

Test Material: Tolclofos methyl

MRID: 48341916

Title: Tolclofos-methyl: Validation of an Analytical Method for the Determination of Residues in Soil.

MRID: 48809102

Title: Independent Laboratory Validation of Valent Method VP-38287 Tolclofos-methyl: Determination of Tolclofos-methyl in Soil.

EPA PC Code: 128905

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto

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Date: 5/6/14

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Date: 5/6/14

Analytical method for tolclofos-methyl in soil

Reports: MRID 48341916. Croucher, A. 2001. Tolclofos-methyl: Validation of an Analytical Method for the Determination of Residues in Soil. Covance Study No.: 333/153. Sumitomo Chemical Company No.: QA-0073. Report prepared by Covance Laboratories Ltd., North Yorkshire, England; sponsored by Sumitomo Chemical Company, Hyogo, Japan; and submitted by Valent U.S.A. Corporation, Walnut Creek, California. Covance Study No.: 333/153. Sumitomo Chemical Company No.: QA-0073. Final report issued October 11, 2001.

MRID 48809102. Pennell, M.F. 2012. Independent Laboratory Validation of Valent Method VP-38287 Tolclofos –methyl: Determination of Tolclofos-methyl in Soil. Report prepared by Critical Path Services, LLC (CPS), Garnet Valley, Pennsylvania; sponsored and submitted by Valent U.S.A. Corporation, Walnut Creek, California. Laboratory Project ID: 12-CPS-006. Final report issued October 5, 2012.

Document No.: MRIDs 48341916 & 48809102

Guideline: 850.6100

Statements: MRID 48341916. The study was conducted compliance with British and OECD GLP (p. 2). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 1i-4).

MRID 48809102. The study was conducted in accordance with the USEPA FIFRA GLP (40 CFR Part 160) (p. 3). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-5).


Classification: This analytical method is classified as Acceptable. The LOQ is less than the lowest toxicological level of concern in water for Tolclofos-methyl noting that:

- The LOQ was arbitrarily selected as the lowest spiked concentration. In the ECM, performance data were not determined at 10xLOQ; and
- method recoveries were corrected for residues of tolclofos-methyl found in the blank samples. The soil matrix was not characterized in the ILV.

PC Code: 128905

Reviewer:

Mohammed A. Ruhman
Senior Scientist, ERB5

Signature: 
Date: 08-01-2022

All page citations refer to MRID 48341916 (ECM) unless otherwise noted.

Executive Summary

This analytical method, Valent Method VP-38287 and Analytical Procedure CLE 333/153-04R, is designed for the quantitative determination of tolclofos-methyl in soil using GC/MS. The method is quantitative for tolclofos-methyl at the stated LOQ of 0.01 mg/kg. The lowest toxicological level of concern in soil was not reported. No major issues were discovered by the independent laboratory.

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						
Tolclofos- methyl	48341916	48809102		Soil	10/11/2001	Valent U.S.A Corporation	GC/MS	0.01 mg/kg

I. Principle of the Method

Samples (50 g) were measured into 500-mL glass jars (p. 11; Appendix 1, p. 26). After fortification, as necessary, samples were mixed with water (100 mL minus the water content of the samples). After 10 minutes, 200 mL of acetone was added. Samples were shaken for 10 minutes then filtered (No. 1 filter paper). 200 mL of the filtrate was decanted into a 500 mL separatory funnel. The filtrate was mixed sequentially with sodium chloride (20 g) and dichloromethane (100 mL). The organic layer was collected in a 500 mL round bottom flask by filtering through sodium sulphate. The separatory funnel and filter cake were rinsed with ethyl acetate (2 x 20 mL). The combined organics were evaporated to dryness via rotary evaporation at 30°C under nitrogen. The residue was reconstituted in 10 mL cyclohexane:ethyl acetate (1:1, v:v). After the solution was mixed with small amount of sodium sulphate, the solution was decanted and 5 mL of the solution was purified using Gel Permeation Chromatography (GPC): Bio-Beads S-X3 (50 g) column eluted with cyclohexane:ethyl acetate (1:1, v:v). Collected GPC samples were evaporated to dryness via rotary evaporation at 30°C under nitrogen. The residue was reconstituted in 5 mL toluene for GC/MS analysis.

Samples were analyzed for tolclofos-methyl by gas chromatography (DB 5MS, 30 m x 0.25 mm, 0.25- μ m column) with positive electron impact tandem mass spectrometry (pp. 11, 14; Appendix 1, p. 27; Appendix 2, p. 31). Three parent-to-daughter ions were monitored: the 301→265 was the quantitative transition; the 301→267 was the confirmatory transition; and the 301→250 was monitored for additional identification. The recoveries of the three transitions were quantified for method confirmation. Injection volume was 1 μ L.

