

**Analytical method for valifenalate, valifenalate acid and p-chlorobenzoic acid in soil**

**Reports:** ECM: EPA MRID No.: MRID 49807289 (Appendix III, pp. 64-198). Ferguson, L-J.C. 2015. ANALYTICAL PHASE REPORT – Terrestrial Field Dissipation of F9170 in Nebraska, USA. Ricerca Document No.: 032967-1. Precision Study Management Study No.: PSM-14-02-05. FMC Tracking No.: 2014EFT-VAL1224. Report prepared by Ricerca Biosciences, LLC, Concord, Ohio, and sponsored and submitted by FMC Corporation, Agricultural Products Group, Ewing, New Jersey; 135 pages. Final report issued November 20, 2015.

ILV: EPA MRID No.: MRID 49807189. Shanmugan, S. 2016. Independent Laboratory Validation (ILV) of an Analytical Method for the Determination of Valifenalate and Two Metabolites (Valifenalate acid and p-Chlorobenzoic Acid) in Soil. FMC Tracking No.: 2015RES-VAL1916. Battelle ID: 100062321. Report prepared by Battelle, Norwell, Massachusetts, and sponsored and submitted by FMC Corporation, Agricultural Products Group, Ewing, New Jersey; 51 pages (including page 1a). Final report issued January 7, 2016.

**Document No.:** MRIDs 49807189 & 49807289

**Guideline:** 850.6100

**Statements:** ECM: The study was conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160; Appendix III, p. 65 of MRID 49807289). Signed and dated GLP and Quality Assurance statements were provided (Appendix III, pp. 65-66). The Data Confidentiality and Authenticity statements were not included.

ILV: The study was conducted in compliance with USEPA FIFRA GLP standards, which are compatible with OECD GLP (p. 3 of MRID 49807189). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). The statement of authenticity was included with the quality assurance statement.

**Classification:** This analytical method is classified as Supplemental. In the ECM, an insufficient number of samples was prepared, and no representative chromatograms were provided. The ILV was not provided with the most difficult matrix with which to validate the method. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures.

**PC Code:** 128200

**EFED Final Reviewer:** Sarah C. Hafner, Ph.D.,  
Chemist

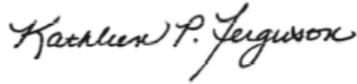
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Date: 5/11/19

**CDM/CSS-Dynamac JV**

Lisa Muto,  
Environmental Scientist

Signature:   
Date: 2/5/18

**Reviewers:** Kathleen Ferguson, Ph.D.,  
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Signature:   
Date: 2/5/18

CDM/CSS-Dynamac JV-altered sections of this Tier II Summary include only the signed cover page, Executive Summary, Deviations and Deficiencies, Reviewer's Verification of Statistical Results, and Conclusions sections. This Tier II Summary may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

## Executive Summary

The analytical method, Ricerca Document No. 032967-1 and Precision Study Management Study No. PSM-14-02-05, is designed for the quantitative determination of valifenalate and valifenalate acid in soil at the stated LOQ of 0.005 mg/kg and of p-chlorobenzoic acid in soil at the stated LOQ of 0.01 mg/kg. The LOQs are less than the lowest toxicological level of concern in soil. Although it was not specifically reported, the reviewer assumed that the method for valifenalate, valifenalate acid, and p-chlorobenzoic acid was validated in the first trial with insignificant modifications to the sample processing and analytical equipment and parameters. The ILV matrix was a sandy loam soil, while the ECM matrix was the loamy sand/sandy loam soil used in the Nebraska terrestrial field dissipation study. The ILV was not provided with the most difficult matrix with which to validate the method given the range of soils (e.g., regarding %OC) used in the four submitted valifenalate terrestrial field dissipation studies. The analytes were identified using two or three ion transitions in the ECM and ILV. All ILV data regarding linearity, repeatability, accuracy, precision, and specificity were satisfactory for all three analytes. In the ECM, an insufficient number of samples was prepared (n=3), and no representative chromatograms were provided; however, specificity was evaluated using representative chromatograms from the concurrent recovery samples. All ECM data regarding repeatability, accuracy, precision, linearity and specificity were satisfactory for all three analytes, but the LODs of the method were not reported within the method appendix. Elsewhere in the MRID, the LOD is reported as 1 ppb (or 0.001 mg/kg) for valifenalate and valifenalate acid and 2 ppb (or 0.002 mg/kg) for p-chlorobenzoic acid.

