

**Test Material:** Picloram and Clopyralid

**MRID:** 49753805

**Title:** Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS

**MRID:** 49753807

**Title:** Independent Laboratory Validation of a Dow AgroSciences Method for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS

**EPA PC Code:** 005101 (Picloram); 117403 (Clopyralid)

**OCSPP Guideline:** 850.6100

**For CDM Smith**

**Primary Reviewer:** Lisa Muto

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**Date:** 6/7/16

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**Date:** 6/7/16

**Analytical method for picloram and clopyralid in soil**

**Reports:** ECM: EPA MRID No.: 49753805. Vincent, T.P. 2013. Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS. Dow AgroSciences Study No.: 120612. ABC Study No.: 68931. Report prepared by ABC Laboratories, Inc., Columbia Missouri, and sponsored and submitted by Regulatory Sciences and Government Affairs, Dow AgroSciences LLC, Indianapolis, Indiana; 133 pages. Final report issued February 20, 2013.

ILV: EPA MRID No. 49753807. Austin, R, and R. Turner. 2014. Independent Laboratory Validation of a Dow AgroSciences Method for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS. Dow AgroSciences Study No.: 140079. Battelle UK Ltd Study No.: YR/14/002. Report prepared by Battelle UK Ltd., Essex, United Kingdom, and sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 63 pages. Final report issued May 19, 2014.

**Document No.:** MRIDs 49753805 & 49753807

**Guideline:** 850.6100

**Statements:** ECM: The study was conducted in accordance with USEPA FIFRA and OECD (1998) Good Laboratory Practices (GLP), as well as Standard Operating Procedures of ABC Laboratories (p. 3 of MRID 49753805). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).  
ILV: The study was conducted in accordance with USEPA, OECD (1998), and UK (1999 and 2004) GLP standards, as well as the UK Department of Health (p. 3; Appendix 3, p. 63 of MRID 49753807). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-4; Appendix 3, p. 63). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).

**Classification:** This analytical method is classified as unacceptable. In the ECM and ILV, no samples were prepared at 10×LOQ. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. In the ECM, analysis of picloram at the LOQ in the loamy sand soil did not meet guideline requirements (means 65-69%). ECM and ILV representative chromatograms showed minor interferences at the LOQ. Finally, the reported LOQ is greater than the lowest toxicological level of concern for both analytes.

**PC Code:** 005101 (Picloram); 117403 (Clopyralid)

**Reviewer:**

Andrew Shelby, Physical Scientist

**Signature:** 

**Date:** September 17, 2018

All page numbers refer to those listed in the upper right-hand corner of the MRIDs.



## Executive Summary

The analytical method, Dow AgroSciences Method 120612, is designed for the quantitation determination of picloram and clopyralid in soil matrices at the LOQ of 0.5 µg/kg using LC/MS/MS. The LOQ is greater than the lowest toxicological level of concern in soil for both analytes. Characterized soil matrices were used in the ECM. The method was validated by the ILV in the first trial using sandy loam soil. Only insignificant modifications of the ECM were reported; however, it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. In the ECM and ILV, no samples were prepared at 10×LOQ. In ECM and ILV chromatograms of clopyralid and picloram, various minor abnormalities were observed, including minor peak tailing, abnormal peak integration due to minor baseline noise around the analyte peak and minor contaminants near the retention time of the analyte. In the ECM, analysis of picloram at the LOQ in the loamy sand soil did not meet guideline requirements (means 65-69%).

**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						
Picloram	49753805	49753807		Soil <sup>1,2</sup>	20/02/2013	Dow AgroSciences LLC	LC/MS/MS	0.50 µg/kg
Clopyralid								

1 For the ECM, loamy sand soil (ABC Designation 484; 87% sand, 8% silt, 5% clay; pH 5.6, percent organic carbon 0.64, percent organic matter 1.1) from Tift County, Georgia, sandy clay loam soil (ABC Designation 485; 53% sand, 18% silt, 29% clay; pH 7.9, percent organic carbon 0.52, percent organic matter 0.90) from Willacy County, Texas, loam soil (ABC Designation 498; 39% sand, 40% silt, 21% clay; pH 6.3, percent organic carbon 1.7, percent organic matter 2.9) from Tehama County, California, and silt loam soil (ABC Designation 508; 23% sand, 58% silt, 19% clay; pH 5.5, percent organic carbon 2.4, percent organic matter 4.0) from Boone County, Missouri were used in the study (pH in 1.1 soil:water ratio; USDA texture classification; p. 14 of MRID 49753805).

