

**Analytical method for dicamba and its metabolite NOA414746 (DCSA) in soil**

**Reports:** ECM: EPA MRID No.: MRID 50784607 (Appendix E, pp. 66-134). Braid, S., and M. Garcia-Alix. 2013. Dicamba. Dicamba - Analytical Method GRM022.06A for the Determination of Dicamba and its Metabolite NOA414746 in Soil. Analytical Method. Report No.: GRM022.06A. Task No.: TK0112263. Report prepared by CEM Analytical Services Ltd. (CEMAS), Berkshire, United Kingdom, sponsored by Syngenta Ltd., Jealott's Hill International Research Centre, Berkshire, United Kingdom, and submitted by BASF Crop Protection, Research Triangle Park, North Carolina; 69 pages. Final report issued August 8, 2013.

ILV: EPA MRID No.: MRID 50784607. Budgeon, Jr., A.D., and A. Li. 2018. Independent Laboratory Validation of Syngenta Analytical Method GRM022.06A: "Dicamba - Analytical Method GRM022.06A for the Determination of Dicamba and its Metabolite NOA414746 in Soil". BASF Study No.: 823033. JRFA Study No.: AU-2018-03. BASF Registration Document No.: 2018/7003759. Report prepared by JRF America, Audubon, Pennsylvania, and sponsored and submitted by BASF Crop Protection, Research Triangle Park, North Carolina; 65 pages. Final report issued July 11, 2018.

**Document No.:** MRID 50784607

**Guideline:** 850.6100

**Statements:** ECM: The study was not conducted in compliance with USEPA FIFRA (40 CFR Part 160) or OECD Principles of Good Laboratory Practices (GLP; Appendix E, p. 68 of MRID 50784607). A signed and dated GLP statement was provided (Appendix E, p. 68). Data Confidentiality, Quality Assurance, and Authenticity statements were not included.

ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 50784607). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Certification of Authenticity statements were provided (pp. 2-5).

**Classification:** This analytical method is classified as **supplemental**. **The ECM should be updated with additional sample clean-up processing to enhance specificity during LC/MS/MS analysis.** The specificity of the method was not supported for dicamba and NOA414746 by ILV representative chromatograms. The specificity of the method was not supported for dicamba by ECM representative chromatograms. The ILV was conducted with a single silt loam soil which was not considered the most difficult matrix with which to validate the method, based on submitted TFD studies. As the study was conducted with a single soil, the ILV soil matrix does not cover the range of soils used in the terrestrial field dissipation studies. ECM soils were inadequately characterized.

**PC Code:** 029801

**EFED Final Reviewer:** Chuck Peck  
Senior Fate Scientist

Signature: 2021.11.09 11:09:10  
Date:  -05'00'

**CDM/CSS-** Lisa Muto, M.S.,

Signature: 

**Dynamac JV** Environmental Scientist  
**Reviewers:**

Date: 05/18/2019

Mary Samuel, M.S.,  
 Environmental Scientist

Signature: 

Date: 05/18/2019

*This Data Evaluation Record 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, BASF Analytical Method GRM022.06A, is designed for the quantitative determination of dicamba and its metabolite NOA414746 (DCSA) in soil at the stated LOQ of 0.0035 mg/kg. There are no toxicological levels of concern in soils for either analyte, so the LOQ is considered sufficient. The ECM used two inadequately characterized soils; the ILV used one characterized soil. As the study was conducted with a single silt loam soil, the ILV soil matrix did not cover the range of soils used in the terrestrial field dissipation studies. Additionally, as the soil used in the ILV had an organic matter content of 1.8%, it is not considered the most difficult matrix, as TFDs were conducted with soils with organic matter content ranging from 1.1 to 3.2%. The ILV validated the ECM in the first trial for both analytes in one soil matrix with what study authors characterized as insignificant modifications to the analytical parameters and equipment. All submitted ILV and ECM data pertaining to precision, repeatability, reproducibility, and linearity was acceptable at the LOQ and 10×LOQ for both analytes. The specificity of the method was not supported for dicamba and NOA414746 in the ILV representative chromatograms and for dicamba in the ECM representative chromatograms since the analyte peak was only identifiable by retention time and baseline noise and contaminants interfered with analyte peak integration and attenuation. **The ECM should be updated with additional sample clean-up processing to enhance specificity during LC/MS/MS analysis.**