**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						
Valifenalate	Appendix III of MRID 49807289 <sup>1</sup>	MRID 49807189 <sup>2</sup>		Soil	20/11/2015	FMC Corporation	LC-MS/MS	0.005 mg/kg
Valifenalate acid								0.01 mg/kg
p-Chlorobenzoic acid								0.01 mg/kg

<sup>1</sup> In the ECM, the test soil was a field soil from Nebraska which was used in a terrestrial field dissipation study. The soil was characterized as loamy sand at 0-6" (83% sand 10% silt 7% clay, pH 6.0 (1:1 soil:water ratio), 0.90% organic carbon) and sandy loam at 6-36" (67-77% sand 12-18% silt 11-15% clay, pH 4.6-6.1 (1:1 soil:water ratio), 0.26-0.93% organic carbon; USDA soil texture classification; Table 4, p. 31 of MRID 49807289).

<sup>2</sup> In the ILV, the sandy loam soil (ID MSL-PF 0-6"; 68% sand 12% silt 20% clay, pH 6.8 (1:1 soil:water ratio), 2.94% organic matter) was provided and characterized by Agvise Laboratories, Northwood, North Dakota (p. 13; Appendix 4, p. 51 of MRID 49807189).

## I. Principle of the Method

For the analysis of valifenalate and its metabolites, soil (10 g aliquots) was fortified then extracted by mechanical shaking for 30 minutes with acetone:0.5N HCl (50:50) followed by centrifugation at approximately 3000 rpm. The supernatant was collected and the pellet re-extracted by mechanical shaking for 30 minutes with acetone:0.5N HCl (20:80) followed by centrifugation at approximately 3000 rpm. The supernatants were combined and an aliquot (10 mL) concentrated to approximately 6.5 mL under nitrogen. The aliquot was then brought back to 10mL in methanol water 50:50 (v/v) with 0.1% formic acid. The sample was diluted 10X in methanol:water 10:90 (v/v) with 0.1% formic acid and transferred to an HPLC vial for LC-MS/MS analysis under positive ionization for valifenalate and valifenalate acid. An HPLC coupled with a SCIEX API 4000 Mass Spectrometer using multiple reaction monitoring (MRM) mode (400°C) was used (Appendix III, pp. 82-85 of MRID 49807289). The following LC conditions were employed: ARMOR C18 column (2.1 mm x 100 mm, 5 µm; column temperature 40°C), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.0-1.5 min. 80:20, 1.7-3.0 min. 5:95, 3.1-5.0 min. 80:20] and injection volume of 20-40 µL. The transitions used for quantitation were m/z 399→155 for valifenalate and m/z 385→116 for valifenalate acid.

Valifenalate MS/MS transitions m/z 399→144 and m/z 399→116 and valifenalate acid transitions m/z 385→186 and m/z 385→144 were monitored for confirmatory purposes. Retention times were *ca.* 3.3 and 3.2 minutes for valifenalate and valifenalate acid, respectively. A separate chromatographic method was used to separate p-chlorobenzoic acid using LC-MS/MS under negative ionization. An HPLC coupled with a SCIEX API 4000 Mass Spectrometer using multiple reaction monitoring (MRM; 550°C) mode was used. The following LC conditions were employed: ARMOR C18 column (2.1 mm x 100 mm, 5 µm; column temperature 40°C), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.0min. 90:10, 2.5-3.0 min. 10:90, 3.1-5.0 min. 90:10] and injection volume of 50 µL. Mass transition m/z 155→111 was used for quantitation, and m/z 155→35 was monitored for confirmation. Retention time was *ca.* 2.6 minutes for p-chlorobenzoic acid.

