Test Material:	Ethephon
MRID:	**49305604**
Title:	Bayer Method ET-002-W13-02: An Analytical Method for the Determination of Residues of Ethephon in Water Using LC/MS/MS
MRID:	**49305603**
Title:	Independent Laboratory Validation of "An Analytical Method for the Determination of Residues of Ethephon and its Metabolite 2-HEPA in Water Using LC/MS/MS"
EPA PC Code:	099801
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lynne Binari **Signature:** 

Date: 12/01/14

Secondary Reviewer: Lisa Muto **Signature:** 

Date: 12/01/14

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 12/01/14

Analytical method for ethephon in water

Reports: ECM: EPA MRID No.: 49305604. Miller, A. 2014. Bayer Method ET-002-W13-02: An Analytical Method for the Determination of Residues of Ethephon in Water Using LC/MS/MS (p. 4). Report prepared, sponsored, and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 17 pages. Final report issued January 23, 2014.

ILV: EPA MRID No. 49305603. Sears, K. 2014. Independent Laboratory Validation of "An Analytical Method for the Determination of Residues of Ethephon and its Metabolite 2-HEPA in Water Using LC/MS/MS". Pyxant Labs Study No.: 2687. Bayer CropScience Study No.: RAETL042. Report prepared by Pyxant Labs Inc., Colorado Springs, Colorado, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 88 pages. Final report issued January 23, 2014.

Document No.: MRIDs 49305604 & 49305603

Guideline: 850.6100

Statements: ECM: The study was considered not required to be conducted in compliance with USEPA Good Laboratory Practice (GLP) standards (p. 3 of MRID 49305604). 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 compliance with USEPA GLP standards (p. 3 of MRID 49305603). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity Certification statements were provided (pp. 2-5).

Classification: This analytical method is classified as supplemental but upgradable upon submission of originating ECM performance data, justification of the procedure used to determine method LOQ, and the LOD of the analyte.

PC Code: 099801

EPA Reviewer:

Ibrahim Abdel-Saheb **Signature:**
Environmental Scientist **Date: 9-10-15**

Executive Summary

This analytical method, Bayer Method ET-002-W13-02, is designed for the quantitative determination of ethephon in water using HPLC/MS/MS. The method is quantitative for ethephon at the stated LOQ of 0.5 µg/L in water. The LOQ is less than the lowest toxicological level of concern in water (2,500 ug/L). The independent laboratory validated the method for analysis of ethephon in surface water after one trial. No major modifications were made by the independent laboratory.

Table 1. Analytical Method Summary[1]

Analyte(s) by Pesticide	MRID		EPA Review	Matrix[2]	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Ethephon	49305604	49305603		Water	23/01/2014	Bayer CropScience	HPLC/MS/MS	0.5 µg/L

1 Originating ECM performance data were not provided.
2 A surface (pond) water matrix was used in the ILV and was characterized (Appendix B, p. 58 of MRID 49305603).

I. Principle of the Method

Water (10 mL) is acidified with 0.050 mL formic acid, fortified with ethephon in 0.1% aqueous formic acid for procedural recoveries, fortified with isotopic *d4*-ethephon (0.1 mL of 1.0 µg/mL solution) as an internal standard, shaken to mix, then analyzed directly by LC/MS/MS (pp. 8-9; Appendix 2, p. 13 of MRID 49305604).

Samples are analyzed for ethephon by HPLC (Phenomenex Aqua C18, 4.6 mm x 150 mm, 3 µm column, 60°C) using an isocratic mobile phase of 0.1% aqueous formic acid:0.1% formic acid in acetonitrile (95:5, v:v) with MS/MS-ESI (AB Sciex API 5500 MS, electrospray ionization, negative ion mode) detection and multiple reaction monitoring (MRM; pp. 6, 9-10 of MRID 49305604). Injection volume is 20 µL. Ethephon is identified using two ion transitions; *m/z* 142.9→107.0 for quantitation (Q) and *m/z* 106.8→78.8 for confirmation (C). The *d4*-ethephon internal standard is quantified using transition *m/z* 146.9→111.0.

The ILV performed the method for analysis of ethephon as written with no major modifications (pp. 16-17, 19 of MRID 49305603). The initial method, Bayer Method ET-002-W13-001, provided to the independent laboratory also included methodology for analysis of ethephon transformation product 2-HEPA (Appendix A, pp. 36-57). However, following one failed trial to validate the method for analysis of 2-HEPA, the study sponsor instructed the independent laboratory to terminate the method validation for 2-HEPA (p. 19; Appendix D, p. 71; Appendix E, pp. 72-86; Appendix F, pp. 87-88).

