

Analytical method for dicamba (dicamba acid) and its degradate DCSA in soil

Reports: ECM: EPA MRID No. 50914301. Jutson, J.I. 2019. Validation of the Analytical Method for the Determination of Dicamba Acid and DCSA Degradate in Soil by LC-MS/MS. Smithers Viscient Study No.: 14166.6105. Report prepared by Smithers Viscient, Wareham, Massachusetts, and sponsored and submitted by Dicamba Registrants Coalition, c/o Wagner Regulatory Associates, Hockessin, Delaware; 91 pages. Final report issued July 11, 2019.

ILV: EPA MRID No. 50931309. Cashmore, A. 2019. Independent Laboratory Validation of Analytical Method 14166.6105 for the Determination of Dicamba Acid and DCSA Degradate in Soil. Study No.: 3202424. Report prepared by Smithers ERS Limited, Harrogate, North Yorkshire, United Kingdom, and sponsored and submitted by Dicamba Registrants Coalition, c/o Wagner Regulatory Associates, Hockessin, Delaware; 93 pages. Final report issued November 18, 2019 (Sponsor signatures dated December 6, 2019; pp. 2-5).

Document No.: MRIDs 50914301 & 50931309

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practices (GLP; 1989), which are accepted by the OECD GLP standards (1998), except that the test and reference substances were non-GLP characterized prior to use in the study (p. 3 of MRID 50914301). 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.

ILV: The study, excluding some characterization of sandy loam test soil, was conducted in accordance with UK GLP standards (1999) as amended by the GLP Regulations (2004) and the OECD GLP (ENV/MC/CHEM(98)17 (Revised 1997, Issued January 1998; p. 3; Appendix 6, p. 93 of MRID 50931309). Studies are suitable for submission to the US FDA/EPA/Japanese regulatory authorities under OECD mutual acceptance of data treaty. Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5). A statement of the authenticity of the study report was also included with the GLP statement.

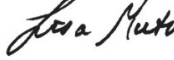
Classification: This analytical method is classified as **supplemental**. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is considered the lowest level of method validation (LLMV) rather than LOQ.

PC Code: 029801

Reviewer: Chuck Peck
Senior Fate Scientist

Signature: _____ Date:  2021.11.09 14:31:26 -05'00'

CDM/CSS-Dynamac JV Lisa Muto, M.S.,
Environmental Scientist

Signature:  Date: 05/1/2020

Reviewers:

Mary Samuel, M.S.,
Environmental Scientist

Signature: 

Date: 05/1/2020

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel. The CDM/CSS-Dynamac JV role does not include establishing Agency policies.

Executive Summary

This analytical method, Smithers Viscient Analytical Method 14166.6105, is designed for the quantitative determination of the dicamba (dicamba acid) and its degradate DCSA (3,6-dichlorosalicylic acid) at 0.05 mg/kg in soil using LC/MS/MS. There are no toxicological levels of concern in soils for either analyte, so the LOQ is considered sufficient. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the reported method LOQ for both analytes in soil. The ECM and ILV validated the method using different characterized soil matrices: sandy loam and loamy sand soils in ECM and sandy loam and silt loam soils in ILV. Based on the TFD studies that have been submitted, it appears that the ILV soil matrices covered the range of soils used in the TFD studies. The ILV validated the method with the first trial as written with insignificant final dilution changes and analytical instrument and equipment modifications. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for dicamba and DCSA.

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						
Dicamba	50914301 ¹	50931309 ²	Supplemental	Soil	11/07/2019	Dicamba Registrants Coalition, c/o Wagner Regulatory Associates	LC/MS/MS	0.05 mg/kg
DCSA								

1 In the ECM, the soil matrices were sandy loam (Batch ID: 24Oct18Soil-A; pH 6.6 (1:1 soil:water ratio); 64% sand, 17% silt, 19% clay, 3.7% organic matter) and loamy sand (05Oct18Soil-D; pH 6.9 (1:1 soil:water ratio); 83% sand, 10% silt, 7% clay, 3.6% organic matter); it was characterized by and obtained from Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification p. 14 of MRID 50914301).

