Test Material:	Picloram and Clopyralid
MRID:	49753804
Title:	Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water and Surface Water by LC-MS/MS
MRID:	49753806
Title:	Independent Laboratory Validation of Dow AgroSciences Method 120611, "Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water and Surface Water by LC-MS/MS"
EPA PC Code:	005101 (Picloram); 117403 (Clopyralid)
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** Lisa Muto

Date: 6/7/16

Secondary Reviewer: Kathleen Ferguson **Signature:** Kathleen P. Ferguson

Date: 6/7/16

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

Date: 6/7/16

Analytical method for picloram and clopyralid in surface, ground and drinking water

Reports: ECM: EPA MRID No.: 49753804. Shaffer, S. 2012. Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water and Surface Water by LC-MS/MS. Dow AgroSciences Study No.: 120611. ABC Study No.: 68631. Report prepared by ABC Laboratories, Inc., Columbia Missouri, and sponsored and submitted by Regulatory Sciences and Government Affairs, Dow AgroSciences LLC, Indianapolis, Indiana; 114 pages. Final report issued December 4, 2012.

ILV: EPA MRID No. 49753806. Austin, R, and R. Turner. 2013. Independent Laboratory Validation of Dow AgroSciences Method 120611, "Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water and Surface Water by LC-MS/MS". Dow AgroSciences Protocol No.: 120613. Battelle Study No.: YR/12/023. Report prepared by Battelle UK Ltd., Essex, United Kingdom, and sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 87 pages. Final report issued April 5, 2013.

Document No.: MRIDs 49753804 & 49753806

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 49753804). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (the signature and date on the QA statement was very faint; 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. 84 of MRID 49753806). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-4; Appendix 3, p. 84). 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 acceptable. In the ECM, 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. ECM and ILV representative chromatograms showed minor interferences at the LOQ.

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

Reviewer:

Andrew Shelby, Physical Scientist **Signature:** 

Date: September 24, 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 120611, is designed for the quantitative determination of picloram and clopyralid in drinking, ground and surface water matrices at the LOQ of 0.05 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water for both analytes. Characterized drinking, ground and surface water matrices were used in the ECM and ILV; however, it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. The ILV study report did not specify the number of trials performed to validate the method; the reviewer assumed that the method was validated in the first trial. Only insignificant modifications of the ECM were reported. In the ECM, no samples were prepared at 10×LOQ. In ECM and ILV chromatograms of picloram, minor abnormalities were observed, including minor peak tailing, abnormal peak integration and baseline noise around the analyte peak.

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	49753804	49753806		Water[1, 2]	04/12/2012	Dow AgroSciences LLC	LC/MS/MS	0.05 µg/L
Clopyralid								

1 For the ECM, ground water (pH 8.37, dissolved organic carbon 5.22 ppm), drinking water (pH 7.62, dissolved organic carbon 5.49 ppm), and surface water (pH 9.30, dissolved organic carbon 14.32 ppm) were used in the study (p. 12 of MRID 49753804). The matrix sources were not reported.

2 For the ILV, ground water (bottled spring water; pH 8.2, dissolved organic carbon 0.7 ppm), drinking water (drinking tap water; pH 8.2, dissolved organic carbon 1.3 ppm), and surface water (pond; pH 8.1, dissolved organic carbon 5.6 ppm) were used in the study (p. 18; Appendix 4, pp. 85-87 of MRID 49753806).

I. Principle of the Method

Samples (100 mL) of water in glass bottles with caps were fortified, as necessary, then acidified with 5 mL of 1N hydrochloric acid to pH 2 (more 1N HCl may be added as needed; pp. 18-19 of MRID 49753804). The study author noted that all steps in the procedure should be carried-out using glass containers. 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 column was dried via vacuum for *ca*. 10 seconds. The sample was applied to the column (*ca*. 2 mL/min rate). The sample bottle was rinsed with 1 mL of 1N HCl which was applied to the column. The sample bottle 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 ≤40°C and reconstituted with methanol:0.1% formic acid (10:90, v:v; 1.0 mL for LOD/LOQ samples and

25.0 mL for 200×LOQ) 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-µm 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 5000 LC/MS/MS (pp. 15-16 of MRID 49753804). The instrumental conditions consisted of an Accucore Phenyl-hexyl column (4.6 x 50 mm, 2.6-µm; column temperature, 30°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 0.00-2.00 min. 79:21, 2.10-3.50 min. 5:95, 3.60-5.60 min. 79:21], MS/MS detection in negative turbo spray (MRM; temperature, 500°C), and injection volume 15 µL. 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. Retention times were observed at *ca*. 1.13 and 1.47-1.48 min. for clopyralid and picloram, respectively (Figures 7-16, pp. 57-66).

