Analytical method for fluroxypyr-MHE and and its metabolites, fluroxypyr acid fluroxypyr-DCP and fluroxypyr-MP, in soil

Reports:	ECM: EPA MRID No.: 50290801 Deziderio, L.Ap.G. 2017. Method Validation for the Determination of Residues of Fluroxypyr-MHE, Fluroxypyr acid, Fluroxypyr-DCP and Fluroxypyr-MP in soil and sediment by Liquid Chromatography with Tandem Mass Spectrometry. Dow AgroSciences Study No.: 160535. Report prepared by Brazil Regulatory Sciences Laboratory, Dow AgroSciences Sementes e Biotecnologia Brasil Ltda, Cravinhos, São Paulo, Brazil, and sponsored, and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 244 pages (including a blank page between pages 176 and 177). Final report issued February 20, 2017. ILV: EPA MRID No. 50290802. Pugh, K. (neé Wiltshire). 2017. Independent Laboratory Validation of an Analytical Method for the Determination of Fluroxypyr and its Metabolites in Soil using LC-MS/MS. DAS PCTR No.: 10002405-000-40402-0001 and Study ID: 160534. Study ID: CEMS-7909. Report prepared by CEM Analytical Services Ltd. (CEMAS), Berkshire, United Kingdom, sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 110 pages. Final report issued April 28, 2017.
Document No.:	MRIDs 50290801 & 50290802
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with NIT-DICLA-035 (INMETRO) and OECD Good Laboratory Practice (GLP) standards (pp. 3-4 of MRID 50290801). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-5). A statement of the authenticity of the study report was included with the quality assurance statement (p. 5). ILV: The study was conducted in accordance with OECD and UK GLP standards (p. 3; Appendix 3, p. 109 of MRID 50290802). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4; Appendix 3, p. 109). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).
Classification:	This analytical method is classified as **UNACCEPTABLE**. In the ILV, no samples were prepared at 10×LOQ; in the ECM, no samples were prepared at 10×LOQ for the analysis of fluroxypyr-MHE and fluroxypyr acid. The specificity of the method was not supported by ECM and ILV representative chromatograms of fluroxypyr acid and fluroxypyr-MP. The specificity of the method was also not supported by ECM representative chromatograms of fluroxypyr-MHE.
PC Code:	128968

EFED Final Reviewer:	Rochelle F. H. Bohaty, PhD, Senior Chemist	Signature: ROCHELLE BOHATY Digitally signed by ROCHELLE BOHATY Date: 2019.09.12 10:41:06 -04'00' Date: September 12, 2019
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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, Dow AgroSciences Study No. 160535, is designed for the quantitative determination of fluroxypyr-MHE and its metabolites, fluroxypyr acid, fluroxypyr-DCP, and fluroxypyr-MP, in soil using LC/MS/MS. The method was established with a LOQ of 0.0004 µg/g[1] for fluroxypyr-MHE and fluroxypyr acid and a LOQ of 0.01 µg/g for fluroxypyr-DCP and fluroxypyr-MP. The respective LOQs are equal to or less than the lowest toxicological level of concern[2] in soil for all four analytes. The ECM performed the method using characterized loam soil, silt loam soil, sandy loam sediment, and sand sediment, as well as an unclassified soil; the ILV validated the method using one characterized loam soil. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and that the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. Analytes were identified using two ion transitions. For fluroxypyr-MHE and fluroxypyr acid, no samples were prepared at 10×LOQ in the ECM and ILV; for fluroxypyr-DCP and fluroxypyr-MP, no samples were prepared at 10×LOQ in the ILV. In the ILV, the method for fluroxypyr-DCP and fluroxypyr-MP was validated in the first trial with insignificant modifications to the analytical instrumentation. The method for fluroxypyr-MHE and fluroxypyr acid was validated in the second trial with modifications to the analytical parameters and instrumentation. The first trial was unsuccessful due to interfering peaks and poor chromatography. All ECM and ILV linearity data was acceptable; all ECM and ILV precision and accuracy data was adequate. The specificity of the method was not supported by ECM and ILV representative chromatograms of fluroxypyr acid and fluroxypyr-MP due to significant baseline noise which interfered with peak attenuation and integration at the LOQ

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[1] LOQ = 0.05 µg/kg = 0.00005 mg/kg; LOD = 0.02 µg/kg
0.00005 mg/kg (s) = [x] kg/ha x 10^6 mg/kg / [0.15 m (depth) x 10^4 m^2/ha x 1.5 x 10^3 kg(s)/m^3(density)]
0.00005 mg/kg(s) = [x] kg/ha x 10^6 mg/kg / 2.25 x 10^6 kg (s)/ha
= 1.1 x 10^{-5} kg/ha = LOQ = 8.0 x 10^{-4} lb/A

[2] Vegetative vigor dicot (cotton) IC_{25} = 0.0012 lb a.i./acre (0.00083 lb a.e./A) (MRID 44080335)

in all matrices; significant baseline noise was also observed in the ECM representative chromatograms of fluroxypyr-MHE.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide[1]	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Fluroxypyr-MHE	50290801	50290802		Soil[2,3]	20/02/2017	Dow AgroSciences LLC	LC/MS/MS	0.0004 µg/g
Fluroxypyr acid								
Fluroxypyr-DCP								0.01 µg/g
Fluroxypyr-MP								

1 Fluroxypyr MHE = Fluroxypyr 1-methylheptyl ester; Fluroxypyr-DCP = Fluroxypyr dichloropyridinol; and Fluroxypyr-MP = Fluroxypyr methoxypyridine.

2 In the ECM, three soil and two sediment matrices were used. Loam soil (M964; 49% sand 40% silt 11% clay, pH 7.2, 0.7% organic carbon) obtained from Hanford, (location nor further specified), silt loam soil (M961; 35% sand 57% silt 8% clay, pH 5.8, 3.9% organic carbon) obtained from Brierlow, (location nor further specified), unclassified soil (M963; uncharacterized) from an unidentified location, sandy loam sediment (M971; 69% sand 18% silt 13% clay, pH 5.9, 2.9% organic carbon) obtained from Emperor Lake (location nor further specified), and sand sediment (M972; 97% sand 3% silt 0% clay, pH 7.7, 1.0% organic carbon) obtained from River Dove, (location nor further specified), were used in this study (USDA soil texture classification; p. 20; Figures 141-152, pp. 220-231 of MRID 50290801). The pH values were based on 1:1 soil:water ratio results.

