Battelle UK

FINAL REPORT

PCNB and metabolites PCA and PCTA: Independent Laboratory Validation in Soil and Sediment

DATA REQUIREMENT AND TEST GUIDELINES

EC Guidance Document, SANCO/3029/99 rev. 4

EC Guidance Document, SANCO/825/00 rev 8.1

OECD, ENV/JM/MONO(2007)17

EPA, OCSPP 850.6100

ABBREVIATIONS AND SYMBOLS

Abbreviation or symbol	Definition
a.i.	active ingredient
AM	analytical method
AU	absorbance units
C	Celsius or Centigrade
ca.	approximately
CAS	Chemical Abstracts Service
CoA	certificate of analysis
conc	concentration
DALA	days after last application
DBA	days before application
d_f	stationary phase film thickness of capillary GC column
EC	European Commission
EPA	Environmental Protection Agency
EU	European Union
FIFRA	the Federal Insecticide, Fungicide, and Rodenticide Act
GC	gas chromatography
GC-MS	gas chromatography with mass spectrometer
GLP	Good Laboratory Practice(s)
i.d.	internal diameter
ILV	Independent Laboratory Validation
IUPAC	International Union of Pure and Applied Chemistry
LOD	limit of detection
LOQ	limit of quantification
MS	mass spectrometry
m/z	mass to charge ratio
NA	not applicable
ND	no peak detected
ng/mL	nanogram per millilitre
o.d.e	oven dried equivalent
OECD	Organisation for Economic Cooperation and Development
PCNB	Pentachloronitrobenzene
PCTA	Pentachlorothioanisole
PCA	Pentachloroaniline
QAU	quality assurance unit
r	correlation coefficient
RSD	relative standard deviation
RT	retention time
s	second
SD	standard deviation
v/v	volume for volume

2. INTRODUCTION

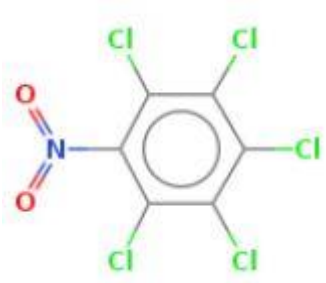
The objective of this study was to independently validate the method described in study 100131902 [1] for the determination of residues of PCNB and metabolites PCTA and PCA in soil and sediment according to OECD, ENV/JM/MONO(2007)17 [2], EC Guidance Document, SANCO/825/00 rev 8.1 [3], EC Guidance Document, SANCO/3029/99 rev.4 [4] and EPA, OCSPP 850.6100 [5].

3. MATERIALS AND METHODS

3.1. Test Items

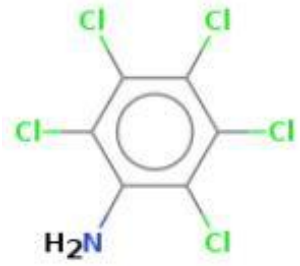
The test items PCNB and metabolites PCTA and PCA were purchased on behalf of the study sponsor by Battelle UK Ltd. The test items were deemed fit for purpose from the beginning to the end of this study following a review of all the characterisation data available for the material, in accordance with the OECD Advisory document 19 on the Management, Characterisation and Use of Test Items [6]. This data included the supplier accreditations, certificates of analyses and safety data sheets. The test item used was characterised at facilities operating under accredited quality standards which are considered a reliable quality standard on which to accept the data.

3.1.1. PCNB

Common name:	Pentachloronitrobenzene (PCNB), Quintozene
Chemical name (IUPAC):	Pentachloronitrobenzene
Chemical name (CAS):	1,2,3,4,5-pentachloro-6-nitrobenzene
CAS registry number:	82-68-8
Molecular formula:	$C_6Cl_5NO_2$
Molecular weight:	295.33
Molecular structure:	
Source:	Sigma-Aldrich
Batch number:	BCBT3735
Physical form/appearance:	Off White Flakes
Purity:	99.7 % (99.7 % purity with < 0.05 % water content)
Expiry date:	30 November 2021
Storage conditions:	Ambient

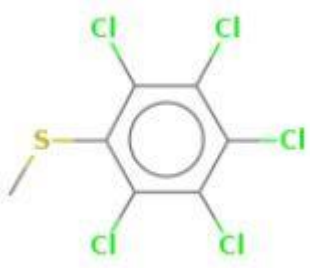
Safety precautions:	Normal handling procedures for pesticides