2 For the ILV, sandy loam soil (Lufa Speyer 2.2; 74% sand, 13% silt, 13% clay; pH 5.4, percent organic matter 3.17) was used (pH in 1.1 soil:water ratio; USDA texture classification; p. 12; Appendix 2, p. 62 of MRID 49753807).

## I. Principle of the Method

Samples ( $5.0 \pm 0.05$  g) of soil in 50-mL glass tubes with caps were fortified, as necessary, then extracted with 25 mL of acetone:1N hydrochloric acid (90:10, v:v) via shaking on a reciprocal shaker for *ca.* 2 hours (pp. 20-22 of MRID 49753805). After centrifugation (3000 rpm for *ca.* 5 min.), the supernatant was decanted into a clean 50-mL glass tube. The soil pellet was extracted again using 10 mL of acetone:1N hydrochloric acid (90:10, v:v) via shaking on a reciprocal shaker for 30 minutes. After centrifugation (3000 rpm for *ca.* 5 min.), the supernatant was decanted into the same 50-mL glass tube. The study author noted that all steps in the procedure should be carried-out using glass containers. The acetone was removed under a gentle stream of nitrogen at room temperature (*ca.* 2 mL will remain in the glass tubes). The volume was adjusted to 8 mL with 1N sodium hydroxide; the sample was mixed via vortexing and sonication. Dichloromethane (*ca.* 8 mL) was added, and the sample was mixed via sonication and vortexing

for 30 seconds. After centrifugation (*ca.* 3000 rpm for *ca.* 5 min.), 6.0 mL of the upper extract layer was transferred into a clean 15-mL glass tube. 6 mL of 1N HCl was added then the sample was purified using solid phase extraction (SPE) procedure (0.2 g Waters HLB SPE column). The SPE column was pre-conditioned with methanol then 1N HCl (5 mL each). The sample was applied to the column (*ca.* 2 mL/min rate). The sample tube was rinsed with 1 mL of 1N HCl which was applied to the column. The sample tube was washed with 5 mL of acetonitrile:1N formic acid (15:85, v:v) which was applied to the column. After drying the column with full vacuum for at least 30 minutes, the analytes were eluted with 14 mL of dichloromethane (the method noted that the SPE columns must be profiled in the presence of matrix and optimized if necessary). The purified sample was evaporated to dryness under a gentle stream of nitrogen at *ca.* 40°C and reconstituted with methanol:0.1% formic acid (10:90, v:v; 1.0 mL for LOD/LOQ samples; higher fortifications should be diluted) via sonication and vortexing. The study author noted that this was a critical step to ensure that all of the residues were dissolved from the sides of the tube: it should be done individually by hand and repeated 2-3 times alternating vortexing and sonication. The final extracts were filtered (0.2- $\mu$ m PTFE syringe filter) and analyzed by liquid chromatography using negative-ion electrospray ionization (ESI) with tandem mass spectrometry.

Samples were analyzed for clopyralid and picloram using an MDS SCIEX API 4000 LC/MS/MS (pp. 17-19 of MRID 49753805). The instrumental conditions consisted of an Accucore Phenylhexyl column (4.6 x 50 mm, 2.6- $\mu$ m; column temperature, 35°C), a mobile phase gradient of (A) water containing 0.01% formic acid and (B) methanol:acetonitrile (60:40, v:v) containing 0.01% formic acid [percent A:B (v:v) at 0.00-3.00 min. 85:15, 3.10-4.40 min. 5:95, 4.50-5.50 min. 85:15], MS/MS detection in negative turbo spray (MRM; temperature, 500°C), and injection volume 50  $\mu$ L. Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively):  $m/z$  189.9  $\rightarrow$  145.9 and  $m/z$  191.9  $\rightarrow$  147.9 for clopyralid and  $m/z$  240.9  $\rightarrow$  196.9 and  $m/z$  238.9  $\rightarrow$  194.9 for picloram. Retention times were observed at *ca.* 1.58-1.59 and 2.30-2.31 min. for clopyralid and picloram, respectively (Figures 7-16, pp. 65-74).

