Analytical method for PCNB (Pentachloronitrobenzene) and its metabolites PCA (Pentachloroaniline) and PCTA (Pentachlorothioanisole) in soil and sediment

Reports: ECM: EPA MRID No.: 51393701. McInerney, K. 2021. Method Development and Validation of Pentachloronitrobenzene and Two Metabolites in One Sediment and Two Soil Matrices. Report prepared by Battelle, Norwell, Massachusetts, monitored by Winding Trails, LLC, Winston-Salem, North Carolina, and sponsored and submitted by AMVAC Chemical Corporation, Los Angeles, California; 229 pages. Battelle Report No.: 100131902. Final report issued January 19, 2021.

ILV: EPA MRID No.: 51472901. Kurkova, R. 2021. PCNB and metabolites PCA and PCTA: Independent Laboratory Validation in Soil and Sediment. Report prepared by Battelle UK Ltd., Essex, United Kingdom, monitored by Winding Trails, LLC, Winston-Salem, North Carolina, and sponsored and submitted by AMVAC Chemical Corporation, Los Angeles, California; 152 pages. Study No.: DN/21/001. Final report issued March 1, 2021.

Document No.: MRIDs 51393701 & 51472901

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 51393701). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). An Authenticity statement was included with the Quality Assurance statement.

ILV: The study was conducted in compliance with OECD GLP embodied in UK GLP Regulations (1999), as amended by UK GLP (2004) and as accepted by the Regulatory Authorities throughout the European Community, the United States of America, and Japan (p. 3 of MRID 51472901). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). An Authenticity statement was included with the GLP and Quality Assurance statements.

Classification: This analytical method is classified as supplemental. The reported limit of quantitation (LOQ) is the lowest level of method validation (LLMV) rather than a true LOQ. No 10 × LOQ representative chromatograms were provided in the ILV, which is a major deficiency. Representative chromatograms from all fortifications should be provided for review. Representative chromatograms for the reagent blank were not included in the ECM or ILV. Inclusion of these chromatograms would also reduce uncertainty in the method.

PC Code: 056502

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This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Battelle Report No. 100131902, is designed for the quantitative determination of PCNB (pentachloronitrobenzene) and its metabolites PCA (pentachloroaniline) and PCTA (pentachlorothioanisole) in soil and sediment at the stated LOQ of 0.01 mg/kg using GC/MS (see **Table 1**). The method is quantitative for PCNB, PCA, and PTCA at 0.01 mg/kg. The LOQ is lower than the lowest toxicological level of concern in soil and sediment for PCNB, PCA and PCTA. For terrestrial plants, the most sensitive toxicity endpoint available in soil and sediment was determined to be the inhibitory concentration of 5% (IC_{05}) for tomato seedling dry weight (USPEA 2020, DP Barcode 451372). The value of this endpoint is 1.3 mg/kg-soil (2.6 lbs a.i./A).[1] Typically, the most sensitive endpoint is the No Observed Adverse Effect Concentration (NOAEC). In this analysis, however, the NOAEC for the most sensitive dicot endpoint, dry weight, is not reliable because it is above the IC_{25}, and so the IC_{05} was chosen for the listed species assessment to be more protective. In sediment, the most sensitive toxicity endpoint available was determined to be 2.4 mg/kg-sediment. Quantitative endpoints from sediment studies were unable to be determined, but sediment conversion assumptions of 0.04 fraction organic carbon and the mean K_{OC} were applied to the most sensitive water column invertebrate endpoint, the NOAEC of 10.2 ug ai/L from the estuarine/marine life-cycle[2], to calculate the desired endpoint (MRID 50769101). The reported LOQ is the lowest level of method validation (LLMV) rather than a true LOQ. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for PCNB, PCA, and PCTA in the tested soil and sediment matrices (0.01 mg/kg-soil or sediment).

PCNB has been identified as having over 80 degradates in the 2006 EPA Reregistration Eligibility decision (USEPA 2006). This method is sufficient in its analysis of residues of concern (ROCs), as latest draft risk assessment identified parent PCNB and PCA as the major degradates observed in soil and sediment fate studies (USEPA 2021). Other methods are readily available for impurities.

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[1] Unit conversion performed using following equation:
[1 lb a.i./acre] × [1/6 inches] × [1/1.5 kg/L] × [4.54 (10^5) mg/lb] × [3.94 inches/dm] × [2.47 (10^{-6}) acres/dm^2] = [0.5 mg a.i./kg soil]. *I.e.*, assuming a 6-inch soil depth and a 1.5 kg/L soil density, [X lbs a.i./acre] = [0.5X mg a.i./kg soil].

[2] To calculate sediment toxicity endpoint (C_{sed}), determined using the below equation, where the NOAEC= 10.2 µg ai/L, the fraction of organic carbon (f_{OC})= 0.04, and mean KOC= 5901 L/kg-$_{OC}$.
C_{sed} = NOAEC x f_{OC} x K_{OC} x (1 mg/1000 µg)

The ECM validated the method using three uncharacterized matrices described only as a freshwater sediment, a sandy loam soil, and a "high organic content" soil. The ILV validated the method using three characterized matrices: sand sediment, sandy loam soil, and clay loam soil. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method and covered the range of soils used in the unknown PCNB terrestrial field dissipation studies. The ILV validated the method for PCNB, PCA, and PCTA in soil and sediment with minor modifications to the analytical parameters that did not warrant an updated ECM. The ILV noted a software issue with some initial analyses and the number of trials required by the ILV to successfully validate the method was not reported. The ILV reported one failed batch per matrix type; however, for each of these failed batches there were five other corresponding acceptable trials that resulted in a satisfactory mean recovery. The ECM was performed by Battelle, Massachusetts while the and ILV were performed by Battelle UK Ltd., United Kingdom. The studies differed in personnel, equipment, and laboratories, though no statement was explicitly included to specify that the scientists who performed the ECM did not contribute to the ILV considering both studies were performed under Battelle. Additionally, it could not be determined if the ILV was performed independently of the ECM since communications between the ILV and ECM or Sponsor were not summarized or included.