Quantitation was obtained using external calibration curves of standards for each analyte. Standard curves were linear over the concentration range 0.05 – 100 ng/mL with correlation coefficients ( $R^2$ ) greater than 0.99.

Reagent blank and blank control specimens of soil were analysed in triplicate and fortified specimens of soil were analysed in sextuplicate for each fortification level. Fortification experiments were performed at the limit of quantitation and at ten times that level.

The ILV performed the ECM method as written, except for the use of a Wrist Action® Shaker, use of vortex to break up the soil pellet prior to second extraction, possible use of a different second extraction solvent (marked 50:0, v/v instead of 20:80, v:v), addition of a filtration step using a Teflon syringe filter prior to final sample dilution for LC-MS/MS analysis, and insignificant modifications to the analytical equipment and parameters (pp. 21-25 of MRID 49807189). The LC-MS/MS instrument and parameters were similar to those of the ECM. Samples were analyzed for valifenalate, valifenalate acid, and p-chlorobenzoic acid using a Shimadzu LC2080 UHPLC coupled with an Applied Biosystems MDS Sciex API 6500 Linear Ion Trap Mass Spectrometer (550°C). A Thermo Betasil C18 column (2.1 mm x 100 mm, 5 µm; column temperature 40°C) was used. The other LC conditions were the same as those reported in the ECM. Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 399→155 and m/z 399→116 for

valifenalate;  $m/z$  385→116 and  $m/z$  385→144 for valifenalate acid; and  $m/z$  155→111 and  $m/z$  155→35 for p-chlorobenzoic acid. Reported retention times were *ca.* 2.2-2.4 for all analytes. No other method modifications were reported.

In the ECM and ILV, the validated Limits of Quantification (LOQs) were 0.005 mg/kg for residues of valifenalate and valifenalate acid in soil and 0.010 mg/kg for residues of p-chlorobenzoic acid in soil (pp. 12, 26 of MRID 49807189; Appendix III, p. 81 of MRID 49807289). In the ILV, the method Limits of Detection (LODs) were 0.0015 mg/kg for residues of valifenalate and valifenalate acid in soil and 0.003 mg/kg for residues of p-chlorobenzoic acid in soil. In the ECM (Appendix III), the LODs were not reported. Elsewhere in the MRID, the LOD is reported as 1 ppb (or 0.001 mg/kg) for valifenalate and valifenalate acid in soil and 2 ppb (or 0.002 mg/kg) for p-chlorobenzoic acid in soil.

## II. Recovery Findings

ECM (Appendix III of MRID 49807289): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of valifenalate and valifenalate acid at fortification levels of 0.005 mg/kg (LOQ) and 0.05 mg/kg (10×LOQ) in one soil matrix and of p-chlorobenzoic acid at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10×LOQ) in one soil matrix; however, an insufficient number of samples was prepared for all fortifications/analytes ( $n = 3$ ; Appendix III, Tables 2-4, pp. 91-93). Only one individual valifenalate acid recovery sample (out of  $n=3$ ) at LOQ exceeded 120% (121%). Three ion pair transitions were monitored for valifenalate and valifenalate acid using LC-MS/MS in positive mode; two ion pair transitions were monitored for p-chlorobenzoic acid using LC-MS/MS in negative mode. The quantification and confirmation ion data was comparable. The test soil was a field soil from Nebraska which was used in a terrestrial field dissipation study. The soil was characterized as loamy sand at 0-6" (83% sand 10% silt 7% clay, pH 6.0 (1:1 soil:water ratio), 0.90% organic carbon) and sandy loam at 6-36" (67-77% sand 12-18% silt 11-15% clay, pH 4.6-6.1 (1:1 soil:water ratio), 0.26-0.93% organic carbon; USDA soil texture classification).

