I. PRINCIPLE OF THE METHOD

The water sample is passed through a C-8 Speedisk™ under vacuum. The pentachloronitrobenzene (PCNB) and its metabolite pentachloroaniline (PCA) (which are retained on the disk) are eluted with toluene and concentrated under nitrogen. The analysis is by capillary GC with an electron capture detector. The quantitation is by external standard method, using a mixture of PCNB and PCA standards to generate the two calibration plots. The LOQ for the method is 0.1 ppb.

II. MATERIALS

A. EQUIPMENT

1. Analytical Evaporator, Model "N-Evap", Organomation Associates, Inc. The evaporator is connected to a house nitrogen line fitted with a filter and a pressure regulator, Balstone Filter Products
2. Balance, analytical, Model 1602MP, Sartorius
3. Vortex mixer, Fisher Scientific
4. Vacuum manifold for SPE disks, 12 port, Supelco
5. Vacuum pump, 1/6 HP, Model 0211-U45N-G8CX, Gast
6. Water purification system, Model Milli-Q, Millipore

Note: Equivalent equipment from other sources can be employed.

B. SUPPLIES

1. Assorted volumetric disposable pipettes, Fisher Scientific
2. Customary analytical laboratory glassware and supplies
3. Filtration suction flask, 0.5L, Fisher Scientific
4. Kolmer evaporation tubes, 10 mL graduated, Fisher Scientific
5. C-8, 50 mm, solid phase extraction disks and reservoirs, Bakerbond Speedisk™, J.T.Baker

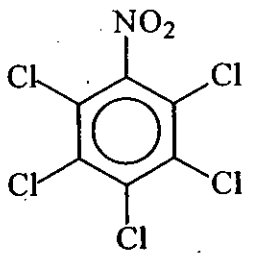
Note: Equivalent supplies from other sources can be employed. It is recommended, however, that SPE disks from J.T. Baker be used.

C. HPLC SOLVENTS

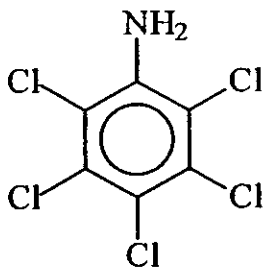
1. Methanol, Fisher Scientific
2. Toluene, Fisher Scientific
3. Water, Milli-Q

Note: HPLC solvents of equivalent purity from other sources can be used.

D. STANDARDS-TEST SUBSTANCES



Chemical name: Pentachloronitrobenzene
Common name: PCNB
CAS number: 82-68-8
Uniroyal Chemical Company Inc. Analytical Standard Code: P-01
Uniroyal Chemical Company Inc. Analytical Standard Lot No.: AC-1398-107
Chemical Purity: 99.8%
Molecular Weight: 295.33
Storage Conditions: Freezer



Chemical Name: Pentachloroaniline
Common Name: None
CAS number: 527-20-8
Uniroyal Chemical Company Inc. Analytical Standard Code: P-02
Uniroyal Chemical Company Inc. Analytical Standard Lot No.: AC-1398-93C
Chemical Purity: 98.7%
Molecular Weight: 265.35
Storage Conditions: Freezer

Standards are archived by the Analytical Services Group of the Crop Protection Division.

E. GC INSTRUMENTATION

E-1 Residue Analysis by GC/Electron Capture Detector

Gas chromatograph: Model HP 6890 equipped with an electron capture detector and an autosampler-injector, Model HP 6763. Instrument parameters and data capture are controlled by a computer with a HP 3365 ChemStation on Windows 95 platform.

Column: DB1701, 30 m X 0.25 mm, 0.05 µm film thickness, J & W Scientific
Carrier Gas: Helium
Makeup Gas: Nitrogen

E-2 Confirmatory Analysis by GC/MSD

Gas chromatograph: Model HP 5890A equipped with a HP Model 5971A mass selective detector and an autosampler-injector, Model HP 6890. Instrument parameters and data capture are controlled by HP Chemstation Software G-1701AA, Rev.A.03.00.

Column: Rtx 200, 30 m X 0.25 mm, 0.05 µm film thickness, Restek Corporation
Carrier Gas: Helium

F. SAFETY AND HEALTH

This method should be performed by trained chemical personnel. Hazards associated with the chemicals used in this analytical method are found in their respective Material Safety Data Sheets (MSDS).

III. ANALYTICAL METHOD

A. WATER SOURCE AND CHARACTERIZATION

This method should be applicable for water from various sources. Pond water from Preston Hill Pond, Middlebury, CT was collected for use in this study. The pond water is characterized by its dissolved organic carbon, pH, and total hardness. The levels of dissolved organic carbon, the pH, and the total hardness of the pond water are shown in Appendix A.

B. PREPARATION OF THE STANDARD SOLUTIONS

B-1a. PCNB Stock Solution

On an analytical balance, accurately weigh approximately 50 mg of PCNB standard into a 50 mL volumetric flask and dilute to volume with toluene. Sonicate the flask. Thus, the concentration of the stock solution is 1 mg/mL. Correct the concentration for the purity of the standard: mg/mL X (percent purity/100).