### ILV

In the ILV, the ECM was performed exactly as written, except for one minor modification of the extraction procedure: the centrifugation speed was reduced to 2000 rpm since the glass tubes cracked at 3000 rpm; separation of extract still occurred at 2000 rpm (p. 16 of MRID 49753807). Two minor modifications of LC/MS/MS conditions: an Agilent 1100 Degasser LC and Applied Biosystems/MDS Sciex API 5000 MS were used, and the injection volume was 100  $\mu$ L (Appendix 1, pp. 55, 60). The two monitored parent-daughter ion transitions per analyte were the same as the ECM. Retention times were observed at *ca.* 1.57 and 2.28 min. for clopyralid and picloram, respectively (Figure 11, p. 39; Figure 18, p. 46). The ILV study report noted the following critical step: that samples must be vortex mixed and sonicated individually three times after SPE clean-up to ensure that all residues are dissolved from the side of the tube (p. 14).

### LOQ/LOD

The LOQ and LOD for both analytes were 0.5  $\mu$ g/kg and 0.15  $\mu$ g/kg, respectively, in the ECM and ILV (pp. 12, 26; Tables 14-15, p. 49 of MRID 49753805; pp. 11, 15 of MRID 49753807).

## II. Recovery Findings

ECM (MRID 49753805): Mean recoveries and relative standard deviations (RSDs) were within guidelines (quantitation and confirmatory HPLC analyses) for analysis of picloram and clopyralid in four soil matrices at fortification levels of 0.50 µg/kg (LOQ) and 1000 µg/kg (2000×LOQ), except for analysis of picloram at the LOQ in the loamy sand soil where the means were 69% and 65% for the quantitation and confirmatory analyses, respectively (Tables 2-13, pp. 31-48; DER Attachment 2). No samples were prepared at 10×LOQ. Performance data (recovery results) of the quantitation HPLC analysis and confirmatory HPLC analysis were comparable. Recoveries from samples fortified at 0.15 µg/kg (LOD) ranged (ions/matrices combined) from 67-121% for clopyralid and 59-97% for picloram (n = 1 for each matrix/analyte; DER Attachment 2). The soil matrices were well characterized (USDA texture classification; p. 14). Loamy sand soil (ABC Designation 484; 87% sand, 8% silt, 5% clay; pH 5.6, percent organic carbon 0.64, percent organic matter 1.1) from Tift County, Georgia, sandy clay loam soil (ABC Designation 485; 53% sand, 18% silt, 29% clay; pH 7.9, percent organic carbon 0.52, percent organic matter 0.90) from Willacy County, Texas, loam soil (ABC Designation 498; 39% sand, 40% silt, 21% clay; pH 6.3, percent organic carbon 1.7, percent organic matter 2.9) from Tehama County, California, and silt loam soil (ABC Designation 508; 23% sand, 58% silt, 19% clay; pH 5.5, percent organic carbon 2.4, percent organic matter 4.0) from Boone County, Missouri were used in the study (pH in 1.1 soil:water ratio).

ILV (MRID 49753807): Mean recoveries and RSDs were within guidelines for analysis of picloram and clopyralid in one soil matrix at fortification levels of 0.50 µg/kg (LOQ) and 1000 µg/kg (2000×LOQ; quantitation and confirmatory HPLC analyses; Tables 10-17, pp. 23-27; DER Attachment 2). No samples were prepared at 10×LOQ. Performance data (recovery results) of the quantitation HPLC analysis and confirmatory HPLC analysis were comparable. Recoveries from samples fortified at 0.15 µg/kg (LOD) ranged (ions combined) from 70-89% for clopyralid and 79-104% for picloram (n = 1 for analyte; DER Attachment 2). The soil matrix was well characterized by Agvise Laboratories, Northwood, North Dakota (USDA texture classification; p. 12; Appendix 2, p. 62). Sandy loam soil (Lufa Speyer 2.2; 74% sand, 13% silt, 13% clay; pH 5.4, percent organic matter 3.17) was used (pH in 1.1 soil:water ratio). The method was validated in the first trial. Only insignificant modifications of the ECM were reported (pp. 16-17).

