Test Material:	Ethephon
MRID:	**49305602**
Title:	Bayer Method ET-001-S13-02: An Analytical Method for the Determination of Residues of Ethephon in Soil and Sediment Using LC/MS/MS
MRID:	**49305601**
Title:	Independent Laboratory Validation of Bayer Method ET 001 S13-01: An Analytical Method for the Determination of Residues of Ethephon and its Metabolite 2-HEPA in Soil and Sediment 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 soil

Reports: ECM: EPA MRID No.: 49305602. Miller, A. 2014. Bayer Method ET-001-S13-02: An Analytical Method for the Determination of Residues of Ethephon in Soil and Sediment Using LC/MS/MS (p. 4). Report prepared, sponsored, and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 18 pages. Final report issued January 16, 2014.

ILV: EPA MRID No. 49305601. Chen, C. 2014. Independent Laboratory Validation of Bayer Method ET 001 S13-01: An Analytical Method for the Determination of Residues of Ethephon and its Metabolite 2-HEPA in Soil and Sediment Using LC/MS/MS. CPS Study No.: 13-CPS-029, Revision No. 1. Bayer CropScience Study No.: RAETL041. Report prepared by Critical Path Services, LLC (CPS), Garnet Valley, Pennsylvania, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 107 pages. Final report issued January 17, 2014. Final report revision issued January 24, 2014.

Document No.: MRIDs 49305602 & 49305601

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 49305602). 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 49305601). 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, the LOD of the analyte, and characterization of the ILV soil matrix.

PC Code: 099801

EPA Reviewer:

Ibrahim Abdel-Saheb
Environmental Scientist

Signature: 
Date: 9-8-2015

Executive Summary

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This analytical method, Bayer Method ET-001-S13-02, is designed for the quantitative determination of ethephon in soil and sediment using HPLC/MS/MS. The method is quantitative for ethephon at the stated **LOQ of 5 µg/kg in soil** (The lowest toxicological level of concern in soil: 5.9 µg/kg a.i./kg in soil, assuming a soil depth of 4 inches); a sediment matrix was not utilized. The independent laboratory validated the method for analysis of ethephon in soil (uncharacterized) 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	49305602	49305601		Soil	16/01/2014	Bayer CropScience	HPLC/MS/MS	5 µg/kg

1 Originating ECM performance data were not provided.

2 The soil matrix used in the ILV was provided by the study sponsor (Bayer CropScience), but was not characterized (p. 13 of MRID 49305601).

I. Principle of the Method

Soil (20 ± 0.05 g) is fortified with ethephon in 0.7% aqueous phosphoric acid for procedural recoveries, then extracted once with 40 mL of 0.7% aqueous phosphoric acid via microwave extractor for 3 minutes at 250 W (pp. 7, 9; Appendix 2, p. 14 of MRID 49305602). Following extraction, the mixture is fortified with isotopic *d4*-ethephon (0.40 mL of 1 µg/mL solution), as an internal standard. An aliquot (1.5 mL) of the fortified extract is centrifuged (10,000 rpm, 5 minutes) for LC/MS/MS analysis.

Samples are analyzed for ethephon by HPLC (Phenomenex Aqua C18, 4.6 mm x 150 mm, 3 µm column, 60°C) using a mobile phase of (A) 0.1% aqueous formic acid and (B) acetonitrile [percent A:B (v:v) at 0.1-3.0 min. 95:5, 3.5-4.5 min. 5:95, 5.0-8.0 min. 95:5] with MS/MS-ESI (AB Sciex API 4000 MS, electrospray ionization, negative ion mode) detection and multiple reaction monitoring (MRM; pp. 6, 9-11 of MRID 49305602). Injection volume is 25 µL. Ethephon is identified using two ion transitions; *m/z* 142.9→106.8 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. 13-16, 18; Tables 2-3, pp. 22-23 of MRID 49305601). The initial method, Bayer Method ET-001-S13-001, provided to the independent laboratory also included methodology for analysis of ethephon transformation product 2-HEPA (Appendix 5, pp. 65-90). However, following two failed trials to validate the method for analysis of 2-HEPA, the study sponsor instructed the independent laboratory to terminate the method validation for 2-HEPA and prepare the ILV report for the successful validation of parent ethephon (p. 18; Appendix 3, pp. 52-53; Appendix 4, pp. 62-64; Appendix 7, pp. 95-107).

The LOQ for ethephon was the same in the ECM and ILV at 5 µg/kg (ng/g, ppb; p. 6 of MRID 49305602; p. 14 of MRID 49305601). A LOD was not reported.

II. Recovery Findings

ECM (MRID 49305602): Originating ECM performance data were not reported.

ILV (MRID 49305601): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of ethephon in soil at fortification levels of 5 µg/kg (ppb, LOQ) and 50 µg/kg (10x LOQ; Table 1, p. 21). 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 the soil matrix at both fortification levels after one trial, with no major modifications (pp. 17-18). The soil matrix was provided by the study sponsor (Bayer CropScience), but was not characterized (p. 13).

Table 2. Initial Validation Method Recoveries for Ethephon in Soil and Sediment

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ethephon	5 (LOQ)		No originating ECM performance data were reported.			
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Table 3. Independent Validation Method Recoveries for Ethephon in Soil[1]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ethephon	Quantitation ion					
	5 (LOQ)	5	92.0-101.6	98.2	3.8	3.9
	50	5	100.0-105.2	102.4	2.2	2.1
	Confirmation ion					
	5 (LOQ)	5	92.4-104.4	98.6	4.4	4.5
	50	5	100.0-114.4	107.4	5.2	4.9

Data (uncorrected recovery results) were obtained from Table 1, p. 21 of MRID 49305601.
1 Uncharacterized (p. 13 of MRID 49305601).

III. Method Characteristics

The LOQ for ethephon was the same in the ECM and ILV at 5 µg/kg (ng/g, ppb; p. 6 of MRID 49305602; p. 14 of MRID 49305601). 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 Soil

		Ethephon
Limit of Quantitation (LOQ)		5 µg/kg
Limit of Detection (LOD)		Not reported.
Linearity (1/x weighting, calibration curve r^2 and concentration range)[1]	ECM:	Q ion: r^2 = 0.9992 C ion: r^2 = 0.9992
	ILV:	Q ion: r^2 = 0.9996 C ion: r^2 = 0.9998
	Range:	0.8-100 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 49305602; p. 14; Table 1, p. 21; Figures 3-6, pp. 27-30; Figures 11-16, pp. 35-40 of MRID 49305601; 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 49305602). 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. 15-17 of MRID 49305302).

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 (5 µg/kg) for ethephon was provided, and a LOD was not reported (p. 6 of MRID 49305602). Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

3. For the ILV, the soil matrix was provided by the study sponsor (Bayer CropScience), but was not characterized (p. 13 of MRID 49305601). A sediment matrix was not provided by the sponsor and not included in the method validation conducted by the independent laboratory. The matrix used in the ILV must be either an equivalent, or more difficult, analytical sample condition as that used in the ECM.

4. It was reported for the ILV that one analyst could extract one set of thirteen samples (one reagent blank, two matrix control samples and ten validation samples) in *ca*. 4 hours, with LC/MS/MS analysis time of *ca*. 6 hours (pp. 17-18 of MRID 49305601).

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)