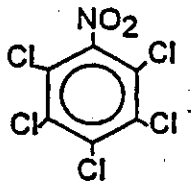
I. Summary

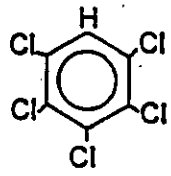
Soil samples were extracted with acetone/hexane. Water (pH > 12) was added to the acetone/hexane extract and the mixture was re-extracted in hexane. The water was then acidified (pH < 1) and once again extracted with hexane. The extract of the acidified water was methylated with diazomethane and combined with the remainder of the sample. The solvent was changed to toluene and the volume of the combined extracts was adjusted. The samples were analyzed for PCNB and metabolites and impurities using GC/ECD.

II. Introduction

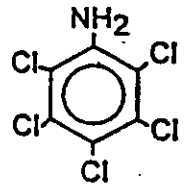
This method was developed by Centre Analytical Laboratories to determine pentachloronitrobenzene (PCNB), pentachlorobenzene (PCB), hexachlorobenzene (HCB), pentachloroaniline (PCA), pentachlorothioanisole (PCTA), pentachlorophenol (PCP), 2,3,4,5-tetrachlorothioanisole sulfone (TCTASOO) and pentachlorothioanisole sulfoxide (PCTASO). The molecular structure of these compounds is shown in Figure 1. The following references were used as a guideline in the initial development of this method: "Determination of Terrazole (5-Ethoxy-3-Trichloromethyl-1,2,4-Thiadiazole) and Terraclor (Pentachloronitrobenezene) and Allied Metabolites in Plant Tissues or Harvest Samples" and "Determination of Terraclor (Pentachloronitrobenzene) and Terrazole (5-Ethoxy-3-Trichloromethyl-1,2,4-Thiadiazole) in Soil".



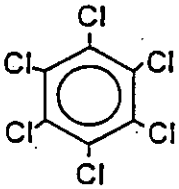
Pentachloronitrobenzene
(PCNB)



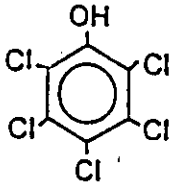
Pentachlorobenzene
(PCB)



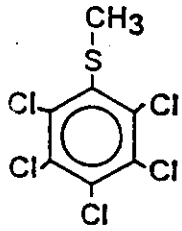
Pentachloroaniline
(PCA)



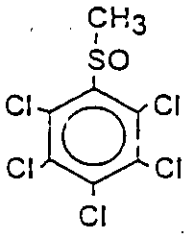
Hexachlorobenzene
(HCB)



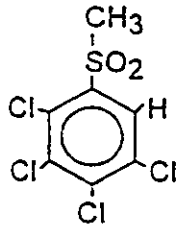
Pentachlorophenol
(PCP)



Pentachlorothioanisole
(PCTA)



Pentachlorothioanisole sulfoxide
(PCTASO)



2,3,4,5-tetrachlorothioanisole sulfone
(TCTASOO)

Figure 1: Molecular structure of PCNB, PCB, PCA, HCB, PCP, PCTA, PCTASO, and TCTASOO

III. Method

A. Chemicals/Supplies

Acetone, residue grade	Baker
1-Decanol	Baker
Diazomethane in diethyl ether solution	Aldrich
Diethyl ether, residue grade	Baker
Dry Ice	Penn State University
n-Hexane, residue grade	Baker
HCB Analytical Standard AC-1194-38C	Uniroyal Chemical Co.
Nitrogen	MG Gases
PCA Analytical Standard AC-1234-4	Uniroyal Chemical Co.
PCB Analytical Standard AC-1166-14	Uniroyal Chemical Co.
PCNB Analytical Standard AC-1261-133	Uniroyal Chemical Co.
PCP Analytical Standard AC-1261-84	Uniroyal Chemical Co.
PCTA Analytical Standard AC-1166-16	Uniroyal Chemical Co.
PCTASO Analytical Standard AGD-1384-005	Uniroyal Chemical Co.
Sodium Hydroxide	Baker
Sulfuric Acid	Baker
TCTASOO Analytical Standard AGD-1384-024	Uniroyal Chemical Co.
Toluene, residue grade	Baker