**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	MRID 50784607	MRID 50784607 <sup>2</sup>	Supplemental	Soil	08/08/2013	BASF Crop Protection	LC/MS/MS	0.0035 mg/kg
NOA414746	(Appendix E) <sup>1</sup>							

1 In the ECM, the Gartenacker loam soil [Sample Reference CCON/033/002; pH: 7.7 (water), 7.2 (in 0.01M CaCl<sub>2</sub>); 3.5% organic matter, 2.0% organic carbon] and 18 Acres sandy clay loam soil [Sample Reference CCON/034/003; pH: 6.4 (water), 5.8 (in 0.01M CaCl<sub>2</sub>); 4.0% organic matter, 2.3% organic carbon] were obtained from Syngenta (Appendix E, Table 1, p. 93 of MRID 50784607). The soil characterization laboratory was not reported.

2 In the ILV, the silt loam soil [Soil 203730; pH 6.4 (in 1:1 water:soil ratio); 21% sand, 57% silt, 22% clay; 1.8% organic matter (Walkley Black), 1.1% organic carbon (Walkley Black)] was collected from Washington, Louisiana, and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 13; Table 8, p. 26; Appendix C, pp. 61-62 of MRID 50784607).

## I. Principle of the Method

Soil (10 g) was weighed and placed in 250-mL round-bottom flasks (Appendix E, pp. 77, 79-81, 134 of MRID 50784607). Samples were extracted by heating at reflux with 50 mL of 0.5M potassium hydroxide solution for 45 minutes. The extracts are allowed to cool to room temperature then centrifuged (4000 rpm for 5 minutes). The supernatant was decanted into a clean centrifuge tube (50 mL). An aliquot (5 mL, equivalent to 1 g of soil) of the extract was acidified to pH 1 with 0.5 mL concentrated HCl, then partitioned four times with 2 mL of diethyl ether. For each extraction the solution was shaken vigorously for about 15 seconds with venting, centrifuged (4000 rpm for 5 minutes), and decanted. The combined diethyl ether fractions were evaporated to dryness using a stream of nitrogen and heating block set to 30°C. The residue was re-dissolved in 1 mL of 0.1M hydrochloric acid and applied to a Phenomenex Strata-X solid phase extraction (SPE; 60 mg, 3 mL size) cartridge which was pre-conditioned with 3 mL each of acetonitrile and ultra-pure water. After adding the sample, the sample flask was rinsed twice with 1 mL ultra-pure water, which was added to the column. The cartridge was washed with 2 x 2 mL of ultra-pure water, then dried under high vacuum for *ca.* 10 minutes. The method noted that the SPE cartridge should not remain dry for extended periods of time since this can cause low recovery of the analytes, which are volatile. The analytes were eluted with 1% acetic acid in acetonitrile (2 x 3.5 mL). The combined elutes were evaporated to dryness using a stream of air or nitrogen and heating block set to 40°C. The residue was re-dissolved in 2 mL of ultra-pure water:acetonitrile (90:10, v:v) and analyzed by liquid chromatography/mass spectrometry (LC/MS/MS, final sample concentration 0.5 g/mL). The method also contained precautions for the use of different SPE cartridges, bottled HPLC grade ultra-pure water for the LC mobile phase, and the use of blank injections after LC analysis of high-concentration samples to prevent carryover (Appendix E, pp. 81-82).

Samples were analyzed for dicamba and NOA414746 (DCSA) using an Agilent 1200 high performance liquid chromatography (HPLC) coupled to an AB Sciex 5500 Q Trap mass spectrometer equipped with TurboIonSpray interface in the negative ion, multiple reaction monitoring (MRM) mode (Appendix E, pp. 83-85 of MRID 50784607). The following LC conditions were used: Waters XSelect CSH<sup>TM</sup> C18 column (3.0 mm x 50 mm, 2.5 µm; oven temperature 30°C), mobile phase of (A) acetonitrile and (B) 0.1% formic acid in ultra-pure water [mobile gradient phase of percent A:B (v:v) at 0.0-2.0 min. 5:95, 2.0-4.1 min. 70:30, 4.1-6.0 min. 5:95], MS temperature 400°C, and injection volume of 40 µL (page 83 of study indicates 40 mL, but this is believed to be an error). Expected retention times were *ca.* 2.7 and 3.8 minutes for dicamba and NOA414746, respectively. Two ion pair transitions were monitored (primary and confirmatory, respectively):  $m/z$  219.0→35 and  $m/z$  221.0→37 for dicamba, and  $m/z$  204.6→125 and  $m/z$  204.6→161 for NOA414746.