ILV (MRID 49807189): Mean recoveries and RSDs were within guidelines for analysis of valifenalate and valifenalate acid at fortification levels of 0.005 mg/kg (LOQ) and 0.05 mg/kg (10×LOQ) in one soil matrix and of p-chlorobenzoic acid at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10×LOQ) in one soil matrix (Tables 1-3, pp. 44-46). Two individual valifenalate acid samples (out of  $n=6$ ) at LOQ were below 70% recovery (68, 69%). Two ion pair transitions were monitored for valifenalate, valifenalate acid and p-chlorobenzoic acid using LC-MS/MS in positive or negative mode; the quantification and confirmation ion data was comparable. Sandy loam soil (ID MSL-PF 0-6"; 68% sand 12% silt 20% clay, pH 6.8 (1:1 soil:water ratio), 2.94% organic matter) was provided and characterized by Agvise Laboratories, Northwood, North Dakota (p. 13; Appendix 4, p. 51). Although it was not specifically reported, the reviewer assumed that the method for valifenalate, valifenalate acid and p-chlorobenzoic acid was validated in the first trial with insignificant modifications to the sample processing and analytical equipment and parameters (pp. 12, 21-26).

**Table 2. Initial Validation Method Recoveries for Valifenalate, Valifenalate acid, and p-Chlorobenzoic acid in Soil**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>NE Loamy Sand (0-6") and Sandy Loam (6-36") Soil<sup>1</sup></b>						
Quantitation ion <sup>2</sup>						
Valifenalate	0.005 (LOQ)	<b>3</b>	92.7-96.0	93.1	2.8	3.0
	0.05	<b>3</b>	85.5-89.4	87.8	2.1	2.3
Valifenalate acid	0.005 (LOQ)	<b>3</b>	102- <b>121</b>	110	10.0	9.1
	0.05	<b>3</b>	103-108	106	2.9	2.7
p-Chlorobenzoic acid	0.01 (LOQ)	<b>3</b>	99.4-112	107	6.6	6.2
	0.1	<b>3</b>	96.1-99.1	97.3	1.6	1.6
Confirmation ion 1 <sup>2</sup>						
Valifenalate	0.005 (LOQ)	<b>3</b>	89.2-92.3	90.9	1.6	1.7
	0.05	<b>3</b>	85.9-88.3	86.9	1.2	1.4
Valifenalate acid	0.005 (LOQ)	<b>3</b>	97.9-110	102	6.9	6.8
	0.05	<b>3</b>	109-111	110	1.2	1.1
p-Chlorobenzoic acid	0.01 (LOQ)	<b>3</b>	88.6-114	104	13.5	13.0
	0.1	<b>3</b>	95.3-101	97.7	2.9	3.0
Confirmation ion 2 <sup>2</sup>						
Valifenalate	0.005 (LOQ)	<b>3</b>	87.9-90.3	89.2	1.2	1.4
	0.05	<b>3</b>	85.3-89.6	88.0	2.4	2.7
Valifenalate acid	0.005 (LOQ)	<b>3</b>	109-108	113	4.5	4.0
	0.05	<b>3</b>	105-110	108	2.5	2.3

Data (uncorrected recovery results; Appendix III, Appendix B, pp. 172-173) were obtained from Appendix III, Tables 2-4, pp. 91-93 of MRID 49807289.

<sup>1</sup> Soil Characteristics for Nebraska Field Site provided below (Table 4, p. 31 of MRID 49807289). Specific soil depth used for method validation was not reported. Soil samples were homogenized prior to fortification (Appendix III, p. 82 of MRID 49807289).

Soil Parameter	Soil Depth in Inches					
	0-6	6-12	12-18	18-24	24-30	30-36
USDA textural class	Loamy Sand	Sandy Loam				
% sand (0.05 to 2.00 mm)	83	77	67	67	71	73
% silt (0.002 to 0.05 mm)	10	12	18	18	16	14
% clay (<0.002 mm)	7	11	15	15	13	13
pH in 1:1 soil: water ratio	6.0	4.6	5.6	6.0	6.1	5.7
% organic carbon	0.90	0.93	0.77	0.39	0.31	0.26
Cation Exchange Capacity (meq/100 g)	8.8	9.9	12.1	11.8	11.1	10.3
Bulk density (gm/cc)	1.34	1.26	1.23	1.25	1.26	1.27
% moisture at ½ bar	10.4	15.4	16.9	16.5	14.6	13.2
% moisture at 15 bar	4.6	6.4	7.7	7.9	6.8	6.1

