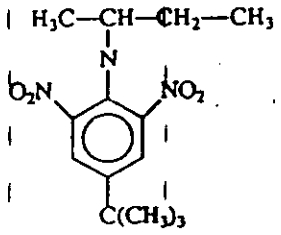
SUMMARY

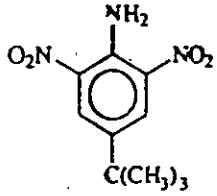
An extraction procedure was developed to extract and quantitate residues of butralin (N-*sec*-butyl-4-*tert*-butyl-2,6-dinitroaniline) and its metabolite, 4-*tert*-butyl-2,6-dinitroaniline in soil. The compounds of interest are extracted from the soil by shaking with dichloromethane. After shaking, the extract is filtered, washed with water and dried by pouring through anhydrous sodium sulfate. The dried extract is taken to about 1-5 mL by rotary evaporation and then to dryness by nitrogen evaporation. The sample is reconstituted in hexane containing 10% ethyl acetate, and then analyzed by gas chromatography with electron capture detection. The amount of compound in the extract is quantitated by comparison to a standard curve that is generated by concurrent analysis of standards ranging from 0.0 to 0.5 µg/mL. The method provides for analysis of samples containing residues of butralin and 4-*tert*-butyl-2,6-dinitroaniline in the range of 0.01 to 0.5 ppm.

INTRODUCTION

Butralin (N-*sec*-butyl-4-*tert*-butyl-2,6-dinitroaniline) is a plant growth regulator registered for use on tobacco around the world and is being registered for use on tobacco in the United States. In addition to butralin, the major metabolite found in soil after application is 4-*tert*-butyl-2,6-dinitroaniline. The structures of the parent and the metabolite of interest are given below:



Butralin



4-*Tert*-butyl-2,6-dinitroaniline

The development and validation of an analytical method for the determination of butralin and its metabolite were conducted to provide quantitation of the subject compound and its metabolites in support of terrestrial field dissipation studies conducted to meet EPA re-registration guideline requirements for products containing butralin as the active ingredient. The method reported herein follows the requirements for Subdivision N, Section 164-1, Terrestrial Field Dissipation.

This study was initiated on July 12, 1993.

This study was completed on November 30, 1994.

No statistical analyses of the data other than mean and standard deviation were performed.

MATERIALS AND METHODS

Apparatus

Balance - Brainweight B300.

Centrifuge - IEC Centra 7.

Vacuum Pump - Model # SA55JXGTD-4144 - Curtin Matheson Scientific.

Nitrogen Evaporator (N-Evap) - Meyer Organomation Model 112.

Vortex-Genie - Catalog No. 12-312 - Fisher Scientific.

Wrist-Action Shaker - Burrell Model 75.

Rotary Evaporator - Buchi RE111 - 461 Water Bath - Brinkman.

Glassware

Graduated Cylinders - 100-mL.

Glass Funnels

Separatory Funnels - 500-mL.

Erlenmeyer Flasks - 250-mL.

Volumetric Pipets - 50-mL

Boiling Flasks - 500-mL.

Pasteur Pipets - Flint Glass.

Gas Chromatograph with an electron capture detector - HP model 5890 with a 7673A autosampler.

GC Column: 30 m, 0.32 mm i.d., 1.0 µm film, DB1701, J&W - Curtin Matheson Scientific.

Reagents and Solvents

Dichloromethane (DCM) - Catalog No. D150-4 - Fisher Scientific.

Hexane - Catalog No. H302-4 - Fisher Scientific.

Ethyl Acetate - Catalog No. E195-4 - Fisher Scientific.

Deionized Ultra-Filtered (DIUF) Water - Catalog No. W2-20 - Fisher Scientific.

Filter Paper - Whatman #4 - Fisher Scientific.

Sodium Sulfate, Anhydrous - Catalog No. 832-140 - Fisher Scientific.

Glass Wool.

Compressed Gases

Helium - zero grade - Central Welding Supplies.

Nitrogen - regular grade - Central Welding Supplies.

Nitrogen - zero grade - Central Welding Supplies.

Standards

Butralin - Lot No. G9-1437, >98% purity, PTRL No. 522-12, Received: 4/4/91

4-*Tert*-butyl-2,6-dinitroaniline - Lot No. R-380-055, 99.7% purity, PTRL No. 522-13, Received 7/29/91.

See Appendix C for certificates of analyses for reference standards.

Solution

10% Ethyl Acetate in Hexane - In a graduated cylinder, measure 2700 mL hexane and pour into a 4L bottle. Add 300 mL ethyl acetate. Add stir bar and stir well.

Soil Extraction Method

1. Weigh a 50 gram soil sample into a 250-mL Erlenmeyer flask with a ground glass top.
2. Add 100 mL dichloromethane (DCM) to the flask, insert the ground glass stopper and place on wrist-action shaker. Shake the sample at moderate speed for 30 minutes.
3. Prepare a glass funnel by plugging the spout with glass wool and place the funnel into a 500-mL separatory funnel.
4. Decant the extract through the funnel into the separatory funnel.
5. Repeat steps 2 and 4, this time adding the soil into the glass funnel.
6. Rinse the erlenmeyer with 25 mL DCM and pour through the soil in the funnel. Discard soil in the funnel.

7. Add 50 mL DIUF water to the separatory funnel and shake vigorously for 1 minute and allow the phases to separate. Filter the DCM layer through a glass funnel containing 2.5 cm of anhydrous sodium sulfate supported by a glass wool plug, into a 500-mL boiling flask.

8. Add 50 mL DCM to the separatory funnel containing the water. Shake for 1 minute and allow the phases to separate. Filter the DCM layer through the same glass funnel containing sodium sulfate used in step 7.

