activity	HPLC profile similar to 1)
4)	sulfatase	5-OH-TBZ (39%) unknowns (10%)	29% (not charact) plus 22% unextractable
5)	6N HCl	5-OH-TBZ	not addressed

Goat Liver (4.77 ppm)

Very little radioactivity was released when liver was treated with protease or ficin. 6N HCl digested greater than 90% of the liver activity with 6% of the liver activity unextractable. The organic extractable fraction accounted for ≈10% (0.49 ppm) of the liver activity, and by HPLC retention time contained 3 major components: BNZ, 5-OH-TBZ, and TBZ (1.8:2.5:4.0 ratio). The peak corresponding to BNZ was isolated and by mass spectroscopy bore some characteristic fragments of an authentic sample of BNZ.

Liver (4.77 ppm)

Treatment

	organic phase	aqueous phase
1) 6N HCl	TBZ, 5-OH-TBZ, BNZ (10%)	83% activity Raney nickel treatment gave a consistent major component that contained no thiazole ring (see below)

The aqueous phase, despite solid phase extraction cleanup attempts, contained multiple components over a wide range of retention time (30-50 min) upon HPLC analysis (Figure 19 of submission). The aqueous phase (after lyophilization) was treated with Raney nickel in an attempt to further elucidate the metabolic profile. Very little TBZ or 5-OH-TBZ was detected in the ethyl acetate extract of the reaction mixture; however, a peak (retention time ≈25 min) appeared consistently in the radiochromatogram. A similar chromatogram was obtained when (cold) 5-OH-TBZ was subjected to Raney nickel treatment in the absence of liver tissue. This component was isolated and examined by high resolution mass spec and NMR, which suggest the rupture of the thiazole ring.

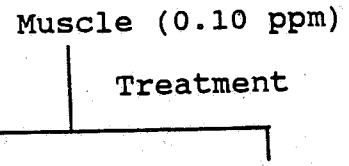
Goat Kidney (1.39 ppm)

Enzymic hydrolysis (glusulase and sulfatase) was not effective in releasing residues and Raney nickel reduction/hydrolysis yielded results that were difficult to interpret. Best results were obtained with 6N HCl hydrolysis. Kidney residue in the organic extractable fraction (23%) consisted of 3 major components, 5-OH-TBZ (8%), BNZ (4%), and TBZ (5%) (by HPLC retention time). The 5-OH-TBZ peak was confirmed by GC/MS, derivatized as TMS. The aqueous-soluble fraction (69%) was found to consist predominantly of BNZ (21%) and 5-OH-TBZ (34%). Tissue-unextractable residues amounted to 8%. There were also unaccounted for residues in the HPLC analysis (7% and 14%).

Muscle (0.10 ppm)

Glusulase hydrolysis was unsuccessful in releasing any of the activity in muscle into the organic phase. After 6N HCl treatment, 30% activity in muscle went into the organic phase, 61% into the aqueous phase, and 9% being unextractable. By HPLC (retention time), the organic-soluble fraction consisted of BNZ (tentative,

7%), 5-OH-TBZ (11%), and TBZ (4%). The aqueous phase was lyophilized and mixed with methanol but the methanol contained little activity to be capable of analysis by HPLC.



1)	6N HCl	TBZ, 5-OH-TBZ, BNZ (22%)	61% activity lyophilized, mixed with methanol; little activity in the methanol phase

Urine

Urine contained 69% of the total recovered activity. Without hydrolysis treatment urine consisted of 2 major components by HPLC, one of which matched the retention time of 5-OH-TBZ, and the other was suspected to be a sulfate or glucuronide conjugate by the nature of its retention time. ß-Glucuronidase did not substantially alter the metabolic profile from untreated urine, but glusulase treatment resulted in a single major component (5-OH-TBZ) in urine by HPLC. Since glusulase is a mixture of ß-glucuronidase and sulfatase, the conjugate was most likely the sulfate. A further piece of supporting evidence was provided by the sulfatase treatment of urine where 5-OH-TBZ was the only component seen in the chromatogram as opposed to 2 components before enzymic hydrolysis.