3.1.2. PCA

Common name:	Pentachloroaniline (PCA)
Chemical name (IUPAC):	2,3,4,5,6-pentachloroaniline
CAS registry number:	527-20-8
Molecular formula:	$C_6H_2Cl_5N$
Molecular weight:	265.35
Molecular structure:	

Source:	Sigma-Aldrich
Batch number:	BCBW9627
Physical form/appearance:	White Powder
Purity:	98.99 % (99.1 % purity - 0.11 % water content)
Expiry date:	31 May 2023
Storage conditions:	Ambient
Safety precautions:	Normal handling procedures for pesticides

3.1.3. PCTA

Common name:	Pentachlorothioanisole (PCTA)
Chemical name (IUPAC):	1,2,3,4,5-pentachloro-6-methylsulfanylbenzene
CAS registry number:	1825-19-0
Molecular formula:	$C_7H_3Cl_5S$
Molecular weight:	296.41
Molecular structure:	

Source:	Chem Service inc.
Batch number:	10830000
Physical form/appearance:	Not recorded
Purity:	99.5 %
Expiry date:	30 June 2022
Storage conditions:	Room Temperature
Safety precautions:	Normal handling procedures for pesticides

3.2. Test System

The soil samples (sandy loam labelled as 18/073 Speyer 2.2 and clay loam labelled as 20/048-Refesol 03-G) and sediment (labelled as 16/002 Swiss Lake sediment) were taken from the Battelle UK stock of control matrices. Characterisation details of the soil samples and sediment are presented in Appendix 3. The soil samples and sediment were stored in a fridge when not in use.

3.3. Method Validation

The determination of PCNB and metabolites PCTA and PCA in soils and sediment were independently validated in terms of linearity, selectivity, accuracy and precision monitoring three ion masses. Additionally, matrix effects were determined.

The control samples were fortified as described in the following table:

Analytes	Matrix	Reagent Blank Replicates	Untreated Control Replicates	Replicates at LOQ Fortification Level (mg/kg)	Replicates at 10xLOQ Fortification Level (mg/kg)
PCNB, PCTA and PCA	Soil (1)	1	2	6 at 0.01	6 at 0.1
	Soil (2)	1	2	6 at 0.01	6 at 0.1
	Sediment	1	2	6 at 0.01	6 at 0.1

LOQ = Limit of Quantification

3.3.1. Linearity

The linearity was investigated with matrix calibration solutions prepared in the range of 3 ng/mL to 120 ng/mL, corresponding to an equivalent sample concentration of 0.003 mg/kg to 0.12 mg/kg.

3.3.2. Specificity

Specificity was confirmed by the use of GC/MS monitoring three ion masses; a highly specific technique. One reagent blank and two untreated replicates were analysed within each batch

to demonstrate that no interferences greater than 30% of the LOQ were present at the retention time of the analyte (except for PCNB ion 293 m/z in sediment where one control was < LOQ).

3.3.3. Accuracy

The accuracy was determined from the analysis of six replicates of fortified control samples at 0.01 mg/kg and at 0.1 mg/kg.

3.3.4. Precision

The precision of the analytical method was determined by measuring the relative standard deviation of the recovery efficiency data at each fortification level.

3.3.5. Limit of Detection

The limit of detection was set at 0.003 mg/kg (30% of the LOQ) for PCNB and metabolites PCTA and PCA in soils and sediment. This was equivalent to the lowest calibration standard.

3.3.6. Matrix Effects

Matrix effects were investigated at the LOQ and 10xLOQ levels by comparing peak areas of solvent standard solutions to peak areas of matrix-matched standard solutions prepared at the same concentration. Experiments assessed whether or not matrix effects were significant (i.e. >20% enhancement or suppression).

3.4. Experimental

The analytical method, consumables and reagents used are presented in Appendix 5.

3.4.1. Principle of the Method

Residues of PCNB and metabolites PCTA and PCA were extracted from two different soils and sediment with acetonitrile by shaking for 15 minutes, followed by addition of QuEChERS extraction salts, shaking for another 15 minutes followed by centrifugation for 10 minutes. An aliquot of 1 ml of acetonitrile was vortexed for 2 minutes in a QuEChERS dSPE tube. A portion of the final extract was taken for final determination by gas chromatography with mass spectrometry (GC-MS), monitoring three ion masses. Full details are presented in Appendix 5.

