

**Test Material:** Trichlorfon

**MRID:** 48855102

**Title:** Bayer method DL-002-S10-03: An analytical method for the determination of residues of trichlorfon and its metabolites in soil and thatch using LC/MS/MS

**MRID:** 48655104

**Title:** Independent laboratory validation - Bayer method DL-002-S10-01 for trichlorfon and its metabolites in soil using LC/MS/MS

**EPA PC Code:** 057901

**OCSPP Guideline:** 850.6100

**For CDM Smith**

**Primary Reviewer:** Lynne Binari

**Signature:** 

**Date:** 12/17/13

**Secondary Reviewer:** Kathleen Ferguson

**Signature:** 


**Date:** 12/17/13

**QC/QA Manager:** Joan Gaidos

**Signature:** 

**Date:** 12/17/13

**Analytical method for trichlorfon, DDVP, desmethyl DDVP (as DCA equivalents) and DCA in soil/thatch**

<b>Reports:</b>	ECM: EPA MRID No. 48655102. Netzband, D.J. 2011. Bayer method DL-002-S10-03: An analytical method for the determination of residues of trichlorfon and its metabolites in soil and thatch using LC/MS/MS. Study No.: DL-002-S10-03. Report prepared by Bayer CropScience, Stilwell, Kansas, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 23 pages. Final report issued July 25, 2011. ILV: EPA MRID No. 48655104. Schierhoff, R. 2011. Independent laboratory validation - Bayer method DL-002-S10-01 for trichlorfon and its metabolites in soil using LC/MS/MS. ABC Study No.: 66769. Bayer Study No.: MEDLY007. Report prepared by ABC Laboratories, Inc., Columbia, Missouri, sponsored and submitted by Bayer CropScience, Stilwell, Kansas; 127 pages. Final report issued August 29, 2011.
<b>Document No.:</b>	MRIDs 48655102 & 48655104
<b>Guideline:</b>	850.6100
<b>Statements:</b>	ECM: Rules and regulations of USEPA FIFRA GLP standards, 40 CFR, Part 160 were considered not to apply (p. 3). Signed and dated Data Confidentiality, GLP and Authenticity Certification statements were provided (pp. 2-4) A Quality Assurance statement was not provided. ILV: The study was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160 (p. 3). Signed and dated Data Confidentiality, GLP, Quality Assurance and Authenticity Certification statements were provided (pp. 2-5).
<b>Classification:</b>	This analytical method is classified as unacceptable. Deficiencies include that no ECM performance data were provided. No justification for selection of the LOQ was provided. It was not defined that the stated LOQ was less than the lowest toxicological level of concern in soil/thatch. <b>(For trichlorfon, the lowest aquatic tox endpoint is NOAEC = 0.0057 ug/L and LOAEC = 0.0086 ug/L based on survival. (MRID 40452601).</b> The ECM did not establish the LOD. The ILV did not validate the finalized version of the ECM (Bayer Method DL-002-S10-03), which uses d <sub>6</sub> -DDVP as an internal standard. The soil/thatch matrix was not characterized.
<b>PC Code:</b>	057901
<b>Reviewer:</b>	Ronald D. Parker, Ph.D. Senior Environmental Engineer, U.S. EPA Date: 01/11/2017 

**All page citations refer to MRID 48655102 (ECM) unless otherwise noted.****Executive Summary**

This analytical method, Bayer Method DL-002-S10-03, is designed for the quantitative determination of trichlorfon and its products DDVP, desmethyl DDVP (as DCA equivalents) and DCA in soil/thatch using LC/MS/MS. The method is quantitative for trichlorfon, DDVP, desmethyl DDVP (as DCA) and DCA at the stated LOQ of 0.25 mg/kg. The lowest toxicological level of concern in soil was not reported. No major issues were discovered by the independent laboratory. The independent laboratory did not validate the finalized version of the method, which includes d<sub>6</sub>-DDVP as an internal standard for trichlorfon and DDVP analyses.

**Table 1. Analytical Method Summary**

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Trichlorfon DDVP Desmethyl DDVP <sup>1</sup> DCA	48655102	48655104 <sup>2</sup>		Soil/thatch	25/07/2011	Bayer CropScience	LC/MS/MS	0.25 mg/kg

<sup>1</sup> Desmethyl DDVP degrades to DCA during extraction and is quantified as DCA equivalents.