The ILV performed the ECM method as written, with what were considered insignificant modifications to the analytical parameters and equipment (pp. 7, 12, 14, 18; Table 6, p. 24 of MRID 50784607). Samples were analyzed for dicamba and NOA414746 using Shimadzu UFLC XR HPLC system coupled with an AB Sciex 4000 API mass spectrometer equipped with TurboSpray ionization in the negative ion, MRM mode. The LC/MS/MS parameters were the same as those of the ECM, except that MS temperature was 500°C. Two ion pair transitions were monitored (primary and confirmatory, respectively):  $m/z$  218.9→35.1 and  $m/z$  220.8→37.0 for dicamba, and  $m/z$  204.8→160.7 and  $m/z$  204.8→124.9 for NOA414746; the quantitation and confirmation ion transition of the ILV were the opposite of those of the ECM for NOA414746. Expected retention times were *ca.* 2.36 and 3.49 minutes for dicamba and NOA414746, respectively.

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.0035 mg/kg for dicamba and NOA414746 in soil (p. 17; Appendix E, pp. 88-89 of MRID 50784607). In the ECM and ILV, the Limit of Detection (LOD) was determined to be 0.6 µg/kg for dicamba and between 0.3 and 0.5 µg/kg for NOA414746 when using a 40 µL injection volume based on the quantitation transition.

## II. Recovery Findings

ECM (Appendix E of MRID 50784607): Dicamba and NOA414746 were fortified at levels of 0.0035 mg/kg (LOQ) and 0.035 mg/kg (10×LOQ) in two soil matrices (Appendix E, Tables 2-5, pp. 94-95). Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%). Two ion pair transitions were monitored for dicamba and NOA414746 using LC/MS/MS in negative mode; the quantification and confirmation ion data were comparable for all analytes/matrices. The Gartenacker loam soil [Sample Reference CCON/033/002; pH: 7.7 (water), 7.2 (in 0.01M CaCl<sub>2</sub>); 3.5% organic matter, 2.0% organic carbon] and 18 Acres sandy clay loam soil [Sample Reference CCON/034/003; pH: 6.4 (water), 5.8 (in 0.01M CaCl<sub>2</sub>); 4.0% organic matter, 2.3% organic carbon] were obtained from Syngenta (Appendix E, Table 1, p. 93). The soil characterization laboratory was not reported.

ILV (MRID 50784607): Dicamba and NOA414746 were fortified at levels of 0.0035 mg/kg (LOQ) and 0.035 mg/kg (10×LOQ) in one soil matrix (Tables 1-4, pp. 19-22). Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD ≤20%). Two ion pair transitions were monitored for dicamba and NOA414746 using LC/MS/MS in negative mode; the quantification and confirmation ion data were comparable. The silt loam soil [Soil 203730; pH 6.4 (in 1:1 water:soil ratio); 21% sand, 57% silt, 22% clay; 1.8% organic matter (Walkley Black), 1.1% organic carbon (Walkley Black)] was collected from Washington, Louisiana, and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 13; Table 8, p. 26; Appendix C, pp. 61-62). The ILV validated the ECM in the first trial for both analytes in one soil matrix with what study authors classified as insignificant modifications to the analytical parameters and equipment (pp. 7, 12, 14, 17-18; Table 6, p. 24).

**Table 2. Initial Validation Method Recoveries for Dicamba and NOA414746 in Soil**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) <sup>1</sup>	Relative Standard Deviation (%)
<b>Gartenacker Loam Soil<sup>2</sup></b>						
Quantitation ion <sup>3</sup>						
Dicamba	0.0035 (LOQ)	5	88-98	94	4	4.8
	0.035	5	74-84	77	4	5.2
NOA414746	0.0035 (LOQ)	5	84-95	90	4	4.4
	0.035	5	78-94	85	7	8.7
Confirmation ion <sup>3</sup>						
Dicamba	0.0035 (LOQ)	5	88-103	93	6	6.6
	0.035	5	74-83	78	3	4.4
NOA414746	0.0035 (LOQ)	5	88-97	93	4	4.2
	0.035	5	79-90	84	4	5.3
<b>18 Acres Sandy Clay Loam Soil<sup>2</sup></b>						
Quantitation ion <sup>3</sup>						
Dicamba	0.0035 (LOQ)	5	80-98	87	9	10.3

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) <sup>1</sup>	Relative Standard Deviation (%)
	0.035	5	76-87	80	4	5.4
NOA414746	0.0035 (LOQ)	5	72-83	76	5	6.4
	0.035	5	72-90	85	7	8.7
Confirmation ion <sup>3</sup>						
Dicamba	0.0035 (LOQ)	5	76-102	89	12	13.6
	0.035	5	74-89	81	6	7.1
NOA414746	0.0035 (LOQ)	5	70-84	76	6	8.3
	0.035	5	71-89	81	8	9.5

Data (recovery results were corrected when residues were quantified in the controls; Appendix E, pp. 85-87) were obtained from of Appendix E, Tables 2-5, pp. 94-95 of MRID 50784607 and DER Attachment 2.