2 In the ILV, the soil matrices were sandy loam (RefeSol 01-A; CS 30/18; pH 6.4 (in water) 5.3 (in CaCl₂); 74% sand, 20% silt, 6% clay, 0.9% organic carbon, 5.3 meq/100 g cation exchange capacity) and silt loam (Newhaven; CS 17/18; pH 6.0 (in water) 5.4 (in CaCl₂); 25% sand, 51% silt, 24% clay, 3.2% organic carbon, 17.4 meq/100 g cation exchange capacity); it was obtained from Smithers ERS, Harrogate, and characterized by Smithers Viscient (ESG), Ltd., Harrogate, United Kingdom (USDA soil texture classification p. 15; Appendix 2, pp. 78-79 of MRID 50931309).

I. Principle of the Method

Samples (10 g, dry weight) of soil were extracted three times with 20 mL of acetonitrile:0.1N hydrochloric acid solution (4:1, v:v) and were placed for 30 minutes on a shaker table (200 rpm)

then centrifuged (3000 rpm for 10 minutes; pp. 19-21 of MRID 50914301). The volume of the combined extracts was adjusted to 100 mL with acetonitrile:0.1N hydrochloric acid solution (4:1, v:v) and mixed well. The recovery samples were further diluted (50xs for LOQ samples and 125xs for 10×LOQ samples) into the calibration range with acetonitrile:purified reagent water (25:75, v:v) prior to LC/MS/MS analysis.

Samples were analyzed for dicamba and DCSA using a Shimadzu LC-20AD HPLC coupled to an AB MDS Sciex 5000 mass spectrometer equipped with an ESI Turbo V interface in the negative ion, multiple reaction monitoring (MRM) mode (pp. 13, 21-22 of MRID 50914301). The following LC conditions were used: Agilent EC-C18 Poroshell 120 column (3.0 mm x 100 mm, 2.7 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 0.20-5.50 min. 75.0:25.0, 5.50-7.01 min. 5.00:95.0, 7.01-9.00 min. 75.0:25.0], MS temperature 550°C, and injection volume of 25.0 μL. Expected retention times were *ca.* 3.6 and 3.1 minutes for dicamba acid (dicamba) and DCSA, respectively. Two ion pair transitions were monitored (primary and confirmatory, respectively): m/z 219.0→175.0 and m/z 221.0→177.0 for dicamba, and m/z 205.0→161.0 and m/z 205.0→125.0 for DCSA.

The ILV performed the ECM method as written, except for the fact that the 10×LOQ samples were diluted 200xs prior to analysis and for insignificant modifications to the analytical parameters and equipment (pp. 11-20; Appendix 3, pp. 80-90 of MRID 50931309). Samples were analyzed for dicamba and DCSA using Shimadzu Nexera series HPLC system coupled with an AB Sciex API 5000 triple quadrupole mass spectrometer equipped with an ESI interface in the negative ion, MRM mode. The LC/MS/MS parameters were the same as those of the ECM, except that MS temperature was 400°C. Two ion pair transitions were monitored (primary and confirmatory, respectively): m/z 218.9→174.4 and m/z 220.9→176.7 for dicamba, and m/z 205.0→161.0 and m/z 205.0→125.0 for DCSA; the monitored ion transitions of the ILV were similar to those of the ECM. Expected retention times were *ca.* 3.2 and 2.6 minutes for dicamba and DCSA, respectively. The ILV noted that the LC column and mobile phases and MS ionization type, polarity, and scan type could not be modified.

The Limit of Quantification (LOQ) for dicamba and DCSA in soil was reported as 0.05 mg/kg in the ECM and ILV (pp. 23-25, 27-30 of MRID 50914301; pp. 23-24 of MRID 50931309). The Limit of Detection (LOD) in soil was calculated as 2-4 μg/kg and 0.4-2 μg/kg for dicamba and DCSA, respectively, in the ECM and 1.11-5.79 μg/kg and 1.33-2.18 μg/kg for dicamba and DCSA in the ILV. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

II. Recovery Findings

ECM (MRID 50914301): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for analysis of dicamba and DCSA in two soil matrices at the LOQ (0.05 mg/kg) and 10×LOQ (0.5 mg/kg; Tables 1-8, pp. 34-41). Recovery results of the quantitative and confirmatory ion transitions were comparable. The soil matrices were sandy loam (Batch ID: 24Oct18Soil-A; pH 6.6 (1:1 soil:water ratio); 64% sand, 17% silt,

19% clay, 3.7% organic matter) and loamy sand (05Oct18Soil-D; pH 6.9 (1:1 soil:water ratio); 83% sand, 10% silt, 7% clay, 3.6% organic matter); it was characterized by and obtained from Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification p. 14).