The LOQ for ethephon was the same in the ECM and ILV at 0.5 µg/L (ng/mL; p. 6 of MRID 49305604; p. 16 of MRID 49305603). A LOD was not reported.

II. Recovery Findings

ECM (MRID 49305604): Originating ECM performance data were not reported.

ILV (MRID 49305603): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of ethephon in surface (pond) water at fortification levels of 0.5 µg/L (ng/mL, LOQ) and 5.0 µg/L (10x LOQ; Tables 1-2, pp. 22-23). Ethephon was identified and quantified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable. The method was validated for ethephon in surface water at both fortification levels after one trial, with no major modifications (p. 19). The water matrix was characterized (p. 12; Appendix B, p. 58).

Table 2. Initial Validation Method Recoveries for Ethephon in Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ethephon	0.5 (LOQ)		No originating ECM performance data were reported.			
	5.0					

Table 3. Independent Validation Method Recoveries for Ethephon in Surface Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ethephon	Quantitation ion					
	0.5 (LOQ)	5	88-109	101	8.5	8.5
	5.0	5	88-103	97	6.2	6.3
	Confirmation ion					
	0.5 (LOQ)	5	86-102	96	6.0	6.2
	5.0	5	81-105	96	8.8	9.2

Data (uncorrected recovery results) were obtained from Tables 1-2, pp. 22-23 of MRID 49305603.

1 Pond water from Colorado, local to Pyxant Labs; matrix characterization was provided (p. 12; Appendix B, p. 58 of MRID 49305603).

III. Method Characteristics

The LOQ for ethephon was the same in the ECM and ILV at 0.5 µg/L (ng/mL; p. 6 of MRID 49305604; p. 16 of MRID 49305603). No justification for the selected LOQ was provided. The LOD for ethephon was not specified in either the ECM or ILV.

Table 4. Method Characteristics for Ethephon in Water

		Ethephon
Limit of Quantitation (LOQ)		0.5 µg/L
Limit of Detection (LOD)		Not reported.
Linearity (1/x weighting, calibration curve r^2 and concentration range)[1]	ECM:	Q ion: r^2 = **0.9908** C ion: r^2 = **0.9922**
	ILV:	Q ion: r^2 = 0.9949 C ion: r^2 = 0.9960
	Range:	0.2-50 ng/mL
Repeatable	ECM:	No performance data.
	ILV:	Yes
Reproducible		ECM did not provide performance data to establish the LOQ.
Specific		Yes

Data were obtained from pp. 6, 8 of MRID 49305604; pp. 15-16; Tables 1-2, pp. 22-23; Figures 5-8, pp. 30-33 of MRID 49305603; DER Attachment 2.

1 ECM and ILV calibration curve r^2 values were derived from reported r values (1/x weighting; DER Attachment 2). Linearity of provided ECM standard curves could not be verified by the reviewer because the individual calibration standard data were not provided.

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

IV. Method Deficiencies and Reviewer's Comments

1. No originating ECM performance data were reported (MRID 49305604). The only results presented in the ECM report were ethephon standard curves (quantitation and confirmation ions, but without individual calibration standard data), a chromatogram of a 50 ng/mL calibration standard (quantitation ion), and a MS spectra (Appendices 3-5, pp. 14-16 of MRID 49305304).

2. The determination of the LOQ and LOD were not based on scientifically acceptable procedures as defined in 40 CFR Part 136, Appendix B. No justification for the selected LOQ (0.5 µg/L) for ethephon was provided, and a LOD was not reported (p. 6 of MRID 49305604). Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

3. For the ECM, linearity (r^2) of the calibration standards was not ≥0.995 (see Table 4 above).

4. For the ILV, the water matrix was obtained by the independent laboratory and was characterized (p. 12; Appendix B, p. 58 of MRID 49305603). However, the matrix used in the ILV must be either an equivalent, or more difficult, analytical sample condition as that used in the ECM.

5. For the ILV, chromatograms of a reagent blank sample were not provided, and chromatograms were provided for only one calibration standard (0.8 ng/mL). In chromatograms of the 0.8 ng/mL calibration standard, untreated control samples, and samples fortified at the LOQ, some baseline noise was observed, but interferences were considered "negligible" (p. 19; Figures 3-8, pp. 28-33 of MRID 49305603).

6. It was reported for the ILV that one analyst could prepare and analyse one set of fourteen samples in *ca*. 4 hours, with initial solution preparation requiring *ca*. 3 hours (p. 20 of MRID 49305603).

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

Ethephon

IUPAC Name: 2-Chloroethylphosphonic acid
CAS Name: (2-Chloroethyl)phosphonic acid
1,2-(2-Chloroethyl)phosphonic acid
CAS Number: 16672-87-0
SMILES String: P(O)(O)(=O)CCCl (EpiSuite 4.0)