EN-CAS METHOD NO. ENC-4/90	AUTHOR (S) R. L. Parkes R. E. Emulsee A. D. Morris	DATE ISSUED: 5/22/91
TITLE: Analytical Method for the Determination of Propanil And Free 3,4-Dichloroaniline in Soil Using Capillary Gas Chromatography	QA APPROVAL HEAD APPROVAL Bert Clayton	REVISIONS

1.0 INTRODUCTION AND SUMMARY

1.1 Scope

This method is used for the determination of propanil and the "free" form of its primary metabolite, 3,4-dichloroaniline (3,4-DCA) in soil. "Free" 3,4-DCA consists of unbound or loosely bound 3,4-DCA which can be extracted from soil by shaking in an appropriate organic solvent (e.g. 1:1 acetone:toulene). It should be noted that a significant portion of the 3,4-DCA produced from propanil in soil becomes strongly or irreversibly bound to the soil. A portion can be freed but this requires hydrolysis in strong base. A procedure to accomplish this is described in EN-CAS Method ENC-9/90.

Method validation results from EN-CAS report 89-0122 PTF, Method Validation for the Determination of Total Dichloroaniline, Propanil and Free Dichloroaniline in Soil, are included in this report (see Tables I to VI). See Figure 1 for a flowchart of the method.

1.2 Principle

Soil samples which may contain propanil and "free" 3,4-DCA are first crushed and homogenized in a Hobart food processor with dry ice. The dry ice is allowed to sublime in a refrigerator or at room temperature before taking a soil subsample. The sample is extracted by first adding acetone to dissolve soil-bound water so that agglomeration of

1.2 Principle (continued)

the soil during extraction is minimized. Toluene is then added in an equal volume of acetone and the sample is shaken on a mechanical shaker. The extracted sample is vacuum filtered and the acetone is removed from the sample by rotary evaporation leaving primarily toluene. The 3,4-DCA is extracted from the toluene by partitioning into 0.5N HCl (saturated with toluene). The resulting organic phase (toluene) contains the propanil, and the aqueous phase contains the 3,4-DCA.

The organic phase is drained through a sodium sulfate pad and evaporated to incipient dryness on a rotary evaporator. Methanol is added to dissolve the propanil residue. The acidic aqueous phase is neutralized and buffered to pH 6.7 - 7.0 and then combined with the methanol from the organic phase. The sample (now containing both propanil and 3,4-DCA) is then loaded onto a pre-conditioned C-18 Mega Bond Elut cartridge. The analytes are eluted from the cartridge with ethyl acetate. The ethyl acetate is dried using anhydrous sodium sulfate. The sample extract is adjusted to a final volume of 10 ml with ethyl acetate and placed into a GC vial. Gas chromatographic analysis is performed using an alkali flame (N/P) detector equipped with a capillary DB-17 or DB-1701 column. A limit of quantitation (LOQ) of 0.01 ppm for either propanil or 3,4-DCA has been achieved for soil.

2.0 APPARATUS

All equipment and apparatus may be replaced by equivalent items from alternate sources.

2.1 16 oz. Nalgene plastic bottles and/or 16 oz. French square bottles

2.2 Buchner funnels, 11 cm

2.3 Side-arm filter flasks, 500 ml

2.4 Whatman GF/C filters, 9.0 cm diameter

2.5 Erlenmeyer flasks, 250 ml, with 24/40 ground glass fittings

2.6 Stoppers, ground glass, 24/40

2.7 Separatory funnels, 500 ml

2.0 APPARATUS (continued)

2.8 Powder funnels, metal, 4 inch diameter

2.9 Centrifuge tubes, 15 ml graduated

2.10 Test tubes, borosilicate glass, 16 x 100 mm, disposable

2.11 Pasteur pipettes, 23 cm

2.12 Glass wool

2.13 C-18 Mega Bond Elut cartridges, 2 gram (Analytichem International)

2.14 Filter paper, 9 cm diameter (Whatman GC/C)

2.15 Reservoirs, 75 ml capacity, Analytichem International

2.16 Luer stopcocks, plastic (Analytichem International)

2.17 Mechanical Shaker (G10 Gyrotory)

2.18 Rotary evaporators (Buchi Rotavapor, model #RE111)

2.19 Air Pump (Neptune Dyna-Pump, Model 4K)

2.20 Hobart homogenizer (model 8142 or 84142)

2.21 pH meter (Corning, Model 106)