B-1b. PCA Stock Solution

On an analytical balance, accurately weigh approximately 50 mg of PCA standard into a 50 mL volumetric flask and dilute to volume with toluene. Sonicate the flask. Thus, the concentration of the stock solution is 1 mg/mL. Correct the concentration for the purity of the standard: mg/mL X (percent purity/100).

B-2a. PCNB Standard Working Solution

Pipette 0.5 mL of PCNB Stock solution B-1a into a 50 mL volumetric flask and dilute to volume with toluene. Thus, the concentration of the solution is 10 µg/mL.

B-2b. PCA Standard Working Solution

Pipette 0.5 mL of PCA Stock solution B-1b into a 50 mL volumetric flask and dilute to volume with toluene. Thus, the concentration of the solution is 10 µg/mL.

B-3a. PCNB Fortification Solution for 1 ppb Recoveries

Pipette 1.0 mL of PCNB working solution B-2a into a 10 mL volumetric flask and dilute to volume with methanol. Thus, the concentration of the solution is 1.0 µg/mL.

B-3b. PCA Fortification Solution for 1 ppb Recoveries

Pipette 1.0 mL of PCA working solution B-2b into a 10 mL volumetric flask and dilute to volume with methanol. Thus, the concentration of the solution is 1.0 µg/mL.

B-3c. PCNB Fortification Solution for 0.1 ppb Recoveries

Pipette 1.0 mL of PCNB working solution B-2a into a 10 mL volumetric flask and dilute to volume with methanol. Pipette 1 mL of this 1.0 µg/mL

solution into a 10 mL volumetric flask and dilute to volume with methanol. Thus, the concentration of the final solution is 0.1 µg/mL.

B-3d. PCA Fortification Solution for 0.1 ppb Recoveries

Pipette 1.0 mL of PCA working solution B-2b into a 10 mL volumetric flask and dilute to volume with methanol. Pipette 1 mL of the 1.0 µg/mL solution into a 10 mL volumetric flask and dilute to volume with methanol. Thus, the concentration of the final solution is 0.1 µg/ml.

B-4a. Calibration Plot for PCNB and PCA in the 1.0 ppb Range

Into five 10 mL volumetric flasks, pipette 1.0, 0.75, 0.5, 0.25, and 0.1 mL of PCNB working solution B-2a and PCA working solution C-2b. Dilute to volume with toluene. Thus, the concentration of the resulting standard solutions are 1.0, 0.75, 0.5, 0.25, and 0.1 µg/mL for PCNB and PCA.

B-4b. Calibration Plot for PCNB and PCA in the 0.1 ppb Range

Pipette 1 mL of PCNB standard working solution B-2a into a 10 mL flask and bring to volume with toluene. Pipette 1 mL of PCA standard working solution B-2b into a 10 mL flask and bring to volume with toluene. Into five 10 mL volumetric flasks, pipette 1.0, 0.75, 0.5, 0.25 and 0.1 mL of each of the above PCNB and PCA solutions. Thus, the concentration of the resulting standard solutions are 0.1, 0.075, 0.05, 0.025, and 0.01 µg/mL for PCNB and PCA..

C. FORTIFICATION OF WATER SAMPLES

C-1 Fortification with PCNB and PCA at the 1.0 ppb level

Measure 500 mL of pond water using a 500 mL graduated cylinder. Decant the sample into a l L glass bottle. Using a 500 µL syringe, add 500 µL each of fortification solutions B-3a and B-3b. Cap the bottle and mix well by shaking.

C-2 Fortification with PCNB and PCA at the 0.1 ppb level

Measure 500 mL of pond water using a 500 mL graduated cylinder. Decant the sample into a 1 L glass bottle. Using a 500 µL syringe, add 500 µL each of fortification solutions B-3c and B-3d.

D. EXTRACTION PROCEDURE

1. Place a C-8 Speedisk™ onto a 500 mL vacuum flask attached to a vacuum pump. Pass 10 mL of toluene through the disk under vacuum and continue to pull the vacuum until the disk is dry.
2. Condition the disk with 10 mL of methanol. Allow the methanol to flow through slowly by gravity. Do not allow the disk to dry.
3. When 3-5 mL of methanol remains on the disk, add 10 ml of HPLC grade water.
4. When 3-5 mL of water remains on the disk, begin adding the 500 mL water sample under full vacuum (15 mm Hg). Leave the disk on full vacuum for 5 minutes after the water has passed through the disk. Then release the vacuum.
5. Transfer the disk to a solid phase extraction manifold and continue drying under full vacuum (15 mm Hg) for an additional 30 minutes.
6. With the manifold port now closed, place a 10 mL Kolmer evaporation tube under the port. Add 10 mL of toluene to the disk. Allow the toluene to soak into the disk for 10 minutes, then open the manifold port so the toluene can be eluted by gravity. Apply slight vacuum to elute any remaining analyte.
7. Concentrate the eluant to 1 mL under a gentle stream of nitrogen at about 50°C . Transfer to an autosampler vial for GC-ECD analysis.