All ILV and ECM data regarding repeatability, accuracy, precision, and linearity were satisfactory for the three ions which were monitored for each analyte, PCNB, PCA, and PCTA, in test soil/sediment matrices. All ECM and ILV data regarding specificity was satisfactory for all analytes in test soil/sediment matrices, except for the ILV data for PCNB in the clay loam soil due to very poor analyte peak resolution. There are some matrix effects at the 263 m/z ion of PCA in sediment, but this data is still acceptable. Additionally, no 10 × LOQ representative chromatograms were provided in the ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide[1]	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Lowest Limit of Method Validation (LLMV)
	Environmental Chemistry Method	Independent Laboratory Validation						
PCNB[1]	51393701[2]	51472901[3]	Sarah Brazeau	Soil/ Sediment	19/01/2021	AMVAC Chemical Corporation	GC/MS	0.01 mg/kg
PCA[1]								
PCTA[1]								

1 PCNB = Pentachloronitrobenzene; PCA = Pentachloroaniline; and PCTA = Pentachlorothioanisole.

2 In the ECM, three matrices were used in the study and described only as a freshwater sediment, a sandy loam soil, and a "high organic" soil (pp. 11, 18-19 of MRID 51393701; "high organic" soil was also called "high organic content" soil). The matrices were reportedly sourced locally to the test facility and the state of Georgia. No soil/sediment characterization was reported. The soil textures could not be verified by the reviewer using USDA-NRCS technical support tools.

3 In the ILV, sand sediment (16/002 Swiss Lake Sediment; 89% sand, 10% silt, 1% clay; pH 6.3 in 1:1 soil:water ratio, pH 5.9 in 0.01M $CaCl_2$; 0.96% organic carbon (Walkley-Black); cation exchange capacity 3.9 meq/100 g), sandy loam soil (18/073 Speyer 2.2; Soil 1; 77% sand, 10% silt, 13% clay; pH 6.1 in 1:1 soil:water ratio, pH 5.8 in 0.01M $CaCl_2$; 1.4% organic carbon (Walkley-Black); cation exchange capacity 7.6 meq/100 g), and clay loam soil (20/048-Refesol 03-G; Soil 2; 28% sand, 39% silt, 33% clay; pH 6.0 in 1:1 soil:water ratio, pH 5.9 in 0.01M $CaCl_2$; 5.1% organic carbon (Walkley-Black); cation exchange capacity 12.8 meq/100 g) were used in the study (pp. 22, 26-27; Appendix 3, pp. 138-142 of MRID 51472901). The matrices were taken from Battelle UK stock of control matrices. The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

I. Principle of the Method

Soil/sediment samples (10 g) were fortified with 50 µL of 1 µg/mL or 10 µg/mL fortification solutions and then extracted with 10 mL of acetonitrile on a GenoGrinder^{TM} for 15 minutes at 1500 rpm (p. 21; Figure 1, p. 51; Appendix 4, p. 228 of MRID 51393701). The sample was combined with one Q-Sep Q110 QuEChERS packet (Restek Part No. 26236) then extracted on a GenoGrinder^{TM} for 15 minutes at 1500 rpm. After centrifugation for 10 minutes at 4000 rpm, a 1-mL aliquot of the acetonitrile layer was transferred to the dispersive solid phase extraction (SPE) tube (Q-Sep QuEChERS d-SPE; Restek Part No. 26242) then shaken for 2 minutes followed by centrifugation at 3500 rpm for 5 minutes. The extract was analyzed by GC/MS. The method noted that matrix-matched calibration samples were also taken through the clean-up steps. Instrumentation and chemicals were described are commercially available, but glassware was not identified.

Samples were analyzed for PCNB, PCA, and PCTA using an Agilent 7890A GC coupled with a 5975C Electronic Ionization MS (SIM; pp. 21-22; Appendix 4, p. 228 of MRID 51393701). The following GC conditions were used: Agilent DB-5 column (30 m × 0.25 mm, 0.25 µm film thickness; injection temperature 250°C), helium carrier gas at 1.0 mL/minute constant flow, temperature program of 120°C hold for 1 minute then ramp 15°C/minute to 275°C hold for 4 minutes, 4 mm focus liner, injection volume of 4.0 µL (pulsed splitless), and MS Source Temperature of 230°C. Monitored ions for each analyte (quantitation, confirmation 1, and confirmation 2, respectively): *m/z* 295, 297, and 293 for PCNB, m/z 265, 267, and 263 for PCA and *m/z* 295, 294, and 246 for PCTA. Reported retention times were *ca*. 9.57, 10.2, and 10.8 minutes for PCNB, PCA, and PCTA, respectively.