ILV (MRID 50931309): Mean recoveries and RSDs met requirements for analysis of dicamba and DCSA in two soil matrices at the LOQ (0.05 mg/kg) and 10×LOQ (0.5 mg/kg; Tables 1-8, pp. 32-39). The soil matrices were sandy loam (ReferSol 01-A; CS 30/18; pH 6.4 (in water) 5.3 (in CaCl₂); 74% sand, 20% silt, 6% clay, 1.6% organic matter) and silt loam (Newhaven; CS 17/18; pH 6.0 (in water) 5.4 (in CaCl₂); 25% sand, 51% silt, 24% clay, 5.5% organic matter); it was obtained from Smithers ERS, Harrogate, and characterized by Smithers Viscient (ESG), Ltd., Harrogate, United Kingdom (USDA soil texture classification p. 15; Appendix 2, pp. 78-79). The method was validated by the ILV with the first trial as written with insignificant final dilution changes and analytical instrument and equipment modifications (pp. 11-20, 24; Appendix 4, p. 91).

Table 2. Initial Validation Method Recoveries for Dicamba and DCSA in Soil¹

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam Soil						
Quantitation ion transition						
Dicamba	0.05 (LOQ)	5	88.0-98.7	94.5	4.01	4.24
	0.50	5	89.3-101	94.5	4.83	5.11
DCSA	0.05 (LOQ)	5	90.9-97.4	94.0	2.32	2.47
	0.50	5	85.5-97.2	91.9	4.66	5.07
Confirmation ion transition						
Dicamba	0.05 (LOQ)	5	86.4-98.4	92.9	4.50	4.84
	0.50	5	86.0-101	94.6	6.26	6.62
DCSA	0.05 (LOQ)	5	85.3-95.8	91.9	4.24	4.62
	0.50	5	85.6-96.5	92.2	4.49	4.86
Loamy Sand Soil						
Quantitation ion transition						
Dicamba	0.05 (LOQ)	5	94.6-101	99.1	2.67	2.69
	0.50	5	98.3-103	101	1.91	1.90
DCSA	0.05 (LOQ)	5	96.7-101	98.7	1.52	1.54
	0.50	5	101-105	103	1.51	1.45
Confirmation ion transition						
Dicamba	0.05 (LOQ)	5	93.9-98.6	96.3	2.01	2.08
	0.50	5	97.9-101	99.4	1.41	1.42
DCSA	0.05 (LOQ)	5	95.3-102	98.3	2.79	2.84
	0.50	5	98.7-103	101	1.85	1.82

Data (uncorrected results, pp. 22-23) were obtained from Tables 1-8, pp. 34-41 of MRID 50914301. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

¹ The soil matrices were sandy loam (Batch ID: 24Oct18Soil-A; pH 6.6 (1:1 soil:water ratio); 64% sand, 17% silt, 19% clay, 3.7% organic matter) and loamy sand (05Oct18Soil-D; pH 6.9 (1:1 soil:water ratio); 83% sand, 10% silt, 7% clay, 3.6% organic matter); it was characterized by and obtained from Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification p. 14). The soil texture was verified by the reviewer using USDA-NRCS technical support tools.

2 Two ion pair transitions were monitored (primary and confirmatory, respectively): m/z 219.0→175.0 and m/z 221.0→177.0 for dicamba, and m/z 205.0→161.0 and m/z 205.0→125.0 for DCSA.

