12-30-98

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Date: 12/30/98

SUBJECT: **Thiabendazole Reregistration.** Residue Analytical Enforcement Methods for Milk, Eggs, and Animal Tissues and Storage Stability Studies for Residues in Milk and Animal Tissues.
DP Barcode No.: D205191. Case: 2760.
PC Code: 060101.
MRID No.: 43251501, 43251502, 43251503, 43251504, 43251505.

FROM: Sherrie L. Mason, Chemist
Reregistration Branch II
Health Effects Division (7509C)

THROUGH: Alan Nielsen, Branch Senior Scientist
Reregistration Branch II
Health Effects Division [7509C]

TO: William Sproat, Chemical Review Manager
Reregistration Branch I
Special Review and Reregistration Division [7508W]

Attached is a review of studies submitted for the residue analytical enforcement methods for milk, eggs, and animal tissues and storage stability studies for residues in milk and animal tissues. The submission was reviewed by Dynamac Corporation under the supervision of HED. This information has undergone secondary review in reregistration branch 2 and is consistent with Agency policies.

The determination of thiabendazole (TBZ), 5-hydroxythiabendazole (5-OH-TBZ), and 5-hydroxythiabendazole sulfate (5-HSO_4-TBZ) determined as 5-OH-TBZ was adequately described for milk and has undergone successful independent laboratory validation and should be sent to the Analytical Chemistry Branch (ACB) for validation by the Agency.

The determination of TBZ, 5-OH-TBZ, and benzimidazole (BNZ) was adequately described for eggs and has undergone successful independent laboratory validation at a combined limit of quantification (LOQ) of 0.15 ppm. As the tolerance for residues in eggs (0.1 ppm) reflects the combined residues of concern, additional data are required to validate the method levels at $\leq$0.03 ppm for each analyte before this

1

method can be validated by the Agency. A new independent laboratory validation will not be required.

The determination of TBZ, 5-OH-TBZ, and BNZ was adequately described and has undergone successful independent laboratory validation at an LOQ of 0.1 ppm for each analyte in liver, kidney, muscle, and skin/fat. The combined LOQ is 0.3 ppm for the residues of concern. Since the tolerance for residues in animal tissues is 0.1 ppm for the combined residues, the method should be validated at levels of 0.05 ppm for each analyte prior to validation by the Agency. A new independent laboratory validation will not be required.

The storage stability data for residues are adequate and indicate that residues of concern are stable at -10°C for up to 2 months in milk, 2.8 months in animal tissue (liver, kidney, and muscle), and 9 weeks in frozen eggs.

EXECUTIVE SUMMARY

Deficiencies in the Tolerance Enforcement method for TBZ, 5-OH-TBZ, and BNZ in Eggs:

The combined LOQ in the independent laboratory validation is 0.15 ppm for the residues of concern. The tolerance for residues in eggs (0.1 ppm) reflects the combined residues of concern and **additional data are required to validate the method levels at ≤0.03 ppm for each analyte.**

Deficiencies in the Tolerance Enforcement method for TBZ, 5-OH-TBZ, and BNZ in Liver, Kidney, Muscle, and Skin/Fat:

The LOQ for each analyte in the independent laboratory validation is 0.1 ppm. The tolerance for residues in animal tissues is 0.1 ppm for the combined residues; therefore, **the method should be validated at levels of 0.05 ppm for each analyte.**

cc: Sherrie L. Mason (RRB2), Thiabendazole Reg. Std. File, Thiabendazole Subject File, RF, LAN. RDI: RRB2 Res. Chem. Team (12/22/1998).

7509C: RRB2: S. Mason: CM#2:Rm 718Q: 703-305-0563: 12/30/98.

2

THIABENDAZOLE
Shaughnessy No. 060101; Case 2760
(CBRS No. 13991; DP Barcode D205191)

Registrant's Response to Residue Chemistry Data Requirements

December 12, 1997

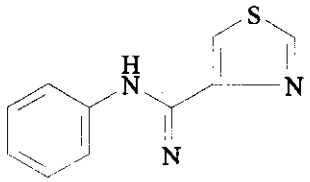
Contract No. 68-D4-0010

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA

Submitted by:
Dynamac Corporation
1910 Sedwick Road
Building 100, Suite B
Durham, NC 27713

3

THIABENDAZOLE



Shaughnessy No. 060101; Case 2760

(CBRS No. 13991; DP Barcode D205191)