2.22 Vac-Elut System (Analytichem International #SPS24)

2.23 Top loading balance, (Fisher Scientific, Model XT-3KD)

2.24 Mettler analytical balance capable of ± 0.00002 g accuracy, for weighing analytical standards

3.0 REAGENTS

3.1 Acetone, pesticide grade

3.2 Toluene, pesticide grade

3.3 Concentrated HCl, ACS Reagent grade

3.4 Sodium Hydroxide (NaOH), ACS reagent or Optima

3.5 Methanol, pesticide grade

3.6 Ethyl Acetate, pesticide grade

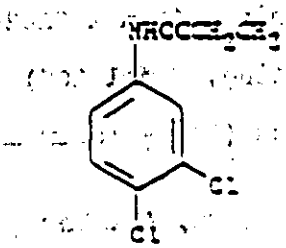
3.7 Potassium Dihydrogen Phosphate (KH_2PO_4), ACS reagent grade

Prepare a 1M KH_2PO_4 solution by dissolving 136.09 g of KH_2PO_4 in 750 ml of H2O, adjusting the pH to 6.5 with 5N NaOH, and bringing the solution up to volume (1000 ml).

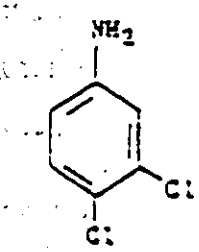
3.8 Sodium Sulfate (Na_2SO_4), Anhydrous, ACS certified, oven baked at 600°F for 2 hours

3.9 Deionized water (Milli-Q system or equivalent)

4.0 TEST SUBSTANCES



Propanil
$C_2H_9CL_6NO$
M.W. 219.09



3,4-Dichloroaniline
$C_6H_5CL_2N$
M.W. 162.03

5.0 PREPARATION OF ANALYTICAL STANDARDS

5.1 Fortification Standards

Weigh 10 mg of active ingredient (i.e., propanil, 3,4-dichloroaniline). Transfer the test substances to separate 100 ml volumetric flasks. Dissolve and dilute to volume with methanol to prepare 100 µg/ml stock solutions. Serially dilute the 100 µg/ml standards to prepare 10 µg/ml, 1.0 µg/ml, and 0.25 µg/ml standard solutions for propanil and 3,4-DCA. Use these solutions to fortify soil control samples in order to monitor procedural recovery. The stock standard solution (100 µg/ml) is stable for at least 12 months. [Note: Store all standard solutions in a freezer at a temperature of -23°C to -27°C.]

5.2 Gas Chromatographic Standards

Use the 100 µg/ml propanil and 3,4-dichloroaniline fortification standards (in methanol) that were prepared for the fortifying solutions to make combined 10 µg/ml and 1.0 µg/ml standard solutions in ethyl acetate. Serially dilute these standards in ethyl acetate to prepare a range of standards from 0.025 µg/ml to 0.25 µg/ml to be used as gas chromatographic (GC) calibration standards. The GC calibration standards are stable for 6 months. [Note: Store all standard solutions in a freezer at a temperature of -23°C to -27°C.]

6.0 ANALYTICAL PROCEDURE

6.1 Sample Processing

Homogenize soil samples using a Hobart food processor and dry ice. Mix the samples for 5-10 minutes until a homogeneous mixture is obtained. Allow the dry ice to sublime in a refrigerator or at room temperature. Weigh a 50 gram subsample into either a 500 ml Nalgene bottle or a 16 oz. French square bottle.