Table 3. Independent Validation Method Recoveries for Dicamba and DCSA in Soil¹

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam Soil						
Quantitation ion transition						
Dicamba	0.05 (LOQ)	5	100-107	104	2.88	2.78
	0.50	5	98.4-103	100	2.22	2.21
DCSA	0.05 (LOQ)	5	88.6-92.4	90.2	1.66	1.84
	0.50	5	79.4-84.2	81.9	1.72	2.10
Confirmation ion transition						
Dicamba	0.05 (LOQ)	5	96.0-107	101	4.11	4.06
	0.50	5	97.0-106	102	3.20	3.14
DCSA	0.05 (LOQ)	5	88.0-97.4	91.0	3.84	4.22
	0.50	5	80.2-85.4	81.9	2.05	2.51
Silt Loam Soil						
Quantitation ion transition						
Dicamba	0.05 (LOQ)	5	99.6-103	101	1.35	1.33
	0.50	5	100-103	101	0.927	0.916
DCSA	0.05 (LOQ)	5	77.0-82.4	79.4	2.27	2.86
	0.50	5	75.8-80.2	78.9	1.80	2.29
Confirmation ion transition						
Dicamba	0.05 (LOQ)	5	97.6-103	101	2.17	2.16
	0.50	5	101-104	102	1.19	1.16
DCSA	0.05 (LOQ)	5	79.4-83.6	81.2	1.58	1.94
	0.50	5	77.4-80.0	78.3	1.12	1.43

Data (uncorrected results, p. 21) were obtained from Tables 1-8, pp. 32-39 of MRID 50931309. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

1 The soil matrices were sandy loam (RefeSol 01-A; CS 30/18; pH 6.4 (in water) 5.3 (in CaCl₂); 74% sand, 20% silt, 6% clay, 0.9% organic carbon, 5.3 meq/100 g cation exchange capacity) and silt loam (Newhaven; CS 17/18; pH 6.0 (in water) 5.4 (in CaCl₂); 25% sand, 51% silt, 24% clay, 3.2% organic carbon, 17.4 meq/100 g cation exchange capacity); it was obtained from Smithers ERS, Harrogate, and characterized by Smithers Viscient (ESG), Ltd., Harrogate, United Kingdom (USDA soil texture classification p. 15; Appendix 2, pp. 78-79). The soil texture was verified by the reviewer using USDA-NRCS technical support tools

2 Two ion pair transitions were monitored (primary and confirmatory, respectively): m/z 218.9→174.4 and m/z 220.9→176.7 for dicamba, and m/z 205.0→161.0 and m/z 205.0→125.0 for DCSA; the monitored ion transitions of the ILV were similar to those of the ECM.

III. Method Characteristics

The LOQ for dicamba and DCSA in soil was reported as 0.05 mg/kg in the ECM and ILV (pp. 23-25, 27-30 of MRID 50914301; pp. 21-24 of MRID 50931309). No justifications or calculations for the LOQ were reported in the ILV or ECM, other than this was the lowest fortification level tested. The LOD in soil was calculated as 0.002-0.004 mg/kg and 0.0004-0.002 mg/kg for dicamba and DCSA, respectively, in the ECM. The LOD was calculated in the ECM using the following equation:

$$\text{LOD} = (3 \times (N_{\text{ctl}}) / (\text{Resp}_{\text{LS}}) \times \text{Conc}_{\text{LS}} \times \text{DF}_{\text{CNTL}})$$

where

LOD is the limit of detection of the analysis

N_{ctl} is the mean noise in height of the control samples (or blanks)

Resp_{LS} is the mean response in height of the two low calibration standards

Conc_{LS} is the concentration of the low calibration standard

DF_{CNTL} is the dilution factor of the control samples (smallest dilution factor used, i.e., 50.0 mL/g).

In the ILV, the LOD in soil was estimated as 0.00111-0.00579 mg/kg for dicamba and DCSA. The LOD was calculated in the ILV using the following equation:

$$\text{LOD } (\mu\text{g/kg}) = 3 \times \text{height of control baseline noise} \times \text{control dilution factor} \times \text{calibration standard concentration } (\mu\text{g/L}) / \text{height of calibration standard peak.}$$

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is considered the lowest level of method validation (LLMV) rather than an LOQ.