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

The Thiabendazole Phase 4 Review (C. Olinger, 2/20/914) required a confirmatory method for determining thiabendazole (TBZ) and 5-hydroxythiabendazole (5-OH-TBZ) in animal commodities and indicated that additional methods would be required if animal metabolism studies identified new residues of concern. After subsequently reviewing the goat and poultry metabolism studies, the HED Metabolism Committee (L. Cheng, 2/14/92) concluded that the residues of concern in animal commodities should be amended to include TBZ, 5-OH-TBZ (free and conjugated), and benzimidazole (BNZ), triggering the requirement for new enforcement methods. In response, Merck has submitted a series of new HPLC fluorescence methods for determining TBZ and its residues of concern in animal commodities (1994; MRIDs 43251501, 43251503, 43251504) along with independent laboratory method validation trials of these methods. In addition to these analytical methods, the registrant has submitted data (1994; MRIDs 43251502 and 43251505) depicting the storage stability of TBZ residues in animal commodities to support the existing animal feeding studies. These submissions are reviewed in this report for adequacy in fulfilling outstanding residue chemistry data requirements. The Conclusions and Recommendations stated in this document pertain only to analytical methodology and the storage stability of residues.

The nature of the residue in plants and animals is adequately understood based on plant metabolism studies on soybean, sugar beet, and wheat, and animal metabolism studies on poultry, and goats. The residues of concern in plants are TBZ and free and conjugated BNZ (L. Cheng; CBRS No. 8192, 3/11/92), and as indicated above, the residues of concern in animal commodities are TBZ, 5-OH-TBZ (free and conjugated), and BNZ.

Tolerances for residues of thiabendazole (2-(4-thiazolyl)benzimidazole) in or on raw agricultural commodities and processed foods and feeds are currently expressed in terms of TBZ per se [40 CFR §180.242(a), §185.5550, and §186.5550(a)]. Tolerances for residues of TBZ in animal commodities are currently expressed as the combined residues of TBZ and its metabolite 5-OH-TBZ [40 CFR §180.242(b)]. Methods are available for determining residues of TBZ per se in or on plant commodities and are listed in PAM, Vol. II, as Methods I, A, B, and C. A method is also available for determining residues of TBZ and 5-OH-TBZ in milk,

and is listed in PAM, Vol. II, as Method D.

Codex Maximum Residue Limits (MRLs) for TBZ are presently expressed in terms of TBZ *per se* in plant commodities and in terms of the combined residues of TBZ and 5-OH-TBZ in animal commodities. Issues regarding the compatibility of the U.S. tolerances and Codex MRLs will be addressed when the reregistration eligibility decision (RED) for TBZ is made.

CONCLUSIONS AND RECOMMENDATIONS

1a. Merck Method M-028.1, a HPLC/fluorescence detection method, is adequate for determining residues of TBZ, 5-OH-TBZ, and the 5-HSO_4-TBZ conjugate (determined as 5-OH-TBZ) in milk. The validated limit of quantitation (LOQ) is 0.05 ppm for TBZ and 5-OH-TBZ, and the limits of detection (LOD) are 0.005 and 0.01 ppm, respectively. As BNZ was not detected in milk in the goat metabolism study, methodology quantifying this metabolite in milk is not necessary. Method M-028.1 has undergone a successful independent laboratory validation, and should be forwarded to the Analytical Chemistry Branch (ACB) for validation.

1b. Merck Method M-025.1 is a HPLC/fluorescence detection method for determining residues of TBZ, 5-OH-TBZ, and BNZ in eggs. This method was adequately validated down to LOQs of 0.05 ppm for each analyte in eggs, for a combined LOQ of 0.15 ppm. The reported method LODs are 0.005 ppm for TBZ and 0.01 ppm for BNZ and 5-OH-TBZ. Method M-025.1 has also undergone a successful independent laboratory validation at the above LOQs.

Before this method can be validated by the Agency, additional data are required validating Method M-025.1 at levels <0.05 ppm for each analyte. As the tolerance for residues in eggs (0.1 ppm) reflects the combined residues of concern, the combined LOQs for the proposed enforcement method must be ≤ 0.1 ppm. Once these data are available this method should be forwarded to ACB for validation. A new independent method validation trial will not be required.