The ILV performed the ECM methods as written, except for minor modifications to the analytical parameters (pp. 13, 23, 31, 32; Appendix 5, pp. 144-152 of MRID 51472901). Additionally, Q-Sep QuEChERS packet (Restek Part No. 25849) was used as an equivalency to Q-Sep Q110 QuEChERS packet (Restek Part No. 26236). Samples were analyzed for PCNB, PCA, and PCTA using an Agilent 6890 N GC coupled with 5975 MSD (EI mode; SIM). The GC/MS parameters were similar to those of the ECM, with the exceptions of the use of Agilent HP-5 MS column (30 m × 0.25 mm, 0.25 µm film thickness; injection temperature 250°C) and Topaz liner (4 mm × 6.5 × 78.5). Monitored ions for each analyte (quantitation, confirmation 1, and confirmation 2, respectively): *m/z* **297**, **295**, and 293 for PCNB, m/z 265, 267, and 263 for PCA and *m/z* **296**, 294, and 246 for PCTA (bolded ions differed slightly from that reported in the ECM). Reported retention times were 7.9 ± 0.5, 8.5 ± 0.5, and 9.2 ± 0.5 minutes for PCNB, PCA, and PCTA, respectively. The ILV modifications did not warrant an updated ECM.

The LLMV for PCNB, PCA, and PCTA in soil and sediment was 0.01 mg/kg in both the ECM and the ILV (pp. 11, 20, 24 of MRID 51393701; pp. 13, 23, 25 of MRID 51472901). The Limit of Detection (LOD) for PCNB, PCA, and PCTA in soil and sediment was set to 0.003 mg/kg in both the ECM and the ILV. The determination of the LOD was not discussed and is therefore a deficiency. As previously discussed, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. Though not a true LOQ, the LLMV is acceptable because reproducible recovery and reliable measurements are shown in the ECM and ILV at both the LLMV and 10xLLMV.

II. Recovery Findings

ECM (MRID 51393701): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of PCNB and its metabolites PCA and PCTA at fortification levels of 0.01 mg/kg (LLMV) and 0.1 mg/kg (10×LLMV) in one sediment matrix and two soil matrices (**Tables 3-11**, pp. 29-37). Three ions were monitored; performance data was comparable between the quantitation and confirmation analyses. Methods were quantitative for each analyte in all matrices. Results are shown in **Table 2**. While within guidelines, RSDs were generally higher in the 10×LLMV fortification and recoveries of the 10×LLMV fortification in the "high organic content" soil were significantly lower than recoveries of the LLMV fortification. Nominal fortification levels reported. Actual fortification levels ranged 0.00984-0.0103 mg/kg and 0.0983-0.102 mg/kg for the LLMV and 10×LLMV fortification levels, respectively (see Reviewer's Comment #9). Three matrices were used in the study and described only as a freshwater sediment, a sandy loam soil, and a "high organic" soil (pp. 11, 18-19; "high organic" soil was also called "high organic content" soil). The matrices were reportedly sourced locally to the test facility and the state of Georgia. No soil/sediment characterization was reported, which is a deficiency. The soil textures could not be verified by the reviewer using USDA-NRCS technical support tools.

ILV (MRID 51472901): Mean recoveries and RSDs were within guidelines for analysis of PCNB and its metabolites PCA and PCTA at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10×LOQ) in one sediment matrix and two soil matrices (pp. 26-30; **Tables 1-27**, pp. 35-61). Parent and two fragment ions were monitored; performance data was comparable between the quantitation and confirmation analyses. Three ions were monitored; performance data was comparable between the quantitation and confirmation analyses. Methods were quantitative for each analyte in all matrices. Results are shown in **Table 3**. The reviewer noted that RSDs were better in recoveries from the 10×LLMV fortification versus the LLMV fortification. The sand sediment (16/002 Swiss Lake Sediment; 89% sand, 10% silt, 1% clay; pH 6.3 in 1:1 soil:water ratio, pH 5.9 in 0.01M $CaCl_2$; 0.96% organic carbon (Walkley-Black); cation exchange capacity 3.9 meq/100 g), sandy loam soil (18/073 Speyer 2.2; Soil 1; 77% sand, 10% silt, 13% clay; pH 6.1 in 1:1 soil:water ratio, pH 5.8 in 0.01M $CaCl_2$; 1.4% organic carbon (Walkley-Black); cation exchange capacity 7.6 meq/100 g), and clay loam soil (20/048-Refesol 03-G; Soil 2; 28% sand, 39% silt, 33% clay; pH 6.0 in 1:1 soil:water ratio, pH 5.9 in 0.01M $CaCl_2$; 5.1% organic carbon (Walkley-Black); cation exchange capacity 12.8 meq/100 g) were used in the study (pp. 22, 26-27; Appendix 3, pp. 138-142). The matrices were taken from Battelle UK stock of control matrices. The soil textures were verified by the reviewer using USDA-NRCS technical support tools. The method for PCNB, PCA, and PCTA in soil and sediment was validated with minor modifications to the analytical parameters, as well as the use of an equivalency for the Q-Sep Q110 QuEChERS packet (pp. 13, 23; Appendix 5, pp. 144-152). The ILV modifications did not warrant an updated ECM. The number of trials required by the ILV to successfully validate the method was not reported and difficult to interpret based on the data provided (pp. 31-32). The ILV reported at least one failed batch each for the validation in each matrix type.

