Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Metolachlor (CGA-24705)

MRID #: 413098-05

Matrix: Soil

Analysis: GC/NPD

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ENVIRONMENTAL CHEMISTRY METHOD

*** 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. 7018-4, 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).

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 3400, 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.

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.

6.2.2 Control samples are fortified with the appropriate spiking solution (6.2.1.3) 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 QUANTITATION

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 6.6.3). This represents a range of 0.40 to 40.0 nanograms (FIGURE 3).

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

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

Equation 2

$$\text{ppm (dry wt)} = \frac{\text{ppm (wet wt)}}{1 - \left[\frac{\text{\% moisture}}{100}\right]}$$

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:

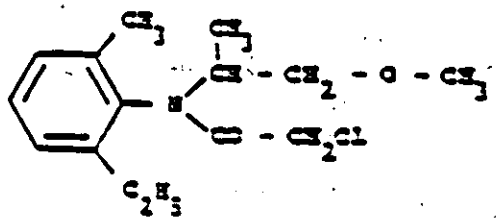
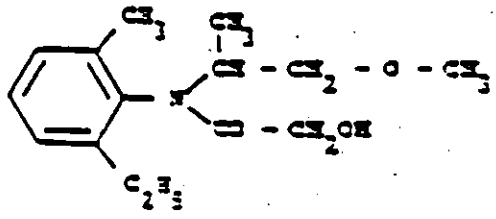
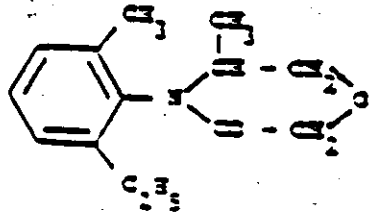
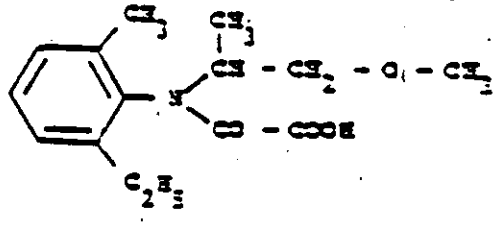
Code number	Structure
CGA 24 705 (Metolachlor) MW 283.81	
CGA 40 172 MW 255.37	
CGA 40 919 MW 223.33	
CGA 51 202 MW 279.35	

