Test Material: Trichlorfon

MRID: 49041701

Title: An Analytical Method for the Determination of Residues of Trichlorfon and its Metabolite DDVP in Water Using LC/MS/MS

MRID: 48958201

Title: Independent Laboratory Validation of Bayer Method DL-004-Q10-01: An Analytical Method for the Determination of Residues of Trichlorfon and its Metabolite DDVP in Water Using LC/MS/MS

EPA PC Code: 057901

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lynne Binari

Signature: Lynne Binari

Date: 3/26/15

Secondary Reviewer: Lisa Muto

Signature: Lisa Muto

Date: 3/26/15

QC/QA Manager: Joan Gaidos

Signature:

Date: 3/26/15

Analytical method for trichlorfon and its transformation product DDVP in water

Reports:	ECM: EPA MRID No.: 49041701. Netzband, D. 2011. An Analytical Method for the Determination of Residues of Trichlorfon and its Metabolite DDVP in Water Using LC/MS/MS. Bayer Method No.: DL-004-W10-01. Report prepared by Bayer CropScience, Environmental Research Section, Stilwell, Kansas, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 16 pages. Final report issued March 31, 2011. ILV: EPA MRID No. 48958201. Pennell, M. 2012. Independent Laboratory Validation of Bayer Method DL-004-W10-01: An Analytical Method for the Determination of Residues of Trichlorfon and its Metabolite DDVP in Water Using LC/MS/MS. CPS Study No.: 12-CPS-007. Bayer Report No.: MEDLN002. Report prepared by Critical Path Services, LLC (CPS), Garnet Valley, Pennsylvania, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 66 pages. Final report issued September 27, 2012.
Document No.:	MRIDs 49041701 & 48958201
Guideline:	850.6100
Statements:	ECM: The study was considered not under the restriction of compliance with USEPA Good Laboratory Practice (GLP) standards (p. 3 of MRID 49041701). Signed and dated Data Confidentiality and GLP statements were provided (pp. 2-3). A signature page was included (p. 4). Quality Assurance and Authenticity Certification statements were not provided. ILV: The study was conducted in compliance with USEPA GLP standards (p. 3 of MRID 48958201). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A signature page was provided, but an Authenticity Certification statement was not provided (p. 5).
Classification:	This analytical method is classified as unacceptable. The determination of the LOQ was not based on scientifically acceptable procedures. LODs were not reported. Water matrices used for the ILV and ECM were not characterized. For the ILV, linearity (r^2) of the trichlorfon calibration curve was not ≥0.995. Adequate performance data were not provided to support the ECM validation.
PC Code:	057901
Reviewer:	Ronald D. Parker, Ph.D., Senior Environmental Engineer, U.S.EPA Date: 01/11/17 Ronald D. Parker

Executive Summary

This analytical method, Bayer Method DL-004-W10-01, is designed for the quantitative determination of trichlorfon and its transformation product DDVP in water using LC/MS/MS. The method is quantitative for the analytes at the stated LOQ of 5.0 µg/L (ng/mL). **The lowest aquatic tox endpoint is a NOAEC of 0.0057 ug/L and LOAEC of 0.0086 ug/L based on reduced survival of aquatic invertebrates (MRID 40452601)** The LOQ is greater than the lowest toxicological level of concern in water. The independent laboratory validated the method for analysis of trichlorfon and DDVP at both fortification levels in surface water after one trial. No major modifications were made by the independent laboratory. For the ILV, the surface water matrix was not characterized, and linearity (r^2) of the trichlorfon calibration curve was not ≥0.995.

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	49041701	48958201		Surface water[1]	31/03/2011	Bayer CropScience	LC/MS/MS	5.0 µg/L (ng/mL)
DDVP								

1 Water matrix was not characterized.

I. Principle of the Method

Water (40 mL) was separately fortified with trichlorfon or DDVP in acetonitrile:0.1% acetic acid in water (50:50, v:v) for procedural recoveries (pp. 7-8 of MRID 49041701). Mixed fortification solutions should not be used because trichlorfon readily degrades to DDVP. Water (40 mL) is acidified with either 10 mL of 0.25% acetic acid in acetonitrile or 10 mL of acetonitrile:0.1% acetic acid in water (20:80, v:v), mixed, and analyzed directly by LC/MS/MS (p. 8; Appendix 3, p. 15).