**Table 2. Initial Validation Method Recoveries for Clopyralid and Picloram in Ground, Drinking and Surface Soil<sup>1,2</sup>**

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Loamy Sand Soil (484)</b>						
Quantitation ion						
Clopyralid	0.15 (LOD)	<b>1</b>	107	--	--	--
	0.5 (LOQ)	5	78-113	90	14.0	15.5
	1000	5	72-75	74	1.5	2.0
Picloram	0.15 (LOD)	<b>1</b>	<b>65</b>	--	--	--
	0.5 (LOQ)	5	64-75	<b>69</b>	4.6	6.7
	1000	5	73-77	75	1.7	2.2
Confirmation ion						
Clopyralid	0.15 (LOD)	<b>1</b>	87	--	--	--
	0.5 (LOQ)	5	68-103	80	13.8	17.2
	1000	5	72-76	74	1.4	1.9
Picloram	0.15 (LOD)	<b>1</b>	<b>62</b>	--	--	--
	0.5 (LOQ)	5	61-69	<b>65</b>	3.1	4.8
	1000	5	73-78	75	2.2	3.0
<b>Sandy Clay Loam Soil (485)</b>						
Quantitation ion						
Clopyralid	0.15 (LOD)	<b>1</b>	99	--	--	--
	0.5 (LOQ)	5	81-96	87	5.7	6.6
	1000	5	75-77	76	0.9	1.1
Picloram	0.15 (LOD)	<b>1</b>	97	--	--	--
	0.5 (LOQ)	5	77-93	87	6.5	7.4
	1000	5	79-89	85	3.7	4.3
Confirmation ion						
Clopyralid	0.15 (LOD)	<b>1</b>	95	--	--	--
	0.5 (LOQ)	5	80-98	87	7.1	8.1
	1000	5	74-77	75	1.0	1.3
Picloram	0.15 (LOD)	<b>1</b>	<b>66</b>	--	--	--
	0.5 (LOQ)	5	74-86	80	4.6	5.7
	1000	5	80-89	85	3.5	4.2
<b>Loam Soil (498)</b>						
Quantitation ion						
Clopyralid	0.15 (LOD)	<b>1</b>	90	--	--	--
	0.5 (LOQ)	5	81-95	89	6.6	7.4
	1000	5	82-93	87	4.5	5.1
Picloram	0.15 (LOD)	<b>1</b>	92	--	--	--
	0.5 (LOQ)	5	73-118	91	16.7	18.3
	1000	5	84-104	94	7.9	8.4
Confirmation ion						
Clopyralid	0.15 (LOD)	<b>1</b>	<b>67</b>	--	--	--
	0.5 (LOQ)	5	74-98	86	10.6	12.4
	1000	5	81-94	87	5.0	5.7

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Picloram	0.15 (LOD)	<b>1</b>	<b>59</b>	--	--	--
	0.5 (LOQ)	5	67-116	85	20.2	23.9
	1000	5	85-104	94	7.4	7.9
<b>Silt Loam Soil (508)</b>						
Quantitation ion						
Clopyralid	0.15 (LOD)	<b>1</b>	121	--	--	--
	0.5 (LOQ)	5	75-109	90	13.7	15.3
	1000	5	68-92	78	9.1	11.6
Picloram	0.15 (LOD)	<b>1</b>	70	--	--	--
	0.5 (LOQ)	5	75-103	85	11.3	13.3
	1000	5	65-97	80	11.9	14.8
Confirmation ion						
Clopyralid	0.15 (LOD)	<b>1</b>	87	--	--	--
	0.5 (LOQ)	5	72-117	88	18.0	20.4
	1000	5	68-91	78	8.4	10.7
Picloram	0.15 (LOD)	<b>1</b>	<b>66</b>	--	--	--
	0.5 (LOQ)	5	66-99	85	15.2	17.8
	1000	5	66-98	81	12.0	14.8

Data (uncorrected recovery results; pp. 22-23) were obtained from Tables 2-13, pp. 31-48 of MRID 49753805 and DER Attachment 2 (LOD % recovery calculations).

