Analytical method for TCVP and its transformation products, TCBA, TCCEol, TCPEol and TCPEone in soil

Reports: Devellis, S.R. 2014. Independent Laboratory Validation (ILV) of the Analytical Method: Method Validation for TCVP, TCBA, TCCEol, TCPEol and TCPEone in Soil by Liquid Chromatography with Mass Spectrometry Detection (14020.6109). Smithers Viscient Study No.: 14020.6110. Bayer HealthCare Study No.: 131101-01 TCVPTF. THMC Study No.: 131101-a TCVPTF. Report prepared by Smithers Viscient, Wareham, Massachusetts; sponsored and submitted by TCVP Task Force c/o SRA Consulting Inc., Cambridge, Maryland; 124 pages. Final report issued March 5, 2014.

Document No.: MRID 49334001

Guideline: 850.6100

Statements: ECM: The study was not conducted in compliance with any GLP standards (Appendix 1, p. 74). An unsigned GLP non-compliance statement was provided (Appendix 1, p. 74). Data Confidentiality, Certification of Authenticity and Quality Assurance statements were not provided.
ILV: The study was conducted in accordance with the USEPA FIFRA GLP (40 CFR Part 160; p. 3). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A statement on the certification of the authenticity of the report was included in the Quality Assurance statement.

Classification: This analytical method is classified as **not acceptable, but upgradeable**. It is unclear whether the ILV was conducted independently of the ECM as defined by the OCSPP guidelines. Both the ILV and ECM were conducted by the same laboratory (Smithers Viscient) and other than the different study directors, it is unclear if the remaining participating personnel were different. The LOD was not reported in the ECM or ILV. The test soils were not fully characterized. A reagent blank was not included in the ECM sample set.

PC Code: 083702

Primary Reviewer: Chuck Peck, Environmental Engineer **Signature:** Chuck Peck 2015.11.09 13:27:16 -05'00'
Date:

Secondary Reviewer: Jim Carleton, Ph.D., Senior Fate Scientist **Signature:** JAMES CARLETON Digitally signed by JAMES CARLETON DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=JAMES CARLETON, dnQualifier=0000001907 Date: 2015.11.09 14:04:48 -05'00'
Date:

Executive Summary

This analytical method, SMV No. 14020.6109, is designed for the quantitative determination of TCVP and its transformation products TCBA, TCCEol, TCPEol and TCPEone in soil using LC/MS/MS. The method is quantitative for TCVP at the stated LOQ of 10.0 µg/kg, although calculated LOQs ranged *ca*. 1 to 4 µg/kg. The lowest toxicological level of concern in soil was not reported. No major modifications were made by the independent laboratory; however, inadequate information was provided to conclusively determine that the ECM and ILV laboratories were two independent laboratory groups as defined by OCSPP guidelines.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
TCVP and its transformation products TCBA, TCCEol, TCPEol and TCPEone	49334401	49334401		Soil	09/06/2013	SRA International, Inc., Cambridge, Maryland	LC/MS/MS	10.0 µg/kg

Calculated LOQs were 1.54 µg/kg (TCVP); 1.70 µg/kg (TCBA); 1.08 µg/kg (TCCEol); 4.05 µg/kg (TCPEol); 3.60 µg/kg (TCPEone; Appendix 1, Appendix 1, pp. 86, 107).

I. Principle of the Method

Methods were validated by fortification of soil samples with mixtures at 10.1 and 101 µg/kg of Mutchler sandy loam soil.

For TCVP and its transformation product TCBA: Samples (5 g, dry weight) were extracted twice with 20.0 mL of acetonitrile:purified reagent water (80:20, v:v) via shaking for 30 minutes on an orbital shaker table at 150 rpm, centrifuged at 3000 rpm for 10 minutes, and then transferred to labelled 50.0-mL volumetric flasks (pp. 19-20; Appendix 1, Appendix 1, pp. 78, 83). The combined extracts were diluted to 50.0 mL with acetonitrile:purified reagent water (80:20, v:v). Samples were further diluted into the standard calibration range using purified reagent water. The high fortification samples were additionally diluted with acetonitrile:purified reagent water (20:80, v:v) to achieve a level within the standard calibration range.