E. CONDITIONS FOR GC-ECD ANALYSIS

Injector Temperature:	250°C
Injector Mode:	Splitless
Detector Temperature:	325°C

Oven Temperature Program:

Initial Temperature:	100°C	
Initial Time:	2.0 minutes	
Ramp: (°C/min)	Final Temp (°C)	Final Time (min.)
30.0	225	5.0
15.0	280	1.0

Injection Volume:	1.0 µL
Retention times:	PCA 7.1 min., PCNB 6.5 min.
Run Time:	15.8 minutes
Combined gas flow:	68.3 mL/min. for injector

Injection Sequence:

a. Start the sequence by injecting the solvent blank (toluene), then the standards, starting with the lowest.
b. Inject a solvent blank then a reagent blank followed by another solvent blank.
c. Inject pond water controls, then a solvent blank.
d. Inject the fortified pond water samples, then a solvent blank.
e. Complete by injecting the standards, starting with the lowest.

Note 1: Reagent blank is 500 mL distilled water which is brought through the entire extraction and concentration procedure identically to the samples.
Note 2: The injection sequence was modified after analyzing the 1 ppb pond water samples. PCA and PCNB standards were combined to reduce the total number of injections.

F. CONFIRMATORY TECHNIQUE

F-1 Method of Confirmation

The method for the confirmation of PCNB and PCA was by GC/MSD. Confirmation was made by comparison of the retention times and mass spectra of the peaks in the extracts from both 1.0 ppb and 0.1 ppb fortified pond water samples with the spectra of the standards.

F-2 Conditions for GC/MSD Analysis

Column head pressure:	10 psi
Injector Temperature:	250°C
Injection Volume:	1.0 µL
Oven Temperature Program:	
Initial Temperature:	100°C
Initial Time:	1.0 min.

Ramp: (°C/min.)	Final Temp.(°C)	Final Time(min.)
12.0	280	6.0

Run Time:	22.0 min.
Equilibration Time:	3.0 min.
MSD Operation Parameters:	
Transfer Line:	280°C
Electron Multiplier Range:	~2000 mV
+eV above Autotune:	0
SIM Settings:	
Group III, PCA	m/z 265
Group II, PCNB	m/z 237, 295
Resolution:	Low
Dwell Time :	50
Solvent Delay (min.):	4.0

G. TIME REQUIRED FOR ANALYSIS

A sample set consists of a reagent blank, two control pond water samples, and five fortified pond water samples. The time required to complete the extraction and concentration of a mixture of PCNB and PCA from pond water is about six

hours. The GC/ECD analysis is carried out overnight using an autosampler injector. The data are processed using Microsoft Excel, version 97, software.

H. CALCULATIONS

H-1 Calibration Plot

Peak areas of the standards (PCA or PCNB) are the dependent variables and the concentrations of the standard solutions, expressed as µg/mL, are the independent variables. They are used to generate a linear regression equation to determine the intercept, the slope, and the linearity of the detector response (coefficient of determination) R^2.

$$Peak\ Area = Intercept + Slope\ X\ (\mu g / mL)_{Std.}$$

H-2 Calculate the amount of PCNB or PCA in the toluene extract

Using the peak area of PCNB or PCA found in the extract, determine the concentration using the following equation:

$$PCNB(\mu g / mL) = \frac{(Peak\ Area_{\ sample} - Intercept)}{Slope}$$

H-3 Calculate the amount (µg) of PCNB and PCA found in the sample

Divide the amount of analyte calculated in section H-2 by the analyte volume, expressed in mL. The analyte volume used in this validation is 1 mL.

$$\mu g = \mu g / mL\ X\ Analyte\ Volume(mL)$$

H-4 Calculate the % Recovery of PCA and PCNB from fortified pond water

Divide the value calculated in section H-3, which is the amount of analyte found in the extract, by the amount of analyte added to the fortified pond water and multiply this value by 100.

$$\%\,Recovery = \frac{\mu g\ Found}{\mu g\ Added} X\,100$$

Example of the calculation method:

A water sample was fortified at the 0.1 ppb level with PCNB and PCA and analyzed on 11/01/99, sample PW#3, sample ID sl110115 (Appendix B).

0.05 µg of PCA and 0.051 µg PCNB were added to a 500 mL sample. PW3 peak area was: 11705 for PCA.

From the PCA linear regression analysis: Intercept =-17.2561 and Slope=326799.2

The µg/mL PCA in the analyte was calculated using the equation:

$$Found, \mu g / mL = \frac{(Peak\ Area - Intercept)}{Slope}$$

{11705-(-17.2561)}/326799.2=.03587 µg/mL

The amount (µg) of PCA in the analyte was calculated as:

Volume of the analyte is 1 mL, therefore:

0.03587 µg/mL X 1 mL=0.03587 µg

The amount (µg) of PCA expected in the 1 mL of analyte:

0.05 µg of PCA was added to a 500 mL sample with a final volume of 1 mL, therefore the expected amount is 0.05 µg.

% Recovery:

$$\frac{\mu g\ found}{\mu g\ added} X\ 100 = \%\,\mathrm{Rec.}$$

(0.03587/0.05) X 100=71.74% Recovery