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

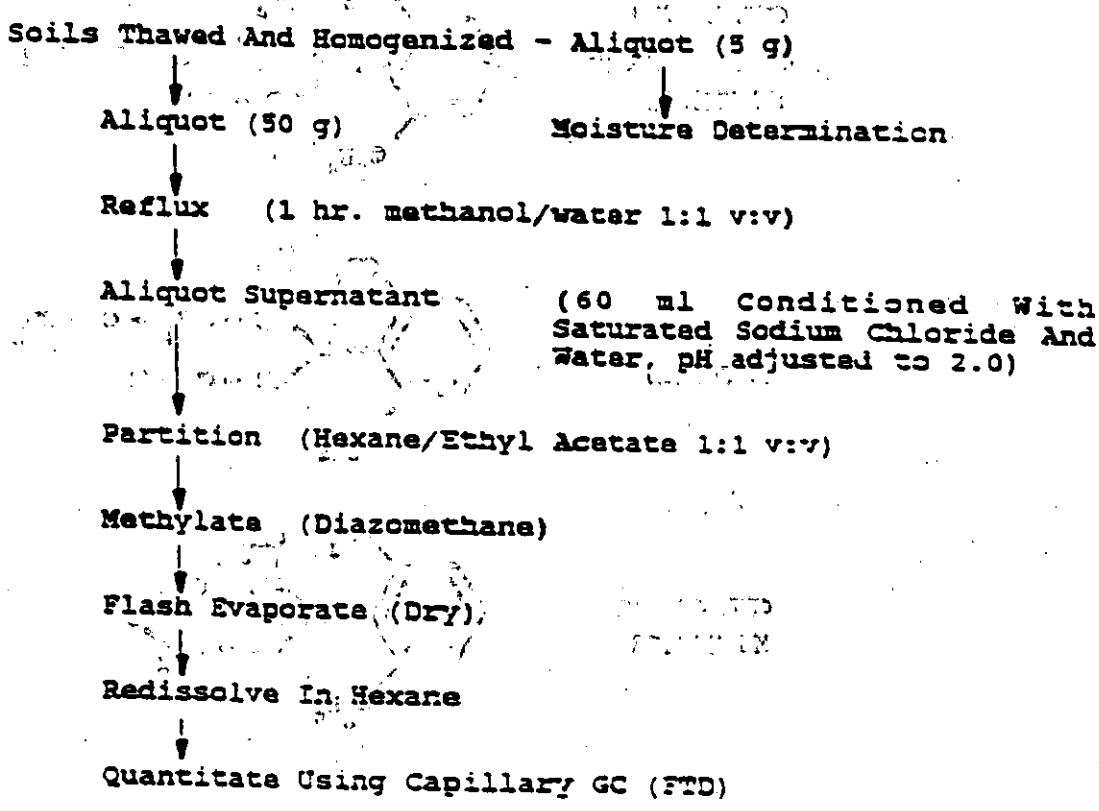
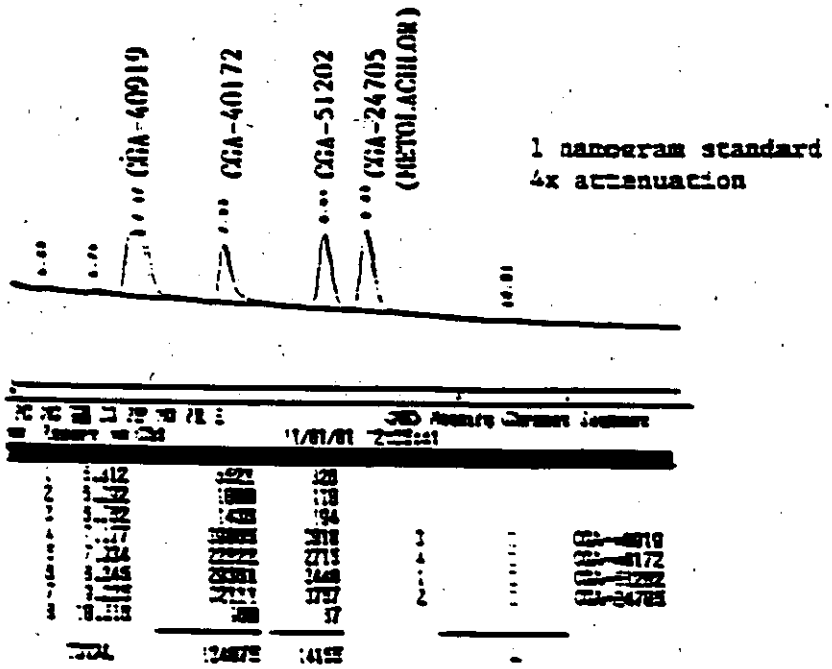


FIGURE 3. TYPICAL STANDARD SOLUTION CHROMATOGRAMS.



