Analytical method for Diflufenican (AE F088657) and its degradates AE 0542291 (Phenoxynicotinamide) and AE B107137 (Diflufenican acid) in soil and sediment

Reports: ECM 1: EPA MRID No.: 51458735. Murphy, I. 2018. An Analytical Method for the Determination of Residues of Diflufenican and its metabolites AE 0542291 and AE B107137 in Soil and Sediment Using LC/MS/MS. Analytical Method. Report prepared, sponsored, and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 26 pages. Bayer Method No.: DC-002-S18-01. Activity ID: RADC0091. Final report issued February 6, 2018.

ECM 2: EPA MRID No.: 51458736. Ali, T. 2019. In House Laboratory Validation of Analytical Method for the Determination of AE F088657 and Its Metabolites: AE 0542291 and AE B107137 in Soil and Sediment by LC/MS/MS. Final Report. Report prepared by Bayer CropScience, Research Triangle Park, North Carolina, and sponsored and submitted by Bayer CropScience, St. Louis, Missouri; 64 pages. Activity/Study ID: RADC0091. Final report issued September 10, 2019.

ILV: EPA MRID No.: 51458737. Miner, P. 2020. "Independent Laboratory Validation of Analytical Method DC-002-S18-01 for the Determination of Residues of Diflufenican and its metabolites AE 0542291 and AE B107137 in Soil". Final Amended Report. Report prepared by Frontage Laboratories, Concord, Ohio, and sponsored and submitted by Bayer CropScience, St. Louis, Missouri; 104 pages. Laboratory Study ID: 037283. Sponsor Study ID: RADC0095. Final report issued September 11, 2019. Amended Report completed July 20, 2020.

Document No.: MRIDs 51458735 & 51458736 & 51458737

Guideline: 850.6100

Statements: ECM 1: The report was not conducted in compliance with USEPA FIFRA Good Laboratory (GLP) standards (40 CFR Part 160) or OECD GLP since it was not a study (p. 3 of MRID 51458735). Signed and dated Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity statements were not included.
ECM 2: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160; 1989; p. 3 of MRID 51458736). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-3, 5). An Authenticity statement was not included.
ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 51458737). 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. ECM 1 MRID 51458735 contained the full method written in a step-wise manner, but no performance data; while ECM 2 MRID 51458736 contained performance data, but only a method summary. Since the reported method 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 LOQ. The ILV (MRID 51458737) was not conducted independently of the internal

validation (ECM 2 MRID 51458736) since the ILV Sponsor Correspondence included the ECM 2 Study Author and ILV technical issues. It could not be determined if the ILV was provided with the most difficult or appropriate matrix with which to validate the method since only one soil was used in the ILV, which was not characterized in the study. No sediment matrix was used in the ILV. The ECM method (Bayer Method No. DC-002-S18-01) should be updated with the ILV modification to adjust the microwave wattage to maintain 70°C, as well as precautions about the LC/MS/MS instrument model needed to provide adequate sensitivity and corrections of the typographical errors in the MS ion transitions. ECM 2 linearity and specificity data only included one soil matrix. No LOD was reported for the method, only an MDL (ECM 2).

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EFED Final Reviewer:	Sheng Lin, Ph.D., Physical Scientist	Signature: Date:	SHENG LIN Digitally signed by SHENG LIN Date: 2022.05.24 13:25:21 -04'00'
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CDM/CSS-Dynamac JV Reviewers:	Mary Samuel, M.S., Environmental Scientist	Signature: Date:	Mary Samuel 09/24/2021

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, Bayer Method No. DC-002-S18-01, is designed for the quantitative determination of diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) in soil and sediment at the stated LOQ of 1.5 ng/g using LC-MS/MS. The LOQ is less than the lowest toxicological level of concern in soil for diflufenican, AE 0542291, and AE B107137. Since the reported method 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. Based on the performance data submitted by the ILV and ECM 2, the LLMV was equivalent to the ECM reported method LOQ for diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) in the tested soil matrices (1.5 ng/g). ECM 1 MRID 51458735 contained the full method written in a step-wise manner, but no performance data; while ECM 2 MRID 51458736 contained performance data, but only a method summary. No LOD was reported for the method, only an MDL (ECM 2).

The ECM validated the method using characterized silt loam and loamy sand soil matrices, as well as characterized loamy sand sediment matrix; the ILV validated the method using one uncharacterized soil matrix, which was sourced from the Sponsor's Iowa terrestrial field dissipation

study (MRID 51458656). No sediment matrix was used in the ILV. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. The one ILV soil matrix did not cover the range of soils used in the four submitted diflufenican terrestrial field dissipation studies; however, the two ECM soils were sourced from diflufenican TFD MRID 51458654 (New York) and MRID 51458655 (Georgia). The ILV (MRID 51458737) was not conducted independently of the internal validation (ECM 2 MRID 51458736) since the ILV Sponsor Correspondence included the ECM 2 Study Author, Tehane Ali, and involved ILV technical issues.

The ILV validated the method (Bayer Method No. DC-002-S18-01) for diflufenican, AE 0542291, and AE B107137 in soil in the third trial as written with a significant modification of the microwave extraction procedure and insignificant modifications to the analytical parameters. The first ILV trial failed due to the inadequate sensitivity of the LC/MS/MS instrument which was used by the ILV. The second trial failed due to the fact that the ILV microwave did not maintain 70°C when the wattage was reduced from 400W to 110W in the 3- to 15-minute interval, as directed in the method (Bayer Method No. DC-002-S18-01). The ILV modified the method by increasing the wattage to maintain 70°C. The ILV modifications warranted an updated ECM since the increase of the wattage for the 3- to 15-minute interval was necessary to maintain 70°C and provide acceptable recoveries. Additionally, the ECM should be updated with precautions about the LC/MS/MS instrument model needed to provide adequate sensitivity, as well as corrected for typographical errors in the MS ion transitions for the quantitation ion transition of AE 0542291 and the confirmation ion transition of AE B107137-d_3.

The method (Bayer Method No. DC-002-S18-01) for diflufenican, AE 0542291, and AE B107137 for sediment was not validated by the ILV since no sediment matrix was included in the ILV validation.

All ILV data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for diflufenican, AE 0542291, and AE B107137 in the test soil matrix. All ECM 2 data regarding repeatability, accuracy, and precision were satisfactory for diflufenican, AE 0542291, and AE B107137 in both test soil matrices; all ECM 2 data regarding linearity and specificity were satisfactory for diflufenican, AE 0542291, and AE B107137 in the loamy sand test soil matrix; no data provided for silt loam soil matrix.

All ECM 2 data regarding repeatability, accuracy, and precision were satisfactory for diflufenican, AE 0542291, and AE B107137 in the test sediment matrix; no data provided for linearity and specificity.