3.4.2. Preparation of Standards

Standard preparation was performed as detailed in analytical method AM 1 v 01 presented in Appendix 5.

3.4.3. GC-MS Analysis

All samples were analysed by gas chromatography coupled with a single mass spectrometer (GC/MS), monitoring three ion masses. Full details of the analytical conditions are presented in Appendix 5.

3.4.4. Time Required for Analysis

The time required for extraction of 20 samples is approximately 7.5 hours. GC/MS analysis can be performed unattended overnight.

3.4.5. Calibration and Calculation

For each analytical batch and analyte at least seven calibration standards were injected covering the range from 30% of the LOQ to 20% above the highest fortification level. Matrix calibration standards were used for quantification. Linear regression calculation was performed by the OpenLab software, with 1/x weighting, using the analyte concentration for the X-axis, versus the analyte peak area for the Y-axis. In the validation batch, correlation co-efficients, r, were ≥ 0.995 demonstrating acceptable performance in terms of linearity.

The concentration of PCNB and metabolites PCTA and PCA in the final sample extracts were made by comparison to the calibration curve using the OpenLab software.

3.5. Computerised Systems

All computerised systems are detailed in the raw data.

Complex computerised equipment used in the study are:

- OpenLab

Prior to use, all computerised systems used within this study were checked in line with the relevant SOP requirements.

4. RESULTS AND DISCUSSION

The objective of this study was to independently validate the method described in study 100131902 [1] for the determination of residues of PCNB and metabolites PCTA and PCA in soil and sediment according to OECD, ENV/JM/MONO(2007)17 [2], EC Guidance Document, SANCO/825/00 rev 8.1 [3], EC Guidance Document, SANCO/3029/99 rev.4 [4] and EPA, OCSPP 850.6100 [5].

Method of Analysis

Residues of PCNB and metabolites PCTA and PCA were extracted from two different soils and sediment with acetonitrile by shaking for 15 minutes, followed by addition of QuEChERS extraction salts, shaking for another 15 minutes followed by centrifugation for 10 minutes. An aliquot of 1 ml of acetonitrile was vortexed for 2 minutes in a QuEChERS dSPE tube. A portion of the final extract was taken for final determination by gas chromatography with mass spectrometry (GC-MS), monitoring three ion masses. The method was validated in terms of linearity (calibration), specificity (selectivity), accuracy (recovery), precision (repeatability) and matrix effects.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) was 0.003 mg/kg (30% of LOQ) for PCNB and metabolites PCTA and PCA in soil and sediment, equivalent to the lowest calibration standard.

The limit of quantification (LOQ) was established at 0.01 mg/kg for PCNB and metabolites PCTA and PCA in soil and sediment, as confirmed by recovery efficiency testing.

Appendix 4: Calculations

The concentration of PCNB and metabolites PCTA and PCA in final sample extracts were made by comparison to the calibration curve using the OpenLab software.

The concentration in each sample were calculated as follows for each analyte:

$$\text{Residue (mg/kg)} = \frac{(C_a \times V_m \times DF) / CF}{W_s}$$

Where:

- C_a = analyte concentration in the final sample extract (ng/mL)
- V_m = extraction volume (mL)
- DF = dilution factor
- CF = conversion factor (1000)
- W_s = sample weight (g)

Procedural recovery data from fortified samples were calculated via the following equation:

$$\text{Recovery [\%]} = \frac{\text{Amount found (mg/kg)}}{\text{Amount spiked (mg/kg)}} \times 100$$

1. SAMPLE PREPARATION

Prepare soils and sediment samples as per SOP and Study Plan.

2. EXTRACTION METHOD(S)

2.1. Matrix Soil and Sediment/PCNB, PCA, PCTA Extraction [1]

STAGE	DETAILS
EXTRACTION	
1	Weigh 10 g of homogenised sample into a 50 mL centrifuge tube
2	Fortify if necessary
3	Add 10 mL of acetonitrile
4	Mechanically shake for 15 minutes
5	Add contents of Q-Sep QuEChERS extraction salt#
6	Mechanically shake for 15 minutes
7	Centrifuge at 4000 rpm for 10 minutes
8	Aliquot 1 ml of acetonitrile (top) layer into Q-Sep QuEChERS dSPE tube##, cap and vortex for 2 minutes
9	***NB: Further 1 mL aliquots of untreated control samples will be needed for preparing matrix-matched calibration***
10	Centrifuge at 3500 rpm for 5 minutes
ANALYSIS	
11	Transfer a portion of the final extract into an autosampler vial.
12	***NB: Further dilution with an appropriate amount of untreated control samples may be needed if the analytes final concentrations are greater than the highest calibration standard***
13	Matrix matched standards used
14	Analysis by GC-MS