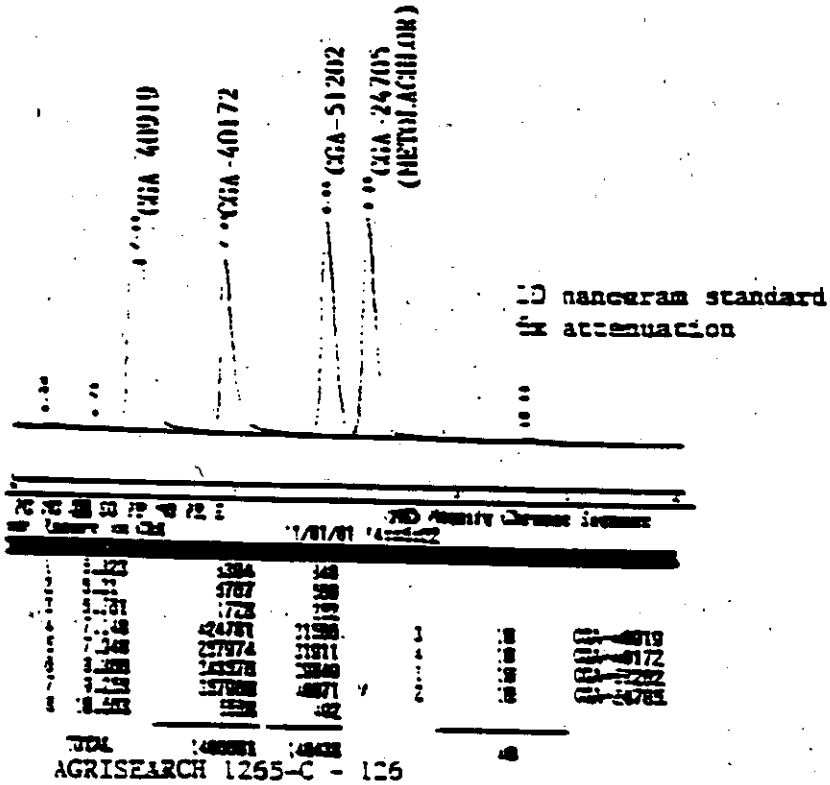


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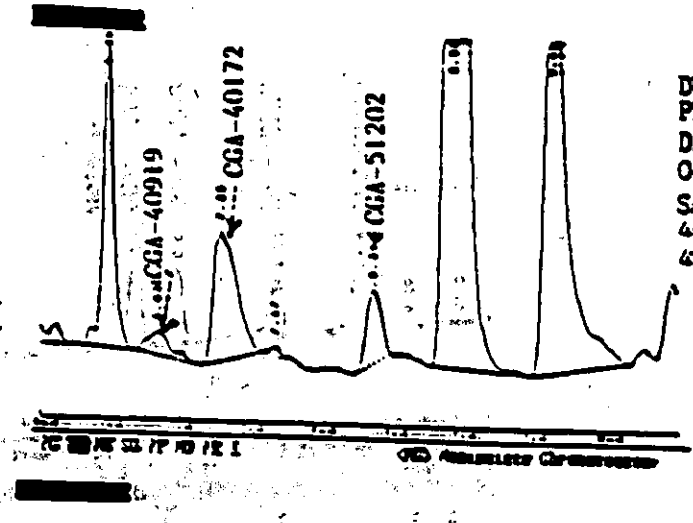
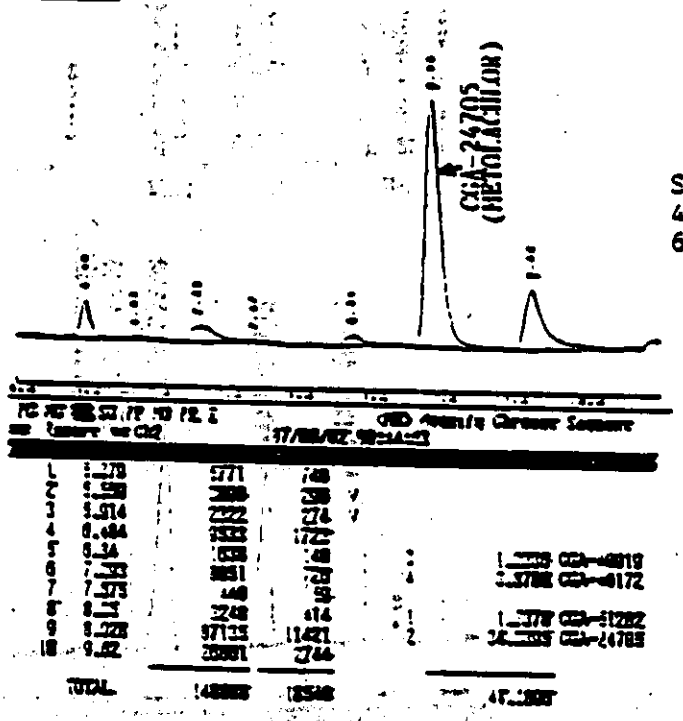
FIGURE 1. TYPICAL SOIL EXTRACT CHROMATOGRAMS





(a) Two attenuation settings of the same injection printed from data storage file to show each peak of interest on scale.