6.2 Extraction

Add 50 ml of acetone, swirl by hand, and wait for 5 minutes. Add 50 ml of toluene to the sample, cap the bottle, and place it on the mechanical shaker for 15 minutes at 200 rpm. [Adding the acetone separately allows the soil moisture to be extracted from the soil and thus prevents the soil from clumping during the shaking step].

Vacuum filter the extract using a Buchner funnel and Whatman #2 (9 cm) filter paper, into a 500 ml side-arm flask. Rinse the Nalgene bottle that contained the sample with 2 x 10 ml aliquots of a 1:1 acetone:toluene solution and add to the side-arm flask. Transfer the solution in the side-arm flask to an Erlenmeyer flask. Rinse the side-arm flask with 10 ml of 1:1 acetone:toluene solution and add to the Erlenmeyer flask. Evaporate the acetone from the sample using a rotary evaporator with a water bath at ambient temperature (the rotary evaporator condenser is cooled by dry ice). [Extreme care must be taken at this point in the procedure due to the volatility of the metabolite (3,4-DCA). The sample should be evaporated just until acetone stops distilling into the collection flask. However, care must also be taken to assure that most of the acetone is removed since an excess of residual acetone will alter the analyte distribution in the partition step which follows. Evaporating to a volume of approximately 70 ml appears to provide a good balance bwtween retention of 3,4-DCA and proper partitioning in the partition step.]

Partition the sample (now mainly toluene) with 3 x 50 ml of 0.5N HCl (saturated with toluene). Retain both the organic layer (top) and the aqueous layer (bottom).

Organic phase (Propanil) - Pass the organic layer through a pad of sodium sulfate approximately one and a half inches thick contained in a funnel. Rinse the pad with 2 x 10 ml toluene. Evaporate the toluene filtrate to dryness using rotary evaporation, keeping the bath temperature at 40°C -50°C. Reconstitute the sample with 5 ml of methanol. Proceed to step 6.3.

6.2 Extraction (continued)

Aqueous phase (3,4-DCA) - Neutralize the aqueous phase to pH 6.7 - 7.0 with 5N NaOH. Use a pH meter to verify the pH. Add 25 ml of 1M Potassium Monohydrogen Phosphate (K_2HPO_4) as a buffer. Proceed to step 6.3.

6.3 Clean-up Step Using C-18 Mega Bond-Elut Cartridges

Combine the methanol reconstituted organic phase (step 6.2) with the neutralized and buffered aqueous phase using 2 x 5 ml methanol as a rinse for the organic phase container. Using a Vac-Elut system, pre-condition the C-18 Mega Bond Elut cartridge with 2 x 10 ml methanol and 2 x 10 ml d.i. water (column should not go dry). Add the sample to the C-18 Mega Bond Elut cartridge and allow it to pass through the cartridge at a flow rate of approximately 5-10 ml/min. Discard the sample load. Elute the analytes with 2 x 4 ml of ethyl acetate at a flow rate of approximately 5-10 ml/min. Pass the eluate through a manually packed micro-column (pasteur pipette with a glass wool plug filled with ~4 cm x 5 mm of sodium sulfate) to dry the samples. Rinse the sodium sulfate with 2 x 1 ml of ethyl acetate. Adjust the sample to a final volume of 10 ml with ethyl acetate. Transfer the extract to a GC vial and analyze by gas chromatography using N/P detection.

6.4 Gas Chromatographic Determinations

Use a 30 m x 0.32 mm, 0.25 µm film thickness capillary DB-17 or DB-1701 column to achieve gas chromatographic separations. Use a Hewlett-Packard Model 5890-A Gas Chromatograph with an alkali flame, nitrogen/phosphorus (N/P) detector to provide adequate sensitivity and selectivity. Gas chromatographic conditions are listed in Section 7.0 of this method.