In the ECM, the LOQ and LOD were 0.01 mg/kg and 0.00295 mg/kg, respectively (p. 14). In the ILV, the LOQ was reported as 0.010 μ g/mL (0.010 ppm; pp. 10, 12 of MRID 48809102). The Method Detection Limit (MDL) was reported as 0.0060 \pm 0.0012 ppm.

II. Recovery Findings

ECM (MRID 48341916): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of tolclofos-methyl in sandy loam soil; however, recoveries were corrected for residues of tolclofos-methyl found in the blank

samples (pp. 11-12, 14-15; Tables 1-3, pp. 17-19; Appendix 5, p. 41). Reviewer-calculated uncorrected recoveries were greater than the corrected recoveries of the study author, most significantly for all recoveries at the LOQ; however, all uncorrected recoveries met guideline requirements (see Reviewer Comment #3). Confirmation of the identified peaks was based on the observation of its three parent-to-daughter ion transitions (p. 15; Appendix 4, pp. 35-40). Quantitative ion and confirmatory ion results were comparable.

ILV (MRID 48809102): Mean recoveries and RSDs were within guideline requirements for analysis of tolclofos-methyl in soil (Penn series soil from Baptistown, New Jersey; soil not characterized; pp. 10-11, 15-16; Tables 4-6, pp. 22-24 of MRID 48809102). The method was validated with the second trial; Trial 1 was repeated due to “instrument equilibration issues” (see Reviewer Comment #5). Quantitative ion and confirmatory ion results were comparable.

Table 2. Initial Validation Method Recoveries for Tolclofos-methyl in Soil*

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy loam soil						
Quantitative ion 301→265						
Tolclofos-methyl	0.01 (LOQ)	5	82-124	95	17.3	18.2
	10.0	5	98-104	102	2.5	2.5
Confirmatory ion 301→267						
Tolclofos-methyl	0.01 (LOQ)	5	80-125	94	18.4	19.6
	10.0	5	99-104	102	2.3	2.3
Confirmatory ion 301→250						
Tolclofos-methyl	0.01 (LOQ)	5	79-120	92	16.7	18.1
	10.0	5	99-104	102	2.5	2.4

Data were obtained from pp. 14-15; Tables 1-3, pp. 17-19 in the study report.

* Recoveries were corrected for residues of tolclofos-methyl found in the blank samples by subtracting the average tolclofos-methyl found in the blank samples (0.0021 to 0.0022 mg/kg for all observed transitions) from the tolclofos-methyl found in the method validation samples (pp. 11-12; Tables 1-3, pp. 17-19; Appendix 1, p. 28). Reviewer-calculated uncorrected method recoveries are reported in Reviewer Comment #3. All uncorrected recoveries were greater than corrected recoveries, most significantly for all recoveries at the LOQ; however, all uncorrected recoveries met guideline requirements.

Table 3. Independent Validation Method Recoveries for Tolclofos-methyl in Soil*

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil (Penn series from New Jersey)						
Quantitative ion 301→265						
Tolclofos-methyl	0.010 (LOQ)	5	58-87	74	11	15
	0.100	5	79-123	97	16	16
Confirmatory ion 301→267						
Tolclofos-methyl	0.010 (LOQ)	5	53-86	71	12	17
	0.100	5	79-124	97	17	18
Confirmatory ion 301→250						
Tolclofos-methyl	0.010 (LOQ)	5	58-89	74	11	17
	0.100	5	80-126	97	17	18

Data were obtained from pp. 10, 15-16 and Tables 4-6, pp. 22-24 of MRID 48809102.

* Reported recoveries are from the “re-injection of Trial 1”. Trial 1 was repeated due to unsatisfactory calibration standard results, which was also referred to as “instrument equilibration issues”.

III. Method Characteristics

The LOQ was the same in the ECM and ILV (0.01 mg/kg; p. 15; MRID 48809102, p. 12) and was arbitrarily set as the lowest fortification level of the spiked samples that achieved acceptable performance data. No other justification or calculation was provided. In the ECM, the LOD of 0.00295 mg/kg was equivalent to the lowest concentration standard (0.01 µg/mL). The signal response at this concentration was greater than 4x the base line noise. In the ILV, the Method Detection Limit (MDL) was measured and reported as 0.0060 ± 0.0012 ppm.

Table 4. Method Characteristics

	Tolclofos-methyl		
	Ion 265	Ion 267	Ion 250
Limit of Quantitation (LOQ)	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg
Limit of Detection (LOD)	0.00295 mg/kg	0.00295 mg/kg	0.00295 mg/kg
Linearity (calibration curve r^2 and concentration range)	$r^2 = 0.997244^{1,2}$ (0.01-0.50 µg/mL)	$r^2 = 0.995981^{1,2}$ (0.01-0.50 µg/mL)	$r^2 = 0.996463^{1,2}$ (0.01-0.50 µg/mL)
Repeatable	Yes	Yes	Yes
Reproducible	Yes	Yes	Yes
Specific	Yes	Yes	Yes

Data were obtained from p. 8; Appendix 3, pp. 32-34.

