# Determination of Terraclor in Crops and Soil by Electron-Capture Gas Chromatography

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An electron-capture gas chromatographic procedure is used to determine trace amounts of Terraclor in celery, flax seed, lettuce, peanuts, peanut hay, peanut shells, potatoes, radishes, strawberries, and soil. The samples are extracted with hexane and the Terraclor in the extract is determined using a gas chromatograph with an electron-capture detector, after removing most of the interferences via a silicic acid column. The method has been used to analyze several aliquots of different samples fortified with varying amounts of Terraclor in the range of 0.010 to 0.270 p.p.m. Recoveries averaged from a low of 74% for leaf lettuce to a high of 119% for peanut hay. Additional data show that the silicic acid cleanup technique can be used to determine Terraclor by the spectrophotometric procedure.

Terraclor is the trade name for pentachloronitrobenzene (PCNB) manufactured and sold by Olin Mathieson Chemical Corp. PCNB is a highly effective and widely used soil fungicide (Eckert, 1962; Gould and Russell, 1965; Olin, 1961). Until recently the presence of Terraclor in crops and soil was detected and determined using the well known spectrophotometric procedure (Ackermann *et al.*, 1958, 1963; Zweig, 1964). This method has been used to determine as little as 3 μg. of Terraclor. The advent of electron-capture detectors to analyze gas chromatographic effluents has enabled quantitation of extremely small amounts of certain chemicals more easily and faster (Gunther, 1964). Since the electron-capture detector is highly sensitive to picogram amounts of Terraclor, the concentration of this chemical in agricultural commodities could be determined using very small amounts of the crop sample. Consequently, techniques were developed to extract and determine Terraclor in several common agricultural products.

## *Experimental*

**Apparatus.** Jarell-Ash 26-700 gas chromatograph with electron-capture detector. Chromatographic tubes (19 × 450 mm.) with Teflon stopcocks. Sonogen (Cole-Parmer, Chicago, Ill.).

**Reagents.** HEXANE. Distill high purity *n*-hexane to eliminate peaks which interfere in the electron-capture gas chromatographic detection of PCNB.

DRY SILICIC ACID. Dry chromatographic grade (100- to 200-mesh) silicic acid overnight in a vacuum oven at 100° ± 5° C. and store in a desiccator.

SILICIC ACID CONTAINING 10% MOISTURE. Weigh 100 grams of the dry silicic acid into a 16-ounce bottle provided with a screw cap. Pipet 10 ml. of distilled water into the bottle, and shake the contents vigorously until the lumps break. Allow the silicic acid to tumble overnight in a Fisher-Kendall mixer and reach equilibrium.

COLUMN FOR ELECTRON-CAPTURE GAS CHROMATOGRAPH. Using a vibrator, pack a 4-foot long, 1/4-inch o.d., U-shaped stainless steel tube with 90- to 100-mesh Anakrom ABS containing a 2% loading of SE-30. Heat the column in an oven at 300° C. for 5 hours with no gas flowing through the column. At this time connect the column to a source of nitrogen to give a flow of 100 ml. per minute and continue to condition it at 300° C. for about 12 hours. At the end of this period connect the column to the electron-capture detector and allow the instrument to stabilize at operating conditions overnight.

TERRACLOR REFERENCE GRADE. Dissolve 10 grams of technical grade Terraclor in 300 ml. of hexane. Slurry 10-gram portions of dry silicic acid with hexane and prepare several chromatographic columns. Pipet 10 ml. of the Terraclor solution into each column and elute with hexane. Discard the first 50 ml. of the effluent and collect the second 50. Combine all the second 50-ml. portions from the columns and evaporate to dryness over a steam bath but avoid overheating. Dry the resulting PCNB crystals overnight in a vacuum oven at 90° C. and store in brown bottles.

TERRACLOR STANDARD SOLUTIONS. Using the reference grade PCNB, prepare standard solutions containing 0.01 μg. of PCNB per ml. of hexane for the gas chromatograph, and 0 to 100 μg. of PCNB per ml. of acetone for recovery standards.

## *Procedure*

**Sample Preparation.** Homogenize the vegetable samples in a Hobart food cutter. Mix the soils as well as seeds in a Patterson-Kelly twin shell blender to ensure uniform subsamples. Shell the peanuts, and break up the shells and kernels into small pieces.

**Extraction.** VEGETABLES AND SEEDS. Weigh a 10-gram sample into an 8-ounce Osterizer jar. Add dropwise 1 ml. of one of the recovery standard solutions. Toss the sample during this addition in order to ensure a uniform distribution. Blow a gentle stream of nitrogen into the jar until the odor of acetone is no longer predominant. At this point add about 30 grams of anhydrous sodium sulfate and 100 ml. of hexane into the jar. Fit the jar with the cutting assembly and blend

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the contents at high speed for 2 minutes while permitting a forceful jet of air to blow at the base of the cutting assembly. Filter the blend through glass wool into a beaker. Run several replicate extractions using a different recovery standard solution each time. Use samples containing 1 ml. of acetone without PCNB as controls to calculate the recovery of PCNB from samples to which 1 ml. of the acetone solution of PCNB is added.