3 In the ILV, the Loam soil (M963; CSR-7909-001; 48% sand 40% silt 12% clay, pH 5.9, 6.0% organic carbon) obtained from the sponsor was used in this study (USDA soil texture classification; p. 14; Figure 5, p. 45 of MRID 50290802). The pH value was based on 1:1 soil:water ratio result. The soil source was not further specified.

I. Principle of the Method

Samples (10.0 ± 0.05 g) of soil or sediment were weighed into 250-mL polypropylene tubes and fortified, as necessary, at the LOD, LOQ, 10×LOQ or 100×LOQ, and 2500×LOQ (p. 18; Appendix I, pp. 233, 241-243 of MRID 50290801). The samples were extracted with 10 mL of acetonitrile:0.5N hydrochloric acid (90:10, v:v), 2 g NaCl, and 5 g $MgSO_4$ by shaking for 60 minutes at *ca*. 180 excursions/minute. After centrifugation for 5 minutes at 2500 rpm, the supernatant (solution A) was removed.

For fluroxypyr-MHE and fluroxypyr acid clean-up, 2 mL of solution A was transferred to a 15-mL falcon tube and mixed with 8 mL of 0.5 N hydrochloric acid via pulse vortex mixing for *ca*. 5 seconds (p. 18; Appendix I, pp. 233, 241-243 of MRID 50290801). A Strata-X solid phase extraction (SPE) cartridge (3 µm Polymeric Reversed Phase; 60 mg, 3 mL) was prepared by conditioning with 3 mL of acetonitrile followed by 3 mL of 0.5 N hydrochloric acid. After the cartridge was dried under vacuum for 5-10 seconds, the extract mixture was applied to the column and pulled through at *ca*. 1 mL/minute. The sample vial was rinsed with 3 mL of acetonitrile:0.5N hydrochloric acid (90:10, v:v) and transferred to the SPE column. All eluates were discarded. The analytes were eluted with 2 mL of acetonitrile at *ca*. 1 mL/minute. A 0.6 mL aliquot of the eluate was transferred to a 2-mL vial and mixed with 0.4 mL of water with 0.2% acetic acid, then analyzed by LC/MS/MS.

For fluroxypyr-DCP and fluroxypyr-MP clean-up, 1 mL of solution A was transferred to a 15-mL falcon tube and mixed with 7 mL of 0.5 N hydrochloric acid via pulse vortex mixing for *ca*. 5 seconds (p. 18; Appendix I, pp. 233, 241-243 of MRID 50290801). A Strata-X solid phase extraction (SPE) cartridge (3 µm Polymeric Reversed Phase; 60 mg, 3 mL) was prepared by conditioning with 3 mL of acetonitrile followed by 3 mL of 0.5 N hydrochloric acid. After the cartridge was dried under vacuum for 10 seconds, the extract mixture was applied to the column and pulled through at *ca*. 1 mL/minute. The sample vial was rinsed with 3 mL of acetonitrile:0.5N hydrochloric acid (90:10, v:v) and transferred to the SPE column. All eluates were discarded. The analytes were eluted with 2 mL of acetonitrile at *ca*. 1 mL/minute. A 0.6 mL aliquot of the eluate was transferred to a 2-mL vial and mixed with 0.6 mL of water with 0.2% acetic acid, then analyzed by LC/MS/MS.

The ECM noted that evaporation procedure must be avoided (Appendix I, p. 243 of MRID 50290801).

Samples were analyzed for analytes by Agilent 1290 Infinity HPLC (Eclipse Plus Phenyl Hexyl column, 3.0 mm x 50 mm, 1.8 µm column; column temperature 50°C) using a mobile phase of (A) water with 0.1% acetic acid and (B) methanol with 0.1% acetic acid [for fluroxypyr-MHE and fluroxypyr acid: percent A:B at 0:0.50 min. 90:10, 2.00-4.00 min. 3:97, 4.10-6.00 min. 90:10; for fluroxypyr-MP and fluroxypyr-DCP: percent A:B at 0:0.50 min. 90:10, 2.50-3.50 min. 3:97, 3.60-6.00 min. 90:10] with AB SCIEX API 6500 MS using MS/MS-ESI (electrospray ionization; temperature 500°C) detection in positive polarity and multiple reaction monitoring (MRM; Appendix I, pp. 239-240 of MRID 50290801). Injection volume was 10 µL. Analytes were identified using two ion transitions (quantitative and confirmatory, respectively): *m/z* 367.025→255.000 and *m/z* 367.025→209.000 for fluroxypyr-MHE; *m/z* 254.910→181.000 and *m/z* 254.910→209.000 for fluroxypyr acid; *m/z* 210.917→196.000 and *m/z* 210.917→112.900 for fluroxypyr-MP; and *m/z* 196.910→152.000 and *m/z* 196.910→144.000 for fluroxypyr-DCP. Expected retention times were *ca*. 3.12, 2.61, 2.86, and 2.46 minutes for fluroxypyr-MHE, fluroxypyr acid, fluroxypyr-MP and fluroxypyr-DCP, respectively (Figures 49-68, pp. 128-147).

In the ILV, the ECM was performed as written, except for insignificant modifications to the analytical instrumentation and method during the analysis of fluroxypyr-MHE and fluroxypyr acid (flow rate increased from 500 to 700 µL/min., injection volume decreased to 5 µL, and mobile phase gradient times were adjusted; pp. 12, 22; Appendix 1, Appendix 1, pp. 85-102; Appendix 2, pp. 104-108 of MRID 50290802). An Agilent 1290 HPLC (Agilent Poroshell 120, Phenyl Hexyl column 50 mm x 2.1 mm, 2.7 µm; column temperature 50°C) coupled with AB SCIEX QTRAP 5500 Q-Trap MS using MS/MS-ESI was used for all analyses. The mobile phase components were the same as the ECM; the gradient program of fluroxypyr-MHE and fluroxypyr acid was adjusted [percent A:B at 0:0.50 min. 90:10, 3.00-4.00 min. 3:97, 4.10-7.00 min. 90:10]. Analytes were identified using two ion transitions (quantitative and confirmatory, respectively): *m/z* 367.1→255.1 and *m/z* 367.1→209.0 for fluroxypyr-MHE; *m/z* 255.1→181.0 and *m/z* 255.1→209.0 for fluroxypyr acid; *m/z* 210.9→196.0 and *m/z* 210.9→112.9 for fluroxypyr-MP; and *m/z* 196.9→152.0 and *m/z* 196.9→144.0 for fluroxypyr-DCP. Expected retention times were *ca*. 3.48, 2.51, 3.97, and 3.31 minutes for fluroxypyr-MHE, fluroxypyr acid, fluroxypyr-MP and fluroxypyr-DCP, respectively. No other modifications to the ECM were reported.

