

\*\*\* AGRISEARCH METHOD \*\*\*

Gas Chromatographic Determination of CGA-24705 (Metolachlor)  
And Its Metabolites In Soil.

1.0 SCOPE

This method is used for the determination of residues of metolachlor, and three metabolites; CGA-51202, CGA-40172, and CGA-40919 in soil (see Figure 1 for structures).

The limit of detection for the method is 0.05 ppm for each chemical.

2.0 PRINCIPLE

Frozen samples are thawed and homogenized. A 5 g aliquot of each sample is dried to determine the percent moisture content. A fifty-gram aliquot of each sample is extracted by refluxing with methanol/glass distilled deionized water (1:1 v:v) for 1 hour. An aliquot of the supernatant is conditioned with sodium chloride saturated distilled water adjusted to pH2 and partitioned with hexane/ethyl acetate (1:1 v:v). The hexane/ethyl acetate fraction is dehydrated with sodium sulfate. Diazomethane is added to the sample to methylate the metabolite CGA-51202. The methylated sample is then flash evaporated, brought to volume in hexane and analyzed by capillary gas chromatography. A flow diagram of the procedure is presented in FIGURE 2.

3.0 REAGENTS

- 3.1 Ethyl Alcohol (AR, denatured), Cat. 7013-4, Mallinckrodt, 95% in glass-distilled deionized water.
- 3.2 Ethyl Ether (product 107; UV cutoff at 208 nm), American Burdick & Jackson.
- 3.3 Ethyl Acetate (product 100, UV cutoff at 252 nm), American Burdick & Jackson.
- 3.4 Hexane, non-spectro (product 230) American Burdick & Jackson.
- 3.5 Methanol (product 230, UV cutoff at 202 nm), American Burdick & Jackson.
- 3.6 Glass distilled deionized water (water).

1

AGRISEARCH 1265-C -116

- 3.7 Sulfuric Acid (AR, analytical reagent), Cat. 2468, Mallinckrodt.
- 3.8 Sodium Chloride (ACS, crystalline), Cat. 7581-12, Mallinckrodt.
- 3.9 Sodium Sulfate, granular (12-60 mesh), suitable for pesticide analysis, J. T. Baker Chemical Co.
- 3.10 Standard Metolachlor CGA-24705 (See FIGURE 1).
- 3.11 Standard CGA-40172 (See FIGURE 1).
- 3.12 Standard CGA-40919 (See FIGURE 1).
- 3.13 Standard CGA-51202 (See FIGURE 1).
- 3.14 Diazomethane Solution (See S.O.P. 13.A.4.0).

#### 4.0 APPARATUS

- 4.1 Oven, Lab-Line 34CO, Imperial III Radiant Heat; 104°C.
- 4.2 Weighing boats, 6.0 cm diameter aluminum.
- 4.3 Top loading balance, American Scientific Products TL 1600s, 0.01 g sensitivity.
- 4.4 Analytical balance, American Scientific Products, S/P 180, 0.1 mg sensitivity.
- 4.5 Flasks, round bottom boiling, 500 ml, 1 liter.
- 4.6 Flasks, volumetric, 5 ml, 10 ml, 50 ml.
- 4.7 Refluxing condensers, water-cooled.
- 4.8 Tygon tubing (R-3603), ID 5/16".
- 4.9 Powder funnels, glass, 100 mm diameter.
- 4.10 Glass wool, Pyrex, 8 micron sliver.
- 4.11 Hamilton Gastight syringes; 10 ul, 100 ul, and 500 ul volumes.
- 4.12 Beakers, glass, 25 ml, 250 ml.
- 4.13 Funnel, separatory, 500 ml, with teflon stopcocks and stoppers.

2  
AGRISEARCH 1265-C - 117

- 4.14 Rotary evaporators with 45°C water bath.
- 4.15 Meter, pH, Beckman Expandomatic with sensorex combination electrode.
- 4.16 Pipettes, glass, disposable, 1 ml, 10 ml.
- 4.17 Pipettes, Pasteur, 2 ml.
- 4.18 Gas chromatograph, Shimadzu Model GC-9A equipped with a flame thermionic detector (FTD-9), using rubidium silicate as the alkali metal salt (nitrogen sensitive).
- 4.19 Column, capillary-type, 0.75 mm x 60 meter glass coil, coated with OV-17; thickness 0.20 microns.
- 4.20 Integrator (Shimadzu - CR4A).