1c. Merck Method M-027 is a HPLC/fluorescence detection method for determining residues of TBZ, 5-OH-TBZ, and BNZ in animal tissues. This method was adequately validated down to LOQs of 0.1 ppm for each analyte in liver, kidney, muscle, and skin/fat; the combined LOQ is 0.3 ppm for the three analytes. The reported method LODs are 0.005 ppm for TBZ and 0.01 ppm for BNZ and 5-OH-TBZ. Method M-027 has also undergone a successful independent laboratory validation at the above LOQs.

Before this method can be validated by the Agency, additional data are required validating Method M-027 at levels <0.05 ppm for each analyte. As the tolerance for residues in animal tissues (0.1 ppm) reflects the combined residues of concern, the combined LOQs for the proposed enforcement method must be ≤ 0.1 ppm. Once these data are available this method should be forwarded to ACB for validation. A new independent method validation trial will not be required.

2a. The submitted storage stability data for residues in milk are adequate and indicate that residues of TBZ, 5-OH-TBZ, and 5-$NaSO_4$-TBZ (sulfate conjugate) are stable in milk at -10 C for up to 2 months.

2b. The submitted storage stability data for residues in animal tissue are adequate and indicate that residues of TBZ and 5-OH-TBZ are stable in liver, kidney, and muscle stored at -10 C for up to 2.8 months.

2c. The available storage stability data support the existing feeding studies. Samples of tissues and eggs from the poultry feeding study were stored for up to 2.5 months prior to analysis, and samples of milk and tissues from the ruminant feeding study were stored up to 1.5 months prior to analysis. In addition to the above data for milk and tissues, data are already available indicating that TBZ and 5-OH-TBZ are stable in frozen eggs for up to 9 weeks.

DETAILED CONSIDERATIONS

Residue Analytical Methods

Merck submitted descriptions of three new HPLC/fluorescence detection methods for determining TBZ residues of concern in milk (MRID 43251501), eggs (MRID 43251503), and animal tissues (MRID 43251504), along with method validation data and independent laboratory method validation trials of these methods. These methods are similar to the current enforcement methods in that residues are extracted with ethyl acetate (EtOAc) and are quantified fluorometrically. However, the new methods employ additional clean-up procedures and utilize HPLC/fluorescence detection for separating and quantifying residues.

Merck Method M-028.1 (MRID 43251501) is for determining TBZ, 5-OH-TBZ, and the 5-HSO_4-TBZ conjugate in milk. The goat metabolism study previously indicated that the major metabolite in milk was 5-HSO_4-TBZ; BNZ was not detected. For Method M-028.1, residues in milk are hydrolyzed at 85-90 C for 4 hours using concentrated HCl, which quantitatively converts 5-HSO_4-TBZ to 5-OH-TBZ. After cooling, the resulting hydrolysate is adjusted to pH 8, and residues are extracted into EtOAc. The EtAOc-soluble residues are then loaded onto a propyl sulfonic acid (PRS) solid phase extraction (SPE) column that has been preconditioned by washing successively with 1% H_3PO_4 in methanol (MeOH)/water (80/20, v/v), MeOH, and EtOAc. After loading, the SPE column is allowed to run dry for 15 minutes, and residues are then eluted from the column using 0.1 M KH_2PO_4 in acetonitrile (ACN)/water (30/70, v/v). Residues of TBZ and 5-OH-TBZ are then analyzed by cation exchange (benzene sulfonic acid column) HPLC using an isocratic mobile phase of ACN/water (20/80, v/v) adjusted to pH 3.8. Residues are quantified using fluorescence detector; the excitation λ and emission λ are 305 and 380 nm for TBZ and 318 and 525 nm for 5-OH-TBZ. The validated LOQ is 0.05 ppm for each analyte in milk, and the estimated LOD is 0.005 ppm for TBZ and 0.01 ppm for 5-OH-TBZ.

6

For method validation, the registrant fortified one set of duplicate control samples of milk with TBZ and 5-OH-TBZ, each at 0.05, 0.4, 2.0 ppm, and another set of duplicate control samples with TBZ and 5-$NaSO_4$-TBZ at the same levels. Results of the method validation are presented in Table 1. Overall method recoveries were 87-103% for TBZ, 98-109% for 5-OH-TBZ, and 96-115% for 5-$NaSO_4$-TBZ (determined as 5-OH-TBZ). Apparent residues of each analyte were <LOD in the four control samples analyzed. Adequate sample calculations, raw data, and representative chromatograms were provided. These data indicate that the HPLC/fluorescence method M-028.1 is adequate for determining residues of TBZ, 5-OH-TBZ and 5-HSO_4-TBZ in milk.

Table 1. Recovery of TBZ and its residues of concern from fortified samples of milk, eggs, and animal tissues using HPLC fluorescence detection methods.

Method No. (MRID)	Matrix	Fortification level (ppm)	% Recovery			
			TBZ	5-OH-TBZ	5-$NaSO_4$-TBZ [a]	BNZ
M-028.1 (43251501)	Milk	0.05	87.1, 91.6, 88.9, 89.7	106, 99.6	96.4, 104	NA= not applicable
		0.4	90.9, 88.3, 91.9, 88.4	109, 105	102, 115	NA
		2.0	95.7, 94.2, 103, 100	101, 97.7	108, 108	NA
M-025.1 (43251503)	Eggs	0.05	90.4, 93.5	84.5, 89.9	NA	95.0, 97.1
		0.1	87.9, 85.9	84.9, 81.4	NA	93.7, 92.5
		0.5	82.4, 73.2	84.8, 78.7	NA	91.3, 89.4
M-027 (43251504)	Poultry liver	0.1	96.9, 100	84.2, 84.1	NA	81.6, 79.3
		0.5	98.3, 92.7	94.5, 91.6	NA	99.8, 101
	Pork kidney	0.1	80.2, 81.4	95.0, 97.1	NA	97.1, 100
		0.5	98.4, 105	81.2, 89.4	NA	98.3, 104
	Poultry muscle	0.1	84.7, 80.2	98.3, 101	NA	86.0, 89.6
		0.5	79.9, 86.4	84.8, 91.3	NA	93.7, 93.3
	Poultry skin/fat	0.1	103, 81.2	86.2, 96.8	NA	85.8, 84.4
		0.5	87.5, 90.3	84.8, 85.5	NA	90.6, 88.3

[a] The 5-$NaSO_4$-TBZ is determined as 5-OH-TBZ.

Method M-028.1 has also undergone an independent method validation trial (MRID 43251501), which was conducted by the Analytical Development Corporation (ADC), Colorado Springs, CO. For each analysis, one set of duplicate control samples of milk was fortified with TBZ and 5-OH-TBZ each at 0.4 and 2.0 ppm, and another set of duplicate control samples was fortified with TBZ and 5-$NaSO_4$-TBZ at the same levels. The current tolerance for the combined residues of TBZ and 5-OH-TBZ in milk is 0.4 ppm.

The first and second attempts at validating the method were unsuccessful due to low recoveries (<70%), and the analytical laboratory reported communicating with the sponsor after each attempt. After the second attempt, the registrant modified the method to specify adjusting the pH of the acid hydrolysate to pH 8 prior to EtOAc extraction, and deleting a MeOH rinse of PRS SPE column following loading of the sample. No problems were reported by the

1

analytical laboratory in conducting the third analysis, and adequate method recoveries were obtained for each analyte (Table 2). This version of the method is also the one described above and validated the registrant. In the third successful method trial, apparent residues of each analyte were <LOD in the four control samples analyzed, except for one sample bearing apparent residues of TBZ at 0.02 ppm. Adequate sample calculations, raw data, representative chromatograms, and standard curves were provided. The analytical laboratory reported that a single sample set (12 samples) required 14.5 person hours for analysis over a 3-day period. The independent laboratory validation for Method M-028.1 is adequate.

Table 2. Independent method validation recovery data for TBZ and its residues of concern from fortified samples of milk, eggs, and animal tissues using HPLC fluorescence detection methods.

Method No. (MRID)	Matrix	Fortification Level (ppm)	% Recovery			
			TBZ	5-OH-TBZ	5-$NaSO_4$-TBZ [a]	BNZ
M-028.1 (43251501)	Milk	0.4	89, 90, 92, 90	83, 81	86, 88	NA [b]
		2.0	88, 92, 96, 98	87, 86	101, 102	NA
M-025.1 (43251503)	Eggs	0.1	75, 81	76, 69	NA	87, 102
		0.5	89, 87	86, 89	NA	103, 102
M-027 (43251504)	Poultry liver	0.1	115, 91, 88, 85	0, 95 82, 77	NA	108, 90 81, 78
		0.5	90, 89	93, 88	NA	92, 90

[a] The 5-$NaSO_4$-TBZ is determined as 5-OH-TBZ.
[b] NA = not applicable.