The Limit of Quantification (LOQ) in the ECM and ILV was reported as 0.0004 µg/g for fluroxypyr-MHE and fluroxypyr acid and 0.01 µg/g for fluroxypyr-DCP and fluroxypyr-MP (pp.

18, 23-24; Tables 26-27, pp. 64-65 of MRID 50290801; pp. 12, 20 of MRID 50290802). The Limit of Detection (LOD) in the ECM and ILV was reported as 0.00012 µg/g for fluroxypyr-MHE and fluroxypyr acid and 0.003 µg/g for fluroxypyr-DCP and fluroxypyr-MP. In the ECM, the LOQ and LOD were calculated as 0.00004-0.00048 µg/g and 0.00001-0.00014 µg/g, respectively, for fluroxypyr-MHE and fluroxypyr acid and 0.00135-0.00615 µg/g and 0.00041-0.00185 µg/g, respectively, for fluroxypyr-DCP and fluroxypyr-MP.

II. Recovery Findings

ECM (MRID 50290801): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of fluroxypyr-MHE and fluroxypyr acid in three soil and two sediment matrices at the fortification levels of 0.0004 µg/g (LOQ), 0.04 µg/g (100×LOQ), and 1.0 µg/g (2500×LOQ), except for the quantitation ion analysis of fluroxypyr acid in the unclassified soil M963 at the LOQ (RSD 32%; n = 6 for all analyses; Tables 11-25, pp. 32-63; DER Attachment 2). For fluroxypyr-MHE and fluroxypyr acid, no samples were prepared at 10×LOQ. Mean recoveries and RSDs were within guideline requirements for analysis of fluroxypyr-DCP and fluroxypyr-MP in three soil and two sediment matrices at the fortification levels of 0.01 µg/g (LOQ), 0.1 µg/g (10×LOQ), and 1.0 µg/g (100×LOQ; n = 6 for all analyses). All analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method. Recoveries for the LOD samples (0.00012 µg/g for fluroxypyr-MHE and fluroxypyr acid; 0.003 µg/g for fluroxypyr-DCP and fluroxypyr-MP) were reviewer-calculated; mean recovery, s.d. and RSDs could not be determined since n = 2. The LOD recoveries were 1-83% for fluroxypyr-MHE, 3-333% for fluroxypyr acid, 107-180% for fluroxypyr-DCP, and 67-130% for fluroxypyr-MP (matrices/ions combined); generally, the LOD recoveries of fluroxypyr-MHE and fluroxypyr acid were more variable and more outside the acceptable recovery range than the LOD recoveries of fluroxypyr-DCP and fluroxypyr-MP. Loam soil (M964; 49% sand 40% silt 11% clay, pH 7.2, 0.7% organic carbon) obtained from Hanford, (location nor further specified), silt loam soil (M961; 35% sand 57% silt 8% clay, pH 5.8, 3.9% organic carbon) obtained from Brierlow, (location not further specified), unclassified soil (M963; uncharacterized) from an unidentified location, sandy loam sediment (M971; 69% sand 18% silt 13% clay, pH 5.9, 2.9% organic carbon) obtained from Emperor Lake (location not further specified), and sand sediment (M972; 97% sand 3% silt 0% clay, pH 7.7, 1.0% organic carbon) obtained from River Dove, (location not further specified), were used in this study (USDA soil texture classification; p. 20; Figures 141-152, pp. 220-231). The pH values were based on 1:1 soil:water ratio results.

ILV (MRID 50290802): Mean recoveries and RSDs were within guideline requirements for analysis of fluroxypyr-MHE and fluroxypyr acid in one soil matrix at the fortification levels of 0.0004 µg/g (LOQ) and 1.0 µg/g (2500×LOQ), except for the confirmation ion analysis of fluroxypyr acid at the LOQ (mean 127%; n = 5 for all analyses; Tables 14-29, pp. 30-39; DER Attachment 2). A confirmatory method is not usually required when LC/MS and GC/MS is the primary method. Mean recoveries and RSDs were within guideline requirements for analysis of fluroxypyr-DCP and fluroxypyr-MP in one soil matrix at the fortification levels of 0.01 µg/g (LOQ) and 1.0 µg/g (100×LOQ; n = 5 for all analyses). No samples were prepared at 10×LOQ All analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were fairly comparable. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method. Recoveries for the LOD samples (0.00012 µg/g for fluroxypyr-MHE and fluroxypyr acid; 0.003 µg/g for fluroxypyr-DCP and fluroxypyr-MP) were reviewer-calculated; mean recovery, s.d. and RSDs could not be determined since n = 1. The LOD recoveries were 50-92% for fluroxypyr-MHE, 125-150% for fluroxypyr acid, 63-76% for fluroxypyr-DCP, and 65-89% for fluroxypyr-MP (ions combined). Loam soil (M963; CSR-7909-001; 48% sand 40% silt 12% clay, pH 5.9, 6.0% organic carbon) obtained from the sponsor was used in this study (USDA soil texture classification; p. 14; Figure 5, p. 45). The pH value was based on 1:1 soil:water ratio result. The soil source was not further specified. The method for fluroxypyr-DCP and fluroxypyr-MP was validated in the first trial with insignificant modifications to the analytical instrumentation (pp. 12, 20, 22; Appendix 1, Appendix 1, pp. 85-102; Appendix 2, pp. 104-108). The method for fluroxypyr-MHE and fluroxypyr acid was validated in the second trial with modifications to the analytical parameters and instrumentation. The first trial was unsuccessful due to interfering peaks and poor chromatography.