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						
Diflufenican (AE F088657)	51458735[1] & 51458736[2]	51458737[3]		Soil and Sediment[4]	06/02/2018 (Method) 10/09/2019 (Internal validation)	Bayer CropScience	LC-MS/MS	1.5 ng/g
AE 0542291 (Phenoxynicotinamide)								
AE B107137 (Diflufenican acid)								

1 ECM 1 MRID 51458735 was a method only study report. No performance data was submitted in this study.

2 In the ECM 2 MRID 51458736 (Internal validation), silt loam soil (33% sand, 56% silt, 11% clay; pH 4.5 in $CaCl_2$ solution; pH 4.8 in aqueous solution; 3.6% organic matter; 2.1% organic carbon) collected from New York (Study MEDC0006; MRID 51458654), loamy sand soil (85% sand, 10% silt, 5% clay; pH 6.2 in $CaCl_2$ solution; pH 6.6 in aqueous solution; 0.96% organic matter; 0.56% organic carbon) collected from Georgia (Study MEDC0007; MRID 51458655), and loamy sand sediment (84.2% sand, 9.3% silt, 6.5% clay; pH 5.3 in $CaCl_2$ solution; pH 5.9 in aqueous solution; 2.2% organic matter; 1.3% organic carbon) collected from North Carolina (Study MEDC0014; MRID 51458651) were used in the study (USDA soil texture classification; pp. 14-15 of MRID 51458736). The soils were collected from two diflufenican terrestrial field dissipation studies. The sediment was collected from a diflufenican aerobic aquatic metabolism study. The soil characterization laboratory was not reported in the study. The soil texture of the silt loam soil was verified by the reviewer using USDA-NRCS technical support tools. The soil texture of the loamy sand soil and sediment was determined by the reviewer using USDA-NRCS technical support tools; in this study, those matrices were designated as sandy loam soil and sand sediment.

3 In the ILV, soil (Soil Sample MEDC0008IA-S001) from the Iowa terrestrial field dissipation (TFD) study (MEDC0008; MRID 51458656) was provided by Bayer CropScience (the Sponsor) and used in the study (p. 14 of MRID 51458737). The soil characterization was not reported in the study. In TFD MRID 51458656, the Iowa soil sample was characterized as silty clay loam (0-45 cm, 60-105 cm), silty clay (45-60 cm), and clay loam (105-120 cm) using USDA soil texture classification (Table 6, p. 36 of MRID 51458656). These soil textures were verified by the reviewer using USDA-NRCS technical support tools.

4 No sediment matrix was used in the ILV.

I. Principle of the Method

Soil/sediment samples (25 ± 0.25 g) were fortified (1.0 µg/mL, 0.1 µg/mL, or 0.01 µg/mL mixed fortification solution) and extracted with *ca*. 50 mL of acetonitrile:water:acetic acid (400:100:3, v:v:v) via microwave extractor at *ca*. 70°C (for 10 samples: 0-3 min. 400W, 3-15 min. 110W; for >10 samples: 0-3 min. +40W for each additional sample) with stirring for 15 minutes (pp. 7-11 of MRID 51458735; pp. 15-16 of MRID 51458736). Mixed internal standard (0.250 mL of 1.0 µg/mL solution) was added with mixing then the solution was cooled to ambient temperature and centrifuged at >12000 rpm for 5 minutes. An aliquot of the supernatant was taken for LC-MS/MS analysis.

Samples were analyzed for diflufenican (AE F088657), AE 0542291 (phenoxynicotinamide), and AE B107137 (diflufenican acid) using a Thermo Vanquish Pumps UHPLC coupled with a Thermo Scientific TSQ Quantiva LC/MS/MS operated in positive API interface-electrospray ionization mode with multiple reaction monitoring (MRM; pp. 16-17 of MRID 51458736). The following LC conditions were used: Phenomenex Luna C18 (2)-HST 100A column (2.0 x 50 mm, 2.5 µm; column temperature 60°C), mobile phase of (A) water:methanol (9:1, v:v) with 10mM ammonium

formate and 120 µL formic acid per liter and (B) water:methanol (1:9, v:v) with 10mM ammonium formate and 120 µL formic acid per liter [mobile gradient phase of percent A:B (v:v) at 0.00-0.50 min. 100:0, 3.00-3.50 min. 0:100, 3:51-5.00 min. 100:0] and injection volume of 10 µL. MS vaporizer temperature was 400°C. Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 395.0→266.0 and *m/z* 395.0→246.0 for diflufenican (AE F088657), *m/z* 283.2→**265.9** and *m/z* 283.2→246.0 for AE 0542291, and *m/z* 284.3→246.0 and *m/z* 284.3→266.0 for AE B107137 [the bolded value was reviewer-determined based on the representative chromatograms for AE 0542291 (see Appendix 2, p. 56 of MRID 51458736 and Appendix 3, p. 19 of MRID 51458735) and the MS scan for AE 0542291 (see Appendix 4, p. 24 of MRID 51458735)]. Reported retention times were *ca*. 3.5, 2.8, and 2.6 minutes for diflufenican (AE F088657), AE 0542291, and AE B107137, respectively (pp. 16-17 of MRID 51458736). Two ion pair transitions were monitored for each internal standard (quantitation and confirmation, respectively): *m/z* 398.0→268.0 and *m/z* 398.0→248.0 for diflufenican-d_3 (AE F088657-d_3), *m/z* 286.3→268.0 and *m/z* 286.3→248.0 for AE 0542291-d_3, and *m/z* 287.2→248.0 and *m/z* **287.2**→268.0 for AE B107137-d_3 [the bolded value was reviewer-determined based on the representative chromatograms for AE B107137-d_3 (see Appendix 2, p. 56 of MRID 51458736 and Appendix 3, p. 23 of MRID 51458735)]. All LC/MS/MS parameters in MRID 51458735 matched those in MRID 51458736; however, many of the mass transition ions were specified to the hundredths and thousandths place (pp. 6, 11-13 of MRID 51458735).

ECM 1 MRID 51458735 and ECM 2 MRID 51458736 should be corrected for typographical errors in the MS ion transitions for the quantitation ion transition of AE 0542291 (reported as *m/z* 283.2→**255.9** or *m/z* 283.243→**255.929**) and the confirmation ion transition of AE B107137-d_3. (reported as *m/z* 284.2→268.0 or *m/z* **284.244**→268; p. 13 of MRID 51458735; p. 17 of MRID 51458736).

