Analytical method for oxyfluorfen and its isomers, RH-34672, RH-42382 and RH-50671, in soil

Reports: ECM: EPA MRID No. 50488801. Turner, R. 2018. Enforcement Method Validation of the Determination of Oxyfluorfen and its Isomers in Soil Matrices by Gas Chromatography with Mass Spectrometry. Dow AgroSciences Protocol No.: 170885. Battelle Study No.: YR/17/020. Report prepared by Battelle UK Ltd., Essex, United Kingdom, sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana;153 pages. Final report issued January 5, 2018.

ILV: EPA MRID No. 50488802. Clark, S. 2018. Independent Laboratory Validation of the "Enforcement Method Validation of the Determination of Oxyfluorfen and its Isomers in Soil Matrices by Gas Chromatography with Mass Spectrometry". Dow AgroSciences Study No.: 170886. EAG Study No.: 86611. Report prepared by Analytical Bio-Chemistry Laboratories, Inc., a wholly owned subsidiary of EAG, Inc., Columbia, Missouri, sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 138 pages. Final report issued January 5, 2018.

Document No.: MRIDs 50488801 & 50488802

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA (40 CFR Part 160), UK, and OECD Good Laboratory Practice (GLP) standards (p. 3; Appendix 3, p. 153 of MRID 50488801). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4; Appendix 3, p. 153). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).
ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 50488802). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was not included.

Classification: This analytical method is classified as **Supplemental**. In the ILV, the linearity of the method for all analytes in loamy sand matrix was not acceptable, and the specificity of the method for RH-50671 in loamy sand matrix was not well-supported. The method linearity and specificity was acceptable in other soils.

PC Code: 111601

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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 Protocol No. 170885, is designed for the quantitative determination of oxyfluorfen and its isomers RH-34672, RH-42382 and RH-50671 in soil at the LOQ of 0.01 mg/kg using GC/MS. The LOQ is greater than the lowest toxicological level of concern in soil (0.0032 mg/kg; USEPA. 2014) for the four analytes. The ECM and ILV were performed using two characterized soil matrices. Analytes were identified using three ions. The ILV validated the method for oxyfluorfen and its isomers in the second trial with insignificant modifications of a few equipment substitutions in the sample processing procedure and the use of a different GC/MS system and GC liner. In the communications with the Sponsor, the reviewer noted that the first trial appeared to have failed due to not filtering matrix-matched standards and the required changing of the GC liner and accompanying O-ring and inlet septa between sets. All ILV and ECM data regarding repeatability, accuracy, and precision were satisfactory for all four analytes. In the ILV, the linearity of the method for all analytes in loamy sand matrix was not acceptable, and the specificity of the method for RH-50671 in loamy sand matrix was not well-supported due to matrix interferences. ECM data regarding linearity and specificity was acceptable.

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						
Oxyfluorfen	50488801	50488802		Soil[1,2]	05/01/2018	Dow AgroSciences LLC	GC/MS	0.01 mg/kg
RH-34672								
RH-42382								
RH-50671								

1 In the ECM, sandy loam soil (Lufa Speyer 2.2; 76% sand 14% silt 10% clay, pH 6.0 in 1:1 soil:water ratio, 1.8% organic carbon) and clay soil (Lufa Speyer 6S; 22% sand 27% silt 51% clay, pH 7.2 in 1:1 soil:water ratio, 1.7% organic carbon) were obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture characterization; p. 16; Appendix 2, pp. 151-152 of MRID 50488801).

2 In the ILV, loamy sand soil (GTS-PF 0-6"; 17-4020; 87% sand 6% silt 7% clay, pH 7.0, 3.0% organic carbon) and clay soil (LAD-SCL-PF 0-6"; 17-4021; 33% sand 26% silt 41% clay, pH 8.4, 1.8% organic carbon) were obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture characterization; p. 14; Table 2, p. 23 of MRID 50488802).

I. Principle of the Method

Soil samples (10.0 g) were fortified in 50 mL vials (Appendix 1, pp. 144, 147 of MRID 50488801). The soil was extracted with water (5 mL) and acetonitrile (10 mL) via vigorous shaking by hand for 2 minutes, then citrate salt mixture (4 g $MgSO_4$, 1 g NaCl, 1 g NaCitrate, 0.5 g disodium citrate sesquihydrate) was added. After vigorous shaking by hand for 2 minutes, the sample was centrifuged for 5 minutes at 4000 rpm, and a 7.5-mL aliquot of the acetonitrile phase was transferred to a clean tube. The solvent was removed using a gentle stream of nitrogen at 40°C. The residue was reconstituted with 0.5 mL of toluene. The extract was filtered (0.2 µm PTFE syringe filter) and transferred to an autosampler vial for analysis by GC/MS.