Table 2. Initial Validation Method Recoveries for Fluroxypyr-MHE and Its Metabolites, Fluroxypyr acid, Fluroxypyr-DCP, and Fluroxypyr-MP, in Soil[1,2,3]

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Loam Soil (M964)					
	Quantitation Ion Transition					
Fluroxypyr-MHE	0.00012 (LOD)	**2**	**1**, **25**	--	--	--
	0.0004 (LOQ)	6	73-76	75	1	2
	0.04	6	71-77	74	2	3
	1	6	70-74	72	2	2
Fluroxypyr acid	0.00012 (LOD)	**2**	**25**, **58**	--	--	--
	0.0004 (LOQ)	6	98-101	100	1	1
	0.04	6	103-115	110	5	5
	1	6	95-111	103	6	6
Fluroxypyr-DCP	0.003 (LOD)	**2**	**133**, **180**	--	--	--
	0.01 (LOQ)	6	108-118	114	5	4
	0.1	6	97-108	102	4	4
	1	6	95-100	98	2	2
Fluroxypyr-MP	0.003 (LOD)	**2**	100, 107	--	--	--
	0.01 (LOQ)	6	80-88	84	3	4
	0.1	6	87-94	90	2	3
	1	6	83-91	86	3	4

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Confirmatory Ion Transition					
Fluroxypyr-MHE	0.00012 (LOD)	**2**	**8**	--	--	--
	0.0004 (LOQ)	6	76-79	77	1	1
	0.04	6	73-78	76	2	2
	1	6	71-74	72	1	2
Fluroxypyr acid	0.00012 (LOD)	**2**	**33**, **50**	--	--	--
	0.0004 (LOQ)	6	93-103	99	4	4
	0.04	6	104-116	110	6	5
	1	6	97-110	103	5	5
Fluroxypyr-DCP	0.003 (LOD)	**2**	**130**, **183**	--	--	--
	0.01 (LOQ)	6	107-119	113	6	5
	0.1	6	98-109	103	4	4
	1	6	94-100	98	2	2
Fluroxypyr-MP	0.003 (LOD)	**2**	80, 90	--	--	--
	0.01 (LOQ)	6	90-99	94	3	3
	0.1	6	87-94	91	3	3
	1	6	82-90	85	3	4
	Silt Loam Soil (M961)					
	Quantitation Ion Transition					
Fluroxypyr-MHE	0.00012 (LOD)	**2**	**33**, 83	--	--	--
	0.0004 (LOQ)	6	82-100	89	7	8
	0.04	6	81-89	86	4	4
	1	6	70-82	76	3	4
Fluroxypyr acid	0.00012 (LOD)	**2**	83	--	--	--
	0.0004 (LOQ)	6	74-100	90	9	10
	0.04	6	98-107	102	3	3
	1	6	89-97	92	3	4
Fluroxypyr-DCP	0.003 (LOD)	**2**	**123**, **170**	--	--	--
	0.01 (LOQ)	6	106-117	112	4	4
	0.1	6	98-105	102	2	2
	1	6	95-98	97	1	1
Fluroxypyr-MP	0.003 (LOD)	**2**	103, **130**	--	--	--
	0.01 (LOQ)	6	96-107	101	4	4
	0.1	6	88-102	97	5	5
	1	6	88-94	91	2	2
	Confirmatory Ion Transition					
Fluroxypyr-MHE	0.00012 (LOD)	**2**	83	--	--	--
	0.0004 (LOQ)	6	73-80	78	2	3
	0.04	6	80-89	85	4	4
	1	6	71-83	77	6	8
Fluroxypyr acid	0.00012 (LOD)	**2**	**167**	--	--	--
	0.0004 (LOQ)	6	80-99	88	7	8
	0.04	6	101-110	105	3	3
	1	6	93-96	95	1	1
Fluroxypyr-DCP	0.003 (LOD)	**2**	**127**, **173**	--	--	--
	0.01 (LOQ)	6	108-117	114	4	4

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.1	6	101-106	104	2	2
	1	6	94-102	97	3	3
Fluroxypyr-MP	0.003 (LOD)	**2**	97, 107	--	--	--
	0.01 (LOQ)	6	97-102	99	2	2
	0.1	6	84-101	95	6	7
	1	6	88-93	91	2	2
	Unclassified Soil (M963)					
	Quantitation Ion Transition					
Fluroxypyr-MHE	0.00012 (LOD)	**2**	**42**, 83	--	--	--
	0.0004 (LOQ)	6	77-99	84	8	9
	0.04	6	74-108	92	13	14
	1	6	72-82	76	3	4
Fluroxypyr acid	0.00012 (LOD)	**2**	**8**, **25**	--	--	--
	0.0004 (LOQ)	6[4]	72-151	93	30	**32**
	0.04	6	91-114	104	10	9
	1	6	88-94	91	2	2
Fluroxypyr-DCP	0.003 (LOD)	**2**	107, **137**	--	--	--
	0.01 (LOQ)	6	101-118	110	6	6
	0.1	6	95-109	103	6	6
	1	6	89-99	95	4	4
Fluroxypyr-MP	0.003 (LOD)	**2**	87	--	--	--
	0.01 (LOQ)	6	84-96	90	6	7
	0.1	6	91-112	101	9	9
	1	6	88-101	94	6	6
	Confirmatory Ion Transition					
Fluroxypyr-MHE	0.00012 (LOD)	**2**	**25**, 83	--	--	--
	0.0004 (LOQ)	6	75-99	84	10	12
	0.04	6	74-108	91	14	15
	1	6	70-85	76	5	7
Fluroxypyr acid	0.00012 (LOD)	**2**	**167**, **333**	--	--	--
	0.0004 (LOQ)	6	80-114	96	12	13
	0.04	6	92-116	105	10	9
	1	6	87-99	93	5	5
Fluroxypyr-DCP	0.003 (LOD)	**2**	120, **137**	--	--	--
	0.01 (LOQ)	6	101-111	108	4	4
	0.1	6	95-116	103	8	7
	1	6	93-101	96	3	3
Fluroxypyr-MP	0.003 (LOD)	**2**	**67**, 113	--	--	--
	0.01 (LOQ)	6	96-104	101	3	3
	0.1	6	87-110	101	9	9
	1	6	89-102	94	4	5