FIGURE 5: TYPICAL CR4A ANALYSIS FILE

** ANALYSIS FILE ** 2:RN1105.

PROCESSING PARAMETERS

WIDTH (sec)	15	SLOPE (uV.min)	100
DRIFT (uV/min)	10000	MIN. AREA (Count)	200
T.DBL (min)	0	STOP. TM (min)	12
ATTEN (2^X mV)	4	SPEED (mm/min)	5
METHOD (0-8)	4	W.B (0:WINDOW 1:BAND)	0
WINDOW (%)	2.5	SPL.WT	100
IS.WT	1	CALIB POINTS (1-8)	5

TIME PROGRAM

0.5 LOCK ON
9.5 LOCK OFF

IDENTIFICATION TABLE

IDNO	Name	Time	Band	Conc	Factor(1)	Factor(2)
1	CGA-51202	8.89		0.4	2.74241E-5	
				1	3.99199E-5	-9.18226
				10	3.34598E-5	0.38906181
				20	2.91379E-5	1.29957
				40	3.11659E-5	-9.08197983
2	CGA-24705	9.28		0.4	2.56941E-5	
				1	3.78682E-5	-9.17787
				10	3.35851E-5	-9.0464654
				20	2.82421E-5	1.53498
				40	0.000028735	1.21272
3	CGA-40919	7.17		0.4	1.52585E-5	
				1	4.36316E-5	-9.743798
				10	2.63777E-5	-9.0542212
				20	2.42704E-5	0.728999
				40	2.53987E-5	-9.145935
4	CGA-48172	7.98		0.4	2.44045E-5	
				1	7.38188E-5	-9.591871
				10	4.32418E-5	-9.3379213
				20	3.68293E-5	1.35065
				40	3.77905E-5	1.346522

OPTION PARAMETERS

PLOT ZERO POSIT (%FS)	10	PLOT CHROMAT (N.Y REV)	0
X.Y MARKERS(X.Y)	3	MARKER INTERVAL(cm)	2
PLOT START TIME (min)	5	PLOT STOP TIME (min)	10
PLOT LENGTH (cm)	10	BASELINE DRAW	1
PEAK TOP COMMENTS	8	2-CHANNEL PLOT	0
MEM SAMPLING (msec)	500	MAX No. of PEAKS	100
MAX No. of SLICES	0	IDENT (0=AB 1=RR 2=MS)	0
QUANTITATE (0=AR 1=HI)	0	CALIB (0=SP 1=LS 2=ML)	2
2-CH CALC (0=NO. 1-4)	0		

PRINT FORMAT

DATE & TIME	0	NOTES	0
CHROMATOGRAM	0	CALCULATION REPORT	0
GROUP DATA (IDNO)	0	GROUP DATA (NAME)	0
PEAK TIME INFO	0	ANALYSIS FILE	0
CALIBRATION DATA	0		

PROTOCOL AMENDMENT NO. 2

PROTOCOL: Metolachlor Field Dissipation Study In California And Iowa

AGRISEARCH PROJECT NO: 1265

TEST MATERIAL: Dual 25G Granule Herbicide

AMENDMENT: Amendment to analytical method for analysis of samples.

REASON FOR ADDITION: Addition of metolachlor metabolite CGA-50720 to analytical method.

PROTOCOL AMENDMENT ACCEPTANCE:

Sponsor: CIBA-GEIGY Corporation
P.O. Box 18300
Greensboro, NC 27419

Richard H. Ross

R.H. Ross, PhD, Sponsor Monitor (Field) — Date 9/19/88

K Balu

K. Balu, PhD, Sponsor Monitor (Analytical) — Date 9/19/88

Testing Facility:

D. Larry Merricks, PhD, Study Director — Date 9-19-88
Agrisearch Incorporated
26 Water Street
Frederick, MD 21701

PROTOCOL AMENDMENT NO. 2
PROJECT NO. 1265

Additions to Agrisearch Method for "Gas Chromatographic Determination of CGA-24705 (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 Figure 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

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_2SO_4 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_2SO_4 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-050720.