Soil samples were analyzed for oxyfluorfen, RH-34672, RH-42382 and RH-50671 using Agilent HP6890N gas chromatograph (Restek Rtx-200 column, 0.32 mm x 60 m, 1.0 µm thickness) using a column temperature program (215°C for 1.0 min., 215°C to 250°C at 5°C/min., and hold for 17 min.) and helium carrier gas coupled with an Agilent 5975 mass spectrometer (source temperature 250°C) using electron ionization in Single Ion Monitoring (SIM) mode (Appendix 1, pp. 148-150 of MRID 50488801). Injection volume was 3 µL. Three ions were monitored for each analyte as follows (quantitation, confirmation 1 and confirmation 2, respectively): *m/z* 361, 317, and 363 for oxyfluorfen, *m/z* 166, 138, and 361 for RH-34672, *m/z* 361, 317, and 363 for RH-42382, and *m/z* 138, 361, and 363 for RH-50671. Observed retention times were *ca*. 15.1 (batches 1 + 5) and 14.8 (batches 9 + 10) minutes for RH-50671, 15.6 (batches 1 + 5) and 15.3 (batches 9 + 10) minutes for RH-42382, 16.8 (batches 1 + 5) and 16.5 (batches 9 + 10) minutes for oxyfluorfen, and 17.3 (batches 1 + 5) and 16.9 (batches 9 + 10) minutes for RH-34672.

In the ILV, the ECM was performed as written, except for a few equipment substitutions in the sample processing procedure and the use of a different GC/MS system and GC liner (pp. 13-16, 19-20 of MRID 50488802). The GC/MS system was an Agilent 7890A (G3440A) gas chromatograph coupled with an Agilent 5975 mass spectrometer; other parameters were the same as the ECM. The same three ions per analyte were monitored as in the ECM. Expected retention times were *ca*. 17.6, 18.0, 16.3, and 15.7 minutes for oxyfluorfen, RH-34672, RH-42382 and RH-50671, respectively. No other modifications to the ECM were reported.

In the ILV, the study author noted the following two critical steps: 1) the matrix-matched standards should be filtered (0.2 µm PTFE syringe filter) prior to use; and 2) for GC/MS analysis, the GC liner and accompanying O-ring and inlet septa were changed between each analytical set (pp. 16-17 of MRID 50488802).

The Limit of Quantification (LOQ) for oxyfluorfen, RH-34672, RH-42382, and RH-50671 in soil was 0.01 mg/kg in the ECM and ILV (p. 19 of MRID 50488801; pp. 13, 18 of MRID 50488802). The Limit of Detection (LOD) for oxyfluorfen, RH-34672, RH-42382, and RH-50671 in soil was reported as 0.003 mg/kg in the ECM and ILV. In the ECM and ILV, LOD and LOQ for determination of oxyfluorfen, RH-34672, RH-42382, and RH-50671 in soil were calculated using the standard deviation from the 0.010 mg/kg recovery results. The calculated values support the LOQ and LOD established for the study.

II. Recovery Findings

ECM (MRID 50488801): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of oxyfluorfen, RH-34672, RH-42382 and RH-50671 in soil matrices at the fortification level of 0.010 mg/kg (LOQ) and 0.10 mg/kg (10×LOQ; Tables 14-37, pp. 38-61 and Tables 38-49, pp. 62-65; DER Attachment 2). Three ions were monitored for each analyte; performance data (recovery results) from primary and confirmatory analyses were comparable. One sample per analyte/matrix was prepared at the LOD, 0.003 mg/kg; sample recoveries ranged 97-107% for oxyfluorfen, 82-145% for RH-34672, 87-110% for RH-42382, and 76-106% for RH-50671 (ions/matrices combined). The sandy loam soil (Lufa Speyer 2.2; 76% sand 14% silt 10% clay, pH 6.0 in 1:1 soil:water ratio, 1.8% organic carbon) and clay soil (Lufa Speyer 6S; 22% sand 27% silt 51% clay, pH 7.2 in 1:1 soil:water ratio, 1.7% organic carbon) were obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture characterization; p. 16; Appendix 2, pp. 151-152).