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Sandy Loam Sediment (M971)					
	Quantitation Ion Transition					
Fluroxypyr-MHE	0.00012 (LOD)	2	2, 33	--	--	--
	0.0004 (LOQ)	6	73-82	77	4	5
	0.04	6	76-91	82	6	7
	1	6	79-90	85	4	5
Fluroxypyr acid	0.00012 (LOD)	2	3, 17	--	--	--
	0.0004 (LOQ)	6	81-89	84	3	3
	0.04	6	88-92	90	2	2
	1	6	88-93	90	2	2
Fluroxypyr-DCP	0.003 (LOD)	2	160, 163	--	--	--
	0.01 (LOQ)	6[4]	114-122	118	3	2
	0.1	6	106-109	107	1	1
	1	6	102-106	104	2	2
Fluroxypyr-MP	0.003 (LOD)	2	90, 107	--	--	--
	0.01 (LOQ)	6	92-96	94	1	2
	0.1	6	96-102	98	2	2
	1	6	91-100	96	3	3
	Confirmatory Ion Transition					
Fluroxypyr-MHE	0.00012 (LOD)	2	42, 50	--	--	--
	0.0004 (LOQ)	6	72-85	80	5	6
	0.04	6	78-90	82	4	5
	1	6	79-90	86	4	5
Fluroxypyr acid	0.00012 (LOD)	2	8, 33	--	--	--
	0.0004 (LOQ)	6	80-85	82	2	2
	0.04	6	90-94	92	2	2
	1	6	89-95	92	2	2
Fluroxypyr-DCP	0.003 (LOD)	2	163, 167	--	--	--
	0.01 (LOQ)	6[4]	113-125	118	4	3
	0.1	6	106-110	108	2	1
	1	6	103-107	105	2	2
Fluroxypyr-MP	0.003 (LOD)	2	110, 113	--	--	--
	0.01 (LOQ)	6	92-100	96	3	3
	0.1	6	92-99	97	2	3
	1	6	94-97	95	1	1
	Sand Sediment (M972)					
	Quantitation Ion Transition					
Fluroxypyr-MHE	0.00012 (LOD)	2	8, 25	--	--	--
	0.0004 (LOQ)	6	73-86	80	6	8
	0.04	6	78-89	84	4	5
	1	6	77-85	80	3	4
Fluroxypyr acid	0.00012 (LOD)	2	8, 17	--	--	--
	0.0004 (LOQ)	6	91-103	98	5	5
	0.04	6	100-109	106	4	3
	1	6	95-106	102	4	4
Fluroxypyr-DCP	0.003 (LOD)	2	133, 147	--	--	--

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.01 (LOQ)	6	114-118	117	2	1
	0.1	6	108-113	110	2	2
	1	6	104-110	107	2	2
Fluroxypyr-MP	0.003 (LOD)	2	93, 117	--	--	--
	0.01 (LOQ)	6	88-100	97	5	5
	0.1	6	95-104	99	4	4
	1	6	93-100	96	2	3
	Confirmatory Ion Transition					
Fluroxypyr-MHE	0.00012 (LOD)	2	1, 25	--	--	--
	0.0004 (LOQ)	6	71-83	78	4	5
	0.04	6	80-96	86	6	7
	1	6	76-82	78	2	3
Fluroxypyr acid	0.00012 (LOD)	2	8, 25	--	--	--
	0.0004 (LOQ)	6	92-106	99	5	6
	0.04	6	100-110	105	4	4
	1	6	96-107	101	4	4
Fluroxypyr-DCP	0.003 (LOD)	2	127, 163	--	--	--
	0.01 (LOQ)	6	113-116	116	1	1
	0.1	6	106-115	110	3	3
	1	6	103-109	106	2	2
Fluroxypyr-MP	0.003 (LOD)	2	97, 117	--	--	--
	0.01 (LOQ)	6	95-99	98	2	2
	0.1	6	92-105	99	6	6
	1	6	94-106	97	5	5

Data (uncorrected recovery results, Figures 13-16, pp. 92-95) were obtained from Tables 11-25, pp. 32-63 of MRID 50290801 and DER Attachment 2.

1 Analytes were identified using two ion transitions (quantitative and confirmatory, respectively): *m/z* 367.025→255.000 and *m/z* 367.025→209.000 for fluroxypyr-MHE; *m/z* 254.910→181.000 and *m/z* 254.910→209.000 for fluroxypyr acid; *m/z* 210.917→196.000 and *m/z* 210.917→112.900 for fluroxypyr-MP; and *m/z* 196.910→152.000 and *m/z* 196.910→144.000 for fluroxypyr-DCP.

2 Loam soil (M964; 49% sand 40% silt 11% clay, pH 7.2, 0.7% organic carbon) obtained from Hanford, (location not further specified), silt loam soil (M961; 35% sand 57% silt 8% clay, pH 5.8, 3.9% organic carbon) obtained from Brierlow, (location not further specified), unclassified soil (M963; uncharacterized) from an unidentified location, sandy loam sediment (M971; 69% sand 18% silt 13% clay, pH 5.9, 2.9% organic carbon) obtained from Emperor Lake (location not further specified), and sand sediment (M972; 97% sand 3% silt 0% clay, pH 7.7, 1.0% organic carbon) obtained from River Dove, (location not further specified), were used in this study (USDA soil texture classification; p. 20; Figures 141-152, pp. 220-231). The pH values were based on 1:1 soil:water ratio results.

3 Recoveries for the 0.00012 µg/g and 0.003 µg/g samples (LOD) were reviewer-calculated based on data from Tables 11-17, pp. 32-55 since the study author did not calculate these recoveries (DER Attachment 2). Mean recovery, s.d. and RSDs could not be determined since n = 2.

4 Recovery statistics for the quantitation ion transition of fluroxypyr acid in unclassified soil M963 and the quantitation and confirmation ion transitions of fluroxypyr-DCP in sandy loam sediment M971 were reviewer-calculated with all recovery values (n = 6) since the study author excluded an outlier from the statistics presented in the study report (n = 5; Table 12, pp. 38-40 and Tables 14-15, pp. 44-49).