Treatment of urine with 6N HCl also produced 5-OH-TBZ as the major radioactive product. A sample of this material was isolated by flash chromatography, purified by preparative TLC, and derivatized as the methyl and TMS compounds. Mass spectra for the methyl derivative are in good agreement; however, those for the TMS derivative are not totally superimposable, particularly the m/z 207 peak.

Feces

Feces contained 28% of the total recovered activity. Among the several hydrolytic procedures, 6N HCl treatment released the most soluble residues: 15% ethyl acetate soluble, 19% aqueous soluble, and 67% unextractable. The organic phase consisted of 5-OH-TBZ (9%), TBZ (1%), and BNZ (2%). The report commented that while BNZ was found as a photolysis product/degradate of TBZ in

plants, its presence in animals as a metabolite has not been previously reported. The aqueous fraction (19%) was not further characterized. Unextractable fraction in feces contained 67% activity.

Metabolism of ^{14}C-Thiabendazole (TBZ) in Poultry

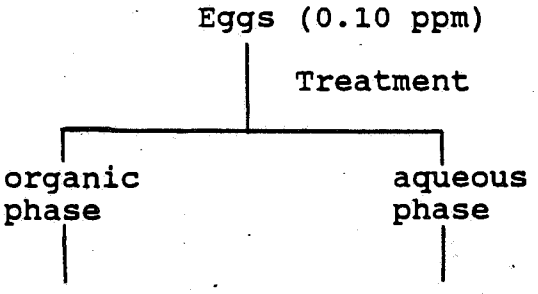
Laying white Leghorn hens (at least 26 weeks old, 1-2 Kg in individual weight, and divided into 5 groups of 5) were dosed with TBZ mixed with cellulose in capsules at 3.19 mg per animal per day for 10 consecutive days. Assuming a daily feed intake of 110 g, the dose is equivalent to 29 ppm TBZ in the diet. Control hens (1 group of 5) were given cellulose capsules only.

Excreta were collected daily, and eggs were collected once in the morning and once in the evening. Tissue samples (liver, kidney, gizzard, heart, abdominal fat, breast muscle, and thigh muscle) were collected from each of the animals upon necropsy, and each tissue type was pooled by treatment group (total of 4 groups).

The hens were sacrificed ca 24 hours of the final dosing of TBZ. Residues in eggs plateaued around day 8.

Eggs (0.10 ppm)

Ethyl acetate extracted little of the egg residue. However, following 6N HCl treatment, residues in eggs partitioned about equally between the organic phase and the aqueous layer. By HPLC 5-OH-TBZ (59%, ca 40 min) was the predominant component along with BNZ (28%, ca 33 min) and TBZ (13%, ca 49 min) in the organic soluble fraction. The identity of these compounds was confirmed by radio TLC with authentic samples after purified samples were obtained from preparative HPLC. The aqueous fraction (<0.05 ppm) showed one major peak (retention time 28 minutes) with a shoulder (26 min) on HPLC (chromatogram not provided) and was not further analyzed.



1)	6N HCl	TBZ (0.007 ppm) BNZ (0.015 ppm) 5-OH-TBZ (0.03 ppm)	<0.05 ppm activity

Chicken Liver (1.47 ppm)

Following 6N HCl digestion, 18% of the liver activity was organic soluble, 75% aqueous soluble, and 7% unextractable. The organic phase contained 3 components which matched the HPLC retention times of BNZ (2%), 5-OH-TBZ (5%), and TBZ (4%) along with 8% unaccounted for during HPLC analysis. HPLC analysis of the aqueous phase showed many components between retention time 20-50 min (chromatogram provided) and additional analysis was not performed. (The report stated that none of the components in the aqueous phase accounted for more than 0.005% of the total recovered radioactivity.)