<sup>2</sup> ILV did not validate finalized version of method (Bayer Method DL-002-S10-03), which used d<sub>6</sub>-DDVP as an internal standard for trichlorfon and DDVP analyses.

**I. Principle of the Method**

**Trichlorfon and DDVP:** Soil/thatch (200 g) is extracted once with methanol:0.5% aqueous HCl (50:50, v:v, 400 mL) by shaking for 20-25 minutes, then allowed to rest for 5 minutes (p. 10). An aliquot (0.5 mL) of the extract is fortified with d<sub>6</sub>-DDVP isotopic internal standard (0.1 mL of 5 µg/mL solution) and diluted to 10.0 mL with 0.1% acetic acid:acetonitrile (80:20, v:v). Samples are analysed by HPLC (Imtakt Unison UK-C18, 3.0 x 75 mm, 3 µm column with in-line filter), using a mobile phase gradient of (A) aqueous 0.1% formic acid and (B) acetonitrile [A:B at 0.0-2.0 min. 75:25 (v:v), 3.5 min. 60:40, 5.5-7.0 min. 5:95, 7.1-10 min. 75:25], with MS/MS-ESI<sup>+</sup> detection and Multiple Reaction Monitoring (MRM; pp. 18, 20 of MRID 48655104).

**Desmethyl DDVP and DCA:** Soil/thatch (200 g) is extracted once with 1.0M aqueous ammonium bicarbonate (400 mL) by shaking for 20-25 minutes, then allowed to rest for 5 minutes (pp. 10-11). An aliquot (1.0 mL) of the extract is fortified with d<sub>2</sub>-DCA isotopic internal standard (0.25 mL of 5 µg/mL solution) and diluted to 25.0 mL with 0.1% acetic acid:acetonitrile (50:50, v:v). Samples are analysed by HPLC (SeQuant ZIC-HILIC, 4.6 x 150 mm, 5 µm, 200 Å column with in-line filter), using a mobile phase gradient of (A) aqueous 0.1% formic acid and (B) acetonitrile [A:B at 0.0 min. 20:80 (v:v), 4.0 min. 35:65, 4.1-6.0 min. 50:50, 6.1-12.0 min. 20:80], with MS/MS-ESI<sup>+</sup> detection and MRM (pp. 19-20 of MRID 48655104).

**All analytes:** The ECM reported a LOQ of 0.25 mg/kg, but no LOD. The ILV used the LOQ of 0.25 mg/kg and estimated the LOD at 0.083 mg/kg (p. 13 of MRID 48655104).

## II. Recovery Findings

ECM (MRID 48655102): No performance data were reported.

ILV (MRID 48655104): Mean recoveries and RSDs were within guideline requirements (Tables 1-4, pp. 30-33). For trichlorfon and DDVP, the method (without d<sub>6</sub>-DDVP internal standard modification) was validated with the first trial (p. 11). For desmethyl DDVP and DCA, the method was validated on the third trial after minor modifications (increased run time, addition of d<sub>2</sub>-DCA internal standard; pp. 11, 19).

**Table 2. Initial Validation Method Recoveries for Analytes in Soil/Thatch**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Trichlorfon	LOQ					
	10 x LOQ					
DDVP	LOQ		No performance data were reported.			
	10 x LOQ					
Desmethyl DDVP	LOQ					
	10 x LOQ					
DCA	LOQ					
	10 x LOQ					

**Table 3. Independent Validation Method Recoveries for Analytes in Soil/Thatch**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Trichlorfon	0.25 (LOQ)	5	100-102	101	1.16	1.15
	2.5	5	107-110	109	1.47	1.35
DDVP	0.25 (LOQ)	5	80-85	83	2.34	2.82
	2.5	5	85-88	87	1.24	1.44
Desmethyl DDVP (as DCA equivalents)	0.25 (LOQ)	5	79-87	83	3.75	4.52
	2.5	5	76-94	87	7.28	8.32
DCA	0.25 (LOQ)	5	101-107	103	2.39	2.31
	2.5	5	99-104	102	1.96	1.93

Data were obtained from Tables 1-4, pp. 30-33 in MRID 48855104.