B. Equipment

Balance	Mettler PE 3000
Centrifuge	Damon/IEC
Centrifuge bottle, teflon 250 ml	Nalgene
Hobart Food Chopper	Hobart Mfg. Co.
Erlenmeyer Flask, 250 ml	Pyrex, Kimax
pH meter	Beckman
Rotary evaporator, Buchi Rotovap	Brinkman
Round bottom flasks, 500 ml	Pyrex, Kimax
Separatory funnel, 250 ml	Nalgene
Standard laboratory equipment:	
beakers, pipets, test tubes etc.	Pyrex, Kimex
TurboVap LV evaporator	Zymark

C. Instrumentation

The gas chromatogragh and integrator models, column type and operating conditions were as follows:

Instrument	Hewlett Packard model 5890 series gas chromatograph
Column	Restek RTX-35, 30 m, 0.53 mm ID, 0.25 um df
Oven	initial temp.100°C , initial time 2 min
	rate A: 5°C/min to 200°C, final time 0 min
	rate B: 20°C/min to 270°C, final time 5 min
Detector	Electron Capture Detector (ECD) temp. 300°C
Injector	Direct Injection temp. 270°C
Carrier Gas Flow	Hydrogen, 10 ml/min
Make-up Flow	Nitrogen, 35 ml/min
Integrator	Shimadzu C-4RA Chromatopac

D. Preparation of Standard and Spiking Solutions

Analytical standards received from the sponsor were used to prepare individual compound stock solutions from which working standard and method day spiking solutions were prepared. Stock solutions of each compound, at a concentration of 1.0 mg/ml, were made by weighing out 10 mg of the analytical standard on an analytical balance, and dissolving it in 10 ml of toluene. The amount of toluene added was corrected considering the percent purity of the standard. For example, if HCB was 99.8% pure then 10 mg would be weighed out and dissolved in 9.98 ml of toluene (10.0 × 0.998). The PCP stock solution was prepared using methanol.

A solution of the combined seven compounds in toluene, at a concentration of 100 µg/ml, was made by adding 2 ml of each of the individual compound stock solutions at 1.0 mg/ml of PCB, HCB, PCNB, PCA, PCTA, TCTASOO and PCTASO, to 6 ml of toluene, so that the final volume was 20 ml. A solution of PCP at 100 µg/ml was made by diluting the 1.0 mg/ml stock solution of PCP ten-fold with methanol.

A Method day spiking solution of the combined seven compounds at a concentration of 10 µg/ml was made by a ten-fold dilution of the 100 µg/ml seven compound solution with toluene. Likewise, PCP day spiking solution at 10 µg/ml was made by a ten-fold dilution with methanol of the 100 µg/ml PCP solution. Fortification of the method day spike samples at a 1 µg level was accomplished by adding 100 µl of the 10 µg/ml spiking solutions to a control sample. Fortification of the method day spike samples at a 10 µg level was done by adding 100 µl of the 100 µg/ml spiking solutions to a control sample.

A 10 µg/ml standard stock solution was made by adding 200 µl of each individual compound stock solutions of PCB, HCB, PCP, PCNB, PCA, PCTA, TCTASOO and PCTASO at 1 mg/ml, and bringing the final volume to 20 ml with toluene. A 1 µg/ml standard stock solution of the combined eight compounds was prepared by diluting the 10 µg/ml standard stock solution ten-fold with toluene. Dilutions of the 10 µg/ml and 1 µg/ml standard stock solutions were made to prepare working 0.100 µg/ml, 0.050 µg/ml, 0.010 µg/ml and 0.003 µg/ml standards.

D. Analytical Procedures

1. Sample Processing

The frozen soil core samples were received in the laboratory. The frozen cores were divided into smaller pieces with a cleaver and rubber mallet, or by other appropriate means. The stones and debris were removed. A Hobart Food Chopper was pre-chilled with dry ice and the frozen soil pieces were put inside. The soil was chopped and homogenized with dry ice. The soil was then placed in sample containers and stored in the freezer where the dry ice was allowed to sublime. The soil samples were kept under freezer conditions (-24°C ± 7°C) until analysis.