ILV

In the ILV, the ECM was performed exactly as written, except for two minor modifications of LC/MS/MS conditions: an Agilent 1100 Degasser LC and Applied Biosystems API 5000 Triple Quadrupole MS were used, and the injection volume was 50 µL (pp. 14, 16-17, 19 of MRID 49753806). The two monitored parent-daughter ion transitions per analyte were the same as the ECM. Retention times were observed at *ca*. 1.32 and 1.73-1.74 min. for clopyralid and picloram, respectively (Figures 9-14, pp. 51-56).

LOQ/LOD

The LOQ and LOD for both analytes were 0.05 µg/L and 0.015 µg/L, respectively, in the ECM and ILV (pp. 11, 18, 22; Tables 12-13, pp. 43-44 of MRID 49753804; pp. 12, 22 of MRID 49753806).

II. Recovery Findings

ECM (MRID 49753804): Mean recoveries and relative standard deviations (RSDs) were within guidelines for analysis of picloram and clopyralid in drinking, ground and surface water matrices at fortification levels of 0.05 µg/L (LOQ) and 10 µg/L (200×LOQ); quantitative and confirmatory HPLC analyses; Tables 2-11, pp. 27-42; DER Attachment 2). No samples were prepared at 10×LOQ. Performance data (recovery results) of the quantitative HPLC analysis and confirmatory HPLC analysis were comparable. Recoveries from samples fortified at 0.015 µg/L (LOD) ranged (ions/matrices combined) from 81-96% for clopyralid and 82-116% for picloram (n = 1 for each matrix/analyte; DER Attachment 2). The water matrices were well characterized (p. 12). Ground water (pH 8.37, dissolved organic carbon 5.22 ppm), drinking water (pH 7.62, dissolved organic carbon 5.49 ppm), and surface water (pH 9.30, dissolved organic carbon 14.32

ppm) were used in the study. The matrix sources were not reported, but dates of collection/characterization were reported as "06 Aug 12/09 Aug 12/17 Aug 12" for all matrices.

ILV (MRID 49753806): Mean recoveries and RSDs were within guidelines for analysis of picloram and clopyralid in drinking (tap), ground (bottled spring water) and surface (pond) water matrices at fortification levels of 0.05 µg/L (LOQ) and 0.50 µg/g (10×LOQ; quantitative and confirmatory HPLC analyses; Tables 2-7, pp. 29-34; DER Attachment 2). Performance data (recovery results) of the quantitative HPLC analysis and confirmatory HPLC analysis were comparable. Recoveries from samples fortified at 0.015 µg/L (LOD) ranged (ions/matrices combined) from 88-133% for clopyralid and 92-137% for picloram (n = 1 for each matrix/analyte; DER Attachment 2). The water matrices were well characterized by Agvise Laboratories, Northwood, North Dakota (p. 18; Appendix 4, pp. 85-87). Ground water (pH 8.2, dissolved organic carbon 0.7 ppm), drinking water (pH 8.2, dissolved organic carbon 1.3 ppm), and surface water (pH 8.1, dissolved organic carbon 5.6 ppm) were used in the study. The drinking water was collected from a "drinking water" tap at Battelle UK Ltd., Ongar. The ground water was obtained from bottled spring water. The surface water was obtained from a pond at Boarded Barns Farm, Ongar, Essex, United Kingdom. The study report did not specify the number of trials performed to validate the method; the reviewer assumed that the method was validated in the first trial (p. 25). Only insignificant modifications of the ECM were reported (pp. 14, 16-17, 19).