Table 3. Independent Validation Method Recoveries for Fluroxypyr-MHE and Its Metabolites, Fluroxypyr acid, Fluroxypyr-DCP, and Fluroxypyr-MP, in Soil[1,2,3]

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[4]	Relative Standard Deviation (%)
	Soil					
	Quantitation ion					
Fluroxypyr-MHE	0.00012 (LOD)	**1**	**50**	--	--	--
	0.0004 (LOQ)	5	93-100	96	3	2.6
	1.0	5	87-93	90	2	2.7
Fluroxypyr acid	0.00012 (LOD)	**1**	**125**	--	--	--
	0.0004 (LOQ)	5	94-**127**	112	15	12.9
	1.0	5	116-**124**	120	3	2.8
Fluroxypyr-DCP	0.003 (LOD)	**1**	76	--	--	--
	0.01 (LOQ)	5	83-89	86	3	3.1
	1.0	5	88-95	92	3	3.2
Fluroxypyr-MP	0.003 (LOD)	**1**	89	--	--	--
	0.01 (LOQ)	5	91-101	95	4	4.6
	1.0	5	98-103	100	2	1.9
	Confirmatory ion					
Fluroxypyr-MHE	0.00012 (LOD)	**1**	92	--	--	--
	0.0004 (LOQ)	5	97-105	100	3	3.1
	1.0	5	86-92	89	2	2.6
Fluroxypyr acid	0.00012 (LOD)	**1**	**150**	--	--	--
	0.0004 (LOQ)	5	**123-130**	**127**[5]	3	2.0
	1.0	5	114-**122**	119	4	3.2
Fluroxypyr-DCP	0.003 (LOD)	**1**	**63**	--	--	--
	0.01 (LOQ)	5	79-87	84	4	4.2
	1.0	5	89-96	93	3	2.8
Fluroxypyr-MP	0.003 (LOD)	**1**	**65**	--	--	--
	0.01 (LOQ)	5	83-93	89	4	4.5
	1.0	5	94-101	97	3	2.9

Data (uncorrected recovery results, pp. 14-17) were obtained from Tables 14-29, pp. 30-39 of MRID 50290802 and DER Attachment 2.

1 Analytes were identified using two ion transitions (quantitative and confirmatory, respectively): *m/z* 367.1→255.1 and *m/z* 367.1→209.0 for fluroxypyr-MHE; *m/z* 255.1→181.0 and *m/z* 255.1→209.0 for fluroxypyr acid; *m/z* 210.9→196.0 and *m/z* 210.9→112.9 for fluroxypyr-MP; and *m/z* 196.9→152.0 and *m/z* 196.9→144.0 for fluroxypyr-DCP.

2 Loam soil (M963; CSR-7909-001; 48% sand 40% silt 12% clay, pH 5.9, 6.0% organic carbon) obtained from the sponsor was used in this study (USDA soil texture classification; p. 14; Figure 5, p. 45). The pH value was based on 1:1 soil:water ratio result. The soil source was not further specified.

3 Recoveries for the 0.00012 µg/g and 0.003 µg/g samples (LOD) was reviewer-calculated based on data from Tables 14-21, pp. 30-37 since the study author did not calculate these recoveries (DER Attachment 2). Mean recovery, s.d. and RSDs could not be determined since n = 1.

4 Standard deviations were reviewer-calculated since these values were not calculated in the study report (see DER Attachment 2). The rules of significant figures were followed.

5 Values were not corrected for residues quantified in the controls (0.00015-0.00017 µg/g, >LOD; Table 17, p. 33). The adjusted percent recovery values which included correction for residues quantified in the controls were 83-91%.

III. Method Characteristics

The LOQ in the ECM and ILV was reported as 0.0004 µg/g for fluroxypyr-MHE and fluroxypyr acid and 0.01 µg/g for fluroxypyr-DCP and fluroxypyr-MP (pp. 18, 23-24, 26; Tables 26-27, pp. 64-65 of MRID 50290801; pp. 12, 20; Tables 10-13, p. 29 of MRID 50290802). The Limit of Detection (LOD) in the ECM and ILV was reported as 0.00012 µg/g for fluroxypyr-MHE and fluroxypyr acid and 0.003 µg/g for fluroxypyr-DCP and fluroxypyr-MP. Following the method of Keith, L. H., *et al*. (see section V. References below), the LOD and LOQ for determination of fluroxypyr-MHE, fluroxypyr acid, fluroxypyr-DCP, and fluroxypyr-MP in soil were calculated in the ECM using the standard deviation from the LOQ recovery results, which corresponded to 0.0004 µg/g recovery results for fluroxypyr-MHE and fluroxypyr acid, and 0.01 µg/g recovery results for fluroxypyr-DCP and fluroxypyr-MP. The LOD was calculated as three times the standard deviation (3*s*), and the LOQ was calculated as ten times the standard deviation (10*s*) of the recovery results. In the ECM, the LOQ and LOD were calculated as 0.00004-0.00048 µg/g and 0.00001-0.00014 µg/g, respectively, for fluroxypyr-MHE and fluroxypyr acid and 0.00135-0.00615 µg/g and 0.00041-0.00185 µg/g, respectively, for fluroxypyr-DCP and fluroxypyr-MP. The calculated LOQ and LOD values supported the Method LOQ and LOD values.