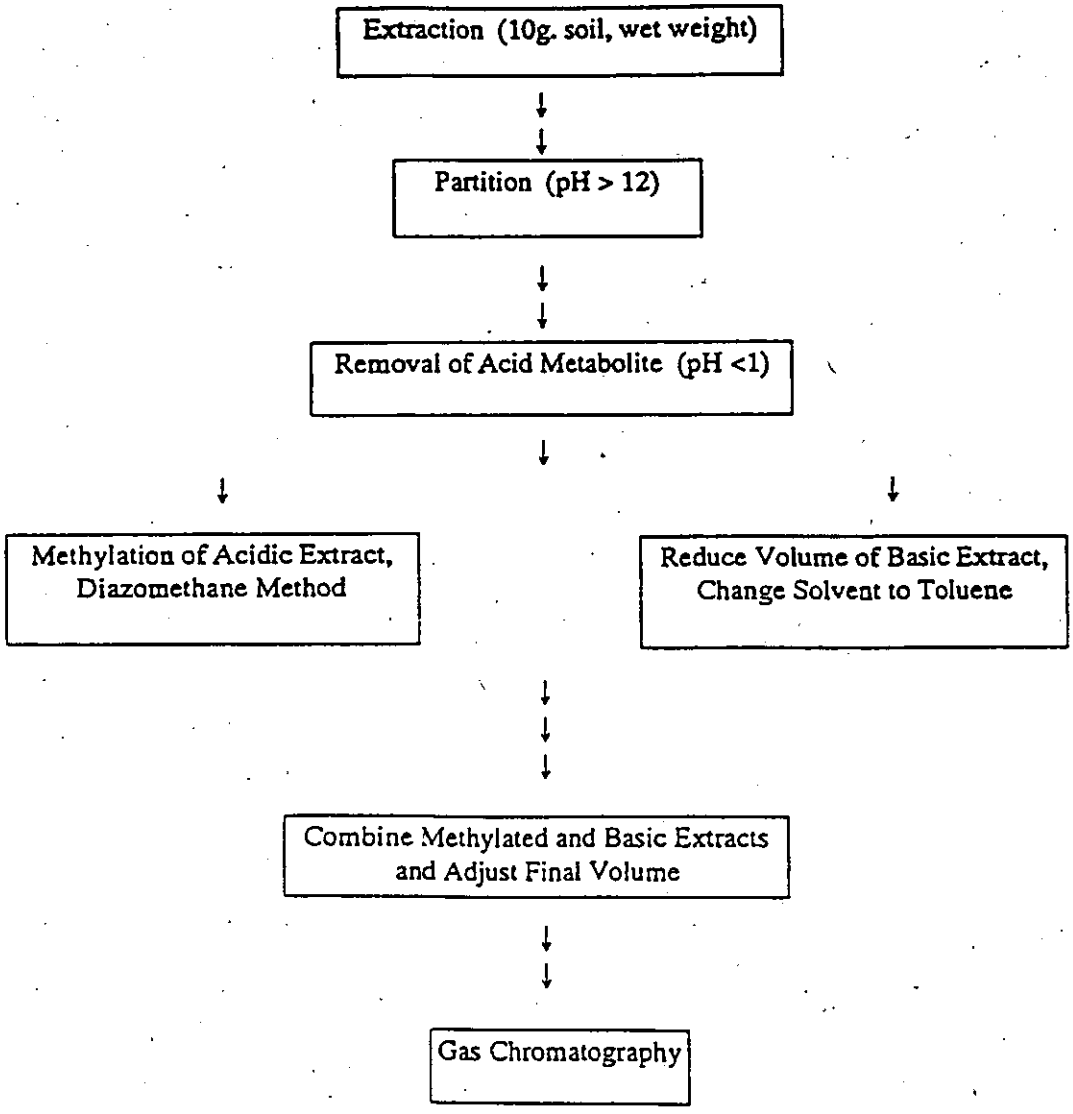
2. Analysis

A flow diagram of the analysis procedure is shown in Figure 2. Detailed explanations of each step are as follows:

Step 1 Extraction (10 g soil, wet weight)

Weigh 10 g of soil in a 250 ml teflon centrifuge bottle. Fortify the two spike samples used to determine extraction method recoveries for the set of samples. Add 100 ml 50:50 v/v acetone:hexane and shake vigorously for 2 min. Centrifuge at 1500 RPM for 5 min. Pour the supernatant into separatory funnel leaving the soil in the bottle. Add another 50 ml of 50:50 v/v acetone:hexane to soil, shake 1 min and centrifuge. Add supernatant to separatory funnel.

