EN-CAS METHOD NO. ENC-5/90	AUTHOR (S) R. L. Parkes R. E. Haulsee	DATE ISSUED: 5/16/91
		REVISIONS
TITLE: Analytical Method for the Determination of Propanil And Free 3,4-Dichloroaniline in Water Using Capillary Gas Chromatography	QA APPROVAL Kelly F. Lyke 5/16/91 MGMT APPROVAL Bert Clayton 5/16/91	

1.0 INTRODUCTION AND SUMMARY

1.1 Scope

This method is used for the determination of solvent extractable propanil and solvent extractable 3,4-dichloroaniline (free 3,4-DCA) in water. "Free" 3,4-DCA consists of unbound or loosely bound 3,4-DCA which can be extracted from water by passing through a C-18 Mega Bond Elut cartridge and eluting with ethyl acetate. It should be noted that some of the 3,4-DCA produced from propanil in water may become strongly or irreversible bound to soil particulates present in water. A portion can be freed, but this requires hydrolysis in strong base. A procedure to accomplish this is described in EN-CAS Method 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 and Water from Rice Fields are included in this report (see Tables I to VI). See Figure 1 for a flowchart of the method.

1.2 Principle

A 100 ml aliquot of water is taken from a water sample and measured into an Erlenmeyer flask. Twenty-five ml of pH 6.5 buffer (1M K_2HPO_4) is added to the sample. The sample is then passed

1.2 Principle (continued)

through a pre-conditioned C-18 Mega Bond Elut cartridge. The analytes which are trapped on the Bond-Elut cartridge are eluted with 2 x 4 ml of ethyl acetate, and collected in a 15 ml disposable test tube. The eluates are dried by passage through a micro-column filled with sodium sulfate and a small plug of glass wool. The sodium sulfate column is then rinsed with ethyl acetate. The sample volume is adjusted to a final volume of 10 ml using ethyl acetate.

Gas chromatographic (GC) analysis is performed using a GC equipped with an alkali flame (N/P) detector and a fused silica capillary DB-17 or DB-1701 column. A limit of quantitation (LOQ) of 0.01 ppm can be achieved for both propanil and 3,4-DCA in water. A flowchart of ENC-5/90 is shown in Figure 1.

2.0 APPARATUS

NOTE: All equipment, apparatus and reagents may be replaced by equivalent items from alternate sources.

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

2.2 Stoppers, plastic, 24/40

2.3 Graduated cylinders, 25 ml and 100 ml

2.4 Centrifuge tubes, graduated, 15 ml

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

2.6 Pasteur pipettes, 23 cm

2.7 Glass wool

2.8 C-18 Mega Bond Elut, 2 gram (Analytichem International, Cat. # 1225-6015)

2.9 Reservoirs, 75 ml capacity (Analytichem International, Cat. # 1213-1012)

2.10 Bond Elut Adaptors, for 75 ml Reservoirs (Analytichem International, Cat. # 1213-1003)

2.0 **APPARATUS** (continued)

2.11 Luer stopcocks, plastic (Analytichem International, Cat. # 1213-1005)

2.12 Luer Lok Syringes, 250 ml, 500 ml, 1000 ml

2.13 Repipetter, 10 ml (Labindustries)

2.14 GC injection vials, 2 ml, with caps

2.15 Scintillation vials, 20 ml

2.16 Parafilm

2.17 Aluminum foil

2.18 Vac-Elut System (Analytichem International #SPS24)

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

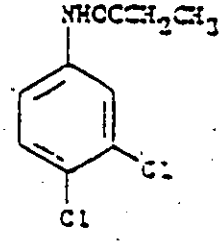
3.0 **REAGENTS**

3.1 Methanol, pesticide grade

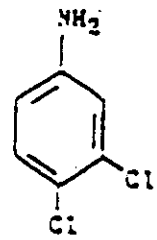
3.2 Ethyl acetate, pesticide grade

3.3 Deionized water (Milli-Q system)

4.0 **TEST SUBSTANCES**



Propanil
$C_9H_9Cl_2NO$
M.W. 218.09



3,4-Dichloroaniline
$C_6H_5Cl_2N$
M.W. 162.03

5.0 PREPARATION OF ANALYTICAL STANDARDS

5.1 Fortification Standards

Weigh 10 mg active ingredient (i.e., propanil, 3,4-DCA) and transfer 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 combined 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 water 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° to -27°C.]