5.0 GAS CHROMATOGRAPHY OPERATION

Temperatures:

Injector: 240°C  
 Column: 200°C

Detector:

Range: 0  
 Attenuation: 5

Gas Flows:

Column Gas: 10 ml/min Helium  
 Reaction gases: 5 ml/min Hydrogen  
 150 ml/min Air

Other Conditions:

Chart speed: 5 mm/min  
 Injection volume: 4 ul  
 Injection solvent: Hexane  
 Retention times (min):

Metolachlor	CGA-24705	9.2 min
	CGA-51202	8.8 min
	CGA-40172	7.9 min
	CGA-40919	7.2 min

## 6.0 PROCEDURE

### 6.1 Soil Preparation

- 6.1.1 Soil samples are removed from the freezer and registered on the sample tracking sheet.
- 6.1.2 The soil samples are allowed to thaw to a workable condition.
- 6.1.3 Each sample is thoroughly homogenized.
- 6.1.4 A 5 gram aliquot is removed, and the percent moisture is determined by weighing, drying the soil in an oven (104±2°C) for 24 hours and reweighing. (See S.O.P. 12.F.2.0).
- 6.1.5 A 50 gram aliquot (WET WEIGHT) is removed from each sample bag and placed in a 1000 ml round bottom flask.

### 6.2 Recovery Sample Fortification

- 6.2.1 Analytical Standards (Metolachlor and each metabolite) to be obtained from CIBA-GEIGY Corporation.
  - 6.2.1.1 A primary standard of each compound is made at a concentration of 5.0 ug/ul (5000 ug/ml).
  - 6.2.1.2 A 5 ml spiking solution (500 ug/ml) is prepared fresh for use each week. This solution is prepared by combining 500 ul of the primary standard for each compound and bringing the spike solution to a final volume of 5 ml.
  - 6.2.1.3 Appropriate control soil samples are fortified with the spiking solution as follows:
    - a. 0.05 ppm spike - use 5 ul of the 500 ug/ml solution.
    - b. 0.1 ppm spike - use 10 ul of the 500 ug/ml solution.
    - c. 1.0 ppm spike - use 100 ul of the 500 ug/ml solution.

AGRISEARCH 1265-C - 119

6.2.2 Control samples are fortified with the appropriate spiking solution (6.2.1.1) prior to extraction, but after sample homogenization. These samples are carried through the entire analytical procedure as a test of method efficiency.

### 6.3 Soil Extraction

6.3.1 Control samples, fortified samples, and test samples are run through the procedure as a set with at least two fortified samples per set of 10 test samples.

6.3.2 All samples are extracted by reflux with 150 ml of 1:1 methanol/water for 1 hour.

6.3.3 The mixture is allowed to cool to room temperature. A 60 ml aliquot of the supernatant (equivalent to 20 g of soil) is removed and placed in a 500 ml separatory funnel.

### 6.4 Partition

6.4.1 Water (100 ml) and 10 ml of saturated sodium chloride solution are added to the separatory funnel containing the 60 ml aliquot of the soil extract. The solution is adjusted to pH2 using 1N  $H_2SO_4$  to aid in the partition of CGA-51202 and CGA-40172 from the aqueous.

6.4.2 The aqueous supernatant is partitioned with 50 ml of a mixture of hexane and ethyl acetate (1:1 v:v). The organic layer is drained into a 250 ml glass beaker. The procedure is repeated two additional times, combining the organic phases.

6.4.3 Approximately 10 g of anhydrous  $Na_2SO_4$  is added to the combined organic phases and the mixture is swirled for 30 seconds.

6.4.4 The  $Na_2SO_4$  is allowed to settle and the organic solvent is carefully decanted into a round bottom flask. The  $Na_2SO_4$  is washed with 20 ml of hexane/ethyl acetate (1:1) and the solvent is decanted into the round bottom flask. This is repeated an additional time.

## 6.5 Methylation

- 6.5.1 The dried hexane/ethyl acetate extract is methylated for a minimum of 30 minutes at room temperature by adding 10 ml of diazomethane solution (S.O.P. 13.A.4.0).
- 6.5.2 The methylated extract is evaporated just to dryness by rotary evaporation at 45°C.
- 6.5.3 The extract is redissolved in 5 ml of hexane for GC analysis.

## 6.6 GC QUANTIFICATION

- 6.6.1 The sample in step 6.5.3 is analyzed by capillary gas chromatography with a GC system using an FTD detector. See Sections 4 and 5 for GC apparatus and operating conditions.
- 6.6.2 The gas chromatographic system should be calibrated with each analytical run. Fresh standard solutions are removed from cold storage and warmed to room temperature prior to each use.
- 6.6.3 Using the spiking solution (500 ug/ml) prepared in 6.2.1.2, make serial dilutions in hexane to obtain GC standards with a range of 0.10 to 10.0 nanograms per ul.
- 6.6.4 Standardize the gas chromatograph by injecting 4 ul aliquots of the diluted GC standards (Section 6.5.3). This represents a range of 0.40 to 40.0 nanograms (FIGURE 1).
- 6.6.5 Control, fortified, and test sample extracts (6.5.3) are positioned in the autosampler tray with analytical standards (6.6.3) so that an analytical standard is measured at least every fifth analysis.