#Q-Sep QuEChERS extraction salt EN 15662 Method (Restek, part number: 25849 is equivalent to part number 26236) – 4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogencitrate sesquihydrate

Q-Sep QuEChERS dSPE tube (Restek, part number: 26242) – 150 mg $MgSO_4$ and 50 mg C18

CRITICAL POINTS	
A	NA

ADDITIONAL INFORMATION	
Analysis Time:	One person can complete the analysis of 20 samples in 1 day, based on a 7.5 hour working day.
Method Stopping Points:	NA
Extract Stability:	Extract stability has been confirmed for 17 days under study ref. [1].
Extract Storage:	Fridge [1].
Sample Stability:	NA
Additional Sample Dilutions	Untreated control samples should be used for any additional sample dilutions are required for samples that are over-range. Full details of any dilutions performed will be included within the raw data.

3. GAS CHROMATOGRAPHY CONDITIONS

GC System:	Agilent 6890 N				
Column:	Agilent HP-5 MS 30 m × 0.25 mm × 0.25 µm or equivalent to DB-5				
Liner (mm)*:	4 mm × 6.5 × 78.5 (Topaz liners splitless, single taper)				
Injection Volume (µL)*:	4				
Inlet Temperature (°C):	250				
Inlet Mode:	Pulsed splitless				
Purge Flow to Split Vent*:	36 mL/min at 1 minute				
Injection Pulse Pressure*:	30 psi until 0.5 minute				
Gas Saver:	20 mL/min after 2 minutes				
Carrier Gas:	Helium				
Mode:	Constant flow				
Flow Rate (mL/min):	1				
Oven:		Rate (°C/min)	Temperature (°C)	Hold Time (min)	Run Time (min)
	Initial	0	120	1	1
	Gradient	15	275	10	21
Equilibration Time:	NA				
Transfer Line Temperature (°C):	300				

* Minor modifications are allowed to optimise instrument performance.

4. MASS SPECTROMETRY CONDITIONS

MS System	5975 MSD
Ionisation Mode:	EI
Electronic Ionisation:	70mV
Scan Type:	SIM
MS Source (°C):	230
MS Quad (°C):	150

Analyte	Transition	SIM ions	Dwell Time (msec)	Expected Retention time (±0.5mins)
PCNB	1#	297	30	7.9
	2#	295	30	
	3#	293	30	
PCA	1#	265	30	8.5
	2#	267	30	
	3#	263	30	
PCTA	1#	296	30	9.2
	2#	294	30	
	3#	246	30	

Transition 1 is to be used for sample quantification, transition 2 and 3 are for confirmation.

5. SYSTEM EQUILIBRATION

A minimum of 3 'prime' injections of the same calibration standard should be injected either at the start of a batch or as a separate equilibration batch to demonstrate acceptable reproducibility. These injections will be labelled as PRIME, the run data will be included within the batch data but is for qualitative purposes only and therefore will not be reported.

6. STOCK SOLUTION PREPARATION

STOCK SOLUTIONS: PCNB, PCA, PCTA	
Preparation:	Weigh in duplicate approximately 5 mg (correct for purity and water content if applicable) of PCNB, PCA, PCTA and dissolve in acetonitrile (approximately 10 mL) to produce a final primary stock concentration of 500 µg/mL.
Labelling:	Label one as '**Calibration standard stock (Cal Stk)**' and the other as '**Fortification standard stock (Fort Stk)**'.
Storage:	Store acetonitrile stock solutions **refrigerated** when not in use and equilibrate to room temperature prior to use.
Stock Stability:	PCNB, PCA, PCTA standards in acetonitrile have been shown to be stable for 56 days [1].