The ILV performed the ECM method (Bayer Method No. DC-002-S18-01) as written with a significant modification of the microwave extraction procedure and insignificant modifications to the analytical parameters (pp. 14-17, 19; Appendix F, pp. 89-104 of MRID 51458737). The temperature of the ILV microwave was determined to "significantly" drop with the wattage change after 3 minutes; therefore, the wattage was increased for this time period "so that the temperature maintain[ed] 70[°]C throughout the duration of the extraction" (Appendix F, p. 90). The microwave extraction procedure was reported as "From 0-3 minutes at 400 W (ambient temperature to approx. 70 °C) and held at 70 ºC from 3-15 minutes" in the ILV analytical methodology (p. 16). The wattage for the 3- to 15-minute interval was not specified. Samples were analyzed for diflufenican (AE F088657), AE 0542291 (phenoxynicotinamide), and AE B107137 (diflufenican acid) using Shimadzu LC-30AD HPLC coupled with an Sciex API 6500 Mass Spectrometer (pp. 16-17). The LC-MS/MS parameters were the same as those of the ECM, with the exception that the LC column was reported as a Luna C18 column (2.0 x 50 mm, 2.5 µm), the MS source temperature was 550°C, and some other minor MS parameter modifications. Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 396→267.000 and *m/z* 396→247.000 for diflufenican (AE F088657), *m/z* 283.063→265.932 and *m/z* 283.063→245.995 for AE 0542291, and *m/z* 284.050→246.078 and *m/z* 284.050→265.889 for AE B107137. These ion transitions were similar to those of ECM 1 and ECM 2, with the ECM 1/ECM 2 corrected quantitation ion transition for AE 0542291. Reported retention times were *ca*. 3.69, 2.97, and 2.72 minutes for diflufenican (AE F088657), AE 0542291, and AE B107137, respectively. Two ion pair transitions were monitored for each internal standard (quantitation and confirmation, respectively): *m/z* 399→267.894 and *m/z* 399→248.000 for diflufenican-d_3 (AE F088657-d_3), *m/z*

286.042→269.000 and *m/z* 286.042→247.930 for AE 0542291-d_3, and *m/z* 287.029→247.937 and *m/z* 287.029→267.000 for AE B107137-d_3. These ion transitions were similar to or fairly similar to those of ECM 1 and ECM 2, with the ECM 1/ECM 2 corrected confirmation ion transition for AE B107137-d_3. The ILV noted that the LC column and mobile phase solvents could not be modified. The ILV modifications warranted an updated ECM since the increase of the wattage for the 3- to 15-minute interval was necessary to maintain 70°C and provide acceptable recoveries (Appendix F, p. 90).

The Limit of Quantification (LOQ) for diflufenican (AE F088657) and its degradates AE 0542291(phenoxynicotinamide) and AE B107137 (diflufenican acid) in soil was 1.5 ng/g in the ECM 1, ECM 2, and ILV (p. 6 of MRID 51458735; pp. 9-10, 25 of MRID 51458736; pp. 6, 19 of MRID 51458737). In the ECM 1, ECM 2, and ILV, the Limit of Detection (LOD) for diflufenican, AE 0542291, and AE B107137 was not reported; however, the Minimum Detection Limit (MDL) was calculated in the ECM 2 (p. 22; Tables 7-8, p. 29 of MRID 51458736). The MDL for soil was determined to be 0.44 ng/g, 0.11 ng/g, and 0.13 ng/g for diflufenican, AE 0542291, and AE B107137, respectively. The MDL for sediment was determined to be 0.54 ng/g, 0.18 ng/g, and 0.17 ng/g for diflufenican, AE 0542291, and AE B107137, respectively. 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 1 (MRID 51458735): MRID 51458735 was a method only study report. No performance data was submitted in this study.

ECM 2 (MRID 51458736): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) at fortification levels of 1.5 ng/g (LOQ) and 15 ng/g (10×LOQ) in two soil matrices and one sediment matrix (Tables 1-6, pp. 27-28). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses, except for the 10×LOQ analysis of AE B107137. The silt loam soil (33% sand, 56% silt, 11% clay; pH 4.5 in $CaCl_2$ solution; pH 4.8 in aqueous solution; 3.6% organic matter; 2.1% organic carbon) collected from New York (Study MEDC0006; MRID 51458654), loamy sand soil (85% sand, 10% silt, 5% clay; pH 6.2 in $CaCl_2$ solution; pH 6.6 in aqueous solution; 0.96% organic matter; 0.56% organic carbon) collected from Georgia (Study MEDC0007; MRID 51458655), and loamy sand sediment (84.2% sand, 9.3% silt, 6.5% clay; pH 5.3 in $CaCl_2$ solution; pH 5.9 in aqueous solution; 2.2% organic matter; 1.3% organic carbon) collected from North Carolina (Study MEDC0014; MRID 51458651) were used in the study (USDA soil texture classification; pp. 14-15). The soils were collected from two diflufenican terrestrial field dissipation studies. The sediment was collected from a diflufenican aerobic aquatic metabolism study. The soil characterization laboratory was not reported in the study. The soil texture of the silt loam soil was verified by the reviewer using USDA-NRCS technical support tools. The soil texture of the loamy sand soil and sediment was determined by the reviewer using USDA-NRCS technical support tools; in this study, those matrices were designated as sandy loam soil and sand sediment.

ILV (MRID 51458737): Mean recoveries and RSDs were within guidelines for analysis of diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) at fortification levels of 1.5 ng/g (LOQ) and 15 ng/g (10×LOQ) in one soil matrix (Table 1, p. 21). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses, except for the LOQ analysis of AE B107137. The soil (Soil Sample MEDC0008IA-S001) from the Iowa terrestrial field dissipation (TFD) study (MEDC0008; MRID 51458656) was provided by Bayer CropScience (the Sponsor) and used in the study (p. 14 of MRID 51458737). The soil characterization was not reported in the study. In TFD MRID 51458656, the Iowa soil sample was characterized as silty clay loam (0-45 cm, 60-105 cm), silty clay (45-60 cm), and clay loam (105-120 cm) using USDA soil texture classification (Table 6, p. 36 of MRID 51458656). These soil textures were verified by the reviewer using USDA-NRCS technical support tools. No sediment matrix was included in the validation.

The method (Bayer Method No. DC-002-S18-01) for diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) for soil was validated in the third trial as written with a significant modification of the microwave extraction procedure and insignificant modifications to the analytical parameters (pp. 14-17, 19; Appendix F, pp. 89-104). The first ILV trial failed due to the inadequate sensitivity of the LC/MS/MS instrument which was used by the ILV (Appendix F, p. 101). The second trial failed due to the fact that the ILV microwave (MARS 6-1 Microwave) did not maintain 70°C when the wattage was reduced from 400W to 110W in the 3- to 15-minute interval, as directed in the method (Bayer Method No. DC-002-S18-01; Appendix F, p. 99). The ILV modified the method by increasing the wattage for the 3- to 15-minute interval to maintain 70°C; however, the actual wattage for this interval was not reported in the ILV (p. 16; Appendix F, p. 90). The ILV modifications warranted an updated ECM since the increase of the wattage for the 3- to 15-minute interval was necessary to maintain 70°C and provide acceptable recoveries. Additionally, the ECM should be updated with precautions about the LC/MS/MS instrument model needed to provide adequate sensitivity, as well as corrected for typographical errors in the MS ion transitions for the quantitation ion transition of AE 0542291 and the confirmation ion transition of AE B107137-d_3.

The method (Bayer Method No. DC-002-S18-01) for diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) for sediment was not validated by the ILV since no sediment matrix was included in the ILV validation.