Table 4. Method Characteristics

		Dicamba	DCSA
Limit of Quantitation (LOQ)*	ECM	0.05 mg/kg	
	ILV		
Limit of Detection (LOD)	ECM	0.002-0.004 mg/kg (SL/LS calc)	0.001 mg/kg (SL calc) 0.0004-0.002 mg/kg (LS calc)
	ILV	0.00253-0.00477 mg/kg (SL calc) 0.00111-0.00579 mg/kg (SIL calc)	0.00133-0.00218 mg/kg (SL calc) 0.00138-0.00159 mg/kg (SIL calc)
Linearity (calibration curve r and concentration range)	ECM ¹	r = 0.9960 (SL Q) r = 0.9955 (SL C) r = 0.9995 (LS Q & C)	r = 0.9950 (SL Q & C) r = 0.9995 (LS Q & C)
	ILV	r = 0.9994 (SL Q) r = 0.9995 (SL C) r = 0.9995 (SIL Q) r = 0.9994 (SIL C)	r = 0.9995 (SL Q) r = 0.9991 (SL C) r = 0.9997 (SIL Q) r = 0.9992 (SIL C)
	Range	0.200-5.00 µg/L	
Repeatable	ECM ²	Yes for LOQ and 10×LOQ in two characterized soil matrices	
	ILV ^{3,4}		

Reproducible		Yes for 0.05 mg/kg (LLMV)* and 0.50 mg/kg in characterized soil matrices
Specific	ECM	Yes, no matrix interferences were observed. Some contamination/baseline noise was observed.
	ILV	Yes, no matrix interferences were observed. Minor baseline noise affected peak integration.

Data were obtained from pp. 23-25, 27-30 (LOQ/LOD); p. 26 (linearity coefficients); Tables 1-8, pp. 34-41 (recovery data); Figures 21-28, pp. 70-77 (calibration curves); Figures 1-20, pp. 50-69 (chromatograms) of MRID 50914301; pp. 21-24 (LOQ/LOD); p. 23 (linearity coefficients); Tables 1-8, pp. 32-39 (recovery data); Figures 1-2, pp. 45-46; Figures 13-14, pp. 52-53; Figures 25-26, pp. 59-60; Figures 37-38, pp. 66-67 (calibration curves); Figures 3-48, pp. 47-72 (chromatograms) of MRID 50931309; DER Attachment 2. Q = quantitative ion transition; C = confirmatory ion transition; SL = Sandy loam; SIL = Silt loam; LS = Loamy sand.

* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

- 1 Reported r values were reviewer-calculated from r² values reported in the study reports (p. 26 of MRID 50914301; DER Attachment 2). Values were reported to four significant figures.
- 2 In the ECM, the soil matrices were sandy loam (Batch ID: 24Oct18Soil-A; pH 6.6 (1:1 soil:water ratio); 64% sand, 17% silt, 19% clay, 3.7% organic matter) and loamy sand (05Oct18Soil-D; pH 6.9 (1:1 soil:water ratio); 83% sand, 10% silt, 7% clay, 3.6% organic matter); it was characterized by and obtained from Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification p. 14 of MRID 50914301).
- 3 In the ILV, the soil matrices were sandy loam (RefeSol 01-A; CS 30/18; pH 6.4 (in water) 5.3 (in CaCl₂); 74% sand, 20% silt, 6% clay, 0.9% organic carbon, 5.3 meq/100 g cation exchange capacity) and silt loam (Newhaven; CS 17/18; pH 6.0 (in water) 5.4 (in CaCl₂); 25% sand, 51% silt, 24% clay, 3.2% organic carbon, 17.4 meq/100 g cation exchange capacity); it was obtained from Smithers ERS, Harrogate, and characterized by Smithers Viscient (ESG), Ltd., Harrogate, United Kingdom (USDA soil texture classification p. 15; Appendix 2, pp. 78-79 of MRID 50931309).
- 4 The ILV validated the method for both analytes in the tested soils with the first trial as written with insignificant final dilution changes and analytical instrument and equipment modifications (pp. 11-20, 24; Appendix 4, p. 91 of MRID 50931309).