7. CALIBRATION STANDARD PREPARATION – MATRIX MATCHED STANDARDS

Dilute the PCNB, PCA, PCTA calibration standard stock solutions as detailed in the table below:

Working Calibration Solution ID	Calibration Standard Concentration (µg/mL)	Volume Taken (mL)	Volume Added (acetonitrile) (mL)	Final Volume (mL)	Intermediate Standard Concentration (µg/mL)
C01x	500	2 mL of each compound	4.0	10	100
C02x	100	1	9.0	10	10
C03x	100	0.12	9.88	10	1.2
C04x	100	0.1	9.90	10	1
C05x	100	0.060	9.94	10	0.6
C06x	10	0.3	9.70	10	0.3
C07x	10	0.1	9.90	10	0.1
C08x	10	0.060	9.94	10	0.060
C09x	10	0.030	9.97	10	0.030

Prepare fresh on the day of extraction

Where: x = Replicate letter (e.g. C01a (1st preparation), C01b (2nd preparation) e.t.c)

NB: Alternative pro-rata volumes may be taken as long as the final composition remains unchanged.

Use the intermediate standard solution to prepare matrix matched calibration standards as detailed in the table below:

Calibration Standard ID	Intermediate Standard Concentration (µg/mL)	Volume Taken (µL)	Volume of Control Final Extract Added* (µL)	Final Volume (mL)	Calibration Standard Concentration (ng/mL)	Sample Concentration (g/mL)	Equivalent Amount in Sample (mg/kg)
AStd 7x	1.2	30	270	0.3	120	1	0.12
AStd 6x	1.0	30	270	0.3	100	1	0.10
AStd 5x	0.6	30	270	0.3	60	1	0.060
AStd 4x	0.3	30	270	0.3	30	1	0.030
AStd 3x	0.1	30	270	0.3	10	1	0.010
AStd 2x	0.060	30	270	0.3	6	1	0.006
AStd 1x	0.030	30	270	0.3	3	1	0.003

Prepare fresh on the day of extraction

LOQ = 0.01 mg/kg

Where: x = Replicate letter (e.g. AStd 1a (1st preparation), AStd1b (2nd preparation) e.t.c) and A = Matrix type; e.g. S1 = Soil 1, S2 = Soil 2, Sed = Sediment, PS = Pure Solvent

* The control final extracts used should match the matrix being extracted.

NB: Alternative pro-rata volumes may be taken as long as the final composition remains unchanged.

Inject AStd 1x to AStd 8x for run calibration.

8. RECOVERY PREPARATION

Dilute the PCNB, PCA, PCTA **fortification standard stock** solutions as detailed in the table below:

Recovery Working Solution ID	Fortification Standard Stock Concentration (µg/mL)	Volume Taken (mL)	Volume Added (acetonitrile) (mL)	Final Volume (mL)	Fortification Standard Concentration (µg/mL)
R01x	500	0.4 mL of each compound	8.8	10	**20**
R02x	20	1	9.0	10	**2**

Prepare fresh on the day of extraction

Where: x = Replicate letter (e.g. R01a (1st preparation), R01b (2nd preparation) e.t.c)

NB: Alternative pro-rata volumes may be taken as long as the final composition remains unchanged.

Weigh 10 g control matrix and fortify as detailed below:

Recovery ID	Fortification Standard Concentration (µg/mL)	Fortification Volume (mL)	Sample Weight (g)	Fortification Level (mg/kg)	Sample Concentration (g/mL)	Equivalent Standard Concentration (ng/mL)
0.01 X Rec n	2	0.05	10	**0.01**	1	10
0.1 X Rec n	20	0.05	10	**0.1**	1	100

Where: X = Control matrix ID; e.g. S1 = Soil 1, S2 = Soil 2, Sed = Sediment n = Replicate number

LOQ = 0.01 mg/kg (10 ng/mL)

NB: Where samples require additional dilution, prepare a dilution recovery (DIL X Rec n) at the approximate concentration level being analysed and dilute as per the samples (greatest dilution) within each batch. The preparation details should be fully documented within the raw data (the fortification volume should not exceed 0.5 mL).

9. SAMPLE CONCENTRATION CALCULATIONS

Extraction	Sample Weighed (g)	Extraction Volume (mL)	Sample Concentration - End of Extraction (g/mL)
	10	10	1
Clean up step	**Volume Taken (mL)**	**Final Volume (mL)**	**Sample Concentration - Final (g/mL)**
	1	1	1

$$\text{Equivalent Sample Concentration [mg/kg]} = \frac{\text{Sample/Standard Concentration (ng/mL)}}{\text{Sample Concentration (g/mL) x 1000}}$$