6.6.6 Each analysis is quantitated on the computing integrator, where the peaks for metolachlor and the three metabolites are integrated by area count and compared to the analytical standards analyzed during the run (See Section 6.6.4 and FIGURE 4). The CR4A Integrator is equipped to provide an integration of a nonlinear standard curve and to store the run for reanalysis. All standards quantitated during the run are recalled from storage and used to construct the standard detection curve. Each control, fortified, and test sample is compared to the standard curve for final quantitation in nanograms (ng). See FIGURE 5 for details of the Shimadzu CR4A analysis file.

#### 6.7 CALCULATIONS

- 6.7.1 The ng value of metolachlor or metabolites from the GC are converted to ppm in wet soil according to Equation 1 - TABLE 1. Note that the weight of the soil aliquot (Section 6.3.3) is used for calculation.
- 6.7.2 The ppm (dry weight) is calculated from the moisture determination (Section 6.1.4) and Equation 2 - TABLE 1.
- 6.7.3 The ppm (metolachlor equivalents) is calculated for each metabolite using each chemical molecular weight and Equation 3 - TABLE 1.

TABLE 1: CALCULATION EQUATIONS

Equation 1

$$\text{ppm (wet wt)} = \frac{\left[ \text{ug found} \times \frac{1 \text{ ug}}{1000 \text{ ng}} \right]}{5000 \text{ ul final volume}} \times 4 \text{ ul injected} \times \text{wt of soil} + \text{aliquot (g)}$$

Equation 2

$$\text{ppm (dry wt)} = \frac{\text{ppm (wet wt)}}{\left[ \frac{\% \text{ moisture}}{100} \right]}$$

Equation 3

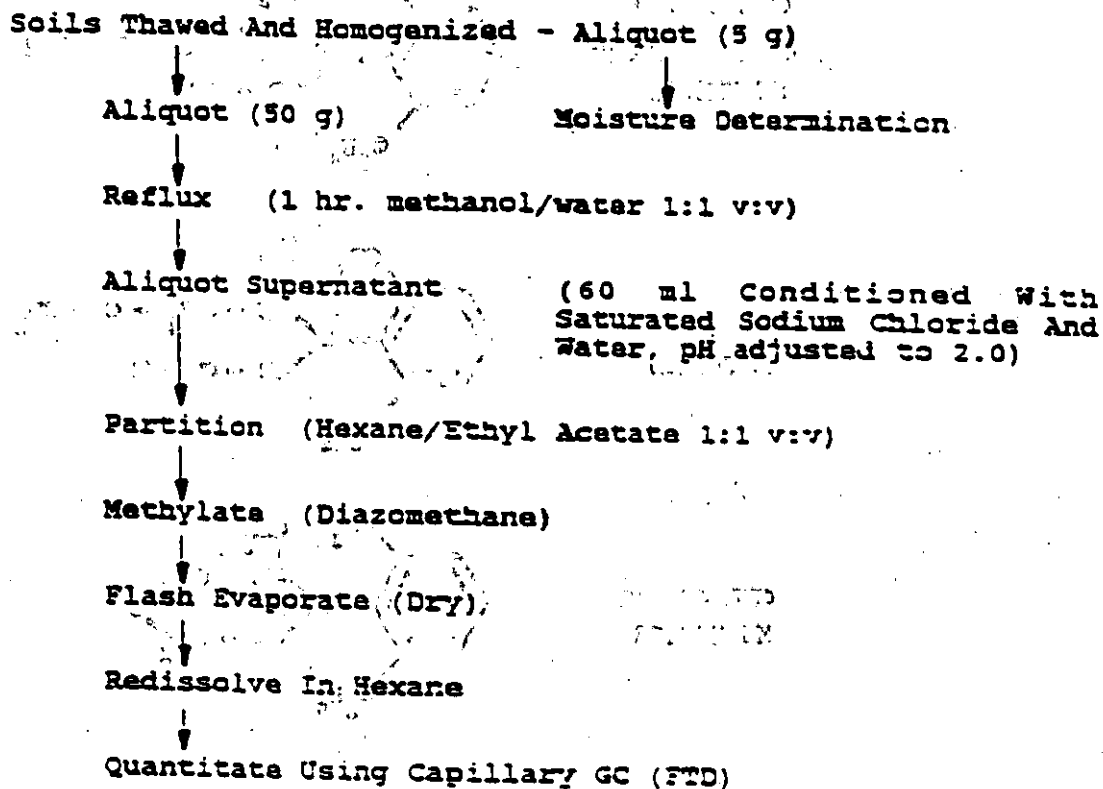
$$\text{ppm (Metolachlor Equivalents)} = \text{ppm (dry wt)} \times \left[ \frac{\text{molecular wt - metolachlor}}{\text{molecular wt - metabolite}} \right]$$