Table 2. Initial Validation Method Recoveries for Diflufenican (AE F088657) and its degradates AE 0542291 (Phenoxynicotinamide) and AE B107137 (Diflufenican acid) in Soil[1,2,3]

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Silt Loam Soil (New York)					
	Quantitation ion transition					
Diflufenican (AE F088657)	1.5 (LOQ)	7	81-119	89	13.3	14.8
	15	5	70-89	83	7.5	9.1
AE 0542291	1.5 (LOQ)	7	82-88	86	2.4	2.8
	15	5	86-89	87	1.3	1.5
AE B107137 (Diflufenican acid)	1.5 (LOQ)	7	80-87	84	2.2	2.6
	15	5	78-85	81	2.6	3.2
	Confirmation ion transition					
Diflufenican (AE F088657)	1.5 (LOQ)	7	83-99	91	6.6	7.2
	15	5	92-102	93	12.8	13.7
AE 0542291	1.5 (LOQ)	7	79-86	83	2.4	2.9
	15	5	83-87	85	1.5	1.8
AE B107137 (Diflufenican acid)	1.5 (LOQ)	7	85-92	88	2.5	2.9
	15	5	89-92	90	1.4	1.6
	Loamy Sand Soil (Georgia)					
	Quantitation ion transition					
Diflufenican (AE F088657)	1.5 (LOQ)	7	91-106	100	5.2	5.2
	15	5	88-104	96	5.8	6.0
AE 0542291	1.5 (LOQ)	7	79-87	84	2.9	3.4
	15	5	79-81	80	0.8	1.0
AE B107137 (Diflufenican acid)	1.5 (LOQ)	7	81-93	86	4.0	4.7
	15	5	79-83	81	1.7	2.1
	Confirmation ion transition					
Diflufenican (AE F088657)	1.5 (LOQ)	7	96-104	99	3.0	3.0
	15	5	85-91	88	3.8	4.3
AE 0542291	1.5 (LOQ)	7	79-87	84	2.9	3.4
	15	5	80-83	81	1.1	1.3
AE B107137 (Diflufenican acid)	1.5 (LOQ)	7	88-101	95	4.6	4.8
	15	5	88-91	90	1.3	1.4
	Loamy Sand Sediment (North Carolina)					
	Quantitation ion transition					
Diflufenican (AE F088657)	1.5 (LOQ)	7	89-119	101	11.2	11.1
	15	5	89-103	96	5.9	6.2
AE 0542291	1.5 (LOQ)	7	84-95	90	3.7	4.1
	15	5	84-90	87	3.1	3.6
AE B107137 (Diflufenican acid)	1.5 (LOQ)	7	86-97	92	3.3	3.6
	15	5	86-93	90	2.9	3.2

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Confirmation ion transition					
Diflufenican (AE F088657)	1.5 (LOQ)	7	94-113	102	6.8	6.7
	15	5	95-107	101	5.3	5.3
AE 0542291	1.5 (LOQ)	7	80-90	84	3.5	4.2
	15	5	74-83	79	4.1	5.1
AE B107137 (Diflufenican acid)	1.5 (LOQ)	7	94-102	97	3.4	3.5
	15	5	101-103	101	0.9	0.9

Data (uncorrected recovery results; pp. 18-19; Appendix 1, pp. 33-38) were obtained from Tables 1-6, pp. 27-28 of MRID 51458736.

1 ECM 1 MRID 51458735 was a method only study report. No performance data was submitted in this study.

2 In the ECM 2 MRID 51458736 (Internal validation), silt loam soil (33% sand, 56% silt, 11% clay; pH 4.5 in $CaCl_2$ solution; pH 4.8 in aqueous solution; 3.6% organic matter; 2.1% organic carbon) collected from New York (Study MEDC0006; MRID 51458654), loamy sand soil (85% sand, 10% silt, 5% clay; pH 6.2 in $CaCl_2$ solution; pH 6.6 in aqueous solution; 0.96% organic matter; 0.56% organic carbon) collected from Georgia (Study MEDC0007; MRID 51458655), and loamy sand sediment (84.2% sand, 9.3% silt, 6.5% clay; pH 5.3 in $CaCl_2$ solution; pH 5.9 in aqueous solution; 2.2% organic matter; 1.3% organic carbon) collected from North Carolina (Study MEDC0014; MRID 51458651) were used in the study (USDA soil texture classification; pp. 14-15 of MRID 51458736). The soils were collected from two diflufenican terrestrial field dissipation studies. The sediment was collected from a diflufenican aerobic aquatic metabolism study. The soil characterization laboratory was not reported in the study. The soil texture of the silt loam soil was verified by the reviewer using USDA-NRCS technical support tools. The soil texture of the loamy sand soil and sediment was determined by the reviewer using USDA-NRCS technical support tools; in this study, those matrices were designated as sandy loam soil and sand sediment.

3 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 395.0→266.0 and *m/z* 395.0→246.0 for diflufenican (AE F088657), *m/z* 283.2→**265.9** and *m/z* 283.2→246.0 for AE 0542291, and *m/z* 284.3→246.0 and *m/z* 284.3→266.0 for AE B107137 [the bolded value was reviewer-determined based on the representative chromatograms for AE 0542291 (see Appendix 2, p. 56 of MRID 51458736 and Appendix 3, p. 19 of MRID 51458735) and the MS scan for AE 0542291 (see Appendix 4, p. 24 of MRID 51458735)].

Table 3. Independent Validation Method Recoveries for Diflufenican (AE F088657) and its degradates AE 0542291 (Phenoxynicotinamide) and AE B107137 (Diflufenican acid) in Soil[1,2]

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Soil					
	Quantitation ion transition					
Diflufenican (AE F088657)	1.5 (LOQ)	5	80.1-94.3	88.3	5.8	6.6
	15	5	90.1-97.8	94.0	3.1	3.3
AE 0542291	1.5 (LOQ)	5	101-110	105.4	4.3	4.1
	15	5	100-108	104.2	2.9	2.7
AE B107137 (Diflufenican acid)	1.5 (LOQ)	5	83.8-106	92.5	8.5	9.2
	15	5	89.4-101	95.2	4.5	4.7
	Confirmation ion transition					
Diflufenican (AE F088657)	1.5 (LOQ)	5	70.2-113	96.0	16.0	16.8
	15	5	87.3-106	95.9	8.5	8.9
AE 0542291	1.5 (LOQ)	5	93.4-104	97.8	4.2	4.3
	15	5	93.7-100	96.3	2.5	2.6
AE B107137 (Diflufenican acid)	1.5 (LOQ)	5	100-126	110	10.5	9.6
	15	5	97.2-101	98.1	1.9	1.9

Data (uncorrected recovery results; p. 18) were obtained from Table 1, p. 21 of MRID 51458737.

1 Soil (Soil Sample MEDC0008IA-S001) from the Iowa terrestrial field dissipation (TFD) study (MEDC0008; MRID 51458656) was provided by Bayer CropScience (the Sponsor) and used in the study (p. 14 of MRID 51458737). The soil characterization was not reported in the study. In TFD MRID 51458656, the Iowa soil sample was characterized as silty clay loam (0-45 cm, 60-105 cm), silty clay (45-60 cm), and clay loam (105-120 cm) using USDA soil texture classification (Table 6, p. 36 of MRID 51458656). These soil textures were verified by the reviewer using USDA-NRCS technical support tools. No sediment matrix was used in the ILV.