- The soil matrices were well characterized (USDA texture classification; p. 14). Loamy sand soil (ABC Designation 484; 87% sand, 8% silt, 5% clay; pH 5.6, percent organic carbon 0.64, percent organic matter 1.1) from Tift County, Georgia, sandy clay loam soil (ABC Designation 485; 53% sand, 18% silt, 29% clay; pH 7.9, percent organic carbon 0.52, percent organic matter 0.90) from Willacy County, Texas, loam soil (ABC Designation 498; 39% sand, 40% silt, 21% clay; pH 6.3, percent organic carbon 1.7, percent organic matter 2.9) from Tehama County, California, and silt loam soil (ABC Designation 508; 23% sand, 58% silt, 19% clay; pH 5.5, percent organic carbon 2.4, percent organic matter 4.0) from Boone County, Missouri were used in the study (pH in 1:1 soil:water ratio).
- Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively):  $m/z$  190.0 → 146.0 and  $m/z$  191.9 → 147.9 for clopyralid and  $m/z$  241.0 → 196.8 and  $m/z$  239.0 → 194.9 for picloram (p. 19).



**Table 3. Independent Validation Method Recoveries for Clopyralid and Picloram in Ground, Drinking and Surface Soil<sup>1,2</sup>**

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Sandy Loam Soil</b>						
Quantitation ion						
Clopyralid	0.15 (LOD)	1	70	--	--	--
	0.5 (LOQ)	5	73-91	85	8	8.8
	1000	5	81-93	89	5	5.3
Picloram	0.15 (LOD)	1	104	--	--	--
	0.5 (LOQ)	5	87-97	94	4	4.4
	1000	5	83-93	90	4	4.6
Confirmation ion						
Clopyralid	0.15 (LOD)	1	89	--	--	--
	0.5 (LOQ)	5	81-90	86	4	4.3
	1000	5	80-92	87	5	5.5
Picloram	0.15 (LOD)	1	79	--	--	--
	0.5 (LOQ)	5	74-93	82	9	8.9
	1000	5	83-95	91	5	5.2

Data (uncorrected results; Figures 7-8, pp. 35-36) were obtained from Tables 10-17, pp. 23-27 of MRID 49753807 and DER Attachment 2 (LOD % recovery calculations; s.d. at LOQ and 10×LOQ).

1 The soil matrix was well characterized by Agvise Laboratories, Northwood, North Dakota (USDA texture classification; p. 12; Appendix 2, p. 62). Sandy loam soil (Lufa Speyer 2.2; 74% sand, 13% silt, 13% clay; pH 5.4, percent organic matter 3.17) was used (pH in 1.1 soil:water ratio)..

2 Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively):  $m/z$  190.0 → 146.0 and  $m/z$  191.9 → 147.9 for clopyralid and  $m/z$  241.0 → 196.8 and  $m/z$  239.0 → 194.9 for picloram (p. 12; Appendix 1, p. 61).

### III. Method Characteristics

In the ECM and ILV, the LOQ and LOD for both analytes were 0.5 µg/kg and 0.15 µg/kg, respectively, in the ECM and ILV (pp. 12, 26, 29; Tables 14-15, p. 49 of MRID 49753805; pp. 11, 15 of MRID 49753807). Following the method of Keith, L. H., *et al.* (see section V. **References** below), the LOD and LOQ for determination of picloram and clopyralid in soil were calculated in the ECM using the standard deviation from the 0.50 µg/kg quantification ion recovery results. The LOD was calculated as three times the standard deviation ( $3s$ ), and the LOQ was calculated as ten times the standard deviation ( $10s$ ) of the recovery results. The calculated values support the LOQ and LOD established for the study and are presented in **Table 4** below.