Samples were analyzed for TCVP and TCBA by HPLC (Acquity BEH C18, 2.1 mm x 50 mm, 1.7 µm column) using a mobile phase gradient of (A) 10 mM ammonium acetate in purified reagent water and (B) acetonitrile [percent A:B at 0.00-0.50 min. 95:5 (v:v), 3.00-4.00 min. 5:95, 4.10-5.00 min. 95:5; pp. 21-22; Appendix 1, Appendix 1, p. 84] with MS/MS detection (ESI) in positive ion mode (TCVP) or negative ion mode (TCBA) and Multiple Reaction Monitoring (MRM). The quantitative transitions were 366.313→126.900 for TCVP and 223.000→ 179.000 for TCBA. No confirmation method was used. Injection volume was 100 µL.

For TCVP transformation products TCCEol, TCPEol and TCPEone: Samples (5 g, dry weight) were extracted twice with 20.0 mL of acetonitrile via shaking for 30 minutes on an orbital shaker table at 150 rpm, centrifuged at 3000 rpm for 10 minutes, and then transferred to labelled 50.0-mL volumetric flasks (pp. 19-20; Appendix 1, pp. 98, 103-104). The combined extracts were diluted to 50.0 mL with acetonitrile. For the analysis of TCCEol, a portion of the extract was reduced to dryness under a gentle stream of nitrogen at room temperature. The residue was reconsitituted in acetonitrile:purified reagent water (20:80, v:v) prior to LC/MS/MS analysis. For the analysis of TCPEol and TCPEone, the extract was not concentrated, but diluted with acetonitrile:purified reagent water (20:80, v:v). After centrifugation to remove solid particles, the samples were analyzed by LC/MS/MS.

Samples were analyzed for TCCEol, TCPEol and TCPEone by HPLC (Acquity BEH C18, 2.1 mm x 50 mm, 1.7 µm column) using a mobile phase gradient of (A) 10 mM ammonium carbonate in purified reagent water and (B) acetonitrile [percent A:B at 0.00-0.50 min. 80:20 (v:v), 3.50-12.00 min. 45:55, 12.10-15.00 min. 80:20; pp. 21-22; Appendix 1, pp. 105-106] with MS/MS detection (APCI) in negative ion mode and Multiple Reaction Monitoring (MRM). The quantitative transition was 202.800 → 160.970 for TCCEol, TCPEol and TCPEone. No confirmation method was used. Injection volume was 100 µL.

II. Recovery Findings

ECM: Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of TCVP and its transformation products TCBA, TCCEol, TCPEol and TCPEone (Appendix 1, Tables 1A-2A, pp. 88-89; Appendix 1, Tables 1B-3B, pp. 109-111). Analyte identification was based on the observation of the MS qualifier ions; no confirmation method was used.

ILV: Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of TCVP and its transformation products TCBA, TCCEol, TCPEol and TCPEone (Tables 1-5, pp. 29-33). Analyte identification was based on the observation of the MS qualifier ions; no confirmation method was used.

Table 2. ECM Recoveries for TCVP and Its Transformation Products in Soil*

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Mutchler Sandy loam soil						
TCVP	10.1 (LOQ)	5	87.8-94.1	90.7	2.5	2.7
	101	5	95.3-99.7	97.7	2.0	2.1
TCBA	10.1 (LOQ)	5	114-118	115.4	1.5	1.3
	101	5	100-111	104.2	4.4	4.3
TCCEol	10.0 (LOQ)	5	85.7-101	93.7	7.4	7.9
	100	5	90.8-100	94.2	3.6	3.9
TCPEol	10.0 (LOQ)	5	94.5-104	100.1	3.7	3.7
	100	5	84.2-92.5	88.8	3.4	3.8
TCPEone	10.0 (LOQ)	5	84.3-90.5	86.9	3.2	3.6
	100	5	81.8-89.6	84.1	3.2	3.8

* Data were obtained from Appendix 1, Appendix 1, Tables 1A-2A, pp. 88-89; Appendix 1, Appendix 1, Tables 1B-3B, pp. 109-111 in the study report. The nominal LOQ was 10.0 µg/kg. Reported values for mean recovery, standard deviation and relative standard deviation were reviewer-calculated because the study authors only provided these values for the entire data set at each fortification level.

Table 3. ILV Method Recoveries for TCVP and Its Transformation Products in Soil*

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Mutchler Sandy loam soil						
TCVP	10.0 (LOQ)	5	75.7-97.9	91.2	9.50	10.4
	100	5	89.8-100	94.6	3.94	4.17
TCBA	10.1 (LOQ)	5	71.7-102	92.4	12.1	13.1
	101	5	77.8-115	97.8	14.8	15.1
TCCEol	10.1 (LOQ)	5	97.2-110	102	5.50	5.40
	101	5	96.1-114	107	7.56	7.10
TCPEol	10.1 (LOQ)	5	102-115	109	5.56	5.12
	101	5	98.3-113	102	6.24	6.11
TCPEone	10.1 (LOQ)	5	91.3-107	97.8	6.73	6.89
	101	5	71.1-88.1	81.0	6.37	7.87

* Data were obtained from Tables 1-5, pp. 29-33 of the study report. The nominal LOQ was 10.0 µg/kg.