Samples are analyzed using either a Finnigan TSQ Quantum Ultra LC/MS/MS with an electrospray interface or an Applied Biosystems API 4000 LC/MS/MS with an electrospray ionization (ESI) interface (pp. 6, 9-10 of MRID 49458109). The following LC conditions were used: Imtakt Unison UK-C18 column (3.0 mm x 75 mm, 3 µm, column temperature 40°C), using an isocratic mobile phase of 0.1% formic acid in methanol:0.1% formic acid in water (60:40, v:v, 0-6.0 minute run time). Injection volume was 50 µL. The following MS/MS conditions were used: electrospray ionization in positive ion mode detection; the scan type was not specified. Analytes are identified using one ion transition. Ion transitions monitored were as follows: *m/z* 257→109 for trichlorfon and *m/z* 221→109 for DDVP. Expected retention times were *ca*. 2.8 and 4.2 minutes for trichlorfon and DDVP, respectively. A confirmatory method was not used.

<u>ILV</u>: Reference substances were supplied by Bayer CropScience (p. 15 of MRID 48958201). The Imtakt Unison HPLC column was also supplied by Bayer CropScience, because the column was on back order, and there were no equivalent columns for substitution (pp. 15-16; Appendix 3, p. 42). Surface water was obtained Brandywine Creek near West Chester, Pennsylvania (p. 11). The independent laboratory performed the method as written with no major modifications (pp. 10, 12-14; Table 2, p. 20; Appendix 5, pp. 51-66). For the ILV, 0.25% acetic acid in acetonitrile was used to acidify water samples. For analysis, an Agilent 1200 HPLC system coupled with an Applied Biosystems API 4000 MS/MS using multiple reaction monitoring (MRM) was employed. Injection volume was reduced to 10 µL. Approximate retention times were 2.66 and 4.16 minutes for trichlorfon and DDVP, respectively (p. 14).

LOQ and LOD: In the ECM and ILV, the LOQ for trichlorfon and DDVP was 5.0 µg/L (ng/mL, 0.005 ppm; p. 6 of MRID 49041701; p. 10 of MRID 48958201). No justification was provided for selection of the LOQ concentration. LODs were not reported in the ECM or ILV.

II. Recovery Findings

ECM (MRID 49041701): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of trichlorfon and its transformation product DDVP in water (uncharacterized) at a fortification level of 5.0 µg/L (ng/mL, LOQ); however, an insufficient number of samples were fortified (n = 3; Appendix 1, p. 13; DER Attachment 2). Fortifications at 10x LOQ were not reported. Analytes were identified and quantified using one ion transition; a confirmatory method was not used.

ILV (MRID 48958201): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of trichlorfon and its product DDVP in surface (creek) water at fortification levels of 5.0 µg/L (ng/mL, 0.005 ppm, LOQ) and 50.0 µg/L (10x LOQ; Table 1, p. 19). The method was validated for both analytes at both fortification levels in the surface water after one trial (pp. 10, 15). The water matrix was not characterized.

Table 2. Initial Validation Method Recoveries for Trichlorfon and Its Transformation Product DDVP in Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Trichlorfon	5.0 (LOQ)	3	87-105	97	9	10
DDVP	5.0 (LOQ)	3	82-106	96	13	13

Data (uncorrected recovery results; recovery range, mean, standard deviation) were obtained from Appendix 1, p. 13 of MRID 49041701. Relative standard deviation (RSD) was determined by the reviewer; the study author did not provide RSDs (DER Attachment 2).

1 The water matrix was neither characterized, nor described (ground, surface, or drinking).

Table 3. Independent Validation Method Recoveries for Trichlorfon and Its Transformation Product DDVP in Surface Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Trichlorfon	5.0 (LOQ)	5	104-110	107	2.9	2.7
	50.0	5	104-108	106	1.9	1.8
DDVP	5.0 (LOQ)	5	102-110	105	3.6	3.4
	50.0	5	89-102	96	4.6	4.8

Data (uncorrected recovery results) were obtained from Table 1, p. 19 of MRID 48958201.

1 Characterization of the water matrix was not provided. Surface water was obtained from Brandywine Creek near West Chester, Pennsylvania (p. 11 of MRID 48958201).

III. Method Characteristics

In the ECM and ILV, the LOQ for trichlorfon and DDVP was 5.0 µg/L (ng/mL, 0.005 ppm; p. 6 of MRID 49041701; p. 10 of MRID 48958201). No justification was provided for selection of the LOQ concentration. LODs were not reported in the ECM or ILV.

Table 4. Method Characteristics for Trichlorfon and Its Transformation Product DDVP in Water

		Trichlorfon	DDVP
Limit of Quantitation (LOQ)		5.0 µg/L (ng/mL, 0.005 ppm)	
Limit of Detection (LOD)		Not reported.	
Linearity (calibration curve r^2 and concentration range)[1]	ECM:	Not reported.	
	ILV:	r^2 = **0.9944**	r^2 = 0.9948
	Range:	1.00-100 ng/mL	
Repeatable	ECM:	Yes at LOQ; however, n = 3 for both analytes. Fortifications at 10x LOQ were not performed.	
	ILV:	Yes at LOQ and 10x LOQ.	
Reproducible		Yes.	
Specific[2]	ECM:	Undetermined; chromatograms were not provided.	
	ILV:	Yes.	