SOILS. Put 50 grams of soil in a 500-ml. Erlenmeyer flask. Pipet 1 ml. of a recovery standard solution into the flask and mix well. Blow nitrogen over the sample until the odor of acetone is not very noticeable. Add 200 ml. of hexane and place the flask in a Sonogen for 30 minutes. Shake the flask manually every 10 minutes during this period. Filter the extract. For wet soils use acetone instead of hexane. Use enough acetone to keep the water content of the extract under 10%. Mix 40 ml. of the acetone, 100 ml. of hexane, and 100 ml. of distilled water in a separatory funnel. Isolate the hexane phase, dry over anhydrous sodium sulfate, and analyze for PCNB.

EXTRACTS FOR SPECTROPHOTOMETRIC PROCEDURE. Weigh 200 grams of sample into a 1/2-gallon Osterizer jar. Fortify the sample with an appropriate amount of PCNB. Add 500 ml. of hexane and about 900 grams of anhydrous sodium sulfate. Blend the contents of the jar in an Osterizer blender for 4 minutes at high speed. Allow the blend to mix and equilibrate on a ball mill roller for about 1/2 hour. Filter the extract and analyze for PCNB.

**Cleanup of Extracts.** Slurry 5 grams of silicic acid containing 10% moisture with hexane and prepare a chromatographic column. Top the column with a 1/2-inch layer of anhydrous sodium sulfate.

Concentrate an aliquot of the extract over a steam bath and transfer it quantitatively into the silicic acid column, elute with hexane, and collect 100 ml. of the effluent. Analyze the effluent directly using the electron-capture gas chromatograph or concentrate it to about 3 ml. and analyze by the spectrophotometric procedure.

**Determination of PCNB by Electron-Capture Gas Chromatography.** Bring the instrument to the following operating conditions:

| | |
|---|---|
| Nitrogen flow | 160 ml. per minute |
| Detector temperature | 210° C. |
| Column oven temperature | 170° C. |
| Injection port temperature | 220° C. |
| Detector standing current | $5 \times 10^{-9}$ ampere at 19 volts |

Inject 2 to 9 μl. of the standard solution containing 0.01 μg. of PCNB per ml. of hexane and obtain the height of the PCNB peaks for each injection. Plot the peak heights against the corresponding microliters and determine the linear portion of the curve. Inject 2 to 9 μl. of the effluent until a response within the linear range is obtained. However, if the effluent does not contain any PCNB, inject about 9 μl. Compare the peak height to that of a standard obtained immediately prior to that of the unknown. Calculate the apparent parts per million of PCNB in the sample using the following equation:

$$C = \frac{l_1 \times g}{P_1} \times \frac{P_2}{l_2} \times \frac{100}{W}$$

where $C$ = apparent p.p.m. PCNB in sample
$l_1$ = microliters of standard injected
$l_2$ = microliters of effluent injected
$g$ = micrograms of PCNB per milliliter of gas chromatographic standard
$P_1$ = peak height in centimeters for $l_1$
$P_2$ = peak height in centimeters for $l_2$
$W$ = grams of sample equivalent to 100 ml. of effluent

If the sample analyzed is a control, $C$ will give the apparent blank, but if the sample has been fortified with PCNB, $C$ will denote the apparent recovery. The average apparent blank is subtracted from the gross recovery to obtain the net recovery for each individual determination.

*Results and Discussion*

Even after the cleanup, the extracts contained materials that interfered with the electron-capture detection of PCNB. But SE-30 columns conditioned as described in the text removed these interferences satisfactorily even after a year of continuous daily use. Unconditioned SE-30 columns produced too much instrument noise and very poor sensitivity. Columns made of other coatings such as DC-11, high vacuum grease, DC-200, and QF-1 did not require any elaborate conditioning like SE-30. However, experience has shown that these columns should be renewed frequently for satisfactory analysis.

Acetone mixed well with green and wet samples. Consequently, all samples used to determine recoveries were fortified with an acetone solution of PCNB. Experimental blanks for such determinations were run using the crop samples to which an equal amount of acetone had been added to compensate for the presence of acetone in the fortified sample.

About 30 grams of anhydrous sodium sulfate tied up most of the water from 10-gram samples to make satisfactory extractions of Terraclor using an Osterizer blender. However, when the samples were wet, acetone was used as a solvent.

A forceful jet of compressed air at the base of the cutting assembly blew away any solvent that leaked into the area, eliminating a fire hazard, and kept the moving parts cool.