Table 2. Initial Validation Method Recoveries for Clopyralid and Picloram in Ground, Drinking and Surface Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground Water						
	Quantitation ion					
Clopyralid	0.015 (LOD)	**1**	94	--	--	--
	0.05 (LOQ)	5	88-93	90	3.6	4.0
	10	5	80-91	86	4.4	5.2
Picloram	0.015 (LOD)	**1**	116	--	--	--
	0.05 (LOQ)	5	94-105	100	3.9	3.9
	10	5	96-101	99	2.0	2.0
	Confirmation ion					
Clopyralid	0.015 (LOD)	**1**	89	--	--	--
	0.05 (LOQ)	5	82-95	92	5.3	5.7
	10	5	79-90	85	5.0	5.9
Picloram	0.015 (LOD)	**1**	109	--	--	--
	0.05 (LOQ)	5	95-100	98	2.1	2.1
	10	5	94-102	98	3.3	3.3
Drinking Water						
	Quantitation ion					
Clopyralid	0.015 (LOD)	**1**	89	--	--	--
	0.05 (LOQ)	5	81-100	92	7.0	7.6
	10	5	74-93	85	8.0	9.3
Picloram	0.015 (LOD)	**1**	93	--	--	--

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.05 (LOQ)	5	96-105	101	3.6	3.6
	10	5	96-101	98	2.2	2.2
	Confirmation ion					
Clopyralid	0.015 (LOD)	**1**	96	--	--	--
	0.05 (LOQ)	5	85-100	94	6.0	6.5
	10	5	76-89	84	6.1	7.2
Picloram	0.015 (LOD)	**1**	97	--	--	--
	0.05 (LOQ)	5	96-100	99	1.7	1.7
	10	5	95-101	98	2.5	2.5
Surface Water						
	Quantitation ion					
Clopyralid	0.015 (LOD)	**1**	94	--	--	--
	0.05 (LOQ)	5	86-97	89	4.7	5.3
	10	5	81-89	86	3.2	3.8
Picloram	0.015 (LOD)	**1**	97	--	--	--
	0.05 (LOQ)	5	85-102	93	6.5	7.0
	10	5	81-100	92	7.3	7.9
	Confirmation ion					
Clopyralid	0.015 (LOD)	**1**	81	--	--	--
	0.05 (LOQ)	5	82-90	86	2.9	3.4
	10	5	79-97	87	7.4	8.5
Picloram	0.015 (LOD)	**1**	82	--	--	--
	0.05 (LOQ)	5	86-100	91	5.7	6.2
	10	5	80-97	91	6.6	7.2

Data (uncorrected recovery results; pp. 19-20) were obtained from Tables 2-11, pp. 27-42 of MRID 49753804 and DER Attachment 2 (LOD % recovery calculations).

1 The water matrices were well characterized (p. 12). Ground water (pH 8.37, dissolved organic carbon 5.22 ppm), drinking water (pH 7.62, dissolved organic carbon 5.49 ppm), and surface water (pH 9.30, dissolved organic carbon 14.32 ppm) were used in the study. The matrix sources were not reported, but dates of collection/characterization were reported as "06 Aug 12/09 Aug 12/17 Aug 12" for all matrices.

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. 16).

Table 3. Independent Validation Method Recoveries for Clopyralid and Picloram in Ground, Drinking and Surface Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground (Bottled Spring) Water						
	Quantitation ion					
Clopyralid	0.015 (LOD)	**1**	92	--	--	--
	0.05 (LOQ)	5	86-90	89	2	2.0
	0.5	5	93-100	98	3	3.1
Picloram	0.015 (LOD)	**1**	92	--	--	--
	0.05 (LOQ)	5	84-95	89	5	5.3
	0.5	5	93-100	96	3	3.2
	Confirmation ion					
Clopyralid	0.015 (LOD)	**1**	88	--	--	--
	0.05 (LOQ)	5	87-91	89	2	2.0
	0.5	5	92-98	94	2	2.4
Picloram	0.015 (LOD)	**1**	96	--	--	--
	0.05 (LOQ)	5	90-100	94	4	4.2
	0.5	5	92-99	97	3	2.9
Drinking (Tap) Water						
	Quantitation ion					
Clopyralid	0.015 (LOD)	**1**	**131**	--	--	--
	0.05 (LOQ)	5	98-101	99	1	1.1
	0.5	5	96-102	99	2	2.4
Picloram	0.015 (LOD)	**1**	**130**	--	--	--
	0.05 (LOQ)	5	99-101	100	1	0.8
	0.5	5	96-102	99	2	2.2
	Confirmation ion					
Clopyralid	0.015 (LOD)	**1**	**133**	--	--	--
	0.05 (LOQ)	5	97-105	101	3	3.0
	0.5	5	97-101	99	2	1.5
Picloram	0.015 (LOD)	**1**	97	--	--	--
	0.05 (LOQ)	5	93-101	97	4	4.2
	0.5	5	98-99	99	1	0.6
Surface (Pond) Water						
	Quantitation ion					
Clopyralid	0.015 (LOD)	**1**	**125**	--	--	--
	0.05 (LOQ)	5	100-106	102	2	2.2
	0.5	5	95-102	98	3	3.2
Picloram	0.015 (LOD)	**1**	112	--	--	--
	0.05 (LOQ)	5	92-100	97	4	4.0
	0.5	5	95-100	97	2	1.8
	Confirmation ion					
Clopyralid	0.015 (LOD)	**1**	**130**	--	--	--
	0.05 (LOQ)	5	102-106	104	2	1.6
	0.5	5	94-104	98	4	4.0