Table 2. Initial Validation Method Recoveries for PCNB and its metabolites PCA and PCTA in Soil and Sediment[1,2]

Analyte	Fortification Level (mg/kg)[3]	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Sandy Loam Soil					
	Quantitation ion					
PCNB	0.01 (LOQ)	7	75-98	83	8.13	9.8
	0.1	7	72-110	85	14.51	17.0
PCA	0.01 (LOQ)	7	71-82	74	3.31	4.5
	0.1	7	71-110	85	14.27	16.8
PCTA	0.01 (LOQ)	7	70-79	73	2.73	3.7
	0.1	7	71-105	82	12.92	15.8
	Confirmation ion 1					
PCNB	0.01 (LOQ)	7	73-94	82	6.18	7.6
	0.1	7	71-108	84	13.98	16.6
PCA	0.01 (LOQ)	7	74-88	78	5.55	7.1
	0.1	7	72-111	86	14.61	17.0
PCTA	0.01 (LOQ)	7	70-81	75	3.70	5.0
	0.1	7	70-104	82	12.37	15.2
	Confirmation ion 2					
PCNB	0.01 (LOQ)	7	75-89	81	5.12	6.3
	0.1	7	72-112	87	14.78	17.1
PCA	0.01 (LOQ)	7	71-81	74	3.0	4.0
	0.1	7	73-113	88	14.69	16.8
PCTA	0.01 (LOQ)	7	72-98	79	8.75	11.0
	0.1	7	70-103	81	12.21	15.0
	"High Organic Content" Soil					
	Quantitation ion					
PCNB	0.01 (LOQ)	7	95-108	101	4.69	4.6
	0.1	7	74-83	79	2.97	3.8
PCA	0.01 (LOQ)	7	80-111	88	10.01	11.4
	0.1	7	70-73	72	1.18	1.7
PCTA	0.01 (LOQ)	7	74-93	82	5.15	6.3
	0.1	7	71-72	72	0.49	0.69
	Confirmation ion 1					
PCNB	0.01 (LOQ)	7	86-112	98	9.87	10.0
	0.1	7	73-84	79	3.37	4.3
PCA	0.01 (LOQ)	7	81-111	91	9.54	10.5
	0.1	7	71-76	73	1.93	2.6
PCTA	0.01 (LOQ)	7	74-96	81	6.85	8.5
	0.1	7	71-73	71	0.70	0.98

	Confirmation ion 2					
PCNB	0.01 (LOQ)	7	93-108	103	4.82	4.7
	0.1	7	73-87	80	4.66	5.8
PCA	0.01 (LOQ)	7	84-116	97	9.29	9.6
	0.1	7	70-72	71	0.93	1.3
PCTA	0.01 (LOQ)	7	85-102	93	6.39	6.9
	0.1	7	72-76	74	1.25	1.7
	Freshwater Sediment					
	Quantitation ion					
PCNB	0.01 (LOQ)	7	73-87	80	4.19	5.2
	0.1	7	75-103	87	9.99	11.5
PCA	0.01 (LOQ)	7	71-91	79	6.76	8.5
	0.1	7	82-114	95	11.41	12.0
PCTA	0.01 (LOQ)	7	71-94	78	7.88	10.1
	0.1	7	76-99	87	8.87	10.2
	Confirmation ion 1					
PCNB	0.01 (LOQ)	7	75-83	78	2.66	3.4
	0.1	7	73-99	84	9.85	11.7
PCA	0.01 (LOQ)	7	72-102	85	10.45	12.2
	0.1	7	84-114	97	10.92	11.2
PCTA	0.01 (LOQ)	7	71-92	78	7.67	9.8
	0.1	7	75-102	87	9.80	11.3
	Confirmation ion 2					
PCNB	0.01 (LOQ)	7	76-99	85	8.42	10.0
	0.1	7	77-104	87	10.32	11.9
PCA	0.01 (LOQ)	7	73-92	79	6.43	8.1
	0.1	7	83-108	95	10.02	10.6
PCTA	0.01 (LOQ)	7	75-102	81	8.87	10.9
	0.1	7	75-100	88	9.30	10.6

Data (uncorrected recovery results; pp. 22-23) were obtained from Tables 3-11, pp. 29-37 of MRID 51393701.

1 Three matrices were used in the study and described only as a freshwater sediment, a sandy loam soil, and a “high organic” soil (pp. 11, 18-19; “high organic” soil was also called “high organic content” soil). The matrices were reportedly sourced locally to the test facility and the state of Georgia. No soil/sediment characterization was reported. The soil textures could not be verified by the reviewer using USDA-NRCS technical support tools.

2 Monitored ions for each analyte (quantitation, confirmation 1, and confirmation 2, respectively): *m/z* 295, 297, and 293 for PCNB, m/z 265, 267, and 263 for PCA and *m/z* 295, 294, and 246 for PCTA.

3 Nominal fortification levels reported. Actual fortification levels ranged 0.00984-0.0103 mg/kg and 0.0983-0.102 mg/kg for the LOQ and 10×LOQ fortification levels, respectively (see Reviewer’s Comment #9; Tables 3-11, pp. 29-37).