2 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 396→267.000 and *m/z* 396→247.000 for diflufenican (AE F088657), *m/z* 283.063→265.932 and *m/z* 283.063→245.995 for AE 0542291, and *m/z* 284.050→246.078 and *m/z* 284.050→265.889 for AE B107137. These ion transitions were similar to those of ECM 1 and ECM 2, with the ECM 1/ECM 2 corrected quantitation ion transition for AE 0542291.

III. Method Characteristics

The LOQ for diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) in soil/sediment was 1.5 ng/g in the ECM 1, ECM 2, and ILV (p. 6 of MRID 51458735; pp. 9-10, 20, 25 of MRID 51458736; pp. 6, 19 of MRID 51458737). In the ECM 2, the LOQ was defined as the lowest fortification level which yields a mean recovery between 70 and 120% with a relative standard deviation of ≤20%, as well as having the blank values below 30%. No justification for the LOQ was reported in ECM 1 and ILV. No LOQ calculations were reported in the ECM 1, ECM 2, or ILV. In the ECM 1, ECM 2, and ILV, the Limit of Detection (LOD) for diflufenican, AE 0542291, and AE B107137 was not reported; however, the Minimum Detection Limit (MDL) was calculated in the ECM 2 using the following equation (p. 22; Tables 7-8, p. 29 of MRID 51458736):

$$MDL = (SD \times t_{0.99})$$

Where, $t_{0.99}$ is the one-tailed t statistic for n -1 and SD is the standard deviation of the analyte recovery measurements at the target LOQ. For the soil MDL, n = 14 and $t_{0.99}$ is 2.650. For the sediment MDL, n = 7 and $t_{0.99}$ is 3.143.

This MDL calculation was in accordance with the EPA Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 (2016); however, it may be preferable to calculate the MDLs for each soil separately.

In ECM 2, the MDL for soil was determined to be 0.44 ng/g, 0.11 ng/g, and 0.13 ng/g for diflufenican, AE 0542291, and AE B107137, respectively. The MDL for sediment was determined to be 0.54 ng/g, 0.18 ng/g, and 0.17 ng/g for diflufenican, AE 0542291, and AE B107137, respectively.

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.

Table 4. Method Characteristics in Soil and Sediment

		Diflufenican (AE F088657)	AE 0542291 (Phenoxynicotinamide)	AE B107137 (Diflufenican acid)
Limit of Quantitation (LOQ)*	ECM 1[1]	1.5 ng/g		
	ECM 2[2]			
	ILV			
Limit of Detection (LOD)	ECM 1	**Not reported**		
	ECM 2	**Not reported**		
		MDL (calc) = 0.44 ng/g (soils) and 0.54 ng/g (sediment)	MDL (calc) = 0.11 ng/g (soils) and 0.18 ng/g (sediment)	MDL (calc) = 0.13 ng/g (soils) and 0.17 ng/g (sediment)
	ILV	**Not reported**		
Linearity (calibration curve r and concentration range)	ECM 1[3,4,5]	r = 0.9978 (Q)	r = 0.9987 (Q)	r = 0.9991 (Q)
	ECM 2[3,5,6]	r = 0.9976 (Q, LSS) r = 0.9953 (C, LSS)	r = 0.9978 (Q, LSS) r = 0.9974 (C, LSS)	r = 0.9971 (Q, LSS) r = 0.9975 (C, LSS)
	ILV[5]	r = 0.9998 (Q, Soil)	r = 0.9986 (Q, Soil)	r = 0.9995 (Q, Soil)
	Range	0.10-50.0 ng/mL		
Repeatable	ECM 1[1]	No performance data submitted. Method only.		
	ECM 2[7]	Yes at LOQ (1.5 ng/g) and 10×LOQ (15 ng/g) (two characterized soil matrices and one characterized sediment matrix)		
	ILV[8,9]	Yes at LOQ (1.5 ng/g) and 10×LOQ (15 ng/g) (one uncharacterized soil matrix; sediment not included)		
Reproducible	Soil	Yes for 1.5 ng/g (LLMV)* and 15 ng/g in soil matrices		
	Sediment[10]	**Could not be determined**. Only one set of performance data was submitted.		
Specific	ECM 1	Yes, no matrix interferences were observed, but **no** representative control chromatograms were provided (matrix undefined).		
	ECM 2	Yes, no matrix interferences were observed, but **only** representative chromatograms of the Georgia loamy sand matrix were provided (no sediment examples provided). Matrix interferences which interfered with LOQ peak attenuation and integration were observed in the confirmation ion of diflufenican.[11]		
	ILV	Yes, matrix interferences were <5% of the LOQ (based on peak area). Baseline noise was noted around the LOQ analyte peak.	Yes, matrix interferences were <5% of the LOQ (based on peak area). Baseline noise and contaminant (RT *ca.* 2.5 min.) were noted around the LOQ analyte peak.	Yes, matrix interferences were <5% of the LOQ (based on peak area). Baseline noise was noted around the LOQ analyte peak.
		Only representative chromatograms of the quantitation ion analysis were provided.[11]		

Data were obtained from p. 6 (LOQ/LOD); p. 9; Appendix 2, p. 17 (calibration curves); Appendix 3, pp. 18-23 (chromatograms) of MRID 51458735; pp. 9-10, 20, 22, 25; Tables 7-8, p. 29 (LOQ/LOD); Tables 1-6, pp. 27-28 (recovery results); Appendix 1, pp. 33-38; Appendix 3, pp. 58-59 (calibration curves); Appendix 2, pp. 39-57 (chromatograms) of MRID 51458736; pp. 6, 19 (LOQ/LOD); Table 1, p. 21 (recovery results); p. 15; Appendix B, Figures B-1 to B-3, pp. 30-32 (calibration curves); Appendix C, Figures C-1 to C-30, pp. 35-64 (chromatograms) of MRID 51458737; DER Excel Attachment. Q = quantitation ion transition; C = confirmation ion transition. LSS = Loamy sand soil (Georgia).