ILV (MRID 50488802): Mean recoveries and RSDs were within guideline requirements for analysis of oxyfluorfen, RH-34672, RH-42382 and RH-50671 in soil matrices at the fortification level of 0.010 mg/kg (LOQ) and 0.10 mg/kg (10×LOQ; Tables 27-50, pp. 30-41 and Tables 51-62, pp. 42-45; DER Attachment 2). Three ions were monitored for each analyte; performance data (recovery results) from primary and confirmatory analyses were comparable. One sample per analyte/matrix was prepared at the LOD, 0.003 mg/kg; sample recoveries ranged 97-112% for oxyfluorfen, 102-121% for RH-34672, 79-113% for RH-42382, and 86-123% for RH-50671 (ions/matrices combined). The loamy sand soil (GTS-PF 0-6”; 17-4020; 87% sand 6% silt 7% clay, pH 7.0, 3.0% organic carbon) and clay soil (LAD-SCL-PF 0-6”; 17-4021; 33% sand 26% silt 41% clay, pH 8.4, 1.8% organic carbon) were obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture characterization; p. 14; Table 2, p. 23). The method for oxyfluorfen and its isomers was validated in the second trial with insignificant modifications of a few equipment substitutions in the sample processing procedure and the use of a different GC/MS system and GC liner (pp. 13-16, 18-20; Appendix II, pp. 130-138). In the communications with the Sponsor, the reviewer noted that the first trial appeared to have failed due to not filtering matrix-matched standards and the required changing of the GC liner and accompanying O-ring and inlet septa between sets.

Table 2. Initial Validation Method Recoveries for Oxyfluorfen and its Isomers, RH-34672, RH-42382, and RH-50671, in Soil[1,2,3]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Sandy Loam Soil (Lufa Speyer 2.2)					
	Quantitation Ion					
Oxyfluorfen	0.003	1	107	--	--	--
	0.01 (LOQ)	5	83-88	86	1.9	2.2
	0.10	5	93-98	95	2.1	2.2
RH-34672	0.003	1	110	--	--	--
	0.01 (LOQ)	5	84-98	91	6.6	7.3
	0.10	5	92-104	97	5.4	5.5
RH-42382	0.003	1	107	--	--	--
	0.01 (LOQ)	5	87-91	89	1.5	1.7
	0.10	5	95-102	98	2.6	2.7
RH-50671	0.003	1	106	--	--	--
	0.01 (LOQ)	5	91-98	95	3.0	3.2
	0.10	5	94-102	98	3.3	3.4
	Confirmation Ion 1					
Oxyfluorfen	0.003	1	102	--	--	--
	0.01 (LOQ)	5	81-89	85	2.9	3.4
	0.10	5	93-98	95	1.9	2.0
RH-34672	0.003	1	**145**	--	--	--
	0.01 (LOQ)	5	85-112	100	10.0	10.0
	0.10	5	91-104	97	5.9	6.1
RH-42382	0.003	1	101	--	--	--
	0.01 (LOQ)	5	82-91	86	3.3	3.8
	0.10	5	95-101	97	2.2	2.2
RH-50671	0.003	1	92	--	--	--
	0.01 (LOQ)	5	88-97	93	3.7	4.0
	0.10	5	95-103	99	3.6	3.6
	Confirmation Ion 2					
Oxyfluorfen	0.003	1	100	--	--	--
	0.01 (LOQ)	5	85-92	88	2.7	3.1
	0.10	5	93-97	95	1.5	1.6
RH-34672	0.003	1	109	--	--	--
	0.01 (LOQ)	5	86-99	93	6.2	6.6
	0.10	5	93-105	98	5.4	5.5
RH-42382	0.003	1	110	--	--	--
	0.01 (LOQ)	5	89-94	91	2.0	2.3
	0.10	5	95-101	98	2.2	2.2
RH-50671	0.003	1	76	--	--	--
	0.01 (LOQ)	5	87-101	95	5.7	6.0
	0.10	5	94-103	98	3.9	4.0

