

ENVIRO-BIO-TECH, LTD.

R.D. #1 BERNVILLE, PA 19506

(215) 488-7664

SOP EBT-208.01

Date: 7-13-88

Replaces: 4-22-87

**STANDARD OPERATING PROCEDURE FOR
THE DETERMINATION OF BROMACIL, TERBACIL AND METABOLITE
RESIDUES IN CROPS AND SOIL**

1.0 PRINCIPLE

Residues of bromacil herbicide (5-bromo-3-*sec*-butyl-6-methyluracil), terbacil herbicide (3-*tert*-butyl-5-chloro-6-methyluracil) and three metabolites in plant tissues and soil are determined by initial extraction with chloroform, cleanup by liquid/liquid partitioning steps, and measurement by halogen-sensitive Hall 700A gas chromatography after formation of silyl derivatives of the metabolites. Method sensitivity is 0.05 ppm for all four compounds relative to a 25-g sample.

2.0 REAGENTS AND APPARATUS

- 2.1 Solvents - All solvents (chloroform, hexane, acetonitrile, ethyl acetate, methanol, and toluene) are HPLC grade from Fisher Scientific Co.
- 2.2 Water - Cloister distilled water (Ephrata, PA) passed through a Barnstead NANOpure water filtering system and collected at 16-18 megohm-cm.
- 2.3 Celite® 545 (J. T. Baker Cat. No. 3371-05).
- 2.4 Sodium Hydroxide (Fisher Scientific Cat. No. S-318).
 - 2.4.1 0.1% NaOH solution - Mix 1.0 g sodium hydroxide in 1000 ml water.
- 2.5 Sodium Sulfate, anhydrous (Mallinckrodt Cat. No. 8624).

The sodium sulfate must be pretreated before using it. 450 grams of sodium sulfate is placed in a beaker and at least 300 ml of methanol added. The solution is mixed well and filtered through a Buchner funnel. The sodium sulfate is returned to the beaker and slurried a second time with another 300 ml of methanol. This slurry is then filtered through the Buchner funnel. The methanol is discarded and the sodium sulfate is dried by drawing air through the Buchner funnel to remove all traces of methanol. The sodium sulfate is then placed in an oven and heated at 135-140°C for 12 hours. Store in a closed container.

- 2.6 Derivatizing Reagent - BSTFA with 1% TMCS (Pierce).
- 2.7 Tracor 565 gas chromatograph with Hall 700A detector in halogen mode for

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terbacil, or Tracor 560 gas chromatograph with Tracor 702 N-P detector for bromacil.

- 2.8 GC column - 6' x 2 mm I. D. Pyrex with 5% XE-60 / 0.2% EPON 1001 on 80-100 mesh Gas Chrom Q for terbacil, or 2' x 2mm I.D. Pyrex with 10% SP-2250 on 100-120 Supelcoport for bromacil.

3.0 PROCEDURE**3.1 Preparation of Standard Solution**

- 3.1.1 Stock Solution (500 µg/ml) - Weigh 50 mg of each standard and transfer to a 100 ml volumetric flask with toluene. Dissolve and bring to volume with toluene.
- 3.1.2 Working Standard - Pipette 0.25 ml of each stock solution into a 100 ml volumetric flask and dilute to volume with toluene to give 1.25 µg/ml each. The working standard is used for recovery studies by adding standard solution by pipette to the appropriate amount of control sample. It is also used for the GC standard curves.

3.2 Extraction

- 3.2.1 Weigh 25 g of sample into a blender cup and add enough chloroform for blending (just under top dent in the cup). Blend for 10 minutes.
- 3.2.2 Filter through a porcelain funnel with Whatman #5 filter paper and Celite (≈ 50 ml) into a 1000 ml filtering flask.
- 3.2.3 Scrape filter cake of substrate and repeat blending and filtering twice. Rinse filter cake after third blend with 50 ml chloroform.
- 3.2.4 Transfer to 1000 ml round-bottomed flask and add 10 ml water. Evaporate off chloroform at 60°C on Roto-vap.
- 3.2.5 Transfer residue (≈ 5 ml H₂O) using several rinses of acetonitrile to a 250 ml separatory funnel (final volume not to exceed 100 ml). Add 50 ml of hexane and shake for 1 minute. Discard hexane. Repeat clean up twice more.
- 3.2.6 Quantitatively transfer acetonitrile to a 500 ml round-bottom flask.

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Evaporate to dryness on Roto-Vap at 60°C.

- 3.2.7 Transfer to 250 ml separatory funnel with 3 x 25 ml rinses of 0.1 % NaOH. Final volume should be less than 80 ml.
- 3.2.8 Add 75 ml ethyl acetate and shake for 2 minutes. After phase separation, drain the ethyl acetate through anhydrous Na₂SO₄ (about 50 ml) in a funnel with glass wool stopper into a 500 ml round-bottomed flask. Repeat extraction 3 more times. Rinse funnel with 25 ml ethyl acetate.
- 3.2.9 Evaporate to about 5 ml on Roto-Vap at 60 °C and transfer to screw-cap graduated centrifuge tube (10 or 15 ml) with ethyl acetate.
- 3.2.10 Evaporate to dryness on N-evap and bring sample up to 1 ml with toluene.
- 3.2.11 Add 150 ml of derivatizing reagent, cap sample and let sit overnight at room temperature (about 15 hours). Derivatization is not necessary when analyzing for bromacil or terbacil parent compound.
- 3.2.12 Just before GC analysis take sample to dryness on N-evap and bring to volume again with toluene.

3.3 Analysis

- 3.3.1 Inject 4 µl volume of sample onto GC column.
- 3.3.2 The standard curve is obtained by injecting known quantities of standard in toluene. 1 ml of standard solution is derivatized according to 3.2.11 and 3.2.12.
- 3.3.3 Recoveries are obtained by adding known amounts of working standard to the appropriate amount of control sample and running through the above procedure.
- 3.3.4 Calculate PPM of each compound present in an unknown sample as follows:
- $$\text{ppm} = \frac{\text{ng (Bromacil, Terbacil, A, B, or C) from STD curve} \times \text{final volume in ml}}{\text{grams of sample} \times \text{volume injected in } \mu\text{l} \times \text{Recovery factor}}$$