Kidney (1.21 ppm)

Protease, sulfatase and 6N HCl hydrolysis were employed for the extraction of TBZ residues in the kidney tissue. Of these, acid hydrolysis led to complete digestion of the tissue. The majority of the kidney residue was organic soluble (77%). By HPLC the organic fraction contained BNZ (≈10%), 5-OH-TBZ (≈33%), and TBZ (≈9%); also 20% of activity unaccounted for. Like the liver, the aqueous fraction (23%) contained many fractions between retention time 25-40 min and was not further analyzed. (The report stated that none of the components in the aqueous phase accounted for more than 0.005% of the total recovered radioactivity; we believe "total recovered radioactivity in kidney" is a typo.)

Thigh muscle (0.09 ppm)

6N Hydrochloric acid hydrolysis rendered practically all activity in the muscle organic soluble (21%) and aqueous soluble (79%). TLC or HPLC did not produce well-resolved chromatograms to yield meaningful results (no chromatograms were submitted) and the report cited low levels of activity and large quantities of interfering matrix materials for causing difficulty in the analysis. The distribution of activity between the organic phase and aqueous phase was very similar to those for the liver.

Excreta

Excreta contained 96% of the administered dose (99.6% of the total recovered activity). Ethyl acetate soluble fraction from an aliquot of excreta without prior treatment and after ß-glucuronidase treatment yielded about the same amount of activity (24%) and the same major component (5-OH-TBZ by HPLC retention time) and the same minor component. Glusulase and sulfatase treatment released 22-44% of the excreta residue into the ethyl acetate soluble fraction the main component of which by HPLC matched the retention time of 5-OH-TBZ. A sample of this compound was isolated by preparative HPLC (after sulfatase and 6N HCl hydrolysis) and whose mass spectrum showed intense peaks at m/z 217

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and 190. The loss of 27 mass unit (extrusion of HCN) was also seen in the mass spectrum of an authentic sample of 5-OH-TBZ.

The metabolism of TBZ in animals was brought before the HED Metabolism Committee on February 12, 1992. Summaries of extraction and characterization of carbon-14 residues in several edible tissues, milk, and eggs were provided to the Committee prior to this meeting. During the meeting the Committee was also informed of the use of TBZ as an anthelmintic in animals (thiabendazole may be fed to horses, cattle, pigs, sheep, and goats in the form of top dressing, feed block, drench or oral paste, or bolus at dosages up to 5 grams per 100 pound body weight, sometimes repeatedly at 2-3 week intervals: 21 CFR §520.2380) and in man (The Merck Index, 10th edition). The Committee concluded (see memo to The Metabolism Committee on Thiabendazole Livestock Metabolism, 2/14/92) that the submitted animal metabolism studies were adequate and the tolerance expression for meat, milk, poultry and eggs should include TBZ, 5-OH-TBZ (free and conjugates), and BNZ.

CONCLUSIONS

1. The major metabolite in goat milk and tissues is the 5-OH-TBZ compound (and its conjugates); BNZ and TBZ are also present. The metabolism of TBZ in the goat is understood.

2. TBZ degrades predominantly to 5-OH-TBZ and its conjugates in hens. Varying amounts of BNZ and unchanged TBZ are also present in eggs and hen tissues. The metabolism of TBZ in hens is understood.

3. The Metabolism Committee recommends that TBZ, 5-OH-TBZ (free and conjugates), and BNZ be included in the tolerance expression for animal commodities. Consequently, the registrant should submit a method that is also capable of measuring conjugates of 5-OH-TBZ in animal tissues, milk, and eggs.

Codex Harmonization

TBZ and the 5-hydroxythiabendazole are being regulated in the animal products under Codex (personal communication with Fred Ives, 1/21/92).

RECOMMENDATION

The registrant should be informed that Guideline 171-4(b) - Nature of the Residue in Animals has been satisfied.

Attachment (to the addressee only): Memorandum of 2/14/92 to the Metabolism Committee

cc (without Attachment):Circ, RF, Thiabendazole (List B) File, Cheng, PIB/FOD
RDI:FSuhre:2/28/92:EZager:2/28/92
H7509C:CBII-RS:LCheng:CM#2:RM810:1/22/92:2/18/92:2/28/9202: