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INTRODUCTION

To support the re-registration of terbacil (Sinbar® Herbicide), a registered product of DuPont Agricultural Products, three field residue experiments were conducted to determine the terbacil residue dissipation patterns in/on field soil. Experiments were conducted in the States of California and Delaware over a three year period and in Illinois over a two year period. Soil samples were collected on 1-day prior to application, day of application, designated Day 15, 30, 60, 90, 120, 160 (not collected from CA site), 180, 360, 540, 720, 900, and 1080 after application from both California and Delaware field sites. Soil sampling intervals from Illinois field site was conducted in the same manner as the California and Delaware field sites up to 720 days after application. Since significant levels of terbacil Metabolites A, B and C were not detected from the aerobic and anaerobic soil metabolism study, terbacil metabolites were only monitored at the day of application through Day 60 and at the last sampling interval (Day 1080) for the California site, at the day of application through Day 60 for the Delaware site and at the day of application through Day 30 for the Illinois site. Terbacil was monitored throughout the entire study.

SOIL FIELD TRIAL PROGRAM

Terbacil soil dissipation residue studies were conducted in Madera, California, Newark, Delaware and Geneseo, Illinois. Untreated control and terbacil aged soil samples were collected from the above three locations. All samples were received frozen and unmacerated at PTRL West and remained frozen until processed or sub-sampled for analysis.

STANDARD REFERENCE MATERIALS

Reference standards of terbacil and Metabolites A, B and C were provided by the E. I. du Pont de Nemours and Company, DuPont Agricultural Products, Wilmington, Delaware. Standards were stored at < 0° C until used for stock solution preparation. Stock solutions of each analyte were prepared at 1000 µg/mL in ethyl acetate. Separate dilutions of 100 µg/mL of each analyte were prepared. Then 5 µg/mL combined analyte solutions in ethyl acetate of terbacil (IN-D732), Metabolite A (IN-G2449), Metabolite B (IN-W2207) and Metabolite C (IN-T2170) were prepared for matrix spiking and standard derivatization for the analysis of terbacil and its

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three metabolites. For terbacil soil analysis, terbacil analyte solutions in ethyl acetate at 50 µg/mL, 10 µg/mL and 1 µg/mL were prepared for matrix spiking. These were stored at < 0° C in amber bottles with Teflon lined caps until used for sample fortification. Standard solutions were allowed to return to room temperature prior to use. The primary reference standard, stock and diluted standard solutions were all stored frozen.

MATRICES

Soil samples were collected inside open-ended plastic cores capped with red and black plastic caps and then packed inside canvas bags. Soil samples were shipped frozen via Federal Express or A.C.D.S. to PTRL West. The soil samples were logged in and frozen on the same day upon arrival at PTRL West. All samples were stored frozen (< 0° C).

ANALYTICAL METHOD SUMMARY

Residue analyses were conducted using the published procedures specified in the Pesticide Analytical Manual Vol. II. 180.209 with additional modifications presented in this analytical method summary report. Brief descriptions of the extraction, derivatization, clean up and chromatographic quantitation of terbacil and its metabolites are summarized below:

Reagents:

Solvents

Chloroform, Optima grade, Fisher Scientific, Santa Clara, CA

Ethyl Acetate, Optima grade, Fisher Scientific, Santa Clara, CA

Acetonitrile, Optima grade, Fisher Scientific, Santa Clara, CA

n-Hexane, Optima grade, Fisher Scientific, Santa Clara, CA

Toluene, Optima grade, Fisher Scientific, Santa Clara, CA

Methanol, Optima grade, Fisher Scientific, Santa Clara, CA

Bis (trimethylsilyl) trifluoroacetamide (BSTFA) plus 1% trimethylchlorosilane (TMCS), Pierce, Rockford, IL

Anhydrous Sodium Sulfate, Certified ACS grade, Fisher Scientific, Santa Clara, CA

NaOH, Certified ACS grade, Fisher Scientific, Santa Clara, CA

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Glassware and Miscellaneous Equipment:

Disposable culture tubes, 16 x 125 mm, CMS catalogue no. 339-317

Florisil Sep-Paks®, Waters Associates, Milford, MA, part no. 51960

Waring blender and blending cups, stainless steel or glass

1000-mL Round bottom flasks

Cotton batting

250-mL Separatory funnels

60° Long stem filtering funnels

250-mL Round bottom flasks

500-mL Round bottom flasks

10-mL Derivatizing vials, Pierce, Rockford, IL

Volumetric flasks of various sizes

Volumetric pipettes, 1-and 4-mL

Analytichem Bond Elut reservoirs and adaptors, Analytichem International, Harbor City, CA

Vacuum filtration adapter, Aldrich, Milwaukee, WI, catalogue no. Z11, 562-2

Rubber adapters

Vacuum evaporator, Buchi Model RE111, Brinkmann Instruments, Inc., Burlingame, CA, with temperature controlled waterbath

Nitrogen evaporator, Meyer Model 111, Organomation Associates, Inc., South Berlin, MA, with temperature controlled waterbath

Glasswool

Pasteur pipettes, 5" and 9"

Food Processor, Regal La Machine I, Model V813, Regalware, Kewaskum, WI

Amber bottles with Teflon lined lids

Drying oven, Fisher Isotemp® 500 Series, Fisher Scientific, Santa Clara, CA

Deionized Water Generator, Barnstead Four Module NanoPure II

Sample Processing:

Soil cores up to the depth of 0–90 cm were collected. Treated soil cores were sectioned into 0-15 cm, 15-30 cm, 30-45 cm and 45-60 cm soil depths and control soil cores were segmented into 0-15 cm, 15-30 cm and 30-60 cm soil depths. The same depth segments within each of the replicate subplots were combined into a homogeneous mixture by vigorous agitation inside a plastic bag. The remaining 60-90 cm treated soil samples and 60-90 cm control soil samples were left uncomposited. These lower soil depth soil samples were preocessed, when needed. All samples were stored frozen in labeled plastic bags until sub-sampled for analysis.

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Isolation:

1. Weigh 10 g of a representative sample into the blender cup and add 150 mL of chloroform. Blend the sample for 5 minutes.

2. Pass the chloroform extract through a cotton plugged funnel into a 1000-mL round bottom flask.

3. Extract twice more with 100-mL portions of chloroform and filter through the cotton as well. Rinse the blender cup and cap with chloroform until all particulate has been removed from the cup.

4. Add 10 mL of water to the combined extracts and evaporate the chloroform in a vacuum rotary evaporator at ~35°C.

Partitioning:

5. Transfer the residue (~5 mL of water) using several volumes of acetonitrile to a 250-mL separatory funnel. (Final volume should be less than 100 mL.)

6. Add 50 mL n-hexane and shake one minute. Allow phases to separate and centrifuge if necessary to ensure separation. Discard hexane and repeat partitioning with two additional portions of hexane.

7. Quantitatively transfer acetonitrile to a 250-mL round bottom flask and evaporate to dryness at ~35°C.

8. Dissolve all residues using several rinses of 0.1% NaOH and transfer to a 250-mL separatory funnel (final volume should be less than 80 mL).

9. Add 75 mL of ethyl acetate and shake for two minutes. Allow phases to separate. Filter the ethyl acetate through a 1 1/2 inch bed of anhydrous sodium sulfate into a 500-mL round bottom flask. Repeat the partition with ethyl acetate three more times and combine the ethyl acetate extracts.

10. Concentrate the combined extracts to 5 mL at ~35°C by roto-evaporation. (For soil analysis monitoring terbacil compound only, steps 11 through 17 and step 24 were eliminated because no derivatization was required for the terbacil compound.)

Derivatization:

11. Quantitatively transfer the concentrate from the 500-mL round bottom flask to a 10-mL derivatizing vial with additional rinses of ethyl acetate.

12. Concentrate to 1 mL under nitrogen at ~35°C.

13. Add 300 µL of BSTFA + 1% TMCS to the derivatizing vial, cap with a Teflon lined lid and shake vigorously for approximately 20 seconds. Allow the derivatization to take place overnight or at least 16 hours at room temperature.

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14. Simultaneously with sample derivatization, prepare a 20 µg/mL standard of all analytes by pipetting 4 mL of the 5 µg/mL standard solutions used for fortification into a 10-mL derivatization vial. Concentrate to 1 mL under nitrogen and derivatize with samples.

Final Sample Clean-up:

15. Prepare a sodium sulfate mini-column for each sample and standard by plugging the bottom of a 5-inch Pasteur pipette with a small amount of glass wool and filling with anhydrous sodium sulfate until the pipette is approximately 1/3 full. Place the mini-columns into 16 x 125 mm disposable culture tubes.

16. After at least 16 hours derivatization, add 1 mL of deionized water and shake 20 seconds. Allow phases to separate. Do not add water to the standards until after completion of the final sample clean-up on the samples to ensure standard stability.

17. Remove the upper phase from the derivatizing vial by Pasteur pipette and place the extract on the top of the sodium sulfate mini-column. Rinse the column twice with 2 mL of ethyl acetate and collect in the culture tube.

18. Concentrate the solution to dryness under nitrogen at ~35°C and resuspend in 10 mL of ethyl acetate/hexane (20:80). Dissolve all the particulate into the solution using a Pasteur pipette.

19. Attach an Analytichem reservoir to a Waters 900-mg Florisil Sep-Pak, on to a vacuum adaptor joined to a 250-mL round bottom flask and attach to a light vacuum.

20. Pre-rinse the Florisil Sep-Pak with 5 mL of 20:80 ethyl acetate/hexane, then add the 10 mL of sample to the reservoir. Allow the sample to be pulled through the Florisil cartridge at approximately 10 mL/min.

21. Rinse the sample tube with 5 mL of 20:80 ethyl acetate/hexane and transfer it to the reservoir. Rinse the culture tube with 5 mL of 10:5:85 methanol/ethyl acetate/toluene and pull it through the cartridge as well, collecting all fractions into the 250-mL round bottom flask.

22. Concentrate the extract to dryness by roto-evaporation at ~35°C and resuspend in 3 mL of ethyl acetate.

23. Transfer the 3 mL of extract to a clean 10-mL centrifuge tube and rinse the flask with several small portions of ethyl acetate. Transfer the rinses to the centrifuge tube as well.

24. Add water to the standard and dry using the mini sodium sulfate column as described in #17 above. A 10-mL centrifuge tube is used for collecting standards instead of a culture tube.

25. Concentrate both samples and standards to 1 mL under nitrogen at ~35°C. (For analysis monitoring terbacil compound only, samples were diluted to 10 mL with the exceptions of the 1.0 ppm and 5.0 ppm fortified soil samples which were diluted to 40 mL. To determine the background for the 40 mL diluted fortified samples, the associated control was also diluted to 40 fold.)

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26. Transfer all samples and standards to GC vials and prepare the following standard dilutions by means of a 1000-µL syringe.

Terbacil and Metabolites A, B and C Analysis:

10 µg/mL standard= 500 µL of 20 µg/mL standard + 500 µL of ethyl acetate

5 µg/mL standard= 500 µL of 10 µg/mL standard + 500 µL of ethyl acetate

2 µg/mL standard= 400 µL of 5 µg/mL standard + 600 µL of ethyl acetate

1 µg/mL standard= 500 µL of 2 µg/mL standard + 500 µL of ethyl acetate

0.5 µg/mL standard= 500 µL of 1 µg/mL standard + 500 µL of ethyl acetate

Terbacil Analysis Only:

1 mL aliquot of the 1 µg/mL terbacil reference standard was pippeted into a GC vial and the following serial dilutions were performed. Normally, two sets of the calibration standards were prepared to cover a sample set.

0.5 µg/mL standard= 500 µL of 1 µg/mL standard + 500 µL of ethyl acetate

0.25 µg/mL standard= 500 µL of 0.5 µg/mL standard + 500 µL of ethyl acetate

0.125 µg/mL standard= 500 µL of 0.25 µg/mL standard + 500 µL of ethyl acetate

27. Generally, inject samples on the GC in the following order for sets monitoring terbacil and its three metabolites: 10 ppm standard, sample, sample, 5 ppm standard, sample, sample, 2 ppm standard, etc. Derivatized samples appear to be stable for at least 18 hours at room temperature.

Generally, inject samples on the GC in the following order for terbacil analysis only: 0.125 ppm standard, sample, sample, 0.25 ppm standard, sample, sample, 0.5 ppm standard, sample, sample, 1 ppm standard, sample, sample, and then repeat the sequence again.