Merck Methods M-025.1 and M-027 (MRIDs 43251503 and 43251504) are for determining TBZ, 5-OH-TBZ, and BNZ and their conjugates in eggs and animal tissues, respectively. These methods are identical to each other and are similar to Method M-028.1 except for differences in the initial extraction procedures. Using these methods, residues in homogenized egg and tissues are hydrolyzed at 90-95 C for 24 hours using 6N HCl. After cooling, the acid hydrolysate is mixed with EtOAc and centrifuged, and the resulting EtOAc fraction is discarded. The hydrolysate is then adjusted to pH 8, and loaded onto a dry Extrelut QE column packed with diatomaceous earth. This column is then attached to the top of a preconditioned PRS SPE column, and residues are eluted onto the SPE column with EtOAc. After loading, the SPE column is washed with EtOAc and then allowed to run dry for 15 minutes. Residues are then eluted from the SPE column using 0.1 M KH_2PO_4 in ACN/water (30/70, v/v). Residues are analyzed by cation exchange HPLC using an isocratic mobile phase of ACN/water (25/75, v/v) with a fluorescence detector. Separate injections are used to determine 5-OH-TBZ and TBZ/BNZ. For analysis of 5-OH-TBZ, the mobile phase pH is adjusted to 3.0 and the excitation and emission wavelengths are 325 and 575 nm. For analysis of TBZ and BNZ, the mobile phase pH is adjusted to 3.4 and the excitation and emission wavelengths are 265 and 380 nm. The validated LOQ is 0.05 ppm for each analyte in eggs and is 0.1 ppm for each analyte in tissues; the estimated LODs in eggs and tissues are 0.005 ppm for TBZ and 0.01 ppm for BNZ and 5-OH-TBZ.

8

For method validation of Method M-025.1, the registrant fortified duplicate control samples of eggs with TBZ, BNZ, and 5-OH-TBZ, each at 0.05, 0.1, 0.5 ppm. Results of the method validation are presented in Table 1. Overall method recoveries from eggs were 73-94% for TBZ, 79-90% for 5-OH-TBZ, and 89-97% for BNZ. Apparent residues of each analyte were <LOD in the two control samples analyzed. Adequate sample calculations, raw data, and representative chromatograms were provided. These data indicate that the HPLC/fluorescence method M-025.1 is adequate for determining residues of TBZ, BNZ, and 5-OH-TBZ in eggs. However, HED notes that the combined LOQs for the residues of concern are 0.15 ppm, which exceeds the current 0.1 ppm tolerance for TBZ residues in eggs.

An independent method validation trial (MRID 43251503) was also conducted on Method M-025.1 by ADC. The analytical laboratory fortified duplicate control samples of eggs with TBZ, BNZ, and 5-OH-TBZ each at 0.1 and 0.5 ppm; the current tolerance for the combined residues of TBZ and 5-OH-TBZ in eggs is 0.1 ppm. Adequate method recoveries were obtained for each analyte (Table 2). Apparent residues of 5-OH-TBZ were <LOD in both control samples. Apparent residues of TBZ and BNZ were also <LOD in one control sample, but the other control sample had apparent residues of TBZ and BNZ at 0.015 and 0.010 ppm, respectively. Adequate sample calculations, raw data, representative chromatograms, and standard curves were provided. The analytical laboratory reported no communications with the sponsor and no problems in conducting the analysis. A single sample set (6 samples) required 39 hours (including 24-hour hydrolysis) for analysis over a 5-day period. The independent laboratory validation for Method M-025.1 is adequate.

For method validation of Method M-027, the registrant fortified duplicate control samples of poultry liver, pork kidneys, poultry muscle, and poultry skin/fat with TBZ, BNZ, and 5-OH-TBZ, each at 0.1 and 0.5 ppm. Results of the method validation are presented in Table 1. Overall method recoveries from animal tissues were 80-105% for TBZ, 81-101% for 5-OH-TBZ, and 79-104% for BNZ. Apparent residues TBZ and BNZ were <LOD in the two control samples of each tissue, and apparent residues 5-OH-TBZ were <LOD in the two control samples of kidneys and skin/fat. However, apparent residues 5-OH-TBZ were 0.011 and 0.016 ppm in the control samples of liver, and 0.011 ppm in the two control samples of muscle. Adequate sample calculations, raw data, and representative chromatograms were provided. These data indicate that the HPLC/fluorescence method M-027 is adequate for determining residues of TBZ, BNZ, and 5-OH-TBZ in animal tissues. However, HED notes that the combined LOQs for the residues of concern are 0.3 ppm, which exceeds the current 0.1 ppm tolerance for TBZ residues in animal tissues.