<sup>2</sup> The transitions used for quantitation were m/z 399→155 for valifenalate, m/z 385→116 for valifenalate acid, and m/z 155→111 for p-chlorobenzoic acid. The transitions used for confirmation were m/z 399→144 and m/z 399→116 for valifenalate, m/z 385→186 and m/z 385→144 for valifenalate acid, and m/z 155→35 for p-chlorobenzoic acid.

**Table 3. Independent Validation Method Recoveries for Valifenalate, Valifenalate acid, and p-Chlorobenzoic acid in Soil**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) <sup>1</sup>	Relative Standard Deviation (%)
<b>Sandy Loam Soil<sup>2</sup></b>						
Quantitation ion <sup>3</sup>						
Valifenalate	0.005 (LOQ)	6	72-84	78	5	6
	0.05	6	75-90	85	6	7
Valifenalate acid	0.005 (LOQ)	6	<b>68</b> -74	72	3	4
	0.05	6	82-90	86	3	3
p-Chlorobenzoic acid	0.01 (LOQ)	6	97-101	99	2	2
	0.1	6	102-110	107	3	3
Confirmation ion <sup>3</sup>						
Valifenalate	0.005 (LOQ)	6	74-87	80	5	6
	0.05	6	80-88	83	3	4
Valifenalate acid	0.005 (LOQ)	6	67-80	71	5	6
	0.05	6	84-89	86	2	2
p-Chlorobenzoic acid	0.01 (LOQ)	6	93-110	102	6	5
	0.1	6	101-107	106	3	3

Data (uncorrected recovery results; pp. 23-24) were obtained from Tables 1-3, pp. 44-46 of MRID 49807189 and DER Attachment 2.

1 Standard deviations were reviewer-calculated since these values were not calculated in the study report (see DER Attachment 2). The rules of significant figures were followed.

2 Sandy loam soil (ID MSL-PF 0-6<sup>3</sup>; 68% sand 12% silt 20% clay, pH 6.8 (1:1 soil:water ratio), 2.94% organic matter) was provided and characterized by Agvise Laboratories, Northwood, North Dakota (p. 13; Appendix 4, p. 51).

3 Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 399→155 and m/z 399→116 for valifenalate; m/z 385→116 and m/z 385→144 for valifenalate acid; and m/z 155→111 and m/z 155→35 for p-chlorobenzoic acid. Only one confirmation ion was monitored for each analyte.

### III. Method Characteristics

In the ECM and ILV, the validated LOQs were 0.005 mg/kg for residues of valifenalate and valifenalate acid in soil and 0.010 mg/kg for residues of p-chlorobenzoic acid in soil (pp. 12, 26 of MRID 49807189; Appendix III, p. 81 of MRID 49807289). In the ILV, the method LODs were 0.0015 mg/kg for residues of valifenalate and valifenalate acid in soil and 0.003 mg/kg for residues of p-chlorobenzoic acid in soil; the LODs were set at 30% of the LOQ. In the ECM, the LODs were not reported. No calculations, justifications, or comparisons to background noise were provided for the LOQs and LODs in the ECM or ILV.