III. Method Characteristics

In the ECM and ILV, the LOQ was nominally 10.0 µg/kg, although the low fortification was performed at either 10.0 or 10.1 µg/kg (pp. 9, 18). The nominal LOQ was validated by calculating LOQ values for each analyte using the detector response of the analyte at the lowest calibration standard concentration and the LOQ of the instrument (p. 25; Appendix 1, Appendix 1, pp. 86, 107-108). In the ECM, the calculated LOQs were 1.54 µg/kg, 1.70 µg/kg, 1.08 µg/kg, 4.05 µg/kg and 3.60 µg/kg for TCVP, TCBA, TCCEol, TCPEol and TCPEone, respectively (Appendix 1, Appendix 1, pp. 78, 98). In the ILV, the calculated LOQs were 1.18 µg/kg, 1.56 µg/kg, 1.12 µg/kg, 4.14 µg/kg and 3.39 µg/kg for TCVP, TCBA, TCCEol, TCPEol and TCPEone, respectively (p. 25). The LOD was not reported in the ECM or ILV.

Table 4. Method Characteristics

		Mutchler Sandy Loam Soil				
		TCVP	**TCBA**	**TCCEol**	**TCPEol**	**TCPEone**
Limit of Quantitation (LOQ)	Nominal	10.0 µg/kg [1]				
	Calculated	1.54 µg/kg	1.70 µg/kg	1.08 µg/kg	4.05 µg/kg	3.60 µg/kg
Limit of Detection (LOD)		Not reported				
Linearity (calibration curve r^2 and concentration range) [2]		r^2 = 0.9995 (0.0354-2.53 µg/L)	r^2 = 0.9899 (0.0354-2.53 µg/L)	r^2 = 0.9918 (0.50-25.0 µg/L)	r^2 = 0.9852 (0.035-2.50 µg/L)	r^2 = 0.9923 (0.0350-2.50 µg/L)
Repeatable		Yes				
Reproducible		Yes				
Specific		Yes				

Data were obtained from Appendix 1, Appendix 1, Tables 1A-2A, pp. 88-89; Tables 1B-3B, pp. 109-111; Figures 5A-6A, pp. 96-97; Figures 9B-11A, pp. 120-122 of the study report.

1 The low fortification was performed at either 10.0 µg/kg or 10.1 µg/kg.

2 ILV calibration curves yielded similar linearity, r^2 = 0.99336-0.99976 (concentration range, 0.035-2.50 µg/L or 0.0354-2.53 µg/L or 0.505-25.3 µg/L; Figures 1-5, pp. 34-38). Reviewer-calculated calibration curves yielded r^2 values of 0.9852-0.9994 for the ECM data and 0.9926-0.9997 for the ILV data (except for ILV TCPEol data which yielded an r^2 of 0.9227; see DER Attachment 2).

IV. Method Deficiencies and Reviewer's Comments

1. It was unclear to the reviewer whether the ILV was conducted independently from the personnel that developed the original ECM. The laboratory which performed the ILV was the same as that which performed the ECM, Smithers Viscient, Wareham, Massachusetts, and the laboratory equipment was the same between the ILV and ECM(pp. 1, 5, 12-13, 21-22; Appendix 1, Appendix 1, pp. 73, 79, 84, 99, 105-106). The study directors/study report authors were different; however, a list of study personnel was not included in the ECM and a statement was not provided to confirm that the scientists who developed the original ECM differed from those who performed the ILV. However, the ILV study author did state that the purpose of the ILV was to confirm that "the original method, developed by one group, can be independently validated by a second group with no major interaction between the two groups" (p. 9). The communication between the two groups was briefly reported as clarification of protocol, acquisition of analytical standards and controls and pre-validation evaluation (p. 23). Without the full list of study personnel and raw communication data, the ILV report cannot be considered "independent" as defined by the OCSPP guidelines. If the laboratory that conducted the validation belonged to the same organization as the originating laboratory, the analysts, study director, equipment, instruments, and supplies of the two laboratories must have been distinct and operated separately and without collusion, and the analysts and study director of the ILV must have been unfamiliar with the method both in its development and subsequent use in field studies.