Table 3. Independent Validation Method Recoveries for PCNB and its metabolites PCA and PCTA in Soil and Sediment[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	Sandy Loam Soil (Soil 1)					
	Quantitation ion					
PCNB	0.01 (LOQ)	6	83-92	88	4	4.1
	0.1	6	81-91	88	4	4.3
PCA	0.01 (LOQ)	6	84-96	90	5	5.5
	0.1	6	87-88	88	1	0.6
PCTA	0.01 (LOQ)	6	83-90	86	2	2.8
	0.1	6	82-85	84	1	1.2
	Confirmation ion 1					
PCNB	0.01 (LOQ)	6	**68**-83	78	6	7.2
	0.1	6	84-87	85	1	1.5
PCA	0.01 (LOQ)	6	89-99	94	3	3.6
	0.1	6	86-88	87	1	0.9
PCTA	0.01 (LOQ)	6	83-87	85	2	1.9
	0.1	6	82-85	84	1	1.2
	Confirmation ion 2					
PCNB	0.01 (LOQ)	6	**61**-91	83	11	13
	0.1	6	82-90	87	3	3.4
PCA	0.01 (LOQ)	6	86-90	88	2	1.9
	0.1	6	86-89	88	1	1.2
PCTA	0.01 (LOQ)	6	71-83	78	5	6.8
	0.1	6	82-84	83	1	1.0
	Clay Loam Soil (Soil 2)					
	Quantitation ion					
PCNB	0.01 (LOQ)	6	75-105	87	11	12
	0.1	6	84-92	88	3	3.3
PCA	0.01 (LOQ)	6	87-94	91	2	2.7
	0.1	6	87-91	89	1	1.6
PCTA	0.01 (LOQ)	6	81-90	84	3	4.1
	0.1	6	79-84	81	2	2.1
	Confirmation ion 1					
PCNB	0.01 (LOQ)	6	92-110	102	6	5.9
	0.1	6	85-90	87	2	2.1
PCA	0.01 (LOQ)	6	82-109	100	10	10
	0.1	6	87-92	89	2	1.8
PCTA	0.01 (LOQ)	6	78-85	82	3	3.7
	0.1	6	80-84	81	2	1.9

	Confirmation ion 2					
PCNB	0.01 (LOQ)	6	87-**127**	100	14	14
	0.1	6	85-90	87	2	2.4
PCA	0.01 (LOQ)	6	88-101	93	6	6.3
	0.1	6	86-92	89	2	2.2
PCTA	0.01 (LOQ)	6	82-93	86	4	4.8
	0.1	6	80-85	82	2	2.3
	Sand Sediment (Swiss Lake)					
	Quantitation ion					
PCNB	0.01 (LOQ)	6	81-102	93	8	8.8
	0.1	6	84-87	86	1	1.4
PCA	0.01 (LOQ)	6	88-97	93	3	3.4
	0.1	6	85-86	86	1	0.6
PCTA	0.01 (LOQ)	6	80-88	84	3	3.4
	0.1	6	78-80	79	1	1.0
	Confirmation ion 1					
PCNB	0.01 (LOQ)	6	76-97	87	9	11
	0.1	6	83-87	85	2	2.2
PCA	0.01 (LOQ)	6	84-94	90	4	4.2
	0.1	6	84-88	86	1	1.6
PCTA	0.01 (LOQ)	6	**69**-90	81	7	8.4
	0.1	6	79-81	80	1	0.8
	Confirmation ion 2					
PCNB	0.01 (LOQ)	6	88-115	101	11	11
	0.1	6	82-88	86	2	2.8
PCA	0.01 (LOQ)	6	74-97	85	8	9.4
	0.1	6	84-87	86	1	1.4
PCTA	0.01 (LOQ)	6	74-87	79	5	6.2
	0.1	6	78-90	79	1	1.2

Data (uncorrected recovery results; Appendix 4, p. 143) were obtained from pp. 26-30; Tables 1-27, pp. 35-61 of MRID 51472901 and DER Excel Attachment.

Bolded values are outside of acceptable limits for the range of recoveries per OCSPP 850.6100 guidelines.

1 The sand sediment (16/002 Swiss Lake Sediment; 89% sand, 10% silt, 1% clay; pH 6.3 in 1:1 soil:water ratio, pH 5.9 in 0.01M $CaCl_2$; 0.96% organic carbon (Walkley-Black); cation exchange capacity 3.9 meq/100 g), sandy loam soil (18/073 Speyer 2.2; Soil 1; 77% sand, 10% silt, 13% clay; pH 6.1 in 1:1 soil:water ratio, pH 5.8 in 0.01M $CaCl_2$; 1.4% organic carbon (Walkley-Black); cation exchange capacity 7.6 meq/100 g), and clay loam soil (20/048-Refesol 03-G; Soil 2; 28% sand, 39% silt, 33% clay; pH 6.0 in 1:1 soil:water ratio, pH 5.9 in 0.01M $CaCl_2$; 5.1% organic carbon (Walkley-Black); cation exchange capacity 12.8 meq/100 g) were used in the study (pp. 22, 26-27; Appendix 3, pp. 138-142). The matrices were taken from Battelle UK stock of control matrices. The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

2 Monitored ions for each analyte (quantitation, confirmation 1, and confirmation 2, respectively): *m/z* **297**, **295**, and 293 for PCNB, m/z 265, 267, and 263 for PCA and *m/z* **296**, 294, and 246 for PCTA (bolded ions differed slightly from that reported in the ECM).