**Table 4. Method Characteristics for Valifenalate, Valifenalate acid, and p-Chlorobenzoic acid in Soil**

		Valifenalate	Valifenalate acid	p-Chlorobenzoic acid
Limit of Quantitation (LOQ)	ECM	0.005 mg/kg		0.01 mg/kg
	ILV			
Limit of Detection (LOD)	ECM	0.001 mg/kg		0.002 mg/kg
	ILV	0.0015 mg/kg		0.003 mg/kg
Linearity (calibration curve $r^2$ and concentration range) <sup>1</sup>	ECM	$r^2 = 0.9998$ (Q)	$r^2 = 0.9992$ (Q)	$r^2 = 0.9994$ (Q)
		0.05-5 ng/mL		0.1-10 ng/mL
	ILV	$r^2 = 0.99818$ (Q) $r^2 = 0.99968$ (C)	$r^2 = 0.99910$ (Q) $r^2 = 0.99864$ (C)	$r^2 = 0.99994$ (Q) $r^2 = 0.99982$ (C)
		0.025-1.5 ng/mL		0.075-5 ng/mL
Repeatable	ECM <sup>2</sup>	Yes at LOQ and 10×LOQ; however, n = <b>3</b> . (characterized loamy sand/sandy loam soil used)		
	ILV <sup>3,4</sup>	Yes at LOQ and 10×LOQ. (characterized sand loam soil used)		
Reproducible		Yes at LOQ and 10×LOQ.		
Specific	ECM	Yes, no matrix interferences were quantified, but some minor baseline noise was observed around the analyte peak. <sup>5</sup> <b>No</b> representative method validation chromatograms provided.		
	ILV	Yes, matrix interferences were <i>ca.</i> 10% of the LOQ (based on peak height). Some minor baseline noise was observed around the analyte peak.		Yes, matrix interferences were <8% of the LOQ (based on peak height). Some minor baseline noise was observed around the analyte peak.

Data were obtained from Appendix III, pp. 80-81, 84; Appendix III, Tables 2-4, pp. 91-93 (recovery results); Appendix III, Figures 1-3, pp. 120-122 (calibration curve); Appendix III, Figures 14-19, pp. 133-138 (chromatograms); Appendix III, Appendix C, Tables C4-C6, pp. 193-197 (correlation coefficients) of MRID 49807289; pp. 12, 19-20, 25-26; Tables 1-3, pp. 44-46 (recovery results); Figures 4-6, pp. 32-34 (calibration curves); Figures 10-12, pp. 38-43 (chromatograms) of MRID 49807189. Q = quantitation ion; C = confirmation ion. All results reported for Q and C ions unless specified otherwise.

1 Correlation coefficients ( $r^2$ ) were reviewer-calculated based on r values (1/x weighted linear regression analysis) reported in the study report (Appendix III, pp. 80, 84 and Figures 1-3, pp. 120-122 of MRID 49807289; Figures 4-6, pp. 32-34 of MRID 49807189; DER Attachment 2). In the ECM, regression equations and calibration curves were only provided for the quantitation ion transition. Matrix-matched solutions were used in the ILV (pp. 19-20, 26 of MRID 49807189).

2 In the ECM, the test soil was a field soil from Nebraska which was used in a terrestrial field dissipation study. The soil was characterized as loamy sand at 0-6" (83% sand 10% silt 7% clay, pH 6.0 (1:1 soil:water ratio), 0.90% organic carbon) and sandy loam at 6-36" (67-77% sand 12-18% silt 11-15% clay, pH 4.6-6.1 (1:1 soil:water ratio), 0.26-0.93% organic carbon; USDA soil texture classification; Table 4, p. 31 of MRID 49807289). Three ion pair transitions were monitored for valifenalate and valifenalate acid using LC/MS/MS in positive mode; two ion pair transitions were monitored for p-chlorobenzoic acid using LC/MS/MS in negative mode.

3 In the ILV, the sandy loam soil (ID MSL-PF 0-6"; 68% sand 12% silt 20% clay, pH 6.8 (1:1 soil:water ratio), 2.94% organic matter) was provided and characterized by Agvise Laboratories, Northwood, North Dakota (p. 13; Appendix 4, p. 51 of MRID 49807189). Three or two ion pair transitions were monitored for valifenalate, valifenalate acid, and p-chlorobenzoic acid using LC/MS/MS in positive or negative mode.

4 Although it was not specifically reported, the reviewer assumed that the method for valifenalate, valifenalate acid, and p-chlorobenzoic acid was validated in the first trial with insignificant modifications to the sample processing and analytical equipment and parameters (pp. 12, 21-26 of MRID 49807189).