* 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 ECM 1 MRID 51458735 was a method only study report. No performance data was submitted in this study.
2 ECM 2 MRID 51458736 (Internal validation) contained the method summary and performance data.
3 ECM 1 and ECM 2 correlation coefficients (r) were reviewer-calculated based on r^2 values reported in the study report (Appendix 2, p. 17 of MRID 51458735; Appendix 1, pp. 33-38 of MRID 51458736; DER Excel Attachment).
4 Only one set of calibration curves were provided, the quantitation ion transition. The matrix used was not specified.
5 In the ECM 1, ECM 2, and ILV, solvent-based calibration standards were used for all analyses (p. 9 of MRID 51458735; p. 24 of MRID 51458736; p. 15 of MRID 51458737). Matrix effects were not studied in ECM 1, ECM 2, and ILV.
6 Only one set of calibration curves were provided, both ion transitions from the Georgia loamy sand soil analysis (Appendix 1, pp. 33-38; Appendix 3, pp. 58-59 of MRID 51458736).
7 In the ECM 2 MRID 51458736 (Internal validation), silt loam soil (33% sand, 56% silt, 11% clay; pH 4.5 in $CaCl_2$ solution; pH 4.8 in aqueous solution; 3.6% organic matter; 2.1% organic carbon) collected from New York (Study MEDC0006; MRID 51458654), loamy sand soil (85% sand, 10% silt, 5% clay; pH 6.2 in $CaCl_2$ solution; pH 6.6 in aqueous solution; 0.96% organic matter; 0.56% organic carbon) collected from Georgia (Study MEDC0007; MRID 51458655), and loamy sand sediment (84.2% sand, 9.3% silt, 6.5% clay; pH 5.3 in $CaCl_2$ solution; pH 5.9 in aqueous solution; 2.2% organic matter; 1.3% organic carbon) collected from North Carolina (Study MEDC0014; MRID 51458651) were used in the study (USDA soil texture classification; pp. 14-15 of MRID 51458736). The soils were collected from two diflufenican terrestrial field dissipation studies. The sediment was collected from a diflufenican aerobic aquatic metabolism study. The soil characterization laboratory was not reported in the study. The soil texture of the silt loam soil was verified by the reviewer using USDA-NRCS technical support tools. The soil texture of the loamy sand soil and sediment was determined by the reviewer using USDA-NRCS technical support tools; in this study, those matrices were designated as sandy loam soil and sand sediment.
8 In the ILV, soil (Soil Sample MEDC0008IA-S001) from the Iowa terrestrial field dissipation (TFD) study (MEDC0008; MRID 51458656) was provided by Bayer CropScience (the Sponsor) and used in the study (p. 14 of MRID 51458737). The soil characterization was not reported in the study. In TFD MRID 51458656, the Iowa soil sample was characterized as silty clay loam (0-45 cm, 60-105 cm), silty clay (45-60 cm), and clay loam (105-120 cm) using USDA soil texture classification (Table 6, p. 36 of MRID 51458656). These soil textures were verified by the reviewer using USDA-NRCS technical support tools. No sediment matrix was used in the ILV.
9 The ILV validated the method (Bayer Method No. DC-002-S18-01) for diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) for soil in the third trial as written with a significant modification of the microwave extraction procedure and insignificant modifications to the analytical parameters (pp. 14-17, 19; Appendix F, pp. 89-104). The first ILV trial failed due to the inadequate sensitivity of the LC/MS/MS instrument which was used by the ILV (Appendix F, p. 101). The second trial failed due to the fact that the ILV microwave (MARS 6-1 Microwave) did not maintain 70°C when the wattage was reduced from 400W to 110W in the 3- to 15-minute interval, as directed in the method (Bayer Method No. DC-002-S18-01; Appendix F, p. 99). The ILV modified the method by increasing the wattage for the 3- to 15-minute interval to maintain 70°C; however, the actual wattage for this interval was not reported in the ILV (p. 16; Appendix F, p. 90). The ILV modifications warranted an updated ECM since the increase of the wattage for the 3- to 15-minute interval was necessary to maintain 70°C and provide acceptable recoveries. Additionally, the ECM should be updated with precautions about the LC/MS/MS instrument model needed to provide adequate sensitivity, as well as corrected for typographical errors in the MS ion transitions for the quantitation ion transition of AE 0542291 and the confirmation ion transition of AE B107137-d_3.
10 Bayer Method No. DC-002-S18-01 for sediment was not validated by the ILV since no sediment matrix was included in the ILV validation.
11 Deviations in the confirmation ion analysis do not affect the specificity of the method since a confirmation method is not usually required when GC/MS and/or LC/MS is used as the primary method to generate study data.

IV. Method Deficiencies and Reviewer's Comments

1. CDM/CSS-Dynamac JV personnel received the following directive from the Environmental Fate and Effects Division regarding the preparation of this DER (email dated 09/17/2021):

   "The bigger issue is that MRID 51458735 should not be cited in the DER if it is redundant and less complete than MRID 51458736, which contains the validation results. There's no requirement that we review every MRID submitted to EPA. We should review the relevant analytical methods that have paired ECM & ILV reports, not every submitted ECM or ILV report."

   However, MRID 51458735 was included in this DER for the following reasons:

   1. ECM 2 MRID 51458736 (Internal validation) contained only a method summary, while ECM 1 MRID 51458735 contained the full method written in a step-wise manner (pp. 10-11 of MRID 51458735; pp. 15-16 of MRID 51458736). In OCSPP guideline 850.6100 states that "The ECM should be clearly written with complete analytical methods that describe the exact procedure, materials, and equipment in sufficient detail to be used by laboratory chemists to review the ECM and its associated ILV results".

      OCSPP guideline 850.6100 could not have been satisfied using only ECM 2 MRID 51458736 since the ECM method (Bayer Method No. DC-002-S18-01) was not completely reproduced in ECM 2 MRID 51458736. Method details such as extraction solvent volumes, microwave procedure, and calibration standard preparation were not included in ECM 2 MRID 51458736. These details were found in ECM 1 MRID 51458735.

   2. The typographical errors in the MS ion transitions (see Reviewer's Comment #9) existed in both the full method ECM 1 MRID 51458735, as well as the internal validation ECM 2 MRID 51458736. These are significant typographical errors, and it is important to note that the typographical error occurred in Bayer Method No. DC-002-S18-01, not the reproduction of the method by the internal validation report.

2. Since the reported method 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 (p. 6 of MRID 51458735; pp. 9-10, 20, 25 of MRID 51458736; pp. 6, 19 of MRID 51458737). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM 2, the LLMV was equivalent to the ECM reported method LOQ for Diflufenican (AE F088657) and its degradates AE 0542291 (Phenoxynicotinamide) and AE B107137 (Diflufenican acid) in the tested soil matrices (1.5 ng/g).

3. The ILV (MRID 51458737) was not conducted independently of the internal validation (ECM 2 MRID 51458736) since the ILV Sponsor Correspondence included the ECM 2 MRID 51458736 Study Author, Study Director, and Laboratory Personnel Tehane Ali (Bayer CropScience (pp. 1, 6 of MRID 51458736; Appendix F, pp. 92-93 of MRID 51458737). Communication with Tehane Ali involved the exchange of the ILV representative chromatograms and standard calibration curves to assess if the LC/MS/MS

instrument (6500) of the ILV delivered adequate sensitivity for the method.