An independent method validation trial (MRID 43251504) was also conducted on Method M-027 by ADC. The analytical laboratory fortified duplicate control samples of liver with TBZ, BNZ, and 5-OH-TBZ each at 0.1 and 0.5 ppm; the current tolerance for the combined residues of TBZ and 5-OH-TBZ in eggs is 0.1 ppm. In a first trial run, the recovery of 5-OH-TBZ from one sample fortified at 0.1 ppm was "0"; therefore, a second run using duplicate samples fortified at 0.1 ppm was also conducted. Aside from the anomalous recovery value for 5-OH-TBZ from the one sample; adequate method recoveries were obtained for each analyte (Table 2). With the exception of one control sample having apparent residues of TBZ

at 0.006 ppm, apparent residues of each analyte were < LOD in both control samples. Adequate sample calculations, raw data, representative chromatograms, and standard curves were provided. The analytical laboratory reported no communications with the sponsor and no problems in conducting the analysis. A single sample set (6 samples) required 40 hours (including 24-hour hydrolysis) for analysis over a 5-day period. The independent laboratory validation for Method M-027 is adequate.

Storage Stability Data

Merck submitted data on the stability of TBZ, 5-OH-TBZ, and 5-$NaSO_4$-TBZ in frozen milk (MRID 53251502) and TBZ and 5-OH-TBZ in frozen tissues (MRID 53251505) to support the existing feeding studies. Samples of tissues and eggs from the poultry feeding study were stored for up to 2.5 months prior to analysis, and samples of milk and tissues from the ruminant feeding study were stored up to 1.5 months prior to analysis. Data are presently available indicating the TBZ and 5-OH-TBZ are stable in frozen animal tissues for up to 4 weeks and in frozen eggs for up to 9 weeks.

To determine the storage stability of residues in milk, one set of control samples was fortified with TBZ at 0.4 ppm and 5-$NaSO_4$-TBZ at 0.2 ppm, and a second set of control samples was fortified with TBZ and 5-OH-TBZ each at 0.4 ppm. After fortification (Day-0), triplicate samples from each set were analyzed along with control samples. After 2 months of storage at -10 C, triplicate samples from each set were analyzed along with control samples and freshly fortified samples. All samples were analyzed using Merck Method M-024, a HPLC/fluorescence detection method that is essentially identical to Merck Method M-028.1 discussed above, except for minor differences in the column clean up procedures. The stated LOD for each analyte in milk is 0.01 ppm. Concurrent method recoveries were adequate and are presented in Table 3, along with the recoveries from stored samples. Apparent residues of each analyte were < LOD in/on six control samples. Adequate sample calculations, raw data, and representative chromatograms were provided.

The submitted storage stability data for residues in milk are adequate and indicate that residues of TBZ, 5-OH-TBZ, and 5-$NaSO_4$-TBZ (sulfate conjugate) are stable in milk at -10 C for up to 2 months.

To determine the storage stability of residues in tissues, control samples of poultry liver and muscle and beef liver were fortified with TBZ and 5-OH-TBZ each at 0.5 ppm. After fortification (Day-0), three or six (kidney) freshly fortified samples of each matrix were analyzed along with control samples. After 2.8 months of storage at -10 C, triplicate samples from each matrix were analyzed along with control samples and freshly fortified samples.

All samples were analyzed using Merck Method M-026, a HPLC/fluorescence detection method. This method is similar to Merck Method M-027 discussed above, except that samples are not acid hydrolyzed and there is no acidic extraction with EtOAc; residues in samples are directly extracted into EtOAc at pH 8. The LOQ and LOD for both analytes are 0.5 and 0.01 ppm, respectively, in all tissues.

- Concurrent method recoveries were adequate and are presented in Table 3, along with the recoveries from stored samples. Apparent residues of each analyte were < LOD in control samples. Adequate sample calculations, raw data, and representative chromatograms were provided. The submitted storage stability data for residues in tissues are adequate and indicate that residues of TBZ and 5-OH-TBZ are stable in animal tissues at -10 C for up to 2.8 months. Although recoveries of 5-OH-TBZ were low in liver (65%) and kidneys (67%) after 2.8 months of storage, these recoveries were similar to the recoveries initially obtained from these samples (liver, 74%; kidneys, 73%).

