Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Thiabendazole

MRID #: 431872-03

Matrix: Soil

Analysis: HPLC/FLD

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ANALYTICAL METHOD

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INTRODUCTION AND SUMMARY

Study Components

"The 'Terrestrial Field Dissipation for Thiabendazole in Soybeans' study (ABC Study No. 38042) is comprised of the following five components: (1) Field Study (38042); (2) Analytical Results for Authentic Field Samples (38042A); (3) Analytical Method (38042M); (4) Freezer Stability (38042S); and (5) the raw data package (38042R)." The Study Compliance Statement located in the Field Study component (38042) covers study components 1 through 5 listed above.

Scope

The analytical methodology used for the residue analysis of thiabendazole (TBZ), and the metabolite benzimidazole (BNZ), in soil was developed by ABC Laboratories, Columbia, Missouri. Initial extraction conditions were optimized based on the results of the aerobic soil metabolism study (MRID #41791201), ABC Study #37639. The analytical method was used to determine thiabendazole and benzimidazole residues in soil at residue levels as low as 0.01 ppm.

Principle

The methodology used in the analysis of thiabendazole and its metabolite benzimidazole was developed at ABC Laboratories. Initial extraction conditions consisted of shaking the soil samples with 1 N KOH/methanol followed by 50:50 6 N hydrochloric acid:dimethylformamide. The extracts were filtered into separatory funnels and buffered to basic pH with sodium hydroxide and sodium carbonate.

The basic extracts were then partitioned against ethyl acetate two times and the organic phases were combined and rotary evaporated. The organic partitions were evaporated to near dryness (~1 mL DMF remains) and the extracts were transferred quantitatively to culture tubes with dilute acetic acid for analysis by high-performance liquid chromatography (HPLC) using fluorescence detection.

The HPLC fluorometric detector was optimized for each compound to accommodate the fluorescence spectra of either thiabendazole or benzimidazole.

MATERIALS

Standard Reference Materials

The following analytical reference standards were used in this study:

Compound	Supplier	Date Received	Purity	ABC Ref.	Lot Number	Storage
Thiabendazole	Merck & Co.	03-20-89	99.8%	PS-3190	L585216-000S141	-20 °C
Benzimidazole	Aldrich	06-07-89	98%	PS-3351	Aldrich 02802JT	Room Temp.

Standards were accurately weighed and dissolved in methanol. Serial dilutions were prepared as indicated in the raw data. Stock and spiking solutions were kept in the freezer and calibration curve solutions were stored in a refrigerator when not in use. Exact copies for preparation of stock solutions and dilution of working standards are located in Appendix VII of the raw data package (38042R).

[Note 1. The purity of the reference standard was determined concurrently with the conduct of the study and was assumed to be 100% in the raw data.]

Reagents

- Methanol—HPLC grade Burdick & Jackson
- Acetone—Pesticide grade Burdick & Jackson
- Ethyl Acetate—Pesticide grade Burdick & Jackson
- Acetic Acid—Reagent grade J.T. Baker
- Potassium Hydroxide—Reagent grade J.T. Baker
- Ammonium Acetate—HPLC grade J.T. Baker
- Sodium Carbonate—Reagent grade J.T. Baker
- Sodium Hydroxide—Reagent grade J.T. Baker
- Hydrochloric Acid—Reagent grade J.T. Baker
- Reagent Grade water—LABCONCO Water Purification
- Buffer Solution for pH Meter Calibration—pH=7 (Fisher Cat. #SB108-500)
- Buffer Solution for pH Meter Calibration—pH=10 (Fisher Cat. #SB116-500)
- Cotton Balls—Generic

PREPARATION OF STANDARD SOLUTIONS

Preparation of Reference Standard Solutions

1. Thiabendazole and Benzimidazole Stock Solutions

   a. Weigh accurately about 25.0 mg of thiabendazole (TBZ) and benzimidazole (BNZ) reference standards and transfer to separate 25-mL volumetric flasks. Dilute each flask to volume with methanol. Mix well. Each solution contains approximately 1000 mcg thiabendazole/mL and 1000 mcg benzimidazole/mL, respectively. Label the flasks "TBZ STOCK SOLUTION 1000 mcg/mL" and "BNZ STOCK SOLUTION 1000 mcg/mL."

[redacted]

b. Transfer approximately 2.0 mL of the "TBZ STOCK SOLUTION 1000 mcg/mL" and approximately 2.0 mL of "BNZ STOCK SOLUTION 1000 mcg/mL" to the same 100-mL volumetric flask. Dilute the flask to the 100-mL mark with methanol. Mix well. The solution contains approximately 20 mcg/mL of TBZ and 20 mcg/mL of BNZ. Label the flask "TBZ/BNZ STOCK SOLUTION 20 mcg/mL."

c. Transfer 5.0 mL of "TBZ/BNZ STOCK SOLUTION 20 mcg/mL" to a 100-mL volumetric flask. Dilute the flask to the 100-mL mark with 10% acetic acid in water. Mix well. The solution contains approximately 1 mcg/mL of TBZ and 1 mcg/mL of BNZ. Label the flask "TBZ/BNZ STOCK SOLUTION 1 mcg/mL."

2. Thiabendazole and Benzimidazole HPLC Calibration Standard Solutions

a. Transfer 25-, 10-, 5-, 2.5-, and 1-mL aliquots of "TBZ/BNZ STOCK SOLUTION 1 mcg/mL" to separate 100-mL volumetric flasks and dilute each flask to the mark with 10% acetic acid in water. Mix well. The solutions contain approximately 0.25, 0.10, 0.050, 0.025, and 0.010 mcg/mL each of TBZ and BNZ, respectively. Label the TBZ/BNZ calibration solutions appropriately.

[Note 2. The actual weights used are documented in the raw data. Stock and spiking solutions were stored in the freezer when not in use.]

Preparation of Thiabendazole and Benzimidazole Fortification Solutions

[Note 3. The actual dilutions used are documented in the raw data.]

3. Transfer 2.0 mL of the "TBZ STOCK SOLUTION 1000 mcg/mL" and 2.0 mL of "BNZ STOCK SOLUTION 1000 mcg/mL" to the same 100-mL volumetric flask. Dilute the flask to the 100-mL mark with methanol. Mix well. The solution contains approximately 20 mcg/mL of TBZ and 20 mcg/mL of BNZ. Label the flask "TBZ/BNZ FORTIFICATION SOLUTION 20 mcg/mL."

4. Transfer 10 mL of "TBZ/BNZ FORTIFICATION SOLUTION 20 mcg/mL" to 50-mL volumetric flask and dilute to the mark with methanol. Mix well. The solution contains approximately 4 mcg/mL of TBZ and 4 mcg/mL of BNZ. Label the flask "TBZ/BNZ FORTIFICATION SOLUTION 4 mcg/mL."

5. Transfer 10 mL of "TBZ/BNZ FORTIFICATION SOLUTION 20 mcg/mL" to a 100 mL volumetric flask and dilute to the mark with methanol. Mix well. The solution contains approximately 2 mcg/mL of TBZ and 2 mcg/mL of BNZ. Label the flask "TBZ/BNZ FORTIFICATION SOLUTION 2 mcg/mL."

Equipment

1. Linear shaker

2. Büchner funnel

3. Rotary evaporator

4. Separatory funnel, 500 mL

5. HPLC equipment (see Instrumentation section)

METHOD OF ANALYSIS

Sample Preparation

At the facilities of ABC Laboratories, 5 soil cores were composited by depth and sample date into a single sample for analysis so that there were three replicate samples (A, B, and C) from the 15 treated cores and one sample from the 5 control cores at each sample date.

A Straub Model 4E grinding mill was used to finely grind and homogenize each sample. The rotating plate of the mill was set to the smallest allowable gap between rotating and stationary plates. Each sample was then passed through the mill three successive times in the presence of enough dry ice to keep the sample frozen. Each sample was then continuously stirred and mixed during grinding. After the final grind, each sample was placed in a prelabeled plastic container and the dry ice allowed to sublime in a small freezer before being returned to the walk-in freezer.

Extraction Procedure

The procedures listed below were followed during this study.

1. Weigh 20 g of soil into an 8-oz Nalgene bottle. Method recovery check samples should be fortified with thiabendazole and benzimidazole at this time.

2. Add 50 mL 1 N methanolic KOH and shake for 1 hour at approximately 180 excursions per minute.[1]

3. Centrifuge the samples at approximately 4000 rpm for 5 minutes. (Caution: Add methanol to balance the bottles to within 0.5 g of each other prior to centrifugation).[1]

4. Decant the supernatant into a 500-mL separatory funnel.[1]

5. Add approximately 50 mL of 1:1 6 N HC1:dimethylformamide (DMF) to the soil sample. Cap and shake for 1 hour at 180 excursions per minute ("Slow" on a linear shaker.)[1]

6. Filter the extracts through a glass-fiber filter paper in a Büchner funnel using water suction after wetting the paper with 5-10 mL water. In the case of slow-filtering samples, 20 mL of Celite is added to the sample prior to filtration with a Whatman #4 filter paper. Wash bottle with 2 X 10 mL 1:1 6 N HC1:DMF and add to filter funnel. Combine the methanolic KOH and HC1:DMF extracts in a 500-mL separatory funnel.[1]

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7. Add 50 mL 4 N NaOH and 50 mL 2 N Na_2CO_3, in that order, slowly, swirling to dissipate heat.

8. Add 100 mL ethyl acetate; shake for 1 minute and allow to separate. Swirling the sample may enhance the separation but an emulsion may persist.

9. Drain the aqueous (lower) layer into a holding vessel. If an emulsion persists add 20 mL of 0.2 N Na_2CO_3, shake 15 seconds, and allow the layers to separate. Drain the aqueous (lower) layer into the same holding vessel.

10. Pass the organic portion through a cotton pledget in a powder funnel into a 500-mL flat bottom flask. Return the aqueous portion to the original separatory funnel.

11. Repeat steps 8-10 for a total of 2 X 100 mL ethyl acetate partitions.[1]

12. Evaporate the ethyl acetate to near dryness on a rotoevaporator with a (30-40 °C) water bath. Approximately 1 mL DMF remains.

13. Rinse the flask with at least 2 X 3 mL portions of 10% acetic acid (v/v) and combine in a 10 mL volumetric flask. Dilute to the 10 mL mark with 10% acetic acid in water. Analyze the solution for TBZ and BNZ by HPLC.

[1] Sample preparation may be stopped and samples stored overnight at room temperature after these steps.

Instrumentation

A Shimadzu 6A HPLC system equipped with autosampler, controller, and pump was usually used in conjunction with either a Varian 2070 spectrofluorometer or a Shimadzu RF-551 programmable spectrofluorometer. Both fluorometers have dual monochrometers to specify the excitation and emission wavelengths. Equipment from other manufacturers may have been used if shown to be functionally equivalent.

Chromatography

Thiabendazole and benzimidazole were separated by the reverse phase HPLC system. However, the fluorometric spectra of thiabendazole and benzimidazole were different to the extent that no benzimidazole peak appeared in the thiabendazole chromatogram when the instrument was set on the thiabendazole wavelengths (and vice versa).

Generally, aliquots of the extracts and calibration standards were transferred to autosampler vials and injected on a chromatograph optimized for one of the analytes. After the analysis, the chromatographic conditions (mobile phase, injection volume, and detector wavelengths) were changed to optimize the system for the other analyte and the same vials were reinjected for that analysis.

Modifications to the following parameters may have been necessary to ensure acceptable chromatography.

Column:	Supelco LC-8-DB, 25 cm × 4.6 mm, 5-μm particle size
Column Temperature:	Ambient
Range of Standard Curve:	250 to 10 ng/mL

Compound: Parameter:	Thiabendazole	Benzimidazole
Mobile Phase: 1 g/L Ammonium Acetate	60% Water 40% Methanol	70% Water 30% Methanol
Injection Volume:	50 μL	10 μL
Excitation Wavelength:	300λ	271λ
Emission Wavelength:	350λ	300λ

Soil Moisture Determination

Soil moisture determinations were performed on each treated sample. Determinations were performed as described in ABC SOP FC 1.7.1. This consisted of weighing the container, then weighing the container plus the wet soil, and then drying at 105-130 °C to a constant weight.

The soil moisture was then calculated by the equation:

% soil moisture =

$$100 \times \frac{\textit{wet wt of sample and container (g)} - \textit{dry wt of sample and container (g)}}{\textit{wet wt of sample and container (g)} - \textit{container wt (g)}}$$

Method of Calculations

The Computer Automated Laboratory System (CALS) or MULTICHROM allows for data acquisition, data analysis, results reporting, and information management.

The CALS or MULTICHROM program measures chromatographic peak areas for standards and samples, then uses the standard concentrations versus peak areas to calculate a regression expression. The analyte concentration in each sample extract is interpolated from the regression curve. The concentration is then converted to parts per million (ppm) of analyte in the sample using the following equation after entering the final volume, dilution factor, and sample weight into MULTICHROM system.

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$$\text{ppm analyte in sample} = \frac{C \times V \times DF}{W}$$

where:

C	=	concentration of analyte in final HPLC assay solution in $\mu g/mL$
V	=	final volume of HPLC assay solution in mL
DF	=	final dilution factor
W	=	weight of sample in g

Spreadsheet Calculations

The ppm levels of analytes in the samples derived from the MULTICHROM data system are entered into a spreadsheet program on a personal computer (Quattro Pro) to calculate the recovery of analyte from fortified samples and to provide correction for recoveries to treated samples. The percent recovery of the analyte from fortified samples corrected for background was calculated as follows:

$$\%\ recovery = \frac{(ppm\ found - ppm\ in\ control)}{theoretical\ ppm\ calculated}$$

The percent recovery of the laboratory fortifications was then used to correct the treated samples for recoveries. No corrections occurred if the percent recovery was equal to or greater than 100%. The residue level in the treated samples was also corrected for moisture content simultaneously as follows:

$$\begin{matrix} ppm\ corrected\ for \\ moisture\ and\ recovery \end{matrix} = \frac{ppm\ found}{\begin{matrix}(avg.\ \%\ rec.\ if < 100\%) \times (1 - \%\ moist.) \\ both\ as\ decimals\end{matrix}}$$

The ppm levels from MULTICHROM are entered into the spreadsheet using all six figures printed on the Results Information Report. All spreadsheet calculations utilize the unrounded results of previous calculations; therefore, the average percent recoveries reported in the data tables may not calculate precisely based on the 3 significant figures reported in the tables. These calculations may be duplicated precisely using the information on the spreadsheet entry printouts in Appendix VII of the raw data package (38042R).

RESULTS AND DISCUSSION

Method Validation

The method was validated by analyzing nontreated soils (control) and nontreated soils fortified at three different fortification levels (0.010, 0.10, and 1.0 ppm) of thiabendazole and benzimidazole. Results of the method validation are given in Table M-1. The overall average recoveries uncorrected for moisture for thiabendazole and benzimidazole was 88 ± 7.9 and 90 ± 8.9%, respectively. The limit of quantitation (LOQ) was set at 0.010 ppm for both thiabendazole and benzimidazole. No values less than this concentration were reported for the actual field samples. Most control soil samples contained <0.01 ppm (estimated) of apparent residues of TBZ and BNZ.

Fortified Samples

Recovery from fortified samples analyzed concurrently with field samples are reported in Table M-2.. The overall average recovery and the standard deviation for each fortification level are also included. Control soil samples were fortified at levels of 0.010, 0.10, and 1.0 ppm for thiabendazole and benzimidazole. The overall average percent recovery for thiabendazole and benzimidazole was 83 ± 11 and 84 ± 10%, respectively.

Accuracy

The accuracy of the analytical method is the statistical agreement of the test results obtained by the analytical method to the true value. Control soil samples were fortified with known amounts of thiabendazole and benzimidazole and analyzed for thiabendazole and benzimidazole residues.

The average percent recoveries TBZ and BNZ from the analysis of soil over the fortification range of 0.01-1 ppm is listed below with the standard deviation and coefficient of variation (CV). The CV is calculated by the following equation.

$$CV = \frac{\sigma \text{ (Standard Deviation of the \% Recoveries)}}{\text{Mean \% Recovery}} X\ 100$$

Precision

The precision of the analytical method is expressed as the relative standard deviation of the test results. The overall precision of the analytical method is calculated from the standard deviation and the mean value of the percent recovery of thiabendazole from fortified control samples using the following equation:

$$\text{Precision (95\% level)} = \frac{2 \times \sigma \text{ (Standard Deviation of the \% Recoveries)}}{\text{Mean \% Recovery}} \times 100$$

The overall accuracy and precision of the analytical method percent recovery for both compounds are tabulated below:

	Statistics				
Analyte	n	Mean	SD	CV	Precision
Thiabendazole	46	83	11	13	27
Benzimidazole	47	84	10	12	24

n = number of observations

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Linearity

The standard calibration curve was shown to be linear over the expected concentration range of the sample solutions analyzed for thiabendazole and benzimidazole.

Limits of Detection and Quantitation

The limit of detection is equal to approximately 2-3 times the background level of a control sample that has been subjected to the analytical procedure. The limit of quantitation is the lowest thiabendazole fortification level for which recovery data are deemed acceptable. The limit of quantitation and detection of the analytical method is 0.01 ppm and 0.005 ppm, respectively, for thiabendazole and benzimidazole in soil.

Limitations

The analytical method is highly specific for detecting thiabendazole and benzimidazole residues in soil. Since the method uses fluorescence detection as a means of quantitating thiabendazole and benzimidazole residues, other pesticides or fungicides which do not have inherent fluorescence are not expected to interfere with the analysis for thiabendazole and benzimidazole.

Confirmation of Residues by Mass Spectroscopy

Residues of the parent compound in treated samples were confirmed to be thiabendazole by gas chromatography/mass spectroscopy. Two treated soil samples and one reagent blank were extracted by the soil method with the exception that the final extract was reconstituted in 2 mL of methanol instead of 10 mL of 10% acetic acid. Samples for analysis were injected on the GC-MS between September 16 and September 19, 1991.

A thiabendazole standard was injected on the GC and the resulting mass spectroscopy data indicates large peaks in the single ion chromatograms (SIC) of ions 201 and 174. Data for the reagent blank for the analysis did not indicate the presence of thiabendazole in this sample. The scan of ions for the retention time of thiabendazole during the analysis of ABC Lab #413.1 (sample ID 182.GA.BG.0-6"C) showed ions 201 and 174 above the background of other ions, indicating the presence of thiabendazole in the extract. Data for ABC Lab #487.1 (ID 542.GA.C.0-6"B) indicated that the ions characteristic of thiabendazole (201 and 174) were present above background levels.

In summary, residues of the parent compound thiabendazole were confirmed in both treated samples analyzed by mass spectroscopy. The reagent blank was found to contain no quantifiable amount of thiabendazole, indicating little possibility of laboratory contamination.

TABLES

TABLE M-1 Method Validation Results for Thiabendazole and Benzimidazole in Georgia Soil

Analytical Lab. # 38042	Sample ID	Spike Level TBZ (ppm)	Spike Level BNZ (ppm)	Date Extracted	Thiabendazole % Recovery	Benzimidazole % Recovery
317.1	Control	0.00	0.00	07/13/90 a	-	-
318.1	Control	0.00	0.00	07/13/90 a	-	-
319.1	Control	0.01	0.01	07/13/90	81%	105%
320.1	Control	0.01	0.01	07/13/90	79%	89%
				Average	80%	97%
321.1	Control	0.1	0.1	07/13/90	90%	96%
322.2	Control	0.1	0.1	07/13/90	84%	80%
				Average	87%	88%
323.1	Control	1.0	1.0	07/13/90	96%	86%
324.1	Control	1.0	1.0	07/13/90	98%	86%
				Average	97%	86%
				Overall Average	88%	90%
				SD	7.9%	8.9%

a Background residues in control samples were below the MLQ (<0.01 ppm).

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TABLE M-2 Recovery from Fortified Control Soil Samples Spiked at 0.010 ppm, 0.10 ppm, and 1.0 ppm

Analytical Lab. # 38042-	Sample ID	Depth	Date Extracted	ppm Spiked	Thiabendazole % Recovery	Benzimidazole % Recovery
351	T1B.	00-06"	04/29/91	0.010	91%	82%
354	T1B.	12-18"	04/29/91	0.010	90%	78%
365	T1B.	18-24"	04/30/91	0.010	86%	78%
368	T1B.	30-36"	04/30/91	0.010	79%	81%
391	T1A.	00-06"	11/19/90	0.010	80%	115%
379	T1A.	00-06"	11/16/90	0.010	73%	73%
416	007.	00-06"	12/19/90	0.010	74%	91%
428	182.	00-06"	12/20/90	0.010	70%	84%
440	182.	12-18"	01/03/91	0.010	88%	103%
463	362.	00-06"	03/20/91	0.010	84%	79%
451	362.	00-06"	01/17/91	0.010	104%	93%
• 484	542.	00-06"	03/27/91	0.010	90%	82%
472	542.	00-06"	03/26/91	0.010	84%	88%
472.1	542.	00-06"	07/17/92	0.010	97%	81%
515	722.	00-06"	05/29/92	0.010	67%	85%
527	722.	00-06"	06/02/92	0.010	58%	85%
539	902.	00-06"	06/04/92	0.010	78%	81%
551	902.	00-06"	06/08/92	0.010	70%	84%
551.1	902.	00-06"	12/11/52	0.010	76%	67%
563	992.	00-06"	08/27/92	0.010	75%	108%
575	992.	00-06"	08/14/92	0.010	107%	73%
587	1082.	00-06"	08/21/92	0.010	70%	79%
599	1082.	00-06"	08/19/92	0.010	70%	69%
				Average	81%	84%
				SD	12%	12%