## III. Method Characteristics

The LOQ was reported as 0.25 mg/kg for each analyte in the ECM, with no justification (p. 6). A LOD was not established.

A set of twenty-three samples required eight person hours for laboratory work, followed by *ca.* 6.5 hours of unattended LC/MS/MS run time (p. 28 of MRID 48655104). Analysis of the four analytes (two extraction sets) would require two 8-hour days, plus two overnight LC/MS/MS runs.

**Table 4. Method Characteristics<sup>1</sup>**

	Trichlorfon	DDVP	Desmethyl DDVP	DCA
Limit of Quantitation (LOQ)	0.25 mg/kg	0.25 mg/kg	0.25 mg/kg	0.25 mg/kg
Limit of Detection (LOD)	-- <sup>2</sup>	--	--	--
Linearity (calibration curve $r^2$ and concentration range) <sup>3</sup>	$r^2 = 1.000$ (n = 1) (0.5-200 ng/mL)	$r^2 = 0.998$ (n = 1) (0.5-200 ng/mL)	$r^2 = \mathbf{0.986-0.993}$ (n = 2) (0.5-100 ng/mL)	$r^2 = 0.996$ (n = 1) (0.5-100 ng/mL)
Repeatable	Yes	Yes	Yes	Yes
Reproducible	Yes	Yes	Yes	Yes
Specific <sup>4</sup>	Yes	Yes	Yes	Yes

Data were obtained from Tables 1-4, pp. 30-33 of MRID 48655104.

1 As verified by the ILV.

2 Not established.

3 Reported r values were converted to  $r^2$  by reviewer (Appendix 4, pp. 118-122 of MRID 48655104; DER Attachment 2).

4 Interferences were <30% of LOQ for test matrix and each analyte (p. 27; Figure 12, pp. 59-61 of MRID 48655104).

#### IV. Method Deficiencies and Reviewer's Comments

##### ECM (MRID 48655102):

1. No performance data were provided. A validation sample set should consist of, at a minimum, a reagent blank, two unspiked matrix control samples, five matrix control samples spike at the LOQ and an additional five matrix control samples spiked at ten times the LOQ (10 x LOQ) for each matrix.
2. Representative chromatograms were not provided for reagent blanks, matrix blanks, standard curves, and spiked samples at the LOQ and 10x LOQ for all analytes.
3. No justification for selection of the LOQ was provided.
4. The lowest toxicological level of concern in soil/thatch was not reported. The established LOQ must be less than toxicological levels of concern.
5. The LOD concentration was not reported. The estimation of the LOD should be based on scientifically acceptable procedures as defined in 40 CFR Part 136, Appendix B (1)(b)(3)(b)(4).
6. The ECM cannot quantify desmethyl DDVP, an organic phosphorus compound, in field samples as the analyte degrades almost completely to DCA during the extraction process (pp. 24-25; Figure 13, p. 63; Figure 14, p. 67; Appendix 4, pp. 120, 122 of MRID 48655104).
7. Characterization of the soil/thatch matrix was not provided. Uncharacterized control soil/thatch was provided by Bayer CropScience to ABC Laboratories for the ILV study (p. 15 of MRID 48655104).
8. The increased LC/MS/MS run times for desmethyl DDVP and DCA analyses used in the ILV were not included in the finalized method (pp. 13, 23 of MRID 48655102; p. 19 of MRID 48655104).

9. Appendix 1 Test and Reference Substances (p. 19) did not include the d<sub>6</sub>-DDVP isotopic internal standard.
10. No reference standard was available for trichlorfon product methyldichloroacetylphosphate (MDAP); however, LC/MS/MS conditions for the analyte were included in the ECM (pp. 7, 14, 16).