6.5 Safety Precautions

Use normal safety precautions, including the wearing of gloves, safety glasses and a fume hood to minimize exposure to the analytes and organic solvents used in this procedure.

6.6 Time Required for Analysis

An experienced technician can process a set of ~10 samples (including controls and recoveries), and prepare for injection on the gas chromatograph in two man-days.

6.7 Measurement Limit

For all of the soil samples validated herein, this method is proven effective to a LOQ of 0.01 ppm for both propanil and 3,4-DCA. Adjust the instrument sensitivity, GC calibration standards and final sample volumes to allow detection of propanil and free 3,4-DCA at 50% of the LOQ.

6.8 Interference and Potential Problems

A loss of 15-20% of 3,4-DCA may occur if more than 50% (70 ml) of the original extraction volume of 1:1 acetone/toluene is removed by rotary evaporation (see step 6.2). Significant loss of 3,4-DCA can also occur by not using the appropriate stopping points in the method. Acceptable stopping points are as follows:

1). After the filtration of the extracted samples (if this stopping point is used, the samples should be refrigerated after the filtration).

2). After the reduction of sample volume following the filtration step.

3). After the sample has been through the C-18 Mega Bond Elut clean-up.

All of these stopping points are designated by footnotes in the flowchart of the method (see Figure 1).

In applying this method to soil from rice fields, it is recommended that the control soils be pre-screened prior to use. Many of these soils have been found to contain bound 3,4-DCA which could, if freed by weathering processes, result in background levels of the analyte. In addition, other crop protection agents applied to rice fields may produce close-lying interferences. The GC conditions have been shown to separate propanil and 3,4-DCA from two commonly applied herbicides, Ordram and Bolero, and a common metabolite, 3,5-DCA, as demonstrated in Figures 15 and 16.

7.0 GAS CHROMATOGRAPHIC ANALYSIS

7.1 Description and Typical Operating Conditions

Instrument:	Hewlett-Packard Model 5890-A Gas Chromatograph with an alkali flame, nitrogen/phosphorus (N/P) detector equipped with a 7673A Automatic Sampler. Collect and process data with a Hewlett-Packard 3396A Integrator.
Column:	Capillary DB-17 or DB-1701 30 m x 0.32 mm, 0.25 µm film thickness (J & W Scientific)
Gases:	Carrier: Helium = 3.80 ml/min. Detector: Hydrogen = 4.20 ml/min. Air = 110 ml/min. Aux He = 20.2 ml/min.
Injection:	2 µl, splitless
Temperatures:	Injector: 250°C Detector: 275°C Column Temperature Program: Initial Oven Temp. = 50°C Initial Time = 1.5 min. Ramp A = 30°C/min. Final Oven Temp. = 195°C Final Time = 2.0 min. Ramp B = 40°C/min. Final Oven Temp. = 240°C Final Time = 2.0 min. Ramp C = 40°C/min. Final Oven Temp. = 275°C Final Time = 10.0 min. Run Length = 33.6 min.
Retention Times:	Free 3,4-DCA = 5.8 min. Propanil = 8.6 min.