5.2 Gas Chromatographic Standards

Use the 100 µg/ml propanil and 3,4-DCA 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 standard solutions from 0.025 µg/ml to 0.25 µg/ml to be used for 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° to -27°C.]

6.0 ANALYTICAL PROCEDURE

6.1 Sample Preparation

Thaw water samples and allow to come to room temperature. Remove a 100 ml aliquot for analysis.

6.2 Extraction

Measure 100 ml of water into an Erlenmeyer flask and add 25 ml of buffer (1M K_2HPO_4). Pre-condition a C-18 Bond Elut cartridge with 2 x 10 ml of methanol and 2 x 10 ml of d.i. water. Place the Bond Elut cartridges onto the Vac-Elut system. Load the sample onto the cartridge, elute the cartridge with 2 x 4 ml of ethyl acetate and collect the eluate in a 15 ml disposable test tube. Pass the eluate through a manually packed micro-column (Pastuer pipette with a glass wool plug

6.2 Extraction (continued)

filled with-4 cm x 5 mm of sodium sulfate). Rinse the sodium sulfate column with 2 x 1 ml of ethyl acetate and collect the sample in a 15 ml graduated centrifuge tube. Adjust the volume to 10 ml with ethyl acetate. Transfer the extract to a GC vial and analyze by gas chromatography using nitrogen/phosphorus (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 N/P detector to provide adequate sensitivity and selectivity. Gas chromatographic conditions are listed in Section 7.0 of this report.

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 approximately 1 man-day.

6.7 Measurement Limit

For all of the water 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

If necessary, the procedure may be stopped after the analytes on the C-18 Mega Bond Eluts have been eluted with ethyl acetate into 15 ml disposable test tubes. The test tubes should then be placed in the freezer under standard freezer conditions. The GC conditions have been shown to separate propanil and 3,4-DCA from two commonly applied herbicides, Bolero and Ordram, and a common crop protection agent metabolite, 3,5-DCA, as demonstrated in Figures 14, 15 and 16.

7.0 GAS CHROMATOGRAPHIC ANALYSIS

7.1 Description and Typical Operating Conditions

Instrument: Hewlett-Packard Model 5890A Gas Chromatograph with an alkali flame, nitrogen/phosphorus (N/P) detector equipped with a 7673A Automatic Sampler. Data was collected and processed with a Hewlett-Packard 3396A integrator.

Column: Capillary DB-17 or DB-1701 column (J & W Scientific) 30 m x 0.32 mm, 0.25 µm film thickness

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 ul, 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
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 DCA = 6.5 min.
Propanil = 11.8 min

7.1 Description and Typical Operating Conditions (continued)

Integrator Parameters: Hewlett-Packard 3396A Integrator

Parameter Definitions	Run 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 IF11, IF12		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 calibration standards in ethyl acetate in concentrations ranging from 0.025 µg/ml to 0.25 µg/ml. 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. 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.

Typical chromatograms illustrating GC calibration standards as well as water controls and water recoveries, from both Louisiana and Arkansas sites are shown in Figures 2 to 11. A typical calibration curve for propanil and free 3,4-DCA is shown in Figure 12 and Figure 13, respectively.

8.0 CALCULATIONS

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

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

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

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

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

For example: EI5133, set 31

$$\text{ng found} = \frac{[2118 \text{ counts} - (-192.02 \text{ counts})]}{37737.16 \text{ counts/ng}} = 0.061 \text{ ng}$$

$$\mu\text{l equiv. injected} = \frac{100 \text{ ml} \times 2.0 \mu\text{l}}{10 \text{ ml} \times 50} = 0.4 \mu\text{l}$$

$$\text{ppm found} = \frac{0.061 \text{ ng}}{0.4 \mu\text{l}} = 0.153 \text{ ppm 3,4-Dichloroaniline}$$

FIGURE 1

Flowchart of Analytical Method 5/90
Water - Procymidone and Free DCA