**Table 4. Method Characteristics**

		Clopyralid	Picloram
Limit of Quantitation (LOQ)	Established	0.50 µg/kg	
	Calculated (ECM)	00.2857-0.6976 µg/kg	0.2312-0.8334 µg/kg
Limit of Detection (LOD)	Established	0.15 µg/kg	
	Calculated (ECM)	0.08571-0.2093 µg/kg	0.06936-0.2500 µg/kg
Linearity (Least squares calibration curve r and concentration range)	ECM <sup>1</sup>	$r^2 = 0.9992$ (Q) $r^2 = 0.9988$ (C)	$r^2 = 0.9996$ (Q & C)
		0.40-50 ng/mL	
	ILV <sup>2</sup>	$r^2 = 0.9988$ (Q) $r^2 = 0.9986$ (C)	$r^2 = 0.9996$ (Q) $r^2 = 0.9992$ (C)
		0.40-50 ng/mL	
Repeatable	ECM <sup>3</sup>	Yes at LOQ and 2000×LOQ (n = 5).	Yes at LOQ and 2000×LOQ (n = 5), except at LOQ in loamy sand soil.
		No samples were prepared at 10×LOQ.	
	ILV <sup>4</sup>	Yes at LOQ and 2000×LOQ (n = 5). No samples were prepared at 10×LOQ.	
Reproducible		Yes at the LOQ. No at 10×LOQ; no samples prepared.	
Specific	ECM	Yes, only minor residues (<20% of the LOD) in the matrix control; minor peak tailing was noted in most of the chromatograms. Also, minor baseline noise which affected peak integration was noted in the LOQ.	
	ILV	Yes, no interferences were observed in the matrix control.	
		Some abnormal peak integration was noted in the LOQ.	A contaminant (RT <i>ca.</i> 2.1 min.; peak height <i>ca.</i> 2×LOQ) was observed near the analyte peak in the confirmation ion chromatograms.

Data were obtained from pp. 12, 20, 22, 26; Tables 2-13, pp. 31-48 (recovery results); Tables 14-15, p. 49 (LOD/LOQ calculations); Figures 3-6, pp. 31-34 (calibration curves); Figures 17-50, pp. 75-108 (chromatograms) of MRID 49753805; pp. 11, 15; Tables 2-9, pp. 20-22 (calibration data); Tables 10-17, pp. 23-27 (recovery results); Figures 3-6, pp. 31-34 (calibration curves); Figures 14-17, pp. 42-45 (clopyralid chromatograms); Figures 21-24, pp. 49-52 (picloram chromatograms) of MRID 49753807; DER Attachment 2. Q = Quantitation HPLC analysis; C = Confirmatory HPLC analysis.

1 ECM standard curves were weighted 1/x. ECM  $r^2$  values are reviewer-generated for both analytes from reported  $r$  values of 0.9996-0.9998 (Q) and 0.9994-0.9998 (C; calculated from data in Figures 3-6, pp. 61-64 of MRID 49753805; see DER Attachment 2).

2 ILV standard curves were weighted 1/x. ILV  $r^2$  values are reviewer-generated for both analytes from reported  $r$  values of 0.9993-0.994 (Q) and 0.9996-0.9998 (C; calculated from data in Figures 3-6, pp. 31-34 of MRID 49753807; see DER Attachment 2).

3 For the ECM, loamy sand soil (ABC Designation 484; 87% sand, 8% silt, 5% clay; pH 5.6, percent organic carbon 0.64, percent organic matter 1.1) from Tift County, Georgia, sandy clay loam soil (ABC Designation 485; 53% sand, 18% silt, 29% clay; pH 7.9, percent organic carbon 0.52, percent organic matter 0.90) from Willacy County, Texas, loam soil (ABC Designation 498; 39% sand, 40% silt, 21% clay; pH 6.3, percent organic carbon 1.7, percent organic matter 2.9) from Tehama County, California, and silt loam soil (ABC Designation 508; 23% sand, 58% silt, 19% clay; pH 5.5, percent organic carbon 2.4, percent organic matter 4.0) from Boone County, Missouri were used in the study (pH in 1.1 soil:water ratio; USDA texture classification; p. 14 of MRID 49753805).

4 For the ILV, sandy loam soil (Lufa Speyer 2.2; 74% sand, 13% silt, 13% clay; pH 5.4, percent organic matter 3.17) was used (pH in 1.1 soil:water ratio; USDA texture classification; p. 12; Appendix 2, p. 62 of MRID 49753807).