1 Values for the standard deviation were calculated by the reviewer (see DER Attachment 2). Rules of significant figures were followed.

2 The Gartenacker loam soil [Sample Reference CCON/033/002; pH: 7.7 (water), 7.2 (in 0.01M CaCl<sub>2</sub>); 3.5% organic matter, 2.0% organic carbon] and 18 Acres sandy clay loam soil [Sample Reference CCON/034/003; pH: 6.4 (water), 5.8 (in 0.01M CaCl<sub>2</sub>); 4.0% organic matter, 2.3% organic carbon] were obtained from Syngenta (Appendix E, Table 1, p. 93). The soil characterization laboratory was not reported.

3 Two ion pair transitions were monitored (primary and confirmatory, respectively):  $m/z$  219.0→35 and  $m/z$  221.0→37 for dicamba, and  $m/z$  204.6→125 and  $m/z$  204.6→161 for NOA414746.

**Table 3. Independent Validation Method Recoveries for Dicamba and NOA414746 in Soil**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Silt Loam Soil <sup>1</sup>						
Quantitation ion <sup>2</sup>						
Dicamba	0.0035 (LOQ)	5	79.5-94.4	87.6	6.3	7.1
	0.035	5	84.0-97.1	87.7	5.4	6.1
NOA414746	0.0035 (LOQ)	5	90.4-106	96.2	6.6	6.8
	0.035	5	85.3-89.7	87.9	1.7	2.0
Confirmation ion <sup>2</sup>						
Dicamba	0.0035 (LOQ)	5	86.5-102	93.0	5.8	6.2
	0.035	5	80.1-87.9	85.2	3.1	3.6
NOA414746	0.0035 (LOQ)	5	85.5-102	94.4	6.1	6.5
	0.035	5	81.1-91.6	86.2	4.4	5.1

Data (uncorrected recovery results; p. 53; Tables 1-4, pp. 19-22) were obtained from Tables 1-4, pp. 19-22 of MRID 50784607.

1 The silt loam soil [Soil 203730; pH 6.4 (in 1:1 water:soil ratio); 21% sand, 57% silt, 22% clay; 1.8% organic matter (Walkley Black), 1.1% organic carbon (Walkley Black)] was collected from Washington, Louisiana, and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 13; Table 8, p. 26; Appendix C, pp. 61-62).

2 Two ion pair transitions were monitored (primary and confirmatory, respectively):  $m/z$  218.9→35.1 and  $m/z$  220.8→37.0 for dicamba, and  $m/z$  204.8→160.7 and  $m/z$  204.8→124.9 for NOA414746; the quantitation and confirmation ion transition of the ILV were the opposite of those of the ECM.



### III. Method Characteristics

In the ECM and ILV, the LOQ was 0.0035 mg/kg for dicamba and NOA414746 in soil (p. 17; Appendix E, pp. 88-89 of MRID 50784607). In the ECM, the LOQ was defined as the lowest fortification level for which mean recoveries were 70-120%, RSD was  $\leq 20\%$ , and blanks did not exceed 30% of the LOQ. Additionally, the response of the analyte peak should be no lower than three times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. In the ILV, the LOQ was defined as the lowest level successfully tested. No calculations were provided for the LOQ in the ECM or ILV. In the ECM and ILV, the LOD was determined to be 0.6  $\mu\text{g}/\text{kg}$  for dicamba and between 0.3 and 0.5  $\mu\text{g}/\text{kg}$  for NOA414746 when using a 40  $\mu\text{L}$  injection volume based on the quantitation transition. In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as three times background noise, and the LOD can vary between runs and from instrument to instrument. In the ILV, the LOD was reported from the ECM without further explanation.