2. The LOD of the method was not reported by the ECM or ILV. The LOD must be reported and justified according to OCSPP guidelines.

3. The test soils used in the ECM and ILV were characterized by soil moisture content and depth of collection; however, origin and percentages of sand, silt, clay and organic matter were not reported (p. 13; Appendix 1, Appendix 1, pp. 81, 100-101). Although the soils of the ECM and ILV were both reported as "Mutchler sandy loam soil", the soil moisture content and depth of collection differed between the study reports.

4. A reagent blank was not included in the ECM (Appendix 1, Appendix 1, Figures 1A-6A, pp. 90-97; Appendix 1, Appendix 1, Figures 1B-11A, pp. 112-122).

5. The LOQ was nominally 10.0 µg/kg, although the low fortification was performed at either 10.0 or 10.1 µg/kg and the calculated LOQs ranged approximately 1 to 4 µg/kg (pp. 9, 25; Appendix 1, Appendix 1, pp. 86, 107-108). Additionally, the lowest toxicological level of concern in soil was not reported.

6. Only one set of performance data were provided in the ILV, suggesting that the ECM was successfully validated by the ILV on the first trial. However, this was not explicitly stated in the ILV.

7. The communication was documented by a summary (p. 23); a detailed log of the communication between the ILV and ECM personnel was not provided.

8. It was reported for the ILV that a single analyst completed a sample set consisting of 20 samples in 8 hours (p. 24).

V. References

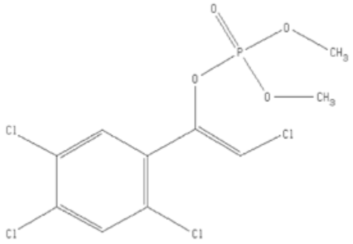
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

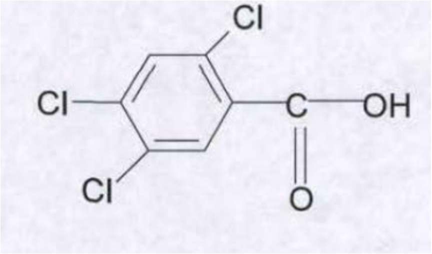
TCVP; Tetrachlorvinphos

IUPAC Name: Tetrachlorvinphos.
CAS Name: Not reported
CAS Number: 22248-79-9
SMILES String: Not reported



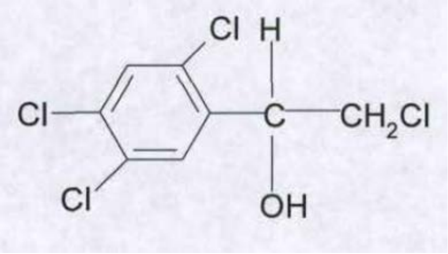
TCBA

IUPAC Name: 2,4,5-Trichlorobenzoic acid.
CAS Name: Not reported
CAS Number: Not listed
SMILES String: Not reported



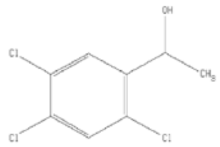
TCCEol

IUPAC Name: 1-(2,4,5-Trichlorophenyl)-2-chloroethanol.
CAS Name: Not reported
CAS Number: Not listed
SMILES String: Not reported



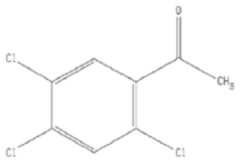
TCPEol

IUPAC Name: 1-(2,4,5-Trichlorophenyl)-ethanol.
CAS Name: Not reported
CAS Number: Not listed
SMILES String: Not reported



TCPEone

IUPAC Name: 2,4,5-Trichloroacetophenone.
CAS Name: Not reported
CAS Number: Not listed
SMILES String: Not reported



Test Material: TCVP and its transformation products: TCBA, TCCEol, TCPEol and TCPEone

MRID: 49334401

Title: Independent Laboratory Validation (ILV) of the Analytical Method: Method Validation for TCVP, TCBA, TCCEol, TCPEol and TCPEone in Soil by Liquid Chromatography with Mass Spectrometry Detection (14020.6109).

EPA PC Code: 083702

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto

Signature: Lisa Muto

Date: 5/29/14

Secondary Reviewer: Dan Hunt

Signature: Dan Hunt

Date: 5/29/14

QC/QA Manager: Joan Gaidos

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Date: 5/29/14