7.1 Description and Typical Operating Conditions (continued)

Integrator Parameters: Hewlett-Packard 3396A Integrator

Parameter Definitions	Rem Parameters	Timetable Events
0. SET BASELINE NOW	ZERO = 20	0.000 INTG / = 2
1. SET BASELINE NEXT VALLEY	ATT 2^ = 1	0.000 INTG / = 8
2. SET BASELINE ALL VALLEYS	CHT SP = 0.0	0.000 INTG / = 9
3. SKIM FROM NEXT PEAK	AR REJ = 0	5.000 CHT SP = 2.0
4. DISABLE AUTO-TANGENT SKIMMING	THRSH = 0	5.005 ZERO = 20
5. EXTEND BASELINE HORIZONTALLY	PK WD = 0.02	5.010 INTG / = -9
6. MEASURE AND UPDATE THRESHOLD		6.500 INTG / = 9
7. TURN OFF RETENTION TIME LABELING		6.505 CHT SP = 0.0
8. TURN ON START/STOP MARKS		8.000 CHT SP = 2.0
9. TURN OFF INTEGRATION		8.005 ATT 2^ = -1
10. INCREMENT THRESHOLD		8.010 ZERO = 20
11. INVERT NEGATIVE PEAKS		8.015 INTG / = -9
12. CLAMP NEGATIVE PEAKS		9.500 INTG / = 9
13. SHOW IP11, IP12		9.505 CHT SP = 0.0
14. START PEAK SUM WINDOW		9.510 ATT 2^ = 10
		9.515 PK WD = 0.02
		9.520 PK WD = 2.5
		9.600 CHT SP = 0.0

7.2 Calibration

Use the combined propanil and 3,4-DCA GC calibration standards in ethyl acetate in concentrations ranging from 0.025 µg/ml to 0.25 µg/ml to calibrate the instrument. Inject appropriate standards at the beginning of the run, after approximately every two or three samples throughout the run, and at the end of the run. A linear regression function is generated using the resulting peak height (obtained from the integrator) vs. nanograms injected (see Figure 12). The correlation coefficient for the line should generally be equal to or greater than 0.990. The sample nanograms found are determined by inserting the sample peak height values into the standard curve linear regression equation.

7.3 Representative Chromatograms

Typical chromatograms illustrating GC calibration standards as well as soil controls, and soil recoveries, from both Louisiana and Arkansas rice field soils are shown in Figures 2 to 11. Typical calibration curves for propanil and free 3,4-DCA are shown in Figure 12 and Figure 13.

8.0 CALCULATIONS

Note: Residue samples may be corrected for procedural recovery and/or procedural recovery may be corrected for control sample background as per client request.

8.1 Calculations for Propanil and Free 3,4-Dichloroaniline in Soil

The ng of analyte found is determined from a standard curve prepared as discussed in section 7.2. The calculation is performed as follows:

$$\text{ng found in injected sample} = \frac{\text{sample peak height} - \text{standard curve y intercept}}{\text{standard curve slope}}$$

$$\text{ng-equiv. injected} = \frac{\text{ng sample extracted} \times \mu\text{l injected}}{\mu\text{l final volume} \times \text{dilution factor}}$$

$$\text{ppm found} = \frac{\text{ng found}}{\text{ng-equiv. injected}}$$

8.1 Calculations for Propanil and Free 3,4-Dichloroaniline in Soil (continued)

Obtain the nanograms (ng) of analyte found by constructing a standard curve using linear regression analysis of GC calibration standards.

For example:

$$\text{ng found} = \frac{[5188 \text{ counts} - (-429.39 \text{ counts})]}{17243.84 \text{ counts/ng}} = 0.326 \text{ ng}$$

$$\text{ng equiv injected} = \frac{50{,}000 \text{ ng} \times 2 \mu\text{l}}{5{,}000 \mu\text{l} \times 10} = 2.0 \text{ ng}$$

$$\text{ppm found} = \frac{0.326 \text{ ng}}{2.0 \text{ ng}} = 0.163 \text{ ppm 3,4-Dichloroaniline}$$

8.2 Soil Moisture Calculations

Determine the percent of moisture in the soil by weighing 10 grams of soil in duplicate, drying overnight at an oven temperature of 110°C, and reweighing the soil. The average of the duplicate soil moistures is to be used in the residue calculation.