IV. Method Deficiencies and Reviewer's Comments

1. Based on the TFD studies that have been submitted, it appears that the ILV soil matrix covers the range of soils used in the terrestrial field dissipation (TFD) studies. In the submitted TFD studies (MRIDs 43651406, 43651405, 43651407, 42754101, and 42754102), four soils were studied: two silt loam soils, two loam soils, and a sandy loam soil. The organic matter content ranged from 1.1 to 3.2% and the pH ranged from 5.7 to 6.9.
2. The communications between the ILV laboratory and Study Monitor (Rachel Hardie, Wagner Regulatory Associates) were summarized and listed but not detailed (pp. 1, 23; Appendix 5, p. 92 of MRID 50914301). Communications involved protocol issue and informing the Study Monitor about the poor instrument sensitivity for dicamba during linearity checks, improved instrument sensitivity for dicamba after instrument re-optimization, and success/results of ILV trial. The reviewer also noted that the ECM laboratory (Smithers Viscient, Wareham) and ILV laboratory (Smithers ERS Limited, Harrogate) were part of the same company, but the reported laboratory personnel differed between the ECM and ILV (p. 6 of MRID 50931309; p. 5 of MRID 50914301). The ILV

reported that the method was provided to the ILV via the ECM on behalf of the Sponsor (p. 12 of MRID 50931309).

OCSPP 850.6100 guidance states that, if the laboratory that conducted the validation belonged to the same organization as the originating laboratory, 1) the analysts, study director, equipment, instruments, and supplies of the two laboratories must have been distinct and operated separately and without collusion, and 2) the analysts and study director of the ILV must have been unfamiliar with the method both in its development and subsequent use in field studies.

3. 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. 23-25, 27-30 of MRID 50914301; pp. 21-24 of MRID 50931309). No justifications or calculations for the LOQ were reported in the ILV or ECM. The LOD was calculated in the ECM using the following equation: $LOD = (3 \times N_{ctl}) / (Res_{PLS}) \times Conc_{LS} \times DF_{CNTL}$, where, LOD is the limit of detection of the analysis, N_{ctl} is the mean noise in height of the control samples (or blanks), Res_{PLS} is the mean response in height of the two low calibration standards, $Conc_{LS}$ is the concentration of the low calibration standard, and DF_{CNTL} is the dilution factor of the control samples (smallest dilution factor used, i.e., 50.0 mL/g). The LOD was calculated in the ILV using the following equation: $LOD (\mu\text{g}/\text{kg}) = 3 \times \text{height of control baseline noise} \times \text{control dilution factor} \times \text{calibration standard concentration} (\mu\text{g}/\text{L}) / \text{height of calibration standard peak}$. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is considered the lowest level of method validation (LLMV) rather than an LOQ.

The Method Detection Limit (MDL) was also calculated in the ECM and ILV as 0.01 mg/kg (pp. 24, 27-30 of MRID 50914301; pp. 24-28 of MRID 50931309). In the ECM, the MDL was defined as the lowest concentration in test samples which can be detected based on the concentration of the low calibration standard and the dilution factor of the control solutions. In the ILV, the MDL was calculated as the initial sample concentration equivalent to the lowest calibration standard (based upon a lowest standard concentration of 0.2 $\mu\text{g}/\text{L}$ and a dilution factor of 50).

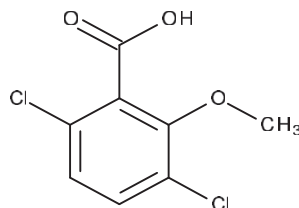
4. In the ECM and ILV, no significant matrix effects were observed (<20%; pp. 25, 27-30; Tables 9-16, pp. 42-49 of MRID 50914301; pp. 24-28; Tables 9-12, pp. 40-43 of MRID 50931309). The ILV noted that the first matrix-assessment attempt was rejected due to poor instrument sensitivity for dicamba; rejected results were not provided.
5. In the ILV, it was reported that “On 10 September 2019, the legal name of Smithers Viscient Ltd was changed to Smithers ERS Limited. The legal entity did not change. Smithers ERS Limited is a business unit of The Smithers Group that is engaged in providing contract research services.” (p. 12 of MRID 50931309).

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**Dicamba (Dicamba acid)**

IUPAC Name: 3,6-Dichloro-o-anisic acid
CAS Name: 3,6-Dichloro-2-methoxybenzoic acid
CAS Number: 1918-00-9
SMILES String: COc1c(Cl)ccc(Cl)c1C(O)=O

**DCSA (3,6-Dichlorosalicylic acid)**

IUPAC Name: 3,6-Dichloro-2-hydroxybenzoic acid
CAS Name: 3,6-Dichlorosalicylic acid
CAS Number: 3401-80-7
SMILES String: O=C(O)c(c(O)ccc1Cl)Cl)c1O