5 Specificity based on concurrent recovery LOQ and 10×LOQ representative chromatograms and quantified residues in the controls of the method validation and concurrent recovery samples. Appendix III, Table 6, p. 95 of MRID 49807289 (quantified residues in the controls of the concurrent recovery samples).

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

- The Appendix III, pp. 64-198 of TFD MRID 49807189 was the ECM for the ILV. The ILV stated that the independent validation was originally for Eurofins method RA034 v07 (MRID 49807188), but followed the method specified in Precision Study Management Amendment 2 (PSM-14-02-05 Amendment 2) because Precision Study Management Amendment 2 is used for field samples and “Eurofins method RA034 v07 MRM transitions were very unusual, used both positive and negative ionization for a given analyte” (p. 26 of MRID 49807189).

Precision Study Management Amendment 2 was contained in Appendix IV, pp. 235-240 (6 pages) of TFD MRID 49807189 as part of the Study Protocol and Amendments for ECM (pp. 200-242). PSM-14-02-05 Amendment 2 was the modified sample processing and analytical parameters for Eurofins method RA034 v07 which were used and rewritten in the ANALYTICAL PHASE REPORT – Terrestrial Field Dissipation of F9170 in Nebraska, USA (Appendix III, pp. 64-198 of MRID 49807289).

- The ILV matrix was a sandy loam soil (ID MSL-PF 0-6”; 68% sand 12% silt 20% clay, pH 6.8 (1:1 soil:water ratio), 2.94% organic matter; p. 13; Appendix 4, p. 51 of MRID 49807189), while the ECM matrix was the loamy sand/sandy loam soil used in the Nebraska terrestrial field dissipation study. The ILV was not provided with the most difficult matrix with which to validate the method given the range of soils and %OC used in the terrestrial field dissipation studies (see table below; MRIDs 48907286, 48907287, 48907288, and 48907289).

**Table 5. Soil Characteristics at the Field Sites Treated with F9170-1 WP (Valifenalate)**

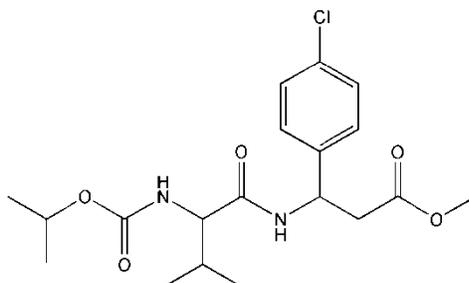
Property		California	New York	Georgia	Nebraska
USDA Textural Classification	0-6 in	Sandy Loam	Silt Loam	Sand	Loamy Sand
% silt		22	60	7	10
% sand		58	25	88	83
% clay		21	15	5	7
USDA Textural Classification	6-12 in	Sandy Loam	Silt Loam	Loamy Sand	Sandy Loam
% silt		25	62	9	12
% sand		58	23	82	77
% clay		18	15	9	11
% Organic Carbon	0-6 in	1.90	2.5	0.43	0.90
Bulk density (g/cc)	0-6 in	1.12	0.97	1.39	1.34
	6-12 in	1.18	1.04	1.33	1.26
	12-18 in	1.20	1.15	1.31	1.23
	18-24 in	1.25	1.17	1.21	1.25
	24-30 in	1.24	1.15	1.29	1.26
	30-36 in	1.23	1.11	1.17	1.27
pH	0-6 in	6.2	5.1	6.6	6.0
Cation Exchange Capacity (meq/100g)	0-6 in	13.1	8.2	4.3	8.8
% moisture at 1.3 bar	0-6 in	19.3	28.6	8.0	10.4
% moisture at 15 bar	0-6 in	9.15	9.9	3.4	4.6

Table 7.3.1-4 from Tier II Summary of MRIDs 49807286 -87 -88 -89.