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

Addition of CGA-50720.

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

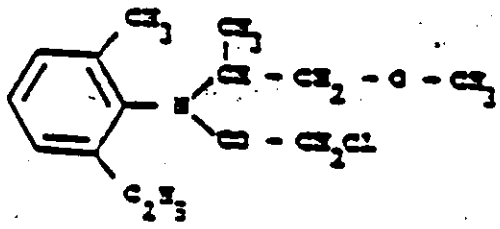
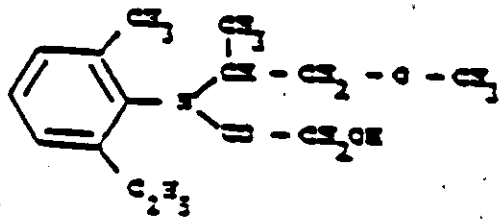
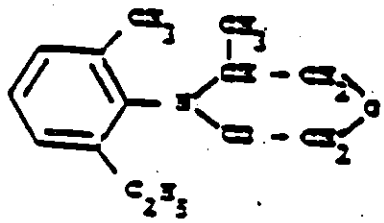
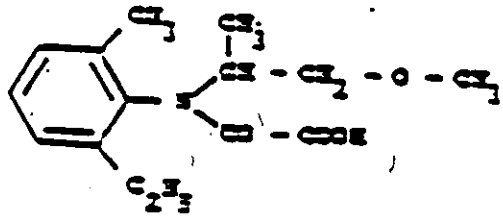
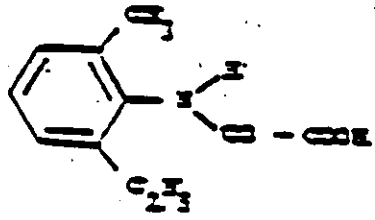
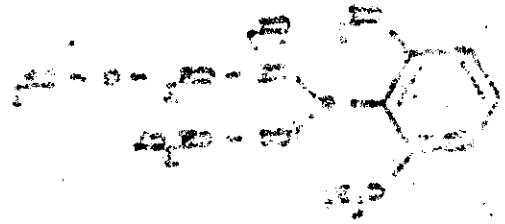
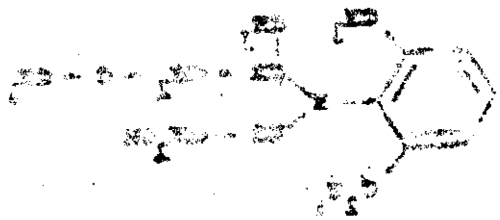
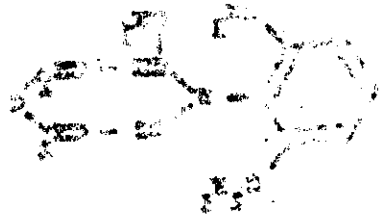
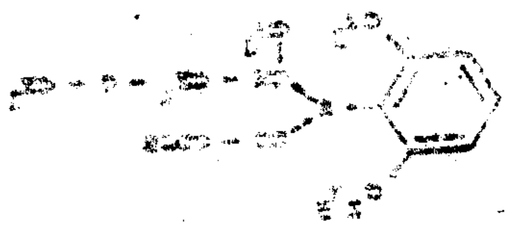
Code number	Structure
CGA 24 705 (Metolachlor) MW 283.81	
CGA 40 172 MW 265.37	
CGA 40 919 MW 233.33	
CGA 51 202 MW 279.35	
CGA 50 720 MW 207.25	

FIGURE 1: STRUCTURES AND CODE NUMBERS FOR [illegible] AND METABOLITES.

Code number	Structure
CGA [illegible] (…olachlor) MW 283.81	
CGA [illegible] MW [illegible]	
CGA [illegible] MW [illegible]	
CGA [illegible] MW [illegible]	
CGA [illegible] MW [illegible]