Analyte	**Fortification Level (mg/kg)**	**Number of Tests**	**Recovery Range (%)**	**Mean Recovery (%)**	**Standard Deviation (%)**	**Relative Standard Deviation (%)**
	Clay Soil (Lufa Speyer 6S)					
	Quantitation Ion					
Oxyfluorfen	0.003	1	99	--	--	--
	0.01 (LOQ)	5	86-93	88	2.9	3.3
	0.10	5	91-100	97	3.5	3.6
RH-34672	0.003	1	82	--	--	--
	0.01 (LOQ)	5	85-99	92	5.2	5.7
	0.10	5	95-109	99	5.8	5.8
RH-42382	0.003	1	109	--	--	--
	0.01 (LOQ)	5	89-97	91	3.4	3.7
	0.10	5	93-104	100	4.1	4.1
RH-50671	0.003	1	98	--	--	--
	0.01 (LOQ)	5	89-103	97	5.4	5.6
	0.10	5	97-107	100	4.2	4.2
	Confirmation Ion 1					
Oxyfluorfen	0.003	1	102	--	--	--
	0.01 (LOQ)	5	84-88	86	1.6	1.8
	0.10	5	91-100	97	3.5	3.6
RH-34672	0.003	1	101	--	--	--
	0.01 (LOQ)	5	80-105	89	10.3	11.6
	0.10	5	93-111	99	7.2	7.3
RH-42382	0.003	1	87	--	--	--
	0.01 (LOQ)	5	85-97	91	5.1	5.6
	0.10	5	93-104	100	4.1	4.1
RH-50671	0.003	1	96	--	--	--
	0.01 (LOQ)	5	93-99	96	2.3	2.4
	0.10	5	97-107	100	4.1	4.1
	Confirmation Ion 2					
Oxyfluorfen	0.003	1	97	--	--	--
	0.01 (LOQ)	5	85-93	89	2.9	3.2
	0.10	5	91-100	97	3.5	3.6
RH-34672	0.003	1	105	--	--	--
	0.01 (LOQ)	5	85-97	91	6.1	6.7
	0.10	5	94-108	98	5.8	5.9
RH-42382	0.003	1	102	--	--	--
	0.01 (LOQ)	5	90-98	93	3.1	3.3
	0.10	5	93-103	100	4.0	4.0
RH-50671	0.003	1	98	--	--	--
	0.01 (LOQ)	5	92-96	93	1.7	1.8
	0.10	5	97-107	100	4.3	4.3

Data (uncorrected recovery results, Tables 14-37, pp. 38-61) were obtained from Tables 14-37, pp. 38-61 and Tables 38-49, pp. 62-65 of MRID 50488801 and DER Attachment 2.

1 Three ions were monitored for each analyte as follows (quantitation, confirmation 1 and confirmation 2, respectively): *m/z* 361, 317, and 363 for oxyfluorfen, *m/z* 166, 138, and 361 for RH-34672, *m/z* 361, 317, and 363 for RH-42382, and *m/z* 138, 361, and 363 for RH-50671.

2 Percent recovery for the 0.003 mg/kg samples was reviewer-calculated based on data from Tables 14-37, pp. 38-61 (DER Attachment 2).

3 The sandy loam soil (Lufa Speyer 2.2; 76% sand 14% silt 10% clay, pH 6.0 in 1:1 soil:water ratio, 1.8% organic carbon) and clay soil (Lufa Speyer 6S; 22% sand 27% silt 51% clay, pH 7.2 in 1:1 soil:water ratio, 1.7% organic carbon) were obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture characterization; p. 16; Appendix 2, pp. 151-152).