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

1. No samples were prepared at 10×LOQ in the ECM or ILV. OSCPP guidelines recommend a minimum of five samples spiked at each fortification level (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.
2. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. In the ILV, sandy loam soil (Lufa Speyer 2.2; 74% sand, 13% silt, 13% clay; pH 5.4, percent organic matter 3.17) was used (pH in 1.1 soil:water ratio; USDA texture classification; p. 12; Appendix 2, p. 62 of MRID 49753807). This soil matrix was not equivalent to the most difficult soil matrix which was used in the ECM: silt loam soil (ABC Designation 508; 23% sand, 58% silt, 19% clay; pH 5.5, percent organic carbon 2.4, percent organic matter 4.0) from Boone County, Missouri were used in the study (pH in 1.1 soil:water ratio; USDA texture classification; p. 14 of MRID 49753805).
3. In the ECM, the analysis of picloram at the LOQ in the loamy sand soil did not meet guideline requirements; the means were 69% and 65% for the quantitation and confirmatory analyses, respectively (Tables 2-13, pp. 31-48 of MRID 49753805). OSCPP guidelines for precision and accuracy require that means and RSDs are 70-120% and ≤20%, respectively.
4. In ECM chromatograms of clopyralid and picloram, minor abnormalities were observed, including minor peak tailing and abnormal peak integration caused by minor baseline noise around the analyte peak (Figures 17-50, pp. 75-108 of MRID 49753805). In the ILV chromatograms of clopyralid, some abnormal peak integration was noted at the LOQ; in ILV chromatograms of picloram, a contaminant (RT *ca.* 2.1 min.; peak height *ca.* 2xLOQ) was observed near the analyte peak in the confirmation ion chromatograms (Figures 14-17, pp. 42-45; Figures 21-24, pp. 49-52 of MRID 49753807). Only minor residues were observed in the control samples of picloram and clopyralid.  
  
A chromatogram of the reagent blank was not included in the ILV (p. 13 of MRID 49753807).
5. The toxicological level of concern was not reported for the analytes in soil. A LOQ above toxicological levels of concern results in an unacceptable method classification.
6. In their calculation sections of the ECM study report, the ECM study authors reported that correlation coefficients were greater than 0.990 ( $r^2 \geq 0.98$ ; p. 23 of MRID 49753805). The reviewer calculated values of 0.9988-0.9996 for all analyte/ion combinations in the ECM; therefore, the reviewer assumed that the statements in the calculation sections were generalizations.
7. The ILV reported that no communications between the ILV and the sponsor occurred (p. 16 of MRID 49753807).

8. In the ECM, the calibration standards and fortification solutions were stable for at least 24 days and 75 days, respectively, when stored under refrigerated conditions (p. 27; Tables 18-21, pp. 52-56 of MRID 49753805). The sample extracts were stable for up to 7 days under refrigeration storage.

In the ECM and ILV, matrix effects were studied (pp. 26-27; Tables 16-17, pp. 50-51 of MRID 49753805; pp. 15-16; Tables 18-19, p. 28 of MRID 49753807). Matrix effects were insignificant ( $\pm 10\%$ ) for all matrices/analytes.

9. It was reported for the ILV that the analytical procedure for one set of 14 samples required approximately 10 hours for preparation (p. 13 of MRID 49753807). Preparation of solutions and reagents required approximately 2 hours. The LC/MS/MS required approximately 2.5 hours. Interpretation of data required approximately 1 hour. The overall time to complete a set of samples was ca. 2 working days.

## V. References

- Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* 1983, 55, 2210-2218 (p. 29 of MRID 49753805).
- 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****Picloram**

**IUPAC Name:** Not reported  
**CAS Name:** 4-Amino-3,5,6-trichloropyridine-2-carboxylic acid  
4-Amino-3,5,6-trichloro-2-pyridinecarboxylic acid  
**CAS Number:** 1918-02-1  
**SMILES String:** Not found

**Clopyralid**

**IUPAC Name:** Not reported  
**CAS Name:** 3,6-Dichloropyridine-2-carboxylic acid  
3,6-Dichloropicolinic acid  
**CAS Number:** 1702-17-6  
**SMILES String:** Not found