Osterizer blending alone was not satisfactory to extract 200-gram samples. In such cases the blends were shaken for about 1/2 hour to obtain more efficient extraction.

Direct evaporation of acetone extracts containing PCNB were not satisfactory. These solutions were partitioned into hexane as a recourse and an aliquot of the hexane phase was analyzed. In the partitioning procedure, the proportion of acetone, hexane, and water was always kept constant eliminating chances of variation in

**Table I. Terraclor Blanks and Recovery Values Obtained with Electron-Capture Gas Chromatography**

| Commodity Analyzed | Terraclor Blanks, P.P.M. | Terraclor Recoveries Added, p.p.m. | Terraclor Recoveries Found, net % |
|---|---|---|---|
| Celery | | | |
| Av. | 0.010 | 0.042 to 0.269 | 87.0 |
| Std. dev. | ±0.004 | | ±16.0 |
| Flax seed | | | |
| Av. | 0.010 | 0.031 to 0.106 | 93.0 |
| Std. dev. | ±0.004 | | ± 2.0 |
| Leaf lettuce | | | |
| Av. | 0.010 | 0.011 to 0.265 | 74.0 |
| Std. dev. | ±0.002 | | ± 7.0 |
| Peanut kernels | | | |
| Av. | 0.020 | 0.011 to 0.255 | 90.0 |
| Std. dev. | ±0.004 | | ± 2.4 |
| Peanut shells | | | |
| Av. | 0.020 | 0.038 to 0.125 | 103.0 |
| Std. dev. | ±0.003 | | ±13.0 |
| Peanut hay | | | |
| Av. | 0.040 | 0.038 to 0.125 | 119.0 |
| Std. dev. | ±0.010 | | ±17.0 |
| Potatoes | | | |
| Av. | 0.005 | 0.010 to 0.270 | 92.0 |
| Std. dev. | 0 | | ±10.0 |
| Radish | | | |
| Av. | 0.020 | 0.031 to 0.102 | 85.0 |
| Std. dev. | ±0.004 | | ±1.0 |
| Strawberries | | | |
| Av. | 0.015 | 0.022 to 0.113 | 88.0 |
| Std. dev. | ±0.004 | | ±22.0 |
| Soil | | | |
| Av. | 0.005 | 0.016 to 0.032 | 87.0 |
| Std. dev. | 0 | | ±14.0 |

**Table II. Terraclor Blanks and Recovery Values Obtained with Spectrophotometric Procedure**

| Commodity Analyzed | Terraclor Blanks, P.P.M. | Terraclor Recoveries Added, p.p.m. | Terraclor Recoveries Found, net % |
|---|---|---|---|
| Celery | | | |
| Av. | 0.070 | 0.054 to 1.610 | 80.0 |
| Std. dev. | ±0.028 | | ±13.0 |
| Potatoes | | | |
| Av. | 0.020 | 0.076 to 0.405 | 93.0 |
| Std. dev. | ±0.007 | | ±11.0 |

the volume of the hexane phase and in the partitioning of PCNB between phases.

Some of the hexane extracts could have been analyzed without any cleanup, but under such conditions, the gas chromatographic column deteriorated faster and the sensitivity of the instrument dropped rapidly. The volume of the extract that needed to be cleaned up was determined by the concentration of Terraclor in the sample. Whenever the amount of Terraclor was negligible, then as much of the extract as was available was concentrated, cleaned up, and analyzed.

Extract concentrates equivalent to 100 grams of sample had been passed through 5-gram silicic acid columns and successfully analyzed by both electron-capture gas chromatographic and spectrophotometric procedures. Therefore, the authors feel that silicic acid effectively removes all significant interferences.

Under the conditions used in these analyses, the electron-capture detector gave about 0.3% noise in a 10-inch full scale recorder, and a full scale response for 150 picograms of PCNB. The retention time for PCNB was 94 seconds as measured between the solvent and the PCNB peaks. Amounts as low as 0.01 p.p.m. of Terraclor had been added to the samples and satisfactorily recovered. The blanks were corrected to the nearest 0.005 p.p.m. and the recoveries were corrected to the nearest 1%. The results obtained with various samples using the electron-capture gas chromatograph are given in Table I. Table II shows the results obtained using the spectrophotometric procedure via the new cleanup technique. The blanks for the spectrophotometric procedure are about 4 to 7 times more than those of the gas chromatographic method. These high blanks definitely limit the application of the spectrophotometric method to detect less than the blank levels of PCNB. The authors have not exploited the maximum sensitivity obtainable with the electron-capture gas chromatograph, since a procedure that would detect 0.01 p.p.m. was the immediate goal.

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*Received for review October 13, 1966. Accepted April 10, 1967.*