Table 3. Independent Validation Method Recoveries for Oxyfluorfen and its Isomers, RH-34672, RH-42382, and RH-50671, in Soil[1,2,3]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Loamy Sand Soil					
	Quantitation Ion					
Oxyfluorfen	0.003	1	100	--	--	--
	0.01 (LOQ)	5	97-100	98	1.2	1.2
	0.10	5	103-106	104	1.1	1.1
RH-34672	0.003	1	103	--	--	--
	0.01 (LOQ)	5	98-101	100	1.3	1.3
	0.10	5	105-108	106	1.1	1.1
RH-42382	0.003	1	99	--	--	--
	0.01 (LOQ)	5	97-102	99	1.8	1.8
	0.10	5	105-107	106	1.0	0.94
RH-50671	0.003	1	93	--	--	--
	0.01 (LOQ)	5	87-100	96	5.0	5.3
	0.10	5	103-105	104	0.84	0.81
	Confirmation Ion 1					
Oxyfluorfen	0.003	1	97	--	--	--
	0.01 (LOQ)	5	95-99	97	1.8	1.8
	0.10	5	103-106	104	1.1	1.1
RH-34672	0.003	1	102	--	--	--
	0.01 (LOQ)	5	91-98	95	3.1	3.3
	0.10	5	104-107	106	1.3	1.2
RH-42382	0.003	1	90	--	--	--
	0.01 (LOQ)	5	96-104	100	3.8	3.8
	0.10	5	106-112	109	2.4	2.2
RH-50671	0.003	1	102	--	--	--
	0.01 (LOQ)	5	99-104	101	1.9	1.9
	0.10	5	102-104	103	1.1	1.1
	Confirmation Ion 2					
Oxyfluorfen	0.003	1	98	--	--	--
	0.01 (LOQ)	5	95-101	98	2.2	2.2
	0.10	5	102-106	104	1.6	1.5
RH-34672	0.003	1	110	--	--	--
	0.01 (LOQ)	5	96-105	100	3.5	3.5
	0.10	5	101-107	103	2.5	2.4
RH-42382	0.003	1	81	--	--	--
	0.01 (LOQ)	5	98-103	100	2.0	2.0
	0.10	5	104-107	105	1.1	1.1
RH-50671	0.003	1	86	--	--	--
	0.01 (LOQ)	5	93-100	97	2.9	3.0
	0.10	5	103-105	104	0.89	0.86

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Clay Soil					
	Quantitation Ion					
Oxyfluorfen	0.003	**1**	112	--	--	--
	0.01 (LOQ)	5	93-97	95	2.0	2.2
	0.10	5	91-93	92	0.89	1.0
RH-34672	0.003	**1**	117	--	--	--
	0.01 (LOQ)	5	95-100	97	2.3	2.4
	0.10	5	93-95	94	0.84	0.89
RH-42382	0.003	**1**	113	--	--	--
	0.01 (LOQ)	5	94-99	96	2.5	2.6
	0.10	5	92-95	94	1.1	1.2
RH-50671	0.003	**1**	119	--	--	--
	0.01 (LOQ)	5	93-100	97	2.5	2.6
	0.10	5	91-93	92	0.71	0.77
	Confirmation Ion 1					
Oxyfluorfen	0.003	**1**	119	--	--	--
	0.01 (LOQ)	5	92-99	94	2.8	2.9
	0.10	5	92-94	93	0.71	0.76
RH-34672	0.003	**1**	**121**	--	--	--
	0.01 (LOQ)	5	82-100	94	7.1	7.6
	0.10	5	91-95	94	1.5	1.6
RH-42382	0.003	**1**	79	--	--	--
	0.01 (LOQ)	5	85-96	90	5.1	5.7
	0.10	5	92-97	95	1.8	1.9
RH-50671	0.003	**1**	114	--	--	--
	0.01 (LOQ)	5	94-99	97	2.1	2.1
	0.10	5	90-93	92	1.1	1.2
	Confirmation Ion 2					
Oxyfluorfen	0.003	**1**	109	--	--	--
	0.01 (LOQ)	5	80-94	90	5.7	6.3
	0.10	5	91-93	92	0.84	0.91
RH-34672	0.003	**1**	112	--	--	--
	0.01 (LOQ)	5	92-99	94	2.8	3.0
	0.10	5	90-93	91	1.3	1.5
RH-42382	0.003	**1**	112	--	--	--
	0.01 (LOQ)	5	94-98	96	1.6	1.6
	0.10	5	92-95	94	1.1	1.2
RH-50671	0.003	**1**	**123**	--	--	--
	0.01 (LOQ)	5	92-98	96	2.3	2.4
	0.10	5	90-92	91	0.84	0.92

Data (uncorrected recovery results, Tables 27-50, pp. 30-41) were obtained from Tables 27-50, pp. 30-41 and Tables 51-62, pp. 42-45 of MRID 50488802 and DER Attachment 2.

1 Three ions were monitored for each analyte as follows (quantitation, confirmation 1 and confirmation 2, respectively): *m/z* 361, 317, and 363 for oxyfluorfen, *m/z* 166, 138, and 361 for RH-34672, *m/z* 361, 317, and 363 for RH-42382, and *m/z* 138, 361, and 363 for RH-50671.