3 Standard deviations were reviewer-calculated since these values were not reported in the study report (DER Excel Attachment). Rules of significant figures were followed.

III. Method Characteristics

As discussed above, the LOQ was established as the LLMV, and in both the ECM and ILV, this value is 0.01 mg/kg for all three analytes in all matrices (pp. 11, 20, 24 of MRID 51393701; pp. 13, 23, 25 of MRID 51472901). The LOD for PCNB, PCA, and PCTA in soil and sediment was set to 0.003 mg/kg in the ECM and the ILV. In both method validations, the LOD was reportedly equivalent to the lowest calibration standard. In the ILV, the LOD value was defined as 30% of the LOQ. No calculations or comparisons to background noise were reported for LOQ or LOD in the ECM or ILV.

Satisfactory repeatability, accuracy, precision, and linearity was achieved for the three ions monitored for each analyte, PCNB, PCA, and PCTA, in test soil/sediment matrices. Specificity was mostly satisfactory, with the exception the ILV study of PCNB in clay loam soil (soil 2). For linearity, the method is satisfactory, with the correlation coefficient (r) ≥ 0.995 in all evaluations. More details are provided in **Table 4**.

Table 4. Method Characteristics in Soil and Sediment

		PCNB	PCA	PCTA
Limit of Quantitation (LOQ)*	ECM	0.01 mg/kg		
	ILV			
Limit of Detection (LOD)	ECM	0.003 mg/kg (30% of the LOQ)		
	ILV			
Linearity (calibration curve r and concentration range) [1]	ECM[2]	r = 0.9960 (Q, SL) r = 0.9955 (C1, SL) r = 0.9955 (C2, SL) r = 0.9965 (Q, HOC) r = 0.9970 (C1, HOC) r = 0.9960 (C2, HOC) r = 0.9955 (Q, FSD) r = 0.9985 (C1, FSD) r = 0.9975 (C2, FSD)	r = 0.9960 (Q, SL) r = 0.9955 (C1, SL) r = 0.9955 (C2, SL) r = 0.9955 (Q, HOC) r = 0.9970 (C1, HOC) r = 0.9965 (C2, HOC) r = 0.9960 (Q, FSD) r = 0.9980 (C1, FSD) r = 0.9965 (C2, FSD)	r = 0.9509 (Q, SL) r = 0.9970 (C1, SL) r = 0.9965 (C2, SL) r = 0.9970 (Q, HOC) r = 0.9970 (C1, HOC) r = 0.9965 (C2, HOC) r = 0.9975 (Q, FSD) r = 0.9960 (C1, FSD) r = 0.9960 (C2, FSD)
	ILV	r = 0.9993 (Q, SL) r = 0.9990 (C1, SL) r = 0.9983 (C2, SL) r = 0.9984 (Q, CL) r = 0.9992 (C1, CL) r = 0.9995 (C2, CL) r = 0.9995 (Q, SSD) r = 0.9995 (C1, SSD) r = 0.9994 (C2, SSD)	r = 0.9998 (Q, SL) r = 0.9995 (C1, SL) r = 0.9998 (C2, SL) r = 0.9994 (Q, CL) r = 0.9979 (C1, CL) r = 0.9995 (C2, CL) r = 0.9997 (Q, SSD) r = 0.9992 (C1, SSD) r = 0.9991 (C2, SSD)	r = 0.9999 (Q, SL) r = 0.9998 (C1, SL) r = 0.9998 (C2, SL) r = 0.9996 (Q, CL) r = 0.9995 (C1, CL) r = 0.9994 (C2, CL) r = 0.9995 (Q, SSD) r = 0.9994 (C1, SSD) r = 0.9999 (C2, SSD)
		3.0-120 ng/mL (0.0030-0.120 mg/kg in samples)		
Repeatable	ECM[3]	Yes at LLMV (0.01 mg/kg) and 10×LLMV (0.1 mg/kg) [two uncharacterized soil matrices (reported as sandy loam and "high organic content" soils) and one uncharacterized sediment matrix (reported as freshwater sediment)]		
	ILV[4,5]	Yes at LLMV (0.01 mg/kg) and 10×LLMV (0.1 mg/kg) [two characterized soil matrices (sandy loam and clay loam) and one characterized sediment matrix (sand sediment)]		
Reproducible		Yes for 0.01 mg/kg (LLMV)* and 0.1 mg/kg in soil and sediment matrices		
Specific	ECM	Yes, no matrix interferences were identified. Matrix interferences were reported as <30% of the LOQ.		
	ILV	Some non-uniform peak integration was noted. Baseline noise/nearby contaminants were significant compared to LOQ peak. **No** 10×LOQ representative chromatograms were provided.		
		Yes for sandy loam and sand sediment, matrix interferences were reported as <30% of the LOQ. **No** for clay loam soil due to poor analyte peak resolution.[6] Matrix interferences were reported as <30% of the LLMV	Yes, matrix interferences were reported as <30% of the LLMV.	

Data were obtained from pp. 11, 20, 24 (LOQ/LOD); Tables 3-11, pp. 29-37 (recovery results); Figures 6-8, pp. 64-188 (chromatograms); p. 19; Figures 9-17, pp. 189-215 (calibration curves) of MRID 51393701; pp. 13, 23, 25 (LOQ/LOD); pp. 26-30; Tables 1-27, pp. 35-61 (recovery results); p. 25; Figures 28-36, pp. 93-119 (calibration curves); Figures 1-27, pp. 66-92 (chromatograms) of MRID 51472901; DER Excel Attachment. Q = quantitation ion; C1 = confirmation ion 1; C2 = confirmation ion 2; SL = Sandy Loam Soil; HOC = "High Organic Content" Soil; FSD = Freshwater Sediment; CL = Clay Loam Soil; SSD = Sand Sediment.

* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 Matrix-matched calibration standards were used in the ECM and ILV (p. 23 of MRID 51472901; pp. 30-32 of MRID 51472901).

2 Reported ECM correlation coefficients (r values) were reviewer-calculated from r^2 values reported in the study report since r values were not reported (see DER Excel Attachment; Figures 9-17, pp. 189-215 of MRID 51393701). The r values were reported to 4 significant figures even though the r^2 values were only reported to 3 significant figures.

3 In the ECM, three matrices were used in the study and described only as a freshwater sediment, a sandy loam soil, and a "high organic" soil (pp. 11, 18-19 of MRID 51393701; "high organic" soil was also called "high organic content" soil). The matrices were reportedly sourced locally to the test facility and the state of Georgia. No soil/sediment characterization was reported. The soil textures could not be verified by the reviewer using USDA-NRCS technical support tools.

4 In the ILV, sand sediment (16/002 Swiss Lake Sediment; 89% sand, 10% silt, 1% clay; pH 6.3 in 1:1 soil:water ratio, pH 5.9 in 0.01M $CaCl_2$; 0.96% organic carbon (Walkley-Black); cation exchange capacity 3.9 meq/100 g), sandy loam soil (18/073 Speyer 2.2; Soil 1; 77% sand, 10% silt, 13% clay; pH 6.1 in 1:1 soil:water ratio, pH 5.8 in 0.01M $CaCl_2$; 1.4% organic carbon (Walkley-Black); cation exchange capacity 7.6 meq/100 g), and clay loam soil (20/048-Refesol 03-G; Soil 2; 28% sand, 39% silt, 33% clay; pH 6.0 in 1:1 soil:water ratio, pH 5.9 in 0.01M $CaCl_2$; 5.1% organic carbon (Walkley-Black); cation exchange capacity 12.8 meq/100 g) were used in the study (pp. 22, 26-27; Appendix 3, pp. 138-142 of MRID 51472901). The matrices were taken from Battelle UK stock of control matrices. The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

5 The ILV validated the method for PCNB, PCA, and PCTA in soil and sediment with minor modifications to the analytical parameters, as well as the use of an equivalency for the Q-Sep Q110 QuEChERS packet (pp. 13, 23; Appendix 5, pp. 144-152 of MRID 51472901). The ILV modifications did not warrant an updated ECM. The number of trials required by the ILV to successfully validate the method was not reported and difficult to interpret based on the data provided (pp. 31-32). The ILV reported at least one failed batch each for the validation in sediment and soil 2 (clay loam soil).

6 PCNB analyte peak was composed of multiple peaks, see Figures 10-12, pp. 75-77 of MRID 51472901. PCNB analyte peak was most irregular in Soil 2 (clay loam) of the three tested matrices.

IV. Method Deficiencies and Reviewer's Comments

1. The reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ (pp. 11, 20, 24 of MRID 51393701; pp. 13, 23, 25 of MRID 51472901). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for PCNB, PCA, and PCTA in the tested soil and sediment matrices (0.01 mg/kg). Evaluations are reproducible at the LLMV, and therefore this work is acceptable.

2. The ECM (Battelle, Massachusetts) and ILV (Battelle UK Ltd., United Kingdom) were part of the same company, but the studies were performed at different laboratories with different personnel and equipment (pp. 1, 5-6; Appendix 4, p. 228 of MRID 51393701; pp. 1, 5-6; Appendix 5, pp. 144-152 of MRID 51472901). Rodney M. Bennett of Winding Trials, LLC, was the Study Monitor for the ECM (Battelle) and the ILV (Battelle UK Ltd.; p. 5 of MRID 51393701; p. 5 of MRID 51472901). A summary of communications was not included in the ILV.

3. There were some noted failures on some trials that were attributed to software failures or a batch run failure. However, subsequent trials showed acceptable results. The ILV reported at least one failed batch each for the validation each matrix. Despite these failed batches, acceptable sample sets were included (5 or more repetitions at the LOQ and 10 x LOQ) at a maximum of three sample sets (2 soils, 1 sediment) per OCSPP 850.6100 guidance.

4. It could not be determined if the ILV soil and sediment matrices covered the range of soils used in the PCNB terrestrial field dissipation studies since no PCNB terrestrial field dissipation studies were submitted for review. In the ILV, sand sediment [1% clay; 0.96% organic carbon (Walkley-Black)], sandy loam soil [13% clay; 1.4% organic carbon (Walkley-Black)], and clay loam soil [33% clay; 5.1% organic carbon (Walkley-Black)] were used (pp. 22, 26-27; Appendix 3, pp. 138-142 of MRID 51472901).

5. In the submitted PCNB foliar field dissipation study MRID 51247501, the amount of residue and dissipation of pentachloronitrobenzene (PCNB) and its metabolites pentachloroaniline (PCA) and pentachlorothioanisole (PCTA) on leaf surfaces was examined under US field conditions in cropped potato plots at two sites near Yuma, Arizona and Ephrata, Washington. The soils in the test sites were characterized as clay loam (38% clay, 0.54% total organic carbon) and loamy sand (5% clay, 0.52% total organic carbon); however, the dissipation of the residues was not studied in the soils (p. 19; Appendix 1, pp. 36-43; and Appendix 4, p. 134 of MRID 51247501).