11

Table 3. Storage stability of TBZ, 5-OH-TBZ, and 5-$NaSO_4$-TBZ in milk and TBZ and 5-OH-TBZ in animal tissues stored at -10 C.

Matrix	Analyte	Fortification level (ppm)	Storage Interval (days)	Percent Recovery	
				Freshly fortified sample	Stored sample
Milk	TBZ	0.4	0	90, 90, 88, 96, 95, 98 (93) [a]	NA
			62 61	93, 93, 92, 99, 96, 96 (95)	92, 92, 91 93, 92, 94 (92)
	5-$NaSO_4$-TBZ	0.2	0	73, 88, 122 (94)	NA
			61	101, 91, 99 (97)	80, 95, 92 (89)
	5-OH-TBZ	0.4	0	73, 77, 74 (75)	NA
			62	86, 88, 88 (87)	89, 83, 85 (86)
Poultry liver	TBZ	0.5	0	85, 87, 87 (86)	NA
			85	90, 89, 91 (90)	89, 91, 89 (90)
	5-OH-TBZ	0.5	0	76, 76, 71 (74)	NA
			85	78, 82, 83 (81)	66, 66, 64 (65)
Poultry muscle	TBZ	0.5	0	87, 85, 88 (87)	NA
			85	92, 88, 89 (90)	91, 89, 90 (90)
	5-OH-TBZ	0.5	0	83, 85, 82 (83)	NA
			85	85, 89, 79 (84)	77, 82, 78 (79)
Beef kidney	TBZ	0.5	0	87, 89, 88, 91, 89, 89 (89)	NA
			84	89, 88, 89	92, 93, 93 (93)
	5-OH-TBZ	0.5	0	66, 75, 70, 77, 77, 72 (73)	NA
			84	86, 84, 87 (86)	54, 73, 73 (67)

[a] Values in parentheses are the average recovery.

AGENCY MEMORANDA CITED IN THIS DOCUMENT

CBRS No. None
DP Barcode: None
Subject: Thiabendazole Livestock (Goat and Poultry) Metabolism. The Metabolism Committee Meeting Held on February 12, 1992.
From: L. Cheng
To: Metabolism Committee, HED
Dated: 2/14/92
MRID(s): None

CBRS No. 8192
DP Barcode: D165718

12

Subject: Thiabendazole Phase V Review. Metabolism Studies: Wheat, Soybean, and Sugar Beet.
From: L. Cheng
To: F. Rubis
Dated: 3/11/92
MRID(s): 41872901 through 41872903

MASTER RECORD IDENTIFICATION NUMBERS

The citations for the MRID documents used in this review are presented below.

43251501 Arenas, R. (1994) Residue Analytical Enforcement Method for Thiabendazole, 5-Hydroxythiabendazole, and the Sulfate Conjugate of 5-Hydroxythiabendazole in Bovine Milk: Lab Project Number: M-028.1. Unpublished study prepared by Merck Research Lab. and Analytical Dev. Corp. 122 p.

43251502 Arenas, R. (1994) Effect of Freezer Storage on the Magnitude of the Residues of Thiabendazole, 5-Hydroxythiabendazole, and the Sulfate Conjugate of 5-Hydroxy thiabendazole in Raw Milk: Lab Project Number: 93857. Unpublished study prepared by Merck Research Lab. 118 p.

43251503 Arenas, R. (1994) Residue Analytical Enforcement Method for Thiabendazole, 5-Hydroxythiabendazole, and Benzimidazole in Chicken Egg: Lab Project Number: M-025.1. Unpublished study prepared by Merck Research Lab. & Analytical Dev. Corp. 120 p.

43251504 Arenas, R. (1994) Residue Analytical Enforcement Method for Thiabendazole, 5-Hydroxythiabendazole, and Benzimidazole in Animal Tissue: Lab Project Number: M-027. Unpublished study prepared by Merck Research Lab. & Analytical Dev. Corp. 169 p.

43251505 Arenas, R. (1994) Effect of Freezer Storage on the Magnitude of the Residues of Thiabendazole and 5-Hydroxythiabendazole in Animal Tissues: Lab Animal Tissues: Lab Project Number: 93897. Unpublished study prepared by Merck Research Lab. 122 p.