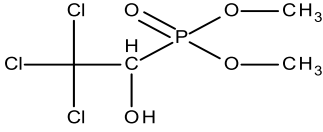
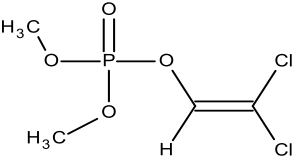
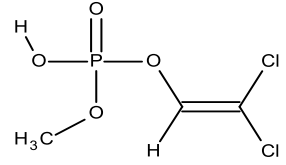
**ILV (MRID 48655104):**

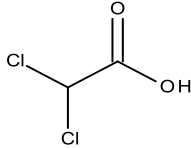
1. For determination of trichlorfon and DDVP in soil/thatch, the finalized version of the ECM (Bayer Method DL-002-S10-03) modified the method to use d<sub>6</sub>-DDVP as an isotopic internal standard for trichlorfon and DDVP analyses. This modification to the method was not included as part of the ILV study.
2. Correlation coefficients (r<sup>2</sup>) for desmethyl DDVP calibration curves were <0.995 (DER Attachment 2).
3. Reagent blank samples were not included in the analyses.
4. Bayer CropScience provided un-homogenized soil/thatch for the first ILV trial and homogenized (method not specified) soil/thatch for the remaining trials (p. 15). The success of trial one for trichlorfon and DDVP, and the lack of success of trials one and two for desmethyl DDVP and DCA indicated that soil/thatch homogenization was not a factor in the success of the method (pp. 15-16).

**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, D.C. EPA 712-C-001.

**DER ATTACHMENT 1. Trichlorfon and Its Environmental Transformation Products. <sup>A</sup>**

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)	Final %AR (study length)
<b>PARENT</b>						
Trichlorfon (C-1147; Dylox; C-1147A)	IUPAC: Dimethyl (RS)-2,2,2-trichloro-1-hydroxyethylphosphonate  CAS: Dimethyl (P)-(2,2,2-trichloro-1-hydroxyethyl)phosphonate  CAS No.: 52-68-6  Formula: C <sub>4</sub> H <sub>8</sub> Cl <sub>3</sub> O <sub>4</sub> P MW: 257.44 g/mol SMILES: COP(=O)(OC)C(O)C(Cl)(Cl)Cl		850.6100 ECM	48655102	NA	NA
			850.6100 ILV	48655104		
Dichlorvos (DDVP; K-1891)	IUPAC: 2,2-Dichlorovinyl dimethyl phosphate  CAS: Phosphoric acid, 2,2-dichloroethenyl diethyl ester  CAS No.: 62-73-7  Formula: C <sub>4</sub> H <sub>7</sub> Cl <sub>2</sub> O <sub>4</sub> P MW: 220.97 g/mol SMILES: O=P(OC)(OC)OC=C(Cl)Cl		850.6100 ECM	48655102	NA	NA
			850.6100 ILV	48655104		
Desmethyl DDVP (DM-DDVP; C-1890)	IUPAC: 2,2-Dichlorovinyl methyl hydrogen phosphate  Formula: C <sub>3</sub> H <sub>5</sub> Cl <sub>2</sub> O <sub>4</sub> P MW: 206.9 g/mol SMILES: COP(=O)(O)OC=C(Cl)Cl		850.6100 ECM	48655102	NA	NA
			850.6100 ILV	48655104		

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)	Final %AR (study length)
Dichloroacetic acid (DCA; K-1894; Metabolite D)	IUPAC: 2,2-Dichloroacetic acid CAS: Acetic acid, 2,2-dichloro CAS No.: 79-43-6 Formula: C <sub>2</sub> H <sub>2</sub> Cl <sub>2</sub> O <sub>2</sub> MW: 128.94 g/mol SMILES: O=C(O)C(Cl)Cl		850.6100 ECM	48655102	NA	NA
			850.6100 ILV	48655104		
<b>MAJOR (&gt;10%) TRANSFORMATION PRODUCTS</b>						
No major transformation products were identified.						
<b>MINOR (&lt;10%) TRANSFORMATION PRODUCTS</b>						
No minor transformation products were identified.						
<b>REFERENCE COMPOUNDS NOT IDENTIFIED</b>						
All compounds used as reference compounds were identified.						

<sup>A</sup> AR means “applied radioactivity”. MW means “molecular weight”. ECM means “Environmental Chemistry Methods”. ILV means “Independent Laboratory Validation”. NA means “not applicable”.