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Picloram	0.015 (LOD)	**1**	**137**	--	--	--
	0.05 (LOQ)	5	93-99	95	2	2.4
	0.5	5	88-93	92	2	2.3

Data (uncorrected results; p. 20) were obtained from Tables 2-7, pp. 29-34 of MRID 49753806 and DER Attachment 2 (LOD % recovery calculations; s.d. at LOQ and 10×LOQ).

1 The water matrices were well characterized by Agvise Laboratories, Northwood, North Dakota (p. 18; Appendix 4, pp. 85-87). Ground water (pH 8.2, dissolved organic carbon 0.7 ppm), drinking water (pH 8.2, dissolved organic carbon 1.3 ppm), and surface water (pH 8.1, dissolved organic carbon 5.6 ppm) were used in the study. The drinking water was collected from a "drinking water" tap at Battelle UK Ltd., Ongar. The ground water was obtained from bottled spring water. The surface water was obtained from a pond at Boarded Barns Farm, Ongar, Essex, United Kingdom.

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. 17).

III. Method Characteristics

In the ECM and ILV, the LOQ and LOD for both analytes were 0.05 µg/L and 0.015 µg/L, respectively (pp. 11, 18, 22, 25; Tables 12-13, pp. 43-44 of MRID 49753804; pp. 12, 22 of MRID 49753806). 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 water were calculated in the ECM using the standard deviation from the 0.05 µg/L quantification ion recovery results. The LOD was calculated as three times the standard deviation (3*s*), and the LOQ was calculated as ten times the standard deviation (10*s*) 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		0.05 µg/L	
	Calculated (ECM)		0.0180-0.0349 µg/L	0.0181-0.0323 µg/L
Limit of Detection (LOD)	Established		0.015 µg/L	
	Calculated (ECM)		0.00540-0.0105 µg/L	0.00543-0.00969 µg/L
Linearity (Least squares calibration curve r and concentration range)	ECM[1]		r^2 = 0.9998 (Q) r^2 = 0.9996 (C)	r^2 = 0.9996 (Q) r^2 = 1.0000 (C)
			1.0-50.0 ng/mL	
	ILV[2]		r^2 = 0.9984 (Q) r^2 = 0.9980 (C)	r^2 = 0.9984 (Q) r^2 = 0.9996 (C)
			1-50 ng/mL	
Repeatable	ECM[3]		Yes at LOQ and 200×LOQ (n = 5). No samples were prepared at 10×LOQ.	
	ILV[4]		Yes at LOQ and 10×LOQ (n = 5).	
Reproducible			Yes at the LOQ and 10×LOQ.	
Specific	ECM	Drinking	Yes, only minor residues (<20% of the LOD) in the matrix control; minor peak tailing was noted in most of the chromatograms.	
		Ground		
		Surface	Yes, only minor residues (<20% of the LOD) in the matrix control; minor peak tailing was noted in most of the chromatograms.	Yes, only minor residues (<20% of the LOD) in the matrix control; minor peak tailing was noted in most of the chromatograms and some abnormal peak integration was noted in the LOQ.
	ILV	Drinking	Yes, only minor residues (<20% of the LOD) in the matrix control.	
		Ground	Yes, no interferences were observed in the matrix control.	Yes, no interferences were observed in the matrix control; some abnormal peak integration was noted in the confirmation ion at the LOQ.
		Surface		Yes, no interferences were observed in the matrix control; some baseline noise around the analyte peak was noted in the confirmation ion at the LOQ.