Data were obtained from p. 6; Appendix 1, p. 13 of MRID 49041701; pp. 10, 14-15; Table 1, p. 19; Figures 5-7, pp. 26-28; Figures 13-15, pp. 34-36 of MRID 48958201; DER Attachment 2.

Linearity is satisfactory when $r^2 \geq 0.995$.

1 ILV r^2 values are from 1/(x*x) weighted linear regression (p. 14; Figure 1, p. 22; Figure 9, p. 30 of MRID 48958201).

2 A confirmatory method was not used; however, a confirmatory method is typically not required where GC/MS and LC/MS methods are used as the primary method.

IV. Method Deficiencies and Reviewer's Comments

1. The determination of the LOQ was not based on scientifically acceptable procedures as defined in 40 CFR Part 136, Appendix B. No justification was provided for selection of the LOQ concentration in the ECM. LODs were not reported in the ECM or ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in water was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

2. For both the ILV and ECM, the water matrices were not characterized. For the ILV, the independent laboratory obtained surface water from Brandywine Creek near West Chester, Pennsylvania (p. 11 of MRID 48958201). Bayer CropScience instructed the independent laboratory to select a surface/ground water matrix over tap water as a more difficult sample matrix (p. 15; Appendix 3, p. 42 of MRID 48958201). The ECM did not report the source of the water matrix used for the validation.

3. For the ILV, linearity (r^2) of the trichlorfon calibration curve (non-matrix matched) was not ≥0.995 (p. 14 of MRID 48958201). The study protocol only required coefficients of determination (r^2) to be ≥0.9801 (Appendix 4, p. 48 of MRID 48958201).

4. Adequate performance data were not provided to support the ECM validation; fortifications at the LOQ were only n = 3, no fortifications at 10x LOQ were reported, and no relevant chromatograms were provided. Standard curve plots with calibration data and regression analyses were not provided. The only chromatograms provided were of desmethyl DDVP and DCA 10 ng/mL standards, not relevant to this ECM (Appendix 4, p. 16 of MRID 49041701).

5. +The ECM description of sample processing was inconsistent between the method text and a procedure flowchart. In section **6.0 PROCEDURE** (p. 8 of MRID 49041701), 0.25% acetic acid in acetonitrile is used to acidify water samples, but in the **Appendix 3 Analytical Scheme for the Analysis of Trichlorfon and DDDVP in Water** flowchart (p. 15) the acidifying solution is reported as acetonitrile:0.1% acetic acid aqueous (20/80, v/v).

6. For the ILV calibration standards, only chromatograms of the 1.00 and 100 ng/mL standards were provided (calibration standard range 1-100 ng/mL; Figures 3-4, pp. 24-25; Figures 11-12, pp. 32-33; Appendix 1, pp. 39-40 of MRID 48958201).

7. The independent laboratory found the Imtakt LC column was on back order for at least two weeks; consequently, Bayer CropScience supplied the independent laboratory with "an unused column in an unopened box" for the ILV (pp. 15-16; Appendix 3, p. 42 of MRID 48958201).

8. A confirmatory method was not employed; however, typically, a confirmatory method is not required where GC/MS and LC/MS methods are used as the primary method(s) to generate study data.

9. It was reported for the ILV that one analyst required approximately 1.1 days to complete one set of twenty-three samples, including extraction and analysis (p. 10 of MRID 48958201). An analyst required *ca*. 3 hours for extraction of one set of twenty-three samples (one reagent blank, two matrix blank samples, and twenty fortified samples), with time of analysis *ca*. 6 hours.

V. References

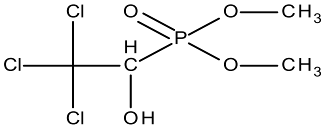
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

Trichlorfon (C-1147; Dylox)

IUPAC Name: Dimethyl (RS)-2,2,2-trichloro-1-hydroxyethylphosphonate
CAS Name: Dimethyl (P)-(2,2,2-trichloro-1-hydroxyethyl)phosphonate
CAS Number: 52-68-6
SMILES String: COP(=O)(OC)C(O)C(Cl)(Cl)Cl



Dichlorvos (DDVP; K-1891)

IUPAC Name: 2,2-Dichlorovinyl dimethyl phosphate
CAS Name: 2,2-Dichloroethenyl dimethyl phosphate
CAS Number: 62-73-7
SMILES String: O=P(OC)(OC)OC=C(Cl)Cl