**Table 4. Method Characteristics for Dicamba and NOA414746 in Soil**

Parameter		Dicamba	NOA414746	
Limit of Quantitation (LOQ)	ECM	0.0035 mg/kg		
	ILV			
Limit of Detection (LOD)	ECM	0.6 $\mu\text{g}/\text{kg}$ for dicamba when using a 40 $\mu\text{L}$ injection volume based on the quantitation transition.	0.3 to 0.5 $\mu\text{g}/\text{kg}$ for NOA414746 when using a 40 $\mu\text{L}$ injection volume based on the quantitation transition.	
	ILV			
Linearity (calibration curve $r^2$ and concentration range)	ECM	Loam	$r^2 = 0.9974$ (Q) $r^2 = 0.9983$ (C)	$r^2 = 0.9999$ (Q & C)
		Sandy Clay Loam	$r^2 = 0.9981$ (Q) $r^2 = 0.9987$ (C)	
	ILV <sup>1</sup>	$r^2 = 0.9978$ (Q) $r^2 = 0.9981$ (C)	$r^2 = 0.9960$ (Q) $r^2 = 0.9950$ (C)	
	Range	0.02-1.0 ng (0.0005-0.025 $\mu\text{g}/\text{mL}$ , 40 $\mu\text{L}$ injection)		
Repeatable	ECM <sup>2</sup>	Yes at LOQ and 10 $\times$ LOQ. (two characterized soil matrices used)		
	ILV <sup>3,4</sup>	Yes at LOQ and 10 $\times$ LOQ. (one characterized soil matrix used)		
Reproducible		Yes at LOQ and 10 $\times$ LOQ.		
Specific	ECM	Loam	No, analyte peak only identifiable by retention time, and baseline noise and contaminants interfered with analyte peak integration and attenuation. <sup>5</sup>	Yes, no matrix interferences were observed, but baseline noise interfered with analyte peak integration and attenuation. Peak tailing observed.
		Sandy Clay Loam		
	ILV	No, analyte peak only identifiable by retention time, and LOQ analyte peak was small compared to contaminants. <sup>6</sup> Matrix interferences were <10% of the LOQ (based on peak area).	No, analyte peak only identifiable by retention time, and LOQ analyte peak was small compared to contaminants. <sup>7</sup> Baseline noise interfered with analyte peak integration and attenuation; peak tailing observed. No matrix interferences were observed	

Data were obtained from p. 17 (ILV LOQ/LOD); Tables 1-4, pp. 19-22 (ILV recovery results); p. 17 ; Figure 1, p. 27, and Figure 14, p. 40 (ILV calibration data & curves); Figures 2-26, pp. 28-52 (ILV chromatograms); Appendix E, pp. 88-89 (ECM LOQ/LOD); Appendix E, Tables 2-5, pp. 94-95 (ECM recovery results); Appendix E, p. 89; Figures 23-30, pp. 120-127 (ECM calibration data); Appendix E, Figures 3-22, pp. 100-119 (ECM chromatograms) of MRID 50784607; DER Attachment 2. Q = quantitation ion; C = confirmation ion. All results reported for Q and C ions unless specified otherwise. All results reported for both test matrices in each study unless specified otherwise.

- 1 Reported ILV  $r^2$  values were reviewer-calculated from  $r$  values provided in the study report (Figure 1, p. 27, and Figure 14, p. 40 of MRID 50784607; DER Attachment 2). Even though reported  $r$  values were given to 11 significant figures, the reviewer only reported  $r^2$  values to 4 significant figures.
- 2 In the ECM, the Gartenacker loam soil [Sample Reference CCON/033/002; pH: 7.7 (water), 7.2 (in 0.01M CaCl<sub>2</sub>); 3.5% organic matter, 2.0% organic carbon] and 18 Acres sandy clay loam soil [Sample Reference CCON/034/003; pH: 6.4 (water), 5.8 (in 0.01M CaCl<sub>2</sub>); 4.0% organic matter, 2.3% organic carbon] were obtained from Syngenta (USDA soil texture classification was not specified; Appendix E, Table 1, p. 93 of MRID 50784607). The soil characterization laboratory was not reported.
- 3 In the ILV, the silt loam soil [Soil 203730; pH 6.4 (in 1:1 water:soil ratio); 21% sand, 57% silt, 22% clay; 1.8% organic matter (Walkley Black), 1.1% organic carbon (Walkley Black)] was collected from Washington, Louisiana, and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 13; Table 8, p. 26; Appendix C, pp. 61-62 of MRID 50784607).
- 4 The ILV validated the ECM in the first trial for both analytes in one soil matrix with only insignificant modifications to the analytical parameters and equipment (pp. 7, 12, 14, 17-18; Table 6, p. 24 of MRID 50784607).
- 5 Based on Appendix E, Figures 5 and 15, pp. 102, 112 of MRID 50784607.
- 6 Based on Figures 10-11, pp. 36-37 of MRID 50784607.
- 7 Based on Figures 23-24, pp. 49-50 of MRID 50784607.