9. Concentrate the extract to 1-5 mL by rotary evaporation at 30-35°C.

10. Further concentrate the extract to just dryness with a gentle stream of nitrogen.

11. Reconstitute the residue by adding 50 mL of 10% ethyl acetate in hexane, capping the flask and swirling to dissolve the residue.

12. Remove a portion of the extract by pasteur pipet and place into an autosample vial for GC analysis.

A flow chart of the extraction procedure is shown in Figure 1.

Analysis by GC/ECD

Instrumentation and operating conditions for the analysis of soil for butralin and 4-*tert*-butyl-2,6-dinitroaniline are as follows:

Instrument: Hewlett Packard 5890 Series II with a 7673A autosampler

Column: J&W DB 1701

Length: 30 m

I.D.: 0.32 mm

Film Thickness: 0.25 µm

Oven Temperature: 150°C hold for 5 minutes
Ramp to 160°C at 20°C/minute
Hold at 160°C for 20 minutes
Ramp to 280°C at 20°C/minute
Hold at 280°C for 2 minutes

Detector: Electron Capture
Temperature: 300°C
Make-up Gas: Nitrogen (total flow = 60 mL/min)

Inlet: Splitless

Injector Temperature: 230°C

Carrier Gas: Helium @ 2.0 mL/min.

Injection Volume: 4 µL

Retention Times: Butralin: approximately 28 minutes
4-*tert*-butyl-2,6-dinitroaniline: approximately 23 minutes

Fortification of Samples

Prepare individual 1.0 mg/mL stock solutions of butralin and 4-*tert*-butyl-2,6-dinitroaniline by accurately weighing each reference standard material into an appropriate vial. Adjust actual weight of the compound for purity (see Calculations) to determine the volume of hexane to be added.

Prepare mixed working fortification solutions at the following levels in hexane for fortification of samples:

ppm level	Concentration of Fortification Solution (µg/mL)
0.01	0.50
0.05	2.50
0.10	5.00
0.50	25.00

To prepare fortified soil samples, add 1.0 mL of the appropriate fortification solution to 50 grams of sample.

Preparation of GC Standard Solutions

Prepare individual 1.0 mg/mL stock solutions of butralin and 4-*tert*-butyl-2,6-dinitroaniline by accurately weighing each reference standard material into an appropriate vial. Adjust actual weight of the compound for purity (see Calculations) to determine the volume of 10% ethyl acetate in hexane to be added.

Prepare mixed working solutions at the following levels in 10% ethyl acetate in hexane for GC standards:

Concentration of Standard Solution (µg/mL)
0
0.01
0.02
0.04
0.10
0.20
0.50

The ab ·/e solutions are analyzed concurrently with samples for quantitation purposes.

CALCULATIONS

Standard or Fortification Solution Preparation:

$$\text{Volume Solvent (mL)} = \frac{(W)\,(P)}{(FC)}$$

Where: W = Milligrams of Neat Standard
P = Purity (decimal form)
FC = Final Concentration (mg/mL)

Calculation of Percent Recovery

Perform linear regression on the plot of standard concentration versus peak area for the GC standard solutions analyzed concurrently with the fortified samples. Determine the intercept and slope, and use the following equation:

$$((PA\text{-}C)/S) \times \frac{FV}{SW}) = \text{Sample Concentration in ppm}$$

PA = Peak Area of Sample
C = Intercept (Constant)
S = Slope (x Coefficient)
FV = Final Volume (50 mL)
SW = Sample Weight (grams)

$$\text{Percent Recovery} = \frac{\text{ppm determined - ppm in control}}{\text{ppm fortified}} \times 100\%$$

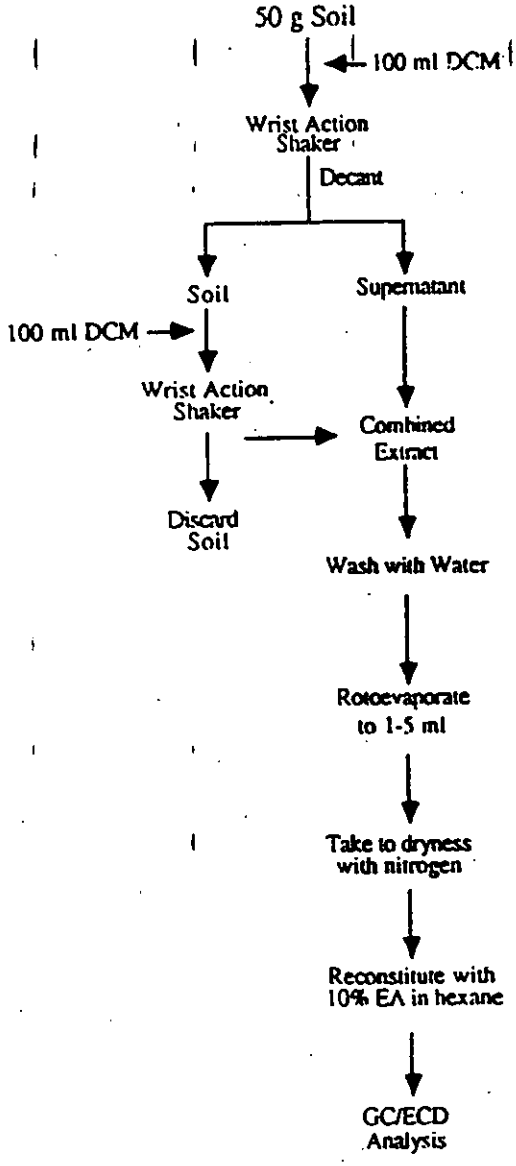


Figure 1. Analysis Scheme for Extraction of Soil.