Chromatography:

Instrumentation:	5890	Hewlett Packard Gas Chromatograph equipped with Electron Capture Detector, Hewlett Packard Company, Wilmington, DE
	3396A	Hewlett Packard Integrator, Hewlett Pac.. rd Company, Wilmington, DE
	7673A	Hewlett Packard Autosampler, Hewlett Packard Company, Wilmington, DE
Column:	HP-5 fused silica column (5% phenyl methyl silicone), 10 m x 0.53 mm id, 2.65 µm film thickness, Hewlett-Packard, Wilmington, DE	
Injector Temperature:	200°C	

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Detector Temperature: 300°C

Oven Temperature:

Initial Temperature	155°C for 16 min.
Initial Ramp	155°C to 200°C at 10°C/min.
Ramp A	200°C to 280°C at 50°C/min.
Final Hold	280°C for 1 min.

Gases: Carrier Gas= Helium at ≈12.5 mL/min.
Makeup Gas= 5% Argon/Methane at ≈29.5 mL/min.

Injection Volume: 1 µL

Representative chromatograms and terbacil calibration curve are shown in Appendix B.

% Moisture Determination for Soil Samples:

Approximately 10 g of each soil sample was placed into an aluminum weighing dish and dried in the oven overnight at approximately 100°C for % moisture determination. The weight of the soil sample before and after oven drying was recorded.

Recovery:

Method recovery was performed with control samples fortified with terbacil at 0.1 ppm (µg/g), 1.0 ppm and 5.0 ppm for each sample set. The diluted standards were fortified on the matrices prior to the initial extraction step. Average and individual recoveries are shown in Table I.

Metabolites A, B and C were monitored during the beginning of this soil dissipation study. Concurrent recoveries were performed with untreated soil samples fortified with Metabolites A, B and C at 0.1 ppm (µg/g), 1.0 ppm and 5.0 ppm. Recovery results are presented in Appendix A: Section 4.

Quantitation:

A calibration curve was generated for each analyte with each sample set from the co-injected standards. The equation of the line based on the peak area of the standard versus concentration injected in nanograms was generated by least squares linear regression calculated by the computer program, Cricketgraph™, version 1.2, MacWarehouse, South Norwalk, CT. The correlation coefficient (r^2) calculated for each set of standards could not be less than 0.95 for

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the data to be considered acceptable. The integrated peak area was plotted versus concentration and fitted by linear regression to the formula y=mx + b where y is peak area and x is concentration injected in nanograms. The dry gross residue concentration of the soil sample was then determined from the y=mx + b formula in nanograms, divided by the volume injected in µL, multiplied by the final volume in mL, multiplied by the % moisture factor and divided by sample weight (10 g).

The equation for the calculation of dry gross residues in soil samples was derived from the % moisture factor. Both the % moisture factor and dry gross residue determination equations are shown below:

$$\%\ \text{Moisture of Soil} = \frac{(\text{Gross Wet Soil Weight-Container Weight})-(\text{Gross Dry Soil Weight-Container Weight})}{(\text{Gross Wet Soil Weight-Container Weight})} \times 100\%$$

$$\%\ \text{Moisture Factor} = \frac{100}{100 - \%\ \text{Moisture of Soil}}$$

The % moisture of soil did not apply to the fortified samples, because the ppm (µg/g) of analyte fortified was based on the weight of moist soil and the purpose of the fortified samples were to determine the method concurrent recoveries on moist soil samples.

Linear regression formula from calibration curve $y=mx + b$

$$\text{Concentration of dry gross residues in soil (ppm)} = \frac{\text{Integrated Peak Area of analyte (y)} - \text{Calibration Intercept (b)}}{\text{Calibration Slope (m)}} \times \frac{\text{Final Volume (mL)}}{\text{Sample Weight (10 g)}} \times \frac{\%\ \text{Moisture Factor}}{\text{Volume Injected (1 µL)}}$$

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The peak areas for fortified samples (y) were corrected for background by subtracting the peak response of the control sample.

% Recovery is determined by:

$$\% \text{ Recovery} = \frac{\text{Concentration in Fortified Matrix } (\mu g/g)}{\text{Concentration Fortified } (\mu g/g)} \times 100\%$$

Spreadsheets showing the concentrations of terbacil analyte in soil and the associated concurrent recoveries are displayed in Appendix A: Sections 1 through 3.