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

1. The specificity of the method was not supported for dicamba and NOA414746 by ILV representative chromatograms. For dicamba and NOA414746, multiple significant contaminants surrounded the LOQ peak, and the analyte peak was only identifiable by retention time (Figures 10-11, pp. 36-37 and Figures 23-24, pp. 49-50 of MRID 50784607). For NOA414746, baseline noise interfered also with analyte peak integration and attenuation; peak tailing observed. **The ECM should be updated with additional sample clean-up processing to enhance specificity during LC/MS/MS analysis.**
2. The specificity of the method was not supported for dicamba by ECM representative chromatograms since the analyte peak was only identifiable by retention time, and baseline noise and contaminants interfered with analyte peak integration and attenuation (Appendix E, Figure 5, p. 102, and Figure 15, p. 112 of MRID 50784607). **The ECM should be updated with additional sample clean-up processing to enhance specificity during LC/MS/MS analysis.**
3. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method since only one uncharacterized homogenized soil matrix was tested. OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs. The organic matter content for the soil used in the ILV was 1.8%. 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%. While a certain number of soil matrices is not specified in the OCSPP guidelines, more than one soil/soil matrix would need to be included in an ILV in order to cover the range of soils used in the terrestrial field

- dissipation studies. The ECM included two soils, while the ILV only included one. The ILV should be a more rigorous test of the method, and therefore, should include at least as many test matrices as the ECM. Additionally, it could not be determined if the ILV soil matrices covered the range of soils used in the terrestrial field dissipation studies.
4. The ECM soils were inadequately characterized. While the ECM soil matrix texture classification was specified as USDA soil texture classification, the soil partition percentages were not reported, so the classification could not be verified (Appendix E, Table 1, p. 93 of MRID 50784607).
  5. The communications between the ILV and BASF Study Monitor (Carlan Downs) were reportedly limited to the results of the first attempt of the ILV (pp. 11, 18 of MRID 50784607). A complete summary list of communication was not provided, but reportedly documented in the study file.
  6. The recovery results reported by the ECM was a summary of results from another study, CEMR-5971-REG (Garcia-Alix., M. 2103; Appendix E, pp. 88, 91 of MRID 50784607).
  7. 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 (p. 17; Appendix E, pp. 88-89 of MRID 50784607). In the ECM, the LOQ was defined as the lowest fortification level for which mean recoveries were 70-120%, RSD was  $\leq 20\%$ , and blanks did not exceed 30% of the LOQ. Additionally, the response of the analyte peak should be no lower than three times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. In the ILV, the LOQ was defined as the lowest level successfully tested. No calculations were provided for the LOQ in the ECM or ILV. In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as three times background noise, and the LOD can vary between runs and from instrument to instrument. In the ILV, the LOD was reported from the ECM without further explanation. Detection limits should not be based on arbitrary values.
  8. In the ECM, it was reported that matrix effects were significant (>20% difference from non-matrix standards) for dicamba and NOA414746; therefore, matrix-matched standards should be used (Appendix E, pp. 88-89; Appendix E, Table 6, p. 96 of MRID 50784607).
  9. In the ECM, it was stated that the final extracts in ultra-pure water:acetonitrile (90:10, v:v) were stable for up to 7 days at *ca.* 4°C (Appendix E, p. 90; Appendix E, Tables 7-8, pp. 96-97 of MRID 50784607).
  10. The time required to complete the method for a set of 13 samples (ten fortified samples, two unfortified samples, one reagent blank) was reported as *ca.* 14 hours (including calculation of results) in the ILV (p. 17 of MRID 50784607).

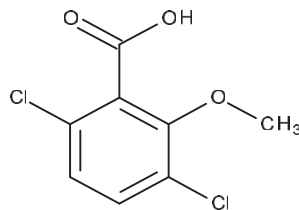


**V. References**

- 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 (SAN 837 H, CGA 57706, BAS183 22 H)**

**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

**NOA414746 (3,6-Dichlorosalicylic acid, DSCA)**

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