Table 4. Method Characteristics

Analyte		**Fluroxypyr-MHE**	**Fluroxypyr acid**	**Fluroxypyr-DCP**	**Fluroxypyr-MP**
Limit of Quantitation (LOQ)	ECM	0.0004 µg/g		0.01 µg/g	
	ECM Calculated	0.00004-0.00031 µg/g (Q) 0.00010-0.00122 µg/g (C)	0.00005-0.00046 µg/g (Q) 0.00007-0.00048 µg/g (C)	0.00152-0.00615 µg/g (Q) 0.00142-0.00575 µg/g (C)	0.00135-0.00561 µg/g (Q) 0.00177-0.00301 µg/g (C)
	ILV	0.0004 µg/g		0.01 µg/g	
Limit of Detection (LOD)	ECM	0.00012 µg/g		0.003 µg/g	
	ECM Calculated	0.00001-0.00009 µg/g (Q) 0.00001-0.00012 µg/g (C)	0.00003-0.00014 µg/g (Q) 0.00002-0.00014 µg/g (C)	0.00046-0.00185 µg/g (Q) 0.00043-0.00173 µg/g (C)	0.00041-0.00168 µg/g (Q) 0.00053-0.00090 µg/g (C)
	ILV	0.00012 µg/g		0.003 µg/g	
Linearity (calibration curve r^2 and concentration range)	ECM[1]	$r^2 = 0.9990-0.9998$ (Q) $r^2 = 0.9988-0.9998$ (C)	$r^2 = 0.9978-0.9998$ (Q) $r^2 = 0.9980-0.9998$ (C)	$r^2 = 0.9990-0.9998$ (Q) $r^2 = 0.9988-0.9996$ (C)	$r^2 = 0.9958-0.9998$ (Q) $r^2 = 0.9974-0.9996$ (C)
	ILV	$r^2 = 1.0000$ (Q) $r^2 = 0.9999$ (C)	$r^2 = 0.9975$ (Q) $r^2 = 0.9981$ (C)	$r^2 = 0.9997$ (Q) $r^2 = 0.9987$ (C)	$r^2 = 0.9976$ (Q) $r^2 = 0.9988$ (C)
	Range	0.1-30 ng/mL		1-30 ng/mL	
Repeatable	ECM[2]	**No** samples prepared at 10×LOQ		Yes at the LOQ, 10×LOQ, and 100×LOQ in three soils and two sediments.	
		Yes at the LOQ, 100×LOQ, and 2500×LOQ in three soils and two sediments.	Yes at the LOQ, 100×LOQ, and 2500×LOQ in two soils and two sediments; in the third soil, yes at 100×LOQ, and 2500×LOQ, but **no** at the LOQ (Q, RSD **32**%).		
	ILV[3,4]	**No** samples prepared at 10×LOQ.			
		Yes at the LOQ and 100×LOQ in one soil.	Yes at the LOQ (Q) and 100×LOQ in one soil, but **no** at the LOQ (C, mean 127%).[5]	Yes at the LOQ and 100×LOQ in one soil.	
Reproducible		Yes at LOQ and 100×LOQ. **Could not be determined for 10×LOQ**.			
Specific	ECM	Representative 10×LOQ chromatograms were **not** presented.			
		Yes, no matrix interferences were observed; however, **significant** baseline noise which interfered with peak attenuation and integration was observed at the LOQ in all matrices.		Yes, no matrix interferences were observed; minor baseline noise which interfered with peak attenuation and integration at the LOQ was observed in some matrices.	Yes, no matrix interferences were observed; however, **significant** baseline noise which interfered with peak attenuation and integration at the LOQ (Q only) was observed in all matrices.
	ILV	Representative LOD chromatograms were **not provided.**			

Analyte		**Fluroxypyr-MHE**	**Fluroxypyr acid**	**Fluroxypyr-DCP**	**Fluroxypyr-MP**
		Yes, no matrix interferences were observed.	Yes, matrix interferences were *ca*. 18% (Q) and *ca*. 38-43% (C) of the LOQ (based on residues quantified). However, **significant** baseline noise which interfered with peak attenuation and integration at the LOQ was observed.[6]	Yes, no matrix interferences were observed.	Yes, matrix interferences were *ca*. 2% (Q) of the LOQ (based on residues quantified). However, **significant** baseline noise which interfered with peak attenuation and integration at the LOQ (Q only) was observed

Data were obtained from pp. 18, 23-24, 26; Tables 2-9, pp. 29-31 (calibration data); Tables 11-25, pp. 32-63 (recovery data); Tables 26-27, pp. 64-65 (calculated LOQ/LOD); Figures 5-12, pp. 84-91 (calibration curves); Figures 21-48, pp. 100-127 (blank and control chromatograms); Figures 49-128, pp. 128-207 (fortification chromatograms) of MRID 50290801; pp. 12, 20; Tables 2-13, pp. 25-29 (calibration data); Tables 14-29, pp. 30-39 (recovery data); Figures 22-37, pp. 62-77 (chromatograms) of MRID 50290802. Q = Quantitation ion transition; C = Confirmation ion transition.

1 Reported correlation coefficients were reviewer-calculated from r values reported in the study report (Tables 2-9, pp. 29-31 of MRID 50290801; DER Attachment 2). Matrix-matched standards were used (pp. 20-21).

2 In the ECM, three soil and two sediment matrices were used. Loam soil (M964; 49% sand 40% silt 11% clay, pH 7.2, 0.7% organic carbon) obtained from Hanford, (location not further specified), silt loam soil (M961; 35% sand 57% silt 8% clay, pH 5.8, 3.9% organic carbon) obtained from Brierlow, (location not further specified), unclassified soil (M963; uncharacterized) from an unidentified location, sandy loam sediment (M971; 69% sand 18% silt 13% clay, pH 5.9, 2.9% organic carbon) obtained from Emperor Lake (location nor further specified), and sand sediment (M972; 97% sand 3% silt 0% clay, pH 7.7, 1.0% organic carbon) obtained from River Dove, (location not further specified), were used in this study (USDA soil texture classification; p. 20; Figures 141-152, pp. 220-231 of MRID 50290801). The pH values were based on 1:1 soil:water ratio results.

3 In the ILV, loam soil (M963; CSR-7909-001; 48% sand 40% silt 12% clay, pH 5.9, 6.0% organic carbon) obtained from the sponsor was used in this study (USDA soil texture classification; p. 14; Figure 5, p. 45 of MRID 50315502). The pH value was based on 1:1 soil:water ratio result. The soil source was not further specified..

4 In the ILV, the method for fluroxypyr-DCP and fluroxypyr-MP was validated in the first trial with insignificant modifications to the analytical instrumentation (pp. 12, 20, 22; Appendix 1, Appendix 1, pp. 85-102; Appendix 2, pp. 104-108 of MRID 50315502). The method for fluroxypyr-MHE and fluroxypyr acid was validated in the second trial with modifications to the analytical parameters and instrumentation. The first trial was unsuccessful due to interfering peaks and poor chromatography.