The communications between the ILV study author (Penny Miner, Frontage Laboratories) and ILV Study Monitor (Derek Netzband, Bayer CropScience) were provided as the email correspondence (pp. 1, 10-11; Appendix F, pp. 89-104 of MRID 51458737). Other personnel involved in the email communications were Chung Lam (Bayer CropScience), Farhad Sayyarpour (Frontage Laboratories Vice President), Tehane Ali (Bayer CropScience; ECM 2 Study Author), Emily Lenz (Frontage Laboratories Associate Scientist I), Kevin Walsh (Frontage Laboratories Scientist), and Audry Miller (Bayer CropScience). Reported communications included: ILV test soil receipt, ILV study results after validation attempts, and discussion of ILV issues. Derek Netzband suggested the use of a Sciex 6500 LC/MS/MS instrument, in place of the Sciex-API 4000 which the ILV laboratory was using during the first failed validation (Appendix F, p. 101 of MRID 51458737). Penny Miner discovered the issue with the microwave temperature maintenance (Appendix F, pp. 89-90 of MRID 51458737).

Additionally, ILV Sponsor Correspondence involved a conference call; no meeting minutes from that conference call were included in the ILV study report (Appendix F, pp. 97-98 of MRID 51458737).

4. Only one soil was used in the ILV, and the soil was not characterized in the study. The ILV soil (Soil Sample MEDC0008IA-S001) from the Iowa terrestrial field dissipation (TFD) study (MEDC0008; MRID 51458656) was provided by Bayer CropScience (the Sponsor; p. 14 of MRID 51458737). The soil characterization was not reported in the study. In TFD MRID 51458656, the Iowa soil sample was characterized as silty clay loam (0-45 cm, 60-105 cm), silty clay (45-60 cm), and clay loam (105-120 cm) using USDA soil texture classification (Table 6, p. 36 of MRID 51458656). These soil textures were verified by the reviewer using USDA-NRCS technical support tools. No sediment matrix was included in the ILV.

5. Even though 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, the one ILV soil matrix (0-15 cm, silty clay loam, 31% clay, 3.1% organic matter; Table 6, p. 36 of MRID 51458656) did not cover the range of soils used in the terrestrial field dissipation studies since four diflufenican (AE F088657) terrestrial field dissipation studies were submitted: MRID 51458654 (New York; 0-15 cm, silt loam, 11% clay, 3.6% organic matter; Table 6, p. 36 of MRID 51458654); MRID 51458655 (Georgia; 0-15 cm, loamy sand, 5% clay, 0.96% organic matter; Table 6, p. 36 of MRID 51458655); MRID 51458656 (Iowa; see above for ILV soil matrix); and MRID 51458657 (Washington State; 0-15 cm, sand, 0% clay, 0.81% organic matter; Table 6, p. 36 of MRID 51458657).

Soils from diflufenican TFD MRID 51458654 (New York) and MRID 51458655 (Georgia) were used in the ECM (pp. 14-15 of MRID 51458736). The ECM sediment matrix was sourced from one of the diflufenican aerobic aquatic metabolism studies (Study MEDC0014; MRID 51458651). The soil texture of the Georgia loamy sand soil and North

Carolina loamy sand sediment was determined by the reviewer using USDA-NRCS technical support tools; in this study (MRID 51458736), those matrices were designated as sandy loam soil and sand sediment. The reviewer-determined soil textures agreed with those reported in the source studies: MRIDs 51458651 & 51458655.

While it could not be determined if the ILV was provided with the most difficult matrices with which to validate the method, the Soil Sample MEDC0008IA-S001) from the Iowa terrestrial field dissipation (TFD) study (MEDC0008; MRID 51458656) contained a high clay and organic matter content in the 0-15 cm sampling depth. 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.

MRID No.s for all terrestrial field dissipation and aerobic aquatic metabolism studies were reviewer-determined using the reported Bayer CropScience Study No.s.

6. The ECM method should be updated with the ILV modification to increase the microwave wattage for the 3- to 15-minute interval, if necessary, to maintain 70°C since this modification was necessary provide acceptable ILV recoveries. Additionally, the ECM should be updated with precautions about the LC/MS/MS instrument model needed to provide adequate sensitivity, as well as corrected for typographical errors in the MS ion transitions for the quantitation ion transition of AE 0542291 and the confirmation ion transition of AE B107137-d_3 (see Reviewer's Comment #9).

7. The ECM 2 only provided representative chromatograms and calibration curves for the Georgia loamy sand matrix (Appendices 1-3, pp. 33-59 of MRID 51458736). No representative chromatograms and calibration curves were provided for the silt loam soil or loamy sand sediment. Correlation coefficients (as r or r^2 values) were not reported for the silt loam soil or loamy sand sediment. The ECM 2 MRID 51458736 study report stated that "the detector response was linear…with correlation coefficients of >0.99 for all samples for all test items" (p. 19). However, the reviewer did not include this data in the review since it was unclear if "correlation coefficients" was referring to r or r^2 values. Supporting chromatograms and linearity data should be provided for all matrices which are tested in order to fully assess the robustness of the method.

8. The justification for selection of diflufenican (AE F088657), and degradates diflufenican-amide (AE 0542291; DFF-amide) and diflufenican-acid (AE B107137; DFF-acid) for soil, sediment, and water methods was described in an accompanying supplemental MRID 51458658 (Shrestha, S. 2021. Justification for Selection of Analytes for Soil, Water Methods and Terrestrial Field Dissipation Studies. Bayer CropScience Report No. US0819. Bayer CropScience LP, St. Louis, Missouri). Residue definition for environmental chemistry methods was determined by assessing the maximum occurrence of each degradate that occurred above 10% of applied in environmental fate laboratory studies, including aerobic soil metabolism, aerobic and anaerobic aquatic metabolism, and soil and aqueous photolysis. DFF-amide was only detected at >10% of the applied in aerobic soil metabolism studies. DFF-acid was detected in at >10% of the applied in aerobic soil metabolism and aerobic and anaerobic aquatic metabolism studies. No major degradates were detected in soil and aqueous photolysis studies. The toxicological significance of any diflufenican degradate was

not reported.

9. The reviewer noted the following significant typographical errors in ECM 1 MRID 51458735 and ECM 2 MRID 51458736: MS ion transitions for the quantitation ion transition of AE 0542291 (reported as *m/z* 283.2→**255.9** or *m/z* 283.243→**255.929**) and the confirmation ion transition of AE B107137-d_3 (reported as *m/z* **284.2**→268.0 or *m/z* **284.244**→268; p. 13 of MRID 51458735; p. 17 of MRID 51458736). The correct MS ion transitions for the quantitation ion transition of AE 0542291 and the confirmation ion transition of AE B107137-d_3 were *m/z* 283.2→**265.9** and *m/z* **287.2**→268.0, respectively. The correct MS ion transitions were reported in the DER and reviewer-determined based on the representative chromatograms for AE 0542291 (see Appendix 2, p. 56 of MRID 51458736 and Appendix 3, p. 19 of MRID 51458735), the MS scan for AE 0542291 (see Appendix 4, p. 24 of MRID 51458735), and representative chromatograms for AE B107137-d_3 (see Appendix 2, p. 56 of MRID 51458736 and Appendix 3, p. 23 of MRID 51458735).

   The MS ion transitions reported in the ILV matched the were similar to those of ECM 1 and ECM 2, with the ECM 1/ECM 2 corrected quantitation ion transition for AE 0542291 and corrected confirmation ion transition of AE B107137-d_3 (p. 17 of MRID 51458737). No comment was reported in the ILV regarding the differences in the MS ion transitions from those reported in the method.