TABLE H-2 (continued) Recovery from Fortified Control Soil Samples Spiked at 0.010 ppm, 0.1 ppm, and 1.0 ppm

Analytical Lab. # 38042-	Sample ID	Depth	Date Extracted	ppm Spiked	Thiabendazole % Recovery	Benzimidazole % Recovery
391.1	T1A.	00-06"	02/04/91	0.10	84%	84%
415	007.	00-06"	12/19/90	0.10	85%	82%
496	028.	00-06"	06/20/91	0.10	93%	104%
427	182.	00-06"	12/20/90	0.10	96%	84%
439	182.	12-18"	01/03/91	0.10	94%	98%
464	362.	00-06"	03/20/91	0.10	87%	88%
452	362.	00-06"	01/17/91	0.10	87%	88%
473	542.	00-06"	03/26/91	0.10	84%	88%
485	542.	00-06"	03/27/[illegible]	0.10	89%	90%
485.1	542.	00-06"	05/01/[illegible]	0.10	85%	81%
516	722.	00-06"	05/29/92	0.10	65%	70%
520	722.	00-06"	06/02/92	0.10	76%	67%
540	902.	00-06"	06/04/92	0.10	73%	80%
552	902.	00-06"	06/08/92	0.10	71%	64%
552.1	902.	00-06"	12/11/92	0.10	84%	93%
564	992.	00-06"	08/27/92	0.10	103%	89%
576	992.	00-06"	08/14/92	0.10	90%	84%
600	1082.	00-06"	08/19/92	0.10	94%	86%
				Average	86%	84%
				SD	9.5%	10%

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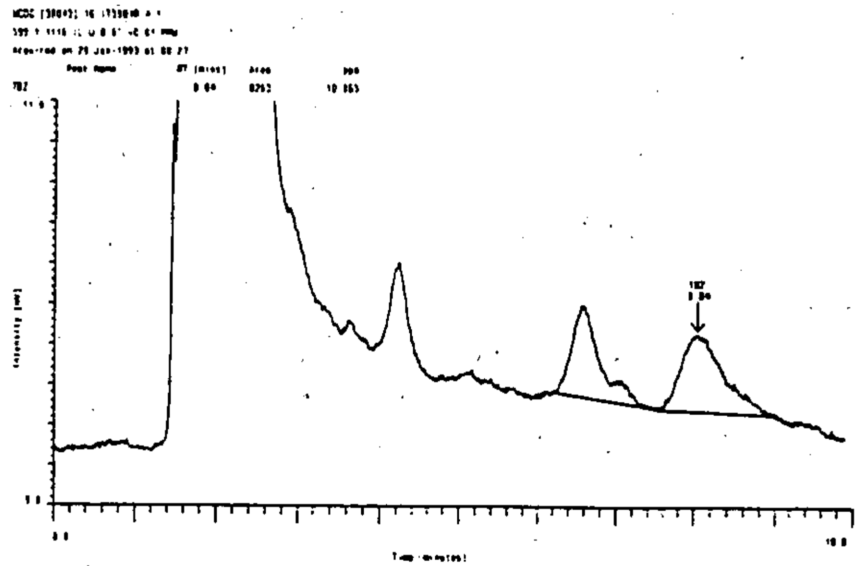
TABLE M-2 (continued) Recovery from Fortified Control Soil Samples Spiked at 0.010 ppm, 0.1 ppm, and 1.0 ppm

Analytical Lab. # 38042-	Sample ID	Depth	Date Extracted	ppm Spiked	Thiabendazole % Recovery	Benzimidazole % Recovery
392	T1A.	00-06"	11/19/90	1.0	72%	87%
380	T1A.	00-06"	11/16/90	1.0	88%	86%
403	007.	00-06"	12/31/90	1.0	93%	92%
473.1	542.	00-06"	07/17/92	1.0	0% a	68%
497	028.	00-06"	06/20/91	1.0	93%	94%
588	1082.	00-06"	08/21/92	1.0	91%	81%
				Average	87%	85%
				SD	8.8%	9.4%
				Overall Average	83%	84%
				Overall SD	11%	11%