Percent moisture in a sample:

$$\frac{\text{wet wt.} - \text{dry wt.}}{\text{wet wt.}} \times 100 = \% \text{ moisture}$$

8.2 Soil Moisture Calculations (continued)

For example:

$$\frac{10.03\ g - 7.02\ g}{10.03\ g} = 0.300 \times 100 = 30\%$$

8.3 Calculation of Soil Residues on a Dry Weight Basis

Dry sample ppm is determined by:

$$\text{ppm dry} = \frac{\text{ppm wet}}{(1 - \text{decimal \% moisture})}$$

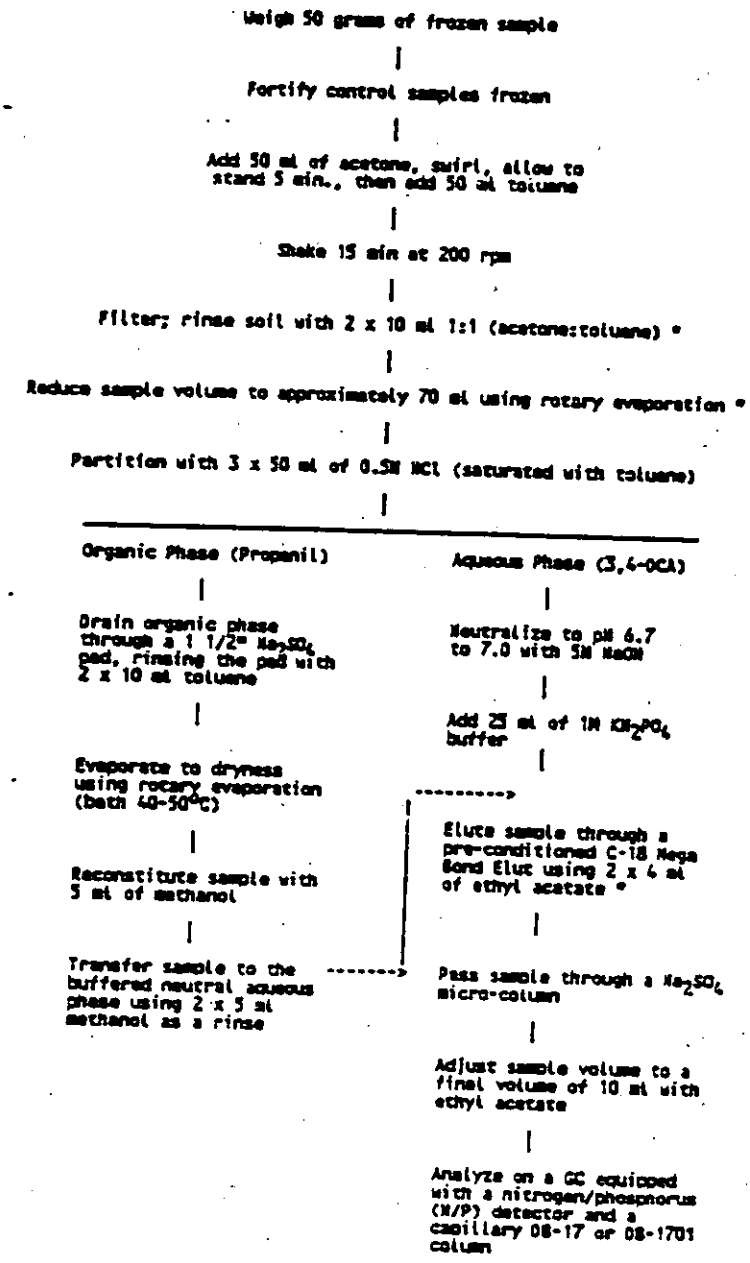
For example:

$$\text{ppm dry} = \frac{0.163\ \text{ppm}}{(1 - 0.31^{*})} = 0.236\ \text{ppm 3,4-Dichloroaniline}$$

* This value is different than the value obtained from the % moisture calculation shown above, because it is an average of the duplicate soil moisture results.

FIGURE 1

Flowchart of Analytical Method ENC-4/90
Soils - Propanil and Free 3,4-DCA



* The procedure may be stopped at this point if necessary. Refrigerate samples at all stopping points.