FIGURE 1: STRUCTURE AND CODE NUMBERS FOR METOLACHLOR AND METABOLITES:

Code number	Structure
CAS 24 705 (Metolachlor) MW 283.81	
CAS 40 172 MW 255.37	
CAS 40 919 MW 233.33	
CAS 51 202 MW 279.35	

FIGURE 2: FLOW DIAGRAM - ANALYSIS OF METOLACHLOR AND METABOLITES FROM SOIL



AGRISEARCH 1265-C - 125

PROTOCOL AMENDMENT NO. 2  
PROJECT NO. 1265

Additions to Agriseach Method for "Gas Chromatographic Determination of CGA-2470J (Metolachlor) and its Metabolites in Soil".

1. ADDITION: 1.0 SCOPE

Add fourth metabolite CGA-50720

2. CHANGE: 2.0 PRINCIPLE

Currently reads: "...adjusted to pH 2..."

Change to: "...adjusted to below pH 2..."

3. CHANGE: 2.0 PRINCIPLE

Currently reads: "...to methylate the metabolite CGA-51202."

Change to: "...to methylate the metabolites CGA-50720 and CGA-51202."

4. ADDITION: 3.0 REAGENTS

3.15 Standard CGA-50720 (See Figura 1).

5. CHANGE/ADDITIONS: 5.0 GAS CHROMATOGRAPHY OPERATION

Temperatures:           Injector/Detector   240°  
                          Column                   195°

Gas Flows:               Column Gas               15 ml/min Helium

Retention Times (Min.):           CGA-50720   4.2  
  CGA-40919   5.3  
  CGA-40172   6.0  
  CGA-51202   6.6  
  Metolachlor = CGA-24705   7.0

AGRISEARCH 1255-C - 130

PROTOCOL AMENDMENT NO. 2  
PROJECT NO. 1265

6. CHANGE: 6.0 PROCEDURE

6.4 Partition

6.4.1 Currently reads: ... "The solution is adjusted to pH 2 using 1N H<sub>2</sub>SO<sub>4</sub> to aid in the partition of CGA-51202 and CGA-40172 from the aqueous."

Change to: ... "The solution is adjusted to pH 1 - 1.5 using 1N H<sub>2</sub>SO<sub>4</sub> to aid in the partition of CGA-50720, CGA-51202, and CGA-40172 from the aqueous."

7. ADDITION: FIGURE 1

Addition of structure, code number and molecular weight for CGA-50720.

8. ADDITION: FIGURE 2 - FLOW DIAGRAM

Addition of pH adjustment to "Aliquot Supernatant"

9. ADDITION: FIGURE 3 - TYPICAL STANDARD SOLUTION CHROMATOGRAMS

Typical standard chromatograms with CGA-50720 added.

10. ADDITION: FIGURE 4 - TYPICAL SOIL EXTRACT CHROMATOGRAMS

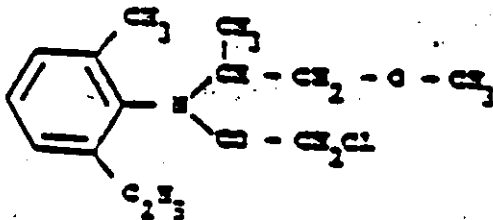
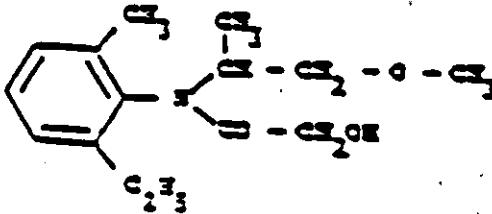
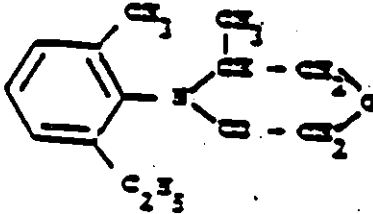
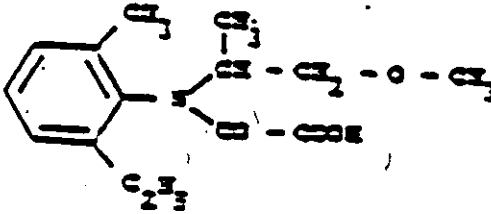
Addition of CGA-50720.

11. ADDITION: FIGURE 5 - TYPICAL CR4A ANALYSIS FILE.

Addition of CGA-50720.

AGRISEARCH 1265-C - 131

FIGURE 1: STRUCTURE AND CODE NUMBERS FOR METOLACHLOR AND METABOLITES.

Code number	Structure
CA 24 709 (Metolachlor) MW 283.91	
CA 40 172 MW 265.37	
CA 40 919 MW 233.33	
CA 51 202 MW 279.35	
CA 50 720 MW 207.25	