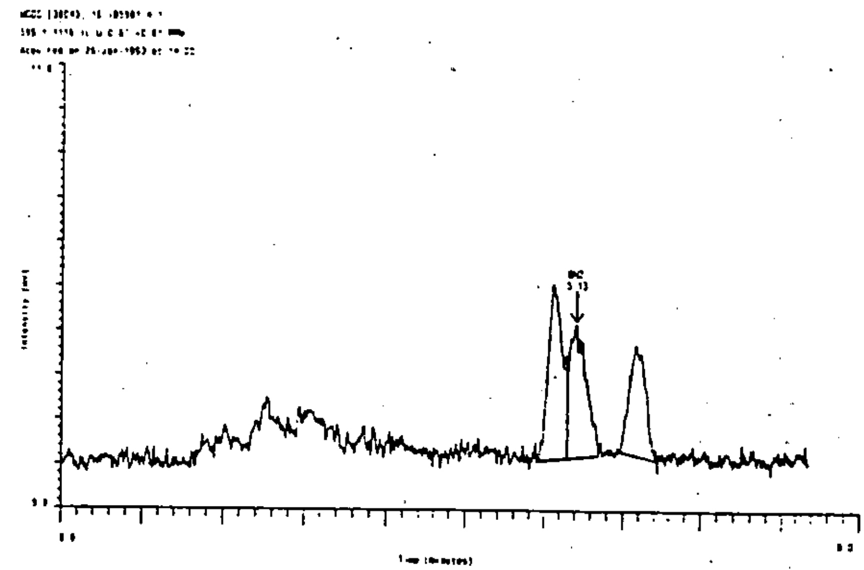
a Data not used in statistics for thiabendazole, a benzimidazole only fortification solution was used.

FIGURES

Figure 1 Representative Chromatograms — 0.01 ppm in Soil

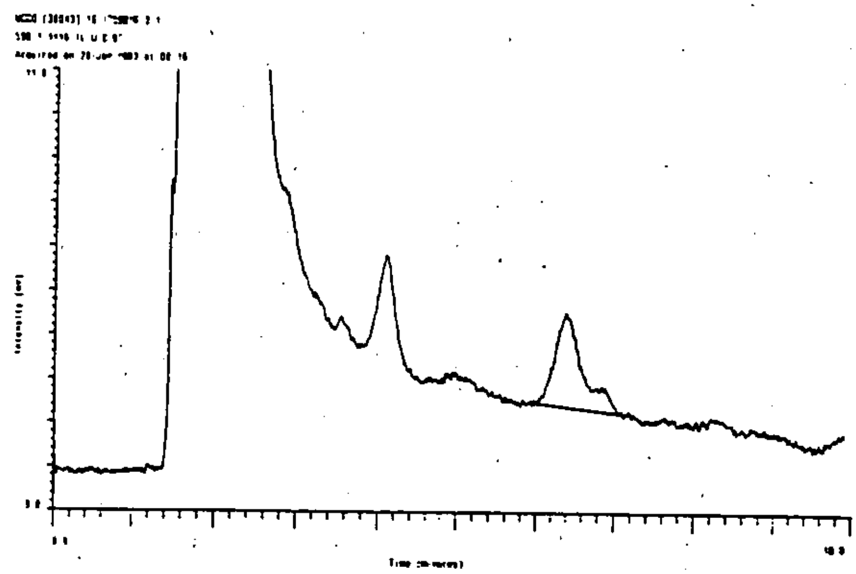


Chromatogram 1 The extract of the fortified control sample 599.1 1116.IL.U.0-6" + 0.01 ppm with the fluorometer optimized for TBZ.

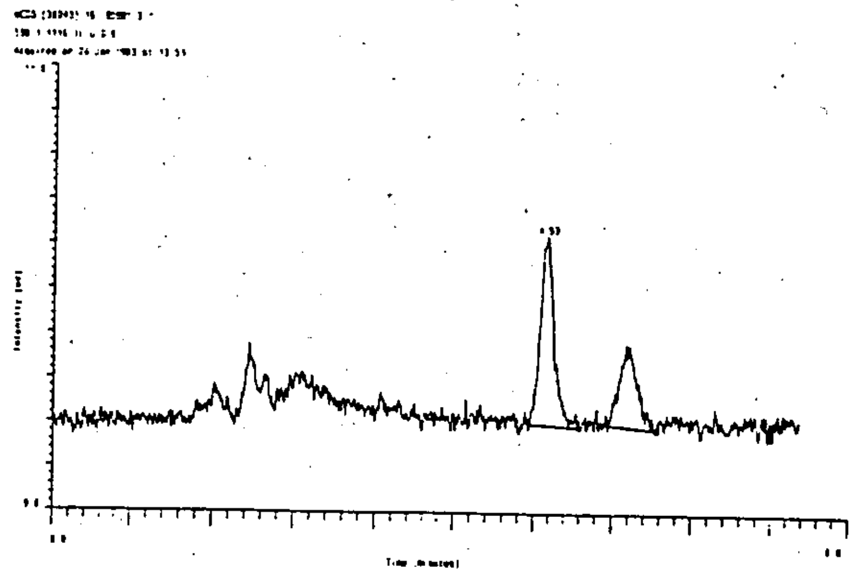


Chromatogram 2 The extract of the fortified control sample 599.1 1116.IL.U.0-6" + 0.01 ppm with the fluorometer optimized for BNZ.