Figure 2: PCNB and Metabolites Analysis Method Flowchart



Step 2 Partition

Add 50 ml distilled/deionized water (pH >12, adjusted with 25% NaOH) to separatory funnel and shake 20 sec. Drain the water/acetone layer into a 250 ml beaker and collect the hexane layer in a 250 ml Erlenmeyer flask. Re-extract the water/acetone layer with 50 ml hexane, shaking for 1 min. Drain the water/acetone layer into the 250 ml beaker and add the remaining hexane layer to the flask. The basic extract in the Erlenmeyer flask contains compounds PCB, HCB, PCNB, PCA, PCTA, TCTASOO, and PCTASO.

Step 3 Removal of Acidic Metabolite

Pour the water/acetone portion back into the separatory funnel and add 10 ml 10 N H_2SO_4 to lower the pH < 1. Add 50 ml hexane, shake vigorously for 1 min and drain the water/acetone layer into the beaker. Pour the hexane layer into a 500 ml round bottom flask. Re-extract the water/acetone with another 50 ml hexane, by shaking for 1 min. Drain the water/acetone layer into the beaker and add the remaining hexane layer to the round bottom flask. The acidic extract in the round bottom flask contains compound PCP.

Step 4 Methylation , Diazomethane Method

Add 10 drops of decanol to the acidic extract to prevent the sample from going to dryness during evaporation and reduce the volume to about 5 ml using a rotary evaporator. Transfer this portion of the sample into a methylation vial, rinsing the round bottom flask with hexane. Further reduce the volume of the sample to 0.5 ml using a TurboVap LV evaporator under nitrogen. Add 0.5 ml diazomethane, or enough to turn the sample yellow, let it stand under a hood for 10 minutes. Evaporate off the diazomethane using the TurboVap, reducing the volume again to 0.5 ml.

Step 5 Combine the Extracts and Adjust the Volume

Rinse a round bottom flask with acetone and transfer the basic extract, prepared in step 2, from the Erlenmeyer flask to the round bottom flask. Reduce the volume to about 5 ml using the rotary evaporator, then add 10 ml toluene. Pour the methylated portion of the sample into the round bottom rinsing the vial with 15 ml toluene. Reduce the volume of the combined extracts to about 5 ml with the rotary evaporator, then and bring the final volume up to 10 ml with toluene. The sample is now ready for GC analysis.

Step 6 Gas Chromatography

Inject a 1 µl aliquot of each eight-component standard in the range of 0.003µg/ml to 0.100 µg/ml into the gas chromatograph. Record the resulting peak areas, or peak heights, and plot this data versus concentration (µg/ml) of the corresponding standard to obtain standard calibration curves. Prepare standard curves for each analysis day.

Inject a 1 µl aliquot of the sample into the gas chromatograph. If necessary, dilute the sample with toluene so that the signal response is within the standard curve range. Record peak areas, or peak heights, and determine the concentration of each component compound relative to the standard curves generated for that day.

IV. Method of Calculation

The peak areas corresponding to the eight compounds (PCB, HCB, PCP, PCNB, PCA, PCTA, TCTASOO, and PCTASO) in the standards were obtained from the chromatograms and regressed versus the concentration of the compounds in the standards. Statistics were generated on a Swan Corporation 386/33 computer using Axum program capable of performing quadratic regression (second order polynomial regression) on the peak areas versus their corresponding concentrations to generate standard curves. The following quadratic equation was used:

$$y = b_0 + b_1 x + b_2 x^2$$

A corrected peak area value, if required, was determined using the following formula:

Peak area in sample corrected = Peak area in sample - Peak area in control

The corrected peak area of each sample was used to calculate the amount in ug/ml of each compound found in the samples analyzed relative to the generated standard curves. The square of the correlation coefficient (R^2) was used to evaluate the fit of the curve. The µg/ml compound found value was then multiplied by the final volume of the sample to yield the ug compound found.

µg compound found = [µg/ml compound found] x [final volume (ml)]

The µg compound found values were converted to ppm compound found value by dividing by the sample weight. The ppm compound found value was then divided by ppm compound added to obtain the percent recovery in fortified method spikes.

If the average percent recovery for the two spiked samples of the set was below 100%, the amount of compound found in the sample was divided by the average recovery of the spikes to give the corrected value. No correction was made for average recoveries above 100%.

µg compound found corrected = µg compound found / average spike recovery

The ppm compound found in the samples was calculated using the µg compound found corrected for percent recoveries divided by sample weight.