Data were obtained from pp. 11, 18, 22, 25; Tables 2-11, pp. 27-42 (recovery results); Tables 12-13, pp. 43-44 (LOD/LOQ calculations); Figures 3-6, pp. 53-56 (calibration curves); Figures 17-42, pp. 67-92 (chromatograms) of MRID 49753804; pp. 12, 22; Tables 2-7, pp. 29-34 (recovery results); Figures 5-8, pp. 47-50 (calibration curves); Figures 15-38, pp. 57-80 (chromatograms) of MRID 49753806; DER Attachment 2. Q = Quantitative 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 0.9997-0.9999 (Q) and 0.9998-1.0000 (C; calculated from data in Figures 3-6, pp. 53-56 of MRID 49753804; 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 0.9992 (Q) and 0.9990-0.9998 (C; calculated from data in Figures 5-8, pp. 47-50 of MRID 49753806; see DER Attachment 2).

3 For the ECM, ground water (pH 8.37, dissolved organic carbon 5.22 ppm), drinking water (pH 7.62, dissolved organic carbon 5.49 ppm), and surface water (pH 9.30, dissolved organic carbon 14.32 ppm) were used in the study (p. 12 of MRID 49753804). The matrix sources were not reported.

4 For the ILV, ground water (bottled spring water; pH 8.2, dissolved organic carbon 0.7 ppm), drinking water (drinking tap water; pH 8.2, dissolved organic carbon 1.3 ppm), and surface water (pond; pH 8.1, dissolved organic carbon 5.6 ppm) were used in the study (p. 18; Appendix 4, pp. 85-87 of MRID 49753806).

IV. Method Deficiencies and Reviewer's Comments

1. In the ECM, no samples were prepared at 10×LOQ. 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. The ILV water characteristics ranged 8.1-8.2 for pH and 0.7-5.6 ppm for dissolved organic carbon, while the ECM water characteristics ranged 7.62-9.30 for pH and 5.22-14.32 ppm for dissolved organic carbon (p. 12 of MRID 49753804; p. 18; Appendix 4, pp. 85-87 of MRID 49753806).

3. In ECM and ILV chromatograms of picloram, minor abnormalities were observed, including minor peak tailing, abnormal peak integration and baseline noise around the analyte peak (Figures 17-42, pp. 67-92 of MRID 49753804; Figures 15-38, pp. 57-80 of MRID 49753806). In the ECM chromatograms of clopyralid, minor peak tailing was observed. 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. 24 of MRID 49753806).

4. The ILV study report did not specify the number of trials performed to validate the method; the reviewer assumed that the method was validated in the first trial.

5. The toxicological level of concern was not reported for the analytes in water. A LOQ above toxicological levels of concern results in an unacceptable method classification.

6. In their calculation sections of their study reports, the ECM and ILV study authors reported that correlation coefficients were greater than 0.990 ($r^2 \geq 0.98$) and 0.996 ($r^2 \geq 0.992$), respectively (p. 20 of MRID 49753804; p. 20 of MRID 49753806). The reviewer calculated values of 0.9980-1.000 for all analyte/ion combinations in the ECM and ILV; 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. 24 of MRID 49753806).

8. In the ECM, the calibration standards and fortification solutions were stable for at least 23-24 days when stored under refrigerated conditions (pp. 23-24; Tables 16-17, pp. 47-48 of MRID 49753804). The sample extracts were stable for up to 15 days under refrigeration storage. In the ILV, the storage stability of the stock and calibration

solutions and sample extracts were also studied (p. 24; Tables 14-17, pp. 41-42 of MRID 49753806). The stock and calibration solutions were stable for at least 29 days when stored under refrigerated conditions. The sample extracts were stable for 8-14 days when stored under refrigerated conditions.

In the ECM and ILV, matrix effects were also studied (p. 23; Tables 14-15, pp. 45-46 of MRID 49753804; p. 23; Tables 8-13, pp. 35-40 of MRID 49753806). Matrix effects were insignificant (±10%) for all matrices/analytes.

9. It was reported for the ILV that the analytical procedure for one set of 14 samples required approximately 7 hours for preparation (p. 24 of MRID 49753806). Preparation of solutions and reagents required approximately 2 hours. The LC/MS/MS was conducted overnight unattended. The interpretation of data required approximately 2 hours. The overall time to complete a set of samples was 1.5 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. 25 of MRID 49753804).

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