Figure 2 Representative Chromatograms — Soil Control

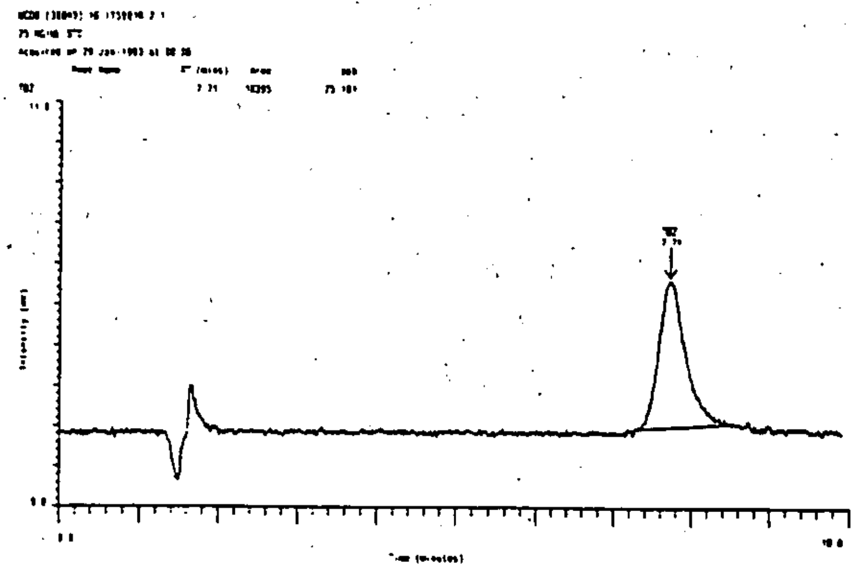


Chromatogram 3 The extract of the control sample 598.1 1116.IL.U.0-6" with the fluorometer optimized for TBZ.

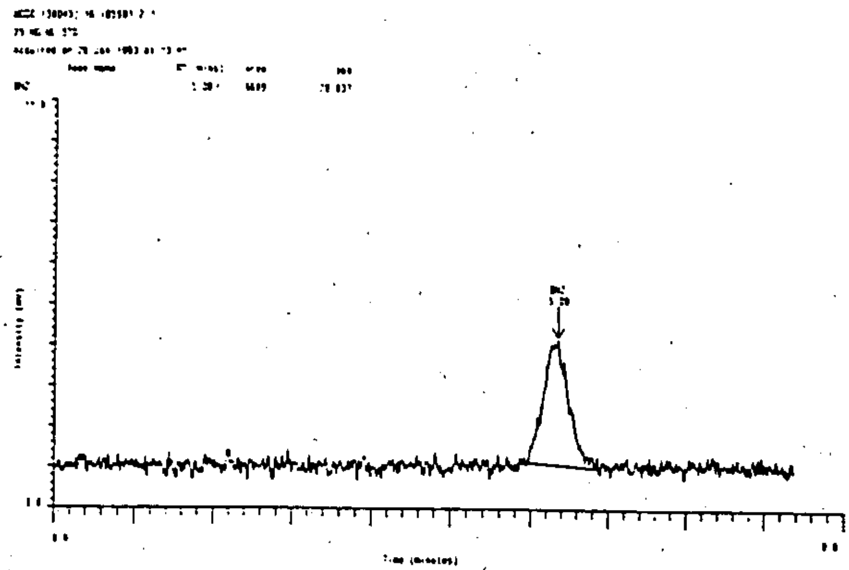


Chromatogram 4 The extract of the control sample 598.1 1116.IL.U.0-6" with the fluorometer optimized for BNZ.

Figure 3 Representative Chromatograms — 25 ng/mL Standard



Chromatogram 5 A 25 ng/ml standard with the fluorometer optimized for TBZ.



Chromatogram 6 A 25 ng/ml standard with the fluorometer optimized for BNZ.