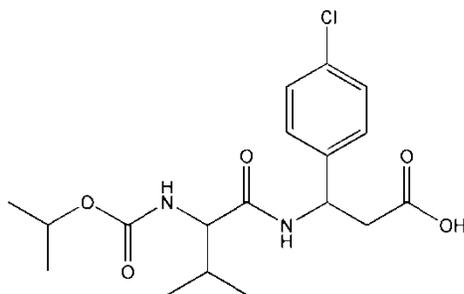
3. In the ECM, an insufficient number of samples was prepared for all fortifications/analytes ( $n = 3$ ; Appendix III, Tables 2-4, pp. 91-93 of MRID 48907289). OCSPP guidelines state that a minimum of five spiked replicates should be analyzed at each concentration (*i.e.*, minimally, the LOQ and  $10\times$  LOQ) for each analyte.
4. In the ECM, no representative chromatograms were provided from the method validation portion of the study. The reviewer assessed specificity based on concurrent recovery LOQ and  $10\times$ LOQ representative chromatograms and quantified residues in the controls of the method validation and concurrent recovery samples.
5. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 12, 26 of MRID 49807189; Appendix III, p. 81 of MRID 49807289). In the ECM, the LODs were not reported in the method (Appendix III) specifically but elsewhere in the MRID. In the ILV, the LODs were set at 30% of the LOQ. No calculations, justifications, or comparisons to background noise were provided for the LOQs and LODs in the ECM or ILV.
6. The communications between the ECM and ILV were not reported or discussed. The ILV did report that the Study Monitor was contacted regarding the change of the method to validate from Eurofins method RA034 v07 (MRID 49807188) to Precision Study Management Amendment 2 (PSM-14-02-05 Amendment 2; p. 25 of MRID 49807189). Detailed communication records were not provided. Study personnel listed in the ILV did not include any ECM personnel (pp. 7-8 of MRID 49807189).
7. In the ILV, the time required to complete the extraction of one set of 13 samples (one reagent blank, two matrix controls and ten fortified samples) was reported as *ca.* 5 hours, followed by *ca.* 2 hours for LC/MS/MS analysis and *ca.* 1 hour for LC/MS/MS evaluation (p. 26 of MRID 49807189). The total time requirement of the method was reported as *ca.* one working day (8 hours) in the ILV.

## V. References

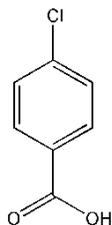
- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

**DER Attachment 1: Chemical Names and Structures****Valifenalate****Valifenalate**

Common Name:	Valifenalate
Chemical Name:	Methyl N-(isopropoxycarbonyl)-L-valyl-(3 <i>R,S</i> )-2-(4-chlorophenyl)-β-alanine
CAS No.:	283159-90-0
Molecular Formula:	C <sub>19</sub> H <sub>27</sub> ClN <sub>2</sub> O <sub>5</sub>
Lot No.:	G019/07 (ID in CoA: IR5885)
Purity:	99.52%
Expiration Date:	Nov 2017
Molecular Weight:	398.88 g/mole
Storage:	Ambient Temperature

**Valifenalate Acid****Valifenalate acid**

Common Name:	Valifenalate acid
Chemical Name:	( <i>R,S</i> )-β-alanine, N-((1-methylethoxy)-L-valyl-3-(4-chlorophenyl) acid)
CAS No.:	NA
Molecular Formula:	C <sub>18</sub> H <sub>25</sub> ClN <sub>2</sub> O <sub>5</sub>
Lot No.:	G029/08 (ID in CoA: IR5839)
Purity:	98.4%
Expiration Date:	Feb 21, 2017
Molecular Weight:	384.86 g/mole
Storage:	Ambient Temperature

**p-Chlorobenzoic acid (PCBA)****p-Chlorobenzoic acid (PCBA)**

Common Name:	p-Chlorobenzoic acid (PCBA)
Chemical Name:	4-Chlorobenzoic acid
CAS No.:	74-11-3
Molecular Formula:	C <sub>7</sub> H <sub>7</sub> ClO <sub>2</sub>
Lot No.:	LC07337V
Purity:	99.2%
Expiration Date:	May 2017
Molecular Weight:	156.57 g/mole
Storage:	Ambient Temperature