6. The three ECM matrices were not characterized and described only as a freshwater sediment, a sandy loam soil, and a “high organic” soil (pp. 11, 18-19 of MRID 51393701; “high organic” soil was also called “high organic content” soil). This lack of characterization is a deficiency. The soil textures could not be verified by the reviewer using USDA-NRCS technical support tools. OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs.

7. One peak (PCNB in clay loam in ILV) had some poor resolution; however, the measured concentrations were repeatable and quantifiable (Figures 10-12, pp. 75-77 of MRID 51472901). The PCNB analyte peak was composed of multiple peaks. Minor irregularities of the PCNB analyte peak were noted in the other two matrices, but the PCNB analyte peak was most irregular in Soil 2 (clay loam) of the three tested matrices.

8. No 10 × LLMV representative chromatograms were provided in the ILV, which is a major deficiency. Representative chromatograms from all fortifications should be provided for review. Representative chromatograms for the reagent blank were not included in the ECM or ILV. Inclusion of these chromatograms would reduce uncertainty in the method.

   The reviewer assumed that the ECM recovery and fortification columns for the 10×LOQ fortification of PCA (*m/z* 265) in sandy loam soil were switched (**Table 4**, p. 30 of MRID 51393701). Other following typographical errors were noted by the reviewer: 1) the 10×LOQ fortification was reported as 0.01 mg/kg, instead of 0.1 mg/kg in Figure 6, p. 99; and 2) the highest calibration level (Calibration Level 7) was reported as 1,200 ng/mL (equivalent to 1.2 mg/kg sample concentration) instead of 120 ng/mL (equivalent to 0.12 mg/kg sample concentration) in Figure 6, p. 82 and Figure 8, p. 165.

9. The determination of the LOD in the ECM and ILV was not based on procedures as defined in 40 CFR Part 136 (pp. 11, 20, 24 of MRID 51393701; pp. 13, 23, 25 of MRID 51472901). In the ECM and ILV, the LOD was reportedly equivalent to the lowest calibration standard. In the ILV, the LOD value was defined as 30% of the LOQ. No calculations or comparisons to background noise were reported for LOQ or LOD in the ECM or ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

10. Significant (>20%) matrix effects for some ions were reported in the ECM (p. 23; **Table 2**, pp. 26-28 of MRID 51472901; pp. 30-32 of MRID 51472901). Matrix-matched calibration standards were used in the ECM and ILV. While there may have been some resolution and matrix effects, they were not enough to compromise the method.

11. In the ECM, stability of PCNB, PCA, and PCTA extracts was reported as 17 days at <6°C but above freezing (pp. 20, 23; **Tables 12-18**, pp. 38-50 of MRID 51393701). Stability of PCNB, PCA, and PCTA acetonitrile solutions was reported as up to 56 days at <6°C but above freezing. Stability of the matrix-matched calibrants was also shown for storage after 16 and 30 days. The ILV used the stability data of the ECM to determine that the sediment samples could be re-vialed for re-analysis (B4RB; p. 32 of MRID 51472901).

The analysis time required to complete one batch of 20 samples was reported as *ca*. 7.5 working hours in the ILV (p. 24; Appendix 5, p. 146 of MRID 51472901). Total time required to complete one batch of 17 samples was reported as 12 working hours in the ECM, not including GC/MS analysis time (p. 21 of MRID 51393701).

V. References

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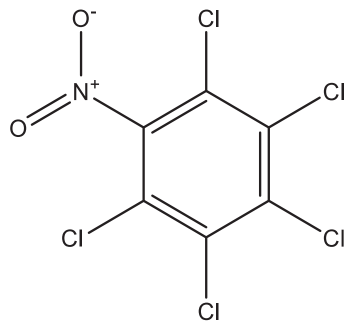
Attachment 1: Chemical Names and Structures

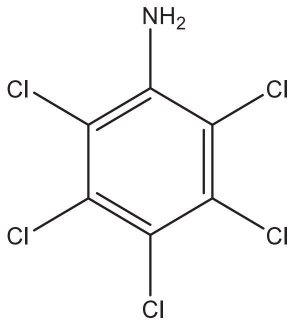
PCNB (Pentachloronitrobenzene)

IUPAC Name:	Pentachloronitrobenzene
CAS Name:	1,2,3,4,5-Pentachloro-6-nitrobenzene
CAS Number:	82-68-8
SMILES String:	O=N(=O)c(c(c(c(c1Cl)Cl)Cl)Cl)c1Cl



PCA (Pentachloroaniline)

IUPAC Name:	2,3,4,5,6-Pentachloroaniline
CAS Name:	Not reported
CAS Number:	527-20-8
SMILES String:	Nc1c(Cl)c(Cl)c(Cl)c(Cl)c1Cl



PCTA (Pentachlorothioanisole)

IUPAC Name: Pentachlorothioanisole
CAS Name: Methyl pentachlorophenyl sulfide
CAS Number: 1825-19-0
SMILES String: ClC1=C(Cl)C(SC)=C(Cl)C(Cl)=C1Cl