10. The stabilities of the final sample extract and calibration solutions were studied in the ECM 2 (pp. 22-24; Tables 9-10, pp. 30-31 of MRID 51458736). The final extracts of the Georgia soils were determined to be stable for up to 7 days at 3.5°C storage. The calibration standards were determined to be stable for up to 35 days with frozen storage (≤18°C).

11. The determinations of the LOQ and LOD in the ECM 1, ECM 2, and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (diflufenican (AE F088657) and its degradates AE 0542291 (phenoxynicotinamide) and AE B107137 (diflufenican acid) in soil was 1.5 ng/g in the ECM 1, ECM 2, and ILV (p. 6 of MRID 51458735; pp. 9-10, 20, 25 of MRID 51458736; pp. 6, 19 of MRID 51458737). In the ECM 2, the LOQ was defined as the lowest fortification level which yields a mean recovery between 70 and 120% with a relative standard deviation of ≤20%, as well as having the blank values below 30%. No justification for the LOQ was reported in ECM 1 and ILV. No LOQ calculations were reported in the ECM 1, ECM 2, or ILV. In the ECM 1, ECM 2, and ILV, the LOD for diflufenican, AE 0542291, and AE B107137 was not reported; however, the Minimum Detection Limit (MDL) was calculated in the ECM 2 using the following equation: MDL = (SD × $t_{0.99}$), where, $t_{0.99}$ is the one-tailed t statistic for n -1 and SD is the standard deviation of the analyte recovery measurements at the target LOQ. For the soil MDL, n = 14 and $t_{0.99}$ is 2.650. For the sediment MDL, n = 7 and $t_{0.99}$ is 3.143 (p. 22; Tables 7-8, p. 29 of MRID 51458736). This MDL calculation was in accordance with the EPA Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 (2016); however, it may be preferable to calculate the MDLs for each soil separately.

    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 the lowest level of method validation (LLMV) rather than an LOQ.

12. The total time required to complete one sample set was reported as one 8-hour day in the ILV (p. 16 of MRID 51458737). The total time required to complete one set of fifteen samples was reported in the ECM as two to three hours with LC/MS/MS performed overnight (p. 24 of MRID 51458736).

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.

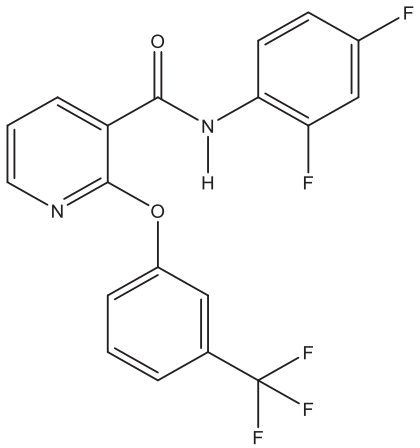
USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 344-347, and Revision 2; 2015 and 2016.

Attachment 1: Chemical Names and Structures

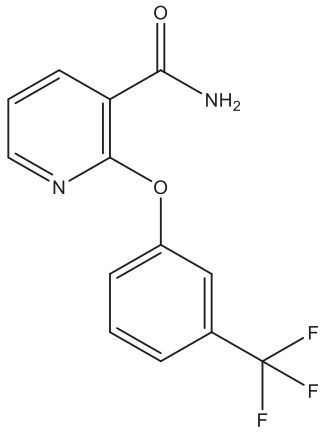
Diflufenican (AE F088657; M&B 38544)

IUPAC Name: 2',4'-Difluoro-2-[(α,α,α-trifluoro-m-tolyl)oxy]nicotinanilide
CAS Name: N-(2,4-difluorophenyl)-2-[3-(trifluoromethyl)phenoxy]- 3-pyridinecarboxamide
CAS Number: 83164-33-4
SMILES String: n1c(Oc2cc(C(F)(F)F)ccc2)c(C(=O)Nc3c(F)cc(F)cc3)ccc1



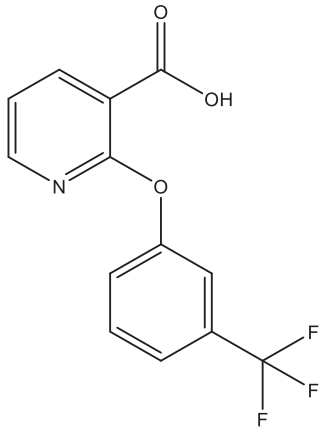
AE 0542291 (M&B 43625; Diflufenican-amide; DFF-amide; Phenoxynicotinamide)

IUPAC Name: 2-(3-Trifluoromethyl)phenoxynicotinamide
CAS Name: 2-(3-Trifluoromethyl)phenoxy-3-pyridinecarboxamide
CAS Number: Not reported
SMILES String: NC(C1=C(OC2=CC=CC(C(F)(F)F)=C2)N=CC=C1)=O



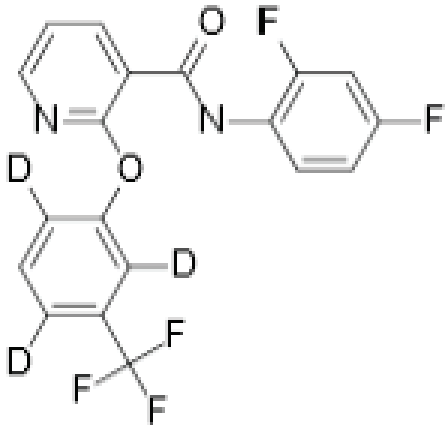
AE B107137 (Diflufenican-acid; M&B 38181; DFF-acid)

IUPAC Name: 2-[3-(Trifluoromethyl)phenoxy]nicotinic acid
CAS Name: 2-(3-Trifluoromethyl)phenoxy-3-pyridinecarboxylic acid
CAS Number: 36701-89-0
SMILES String: OC(C1=CC=CN=C1OC2=CC=CC(C(F)(F)F)=C2)=O



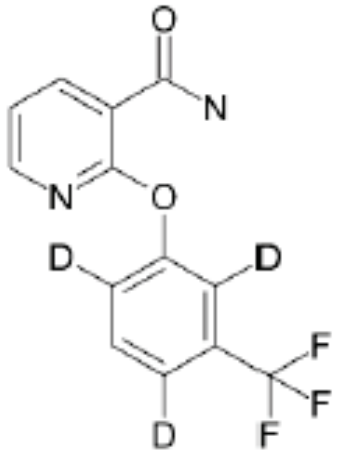
Diflufenican-d_3

IUPAC Name: Not reported
CAS Name: Not reported
CAS Number: Not reported
SMILES String: Not found



AE 0542291-d_3

IUPAC Name: Not reported
CAS Name: Not reported
CAS Number: Not reported
SMILES String: Not found



Diflufenican acid-d_3 (AE B107137- d_3)

IUPAC Name: Not reported
CAS Name: Not reported
CAS Number: Not reported
SMILES String: Not found