1 Reviewer-reported r^2 values contain a degree of uncertainty due to poor resolution of the chromatogram raw data in the report. The study author did not report individual r^2 values in the study report.

2 ILV calibration curves displayed linearity, $r^2 = 0.9876-0.9888$, for its soil in the concentration range of 0.020-0.500 µg/mL (p. 15; Figures 1-3, pp. 30-32 of MRID 48809102).

IV. Method Deficiencies and Reviewer’s Comments

1. The LOQ in the ECM and ILV was set at the lowest fortification level of the spiked samples that achieved acceptable performance data. No comparison was made to chromatogram background levels. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in soil was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification. The estimation of the LOD in the ECM was based on scientifically acceptable procedures as defined in 40 CFR Part 136.
2. In the ECM, mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of tolclofos-methyl in sandy loam soil; however, fortifications were performed at the LOQ and 1000×LOQ instead of at the LOQ and 10×LOQ as recommended in the guidelines. In the ILV, fortifications were performed at the LOQ and 10×LOQ as recommended.

3. In the ECM, method recoveries were corrected for residues of tolclofos-methyl found in the blank samples by subtracting the average tolclofos-methyl found in the blank samples (0.0021 to 0.0022 mg/kg for all observed transitions) from the tolclofos-methyl found in the method validation samples (pp. 11-12; Tables 1-3, pp. 17-19; Appendix 1, p. 28). The reviewer calculated the following uncorrected method recoveries in Table 5 using data obtained from Tables 1-3, pp. 17-19 of the study report (see DER Attachment 2). All reviewer-calculated uncorrected recoveries met OCSP Guideline 850.6100 criteria with average recoveries of 70-110% and a RSD \leq 20%. In the ILV, sample recoveries were not corrected (Appendix 2, pp. 56-57 of MRID 48809102).

Table 5. Reviewer-calculated Initial Validation Method Recoveries for Tolclofos-methyl in Soil (Uncorrected)

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy loam soil						
Quantitative ion 301→265						
Tolclofos-methyl	0.01 (LOQ)	5	103-145	116	17	15
	10.0	5	98-104	102	3	3
Confirmatory ion 301→267						
Tolclofos-methyl	0.01 (LOQ)	5	102-147	116	18	16
	10.0	5	99-104	102	2	2
Confirmatory ion 301→250						
Tolclofos-methyl	0.01 (LOQ)	5	101-142	114	17	15
	10.0	5	99-104	102	3	3

Data were obtained from pp. 11-12; Tables 1-3, pp. 17-19 in the study report.

4. The soil used in the ILV was not characterized. The soil was described as a Penn series soil from Baptistown, New Jersey and was not further characterized (p. 11 of MRID 48809102). Test soils used in the ECM were adequately characterized (Appendix 5, p. 41).
5. The ILV reported that the validation was performed and succeeded on the first trial for soil; however, the reviewer considered that the method was validated with the second trial since the study author reported that Trial 1 was repeated due to “instrument equilibration issues” (p. 10 of MRID 48809102). Furthermore, the final data for the study report was based on the “re-injection of Trial 1”, as opposed to “Trial 1” (Tables 4-6, pp. 22-24 of MRID 48809102).
6. In the ECM, interferences in the control matrix were $<$ 30% of the LOQ at or near the retention time of tolclofos-methyl (pp. 13, 15; Tables 1-3, pp. 17-19; Appendix 4, p. 38). The detected tolclofos-methyl (range, 0.0014-0.0029 mg/kg) was attributed to laboratory background from the 10.0 mg/kg fortification. The reviewer believed that the laboratory glassware cleaning procedure or order of samples analysis should have been modified to decrease or eliminate these background interferences. These interferences were not observed in the ILV (pp. 15-16 of MRID 48809102).

7. The communication between the ILV and ECM was documented (p. 16 and Appendix 3, pp. 58-60 of MRID 48809102).
8. It was reported for the ILV that a single analyst completed a sample set consisting of 13 samples in *ca.* 16.0 hours (p. 16 of MRID 48809102).

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**Tolclofos-methyl [TM; V-10178; Rizolex]****IUPAC Name:** O-2,6-Dichloro-p-tolyl O,O-dimethyl phosphorothioate.**CAS Name:** O-(2,6-Dichloro-4-methylphenyl) O,O-dimethyl phosphorothioate.**CAS Number:** 57018-04-9.**SMILES String:** C1C(C(H)(H)H)=CC(Cl)=C(OP(=S)(OC(H)(H)H)OC(H)(H)H)C=1Cl
(EpiSuite 4.0).