5 A confirmatory method is not usually required when LC/MS and GC/MS is the primary method to generate study data.

6 Based on Tables 16-17, pp. 32-33 and Figures 27-28, pp. 67-68 of MRID 50290802.

IV. Method Deficiencies and Reviewer's Comments

1. For fluroxypyr-MHE and fluroxypyr acid, no samples were prepared at 10×LOQ in the ECM and ILV (Tables 11-25, pp. 32-63 of MRID 50290801; Tables 2-13, pp. 25-29 of MRID 50290802). For fluroxypyr-DCP and fluroxypyr-MP, no samples were prepared at 10×LOQ in the ILV. OCSPP guidelines state that a minimum of five spiked replicates should be analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.

2. Only one soil, and no sediment, was used in the ILV, whereas three soil matrices and two sediment matrices were used in the ECM (p. 14; Figure 5, p. 45 of MRID 50290802). The ILV should provide a more challenging and comprehensive test of the ECM method than the ECM. Additionally, it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and that the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies.

   In the ECM, the soil description and characterization was not included for soil M963 (p. 20; Figures 141-152, pp. 220-231 of MRID 50290801).

3. The specificity of the method was not supported by ECM and ILV representative chromatograms of fluroxypyr acid and fluroxypyr-MP due to significant baseline noise which interfered with peak attenuation and integration at the LOQ in all matrices (Figures 49-128, pp. 128-207 of MRID 50290801; Figures 22-37, pp. 62-77 of MRID 50290802). Additionally, the reviewer noted that ILV representative chromatograms of fluroxypyr acid showed matrix interferences of *ca*. 18% (Q) and *ca*. 38-43% (C) of the LOQ. The residues quantified in the controls of the confirmation ion (0.00015-0.00017 µg/g, >LOD) appeared to cause the performance data to be unacceptable (Tables 16-17, pp. 32-33; Figures 27-28, pp. 67-68 of MRID 50290802).

   The specificity of the method was not supported by ECM representative chromatograms of fluroxypyr-MHE due to significant baseline noise which interfered with peak attenuation and integration at the LOQ in all matrices (Figures 49-128, pp. 128-207 of MRID 50290801).

   In the ILV, representative LOD chromatograms were not provided; in the ECM, representative 10×LOQ chromatograms were not provided. Representative chromatograms from all matrices and fortifications should be provided for review.

4. Performance data was not satisfactory for the ECM quantitation ion analysis of fluroxypyr acid in the unclassified soil M963 at the LOQ (RSD 32%) and for the ILV confirmation ion analysis of fluroxypyr acid at the LOQ (mean 127%; Tables 11-25, pp. 32-63 of MRID 50290801; Tables 2-13, pp. 25-29 of MRID 50290802). OCSPP guideline requirements state that the mean recovery is 70-120% and the RSD is ≤20%. The reviewer did not consider these guideline deviations to be significant since the ECM performance data deviation occurred in only one of the five matrices tested and the ILV performance data deviation occurred in the confirmation ion analysis which is not necessary required when LC/MS is used as the primary method to generate study data.

5. The matrix effects were found to be significant (>20%) for the analytes in the test matrices in the ECM and ILV (quantitation and confirmatory transitions; p. 25; Tables 44-47, pp. 76-79 of MRID 50290801; p. 21; Tables 30-33, p. 40 of MRID 50290802). Matrix-matched standards were used in the ECM and ILV.

6. In the ECM, LOD chromatograms of fluroxypyr-MHE and fluroxypyr acid showed peak just barely distinguishable above baseline noise and nearby contaminant peaks (Figures 49-58, pp. 128-137 of MRID 50290801).

7. In the ECM, the stock, fortification, and calibration solutions were found to be stable up to 35 days of storage at *ca*. 4°C (p. 24; Tables 28-39, pp. 66-71 of MRID 50290801). The final sample extracts were found to be stable up to 5 days at *ca*. 4°C (p. 25; Tables 40-43, pp. 72-75).

8. The reviewer noted a significant typographical error in the ECM: 10×LOQ was listed in the fortification table for fluroxypyr-MHE and fluroxypyr acid, instead of 100×LOQ (Appendix I, p. 241of MRID 50290801).

9. The ILV detailed the communications between the Study Director and the Study Monitor (p. 22; Appendix 4, p. 110 of MRID 50290802). The communication mainly involved the communication of the failed ILV trial and ECM chromatogram exchange.

10. It was reported for the ILV that one sample set (1 reagent blank, 1 control, a minimum of three fortified controls and associated samples) required *ca*. 8 hours for one technician to prepare with LC/MS/MS performed unattended overnight (p. 19 of MRID 50290802). Evaluation of the LC/MS/MS results was completed the following day, so a sample set was completely processed and evaluated in *ca*. 2 working days.

V. References

1. Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* 1983, *55*, 2210-2218 (p. 26 of MRID 50290801).

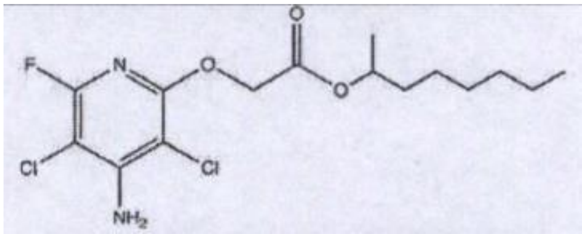
2. U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

3. 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

DER Attachment 1: Chemical Names and Structures

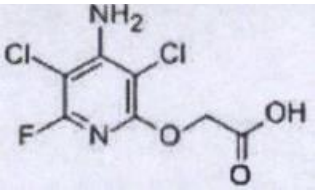
Fluroxypyr-MHE (Fluroxypyr 1-MHE; Fluroxypyr 1-meptyl)

IUPAC Name:	((4-Amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy)acetic acid 1-methylheptyl ester
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	Not found



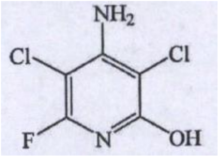
Fluroxypyr acid

IUPAC Name:	((4-Amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy)acetic acid
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	Not found



Fluroxypyr-DCP (Fluroxypyr-2-pyridinol; X061784)

IUPAC Name:	4-Amino-3,5-dichloro-6-fluoro-2-pyridinol
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	Not found



Fluroxypyr-MP (DMP fluroxypyr metabolite; X61420)

IUPAC Name:	4-Amino-3,5-dichloro-6-fluoro-2-methoxypyridine
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	Not found