2 Percent recovery for the 0.003 mg/kg samples was reviewer-calculated based on data from Tables 27-50, pp. 30-41 (DER Attachment 2).

3 The loamy sand soil (GTS-PF 0-6”; 17-4020; 87% sand 6% silt 7% clay, pH 7.0, 3.0% organic carbon) and clay soil (LAD-SCL-PF 0-6”; 17-4021; 33% sand 26% silt 41% clay, pH 8.4, 1.8% organic carbon) were obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture characterization; p. 14; Table 2, p. 23).

III. Method Characteristics

The LOQ for oxyfluorfen, RH-34672, RH-42382, and RH-50671 in soil was 0.01 mg/kg in the ECM and ILV (p. 19 of MRID 50488801; pp. 13, 18 of MRID 50488802). The LOD for oxyfluorfen, RH-34672, RH-42382, and RH-50671 in soil was reported as 0.003 mg/kg in the ECM and ILV. Following the method of Keith, L. H., *et al*. (see section V. References below), the LOD and LOQ for determination of oxyfluorfen, RH-34672, RH-42382, and RH-50671 in soil were calculated in the ECM using the standard deviation from the 0.010 mg/kg recovery results. The LOD was calculated as three times the standard deviation (3*s*), and the LOQ was calculated as ten times the standard deviation (10*s*) of the recovery results. In the ECM, the LOD and LOQ were calculated as 0.000477-0.000876 mg/kg and 0.00159-0.00292 mg/kg, respectively, for oxyfluorfen, 0.00163-0.00315 mg/kg and 0.00544-0.0105 mg/kg, respectively, for RH-34672, 0.000522-0.00153 mg/kg and 0.00174-0.00511 mg/kg, respectively, for RH-42382, and 0.000498-0.00167 mg/kg and 0.00166-0.00558 mg/kg, respectively, for RH-50671 (Tables 50-53, pp. 66-69 of MRID 50488801). In the ILV, the LOD and LOQ for determination of oxyfluorfen, RH-34672, RH-42382, and RH-50671 in soil were calculated using the standard deviation from the 0.010 mg/kg recovery results, as well, but the LOD was calculated as the standard deviation multiplied by the t-statistic (3.747) and the LOQ was calculated as three times the LOD result. In the ILV, the LOD and LOQ were calculated as 0.000485-0.00212 mg/kg and 0.00146-0.00636 mg/kg, respectively, for oxyfluorfen, 0.000479-0.00256 mg/kg and 0.00144-0.00769 mg/kg, respectively, for RH-34672, 0.000594-0.00190 mg/kg and 0.00178-0.00569 mg/kg, respectively, for RH-42382, 0.000737-0.00198 mg/kg and 0.00221-0.00594 mg/kg, respectively, for RH-50671 (Tables 63-66, pp. 46-47 of MRID 50488802). The calculated values support the LOQ and LOD established for the study.

Table 4. Method Characteristics

Analyte			Oxyfluorfen	RH-34672	RH-42382	RH-50671
Limit of Quantitation (LOQ)	ECM	Method	0.010 mg/kg			
		Calculated[1]	0.00159-0.00292 mg/kg	0.00544-0.0105 mg/kg	0.00174-0.00511 mg/kg	0.00166-0.00558 mg/kg
	ILV	Method	0.010 mg/kg			
		Calculated[1]	0.00146-0.00636 mg/kg	0.00144-0.00769 mg/kg	0.00178-0.00569 mg/kg	0.00221-0.00594 mg/kg
Limit of Detection (LOD)	ECM	Method	0.003 mg/kg			
		Calculated[1]	0.000477-0.000876 mg/kg	0.00163-0.00315 mg/kg	0.000522-0.00153 mg/kg	0.000498-0.00167 mg/kg
	ILV	Method	0.003 mg/kg			
		Calculated[1]	0.000485-0.00212 mg/kg	0.000479-0.00256 mg/kg	0.000594-0.00190 mg/kg	0.000737-0.00198 mg/kg
Linearity (calibration curve r^2 and concentration range)	ECM	Sandy Loam	r^2 = 0.99918 (Q) r^2 = 0.99949 (C1) r^2 = 0.99931 (C2)	r^2 = 0.99971 (Q) r^2 = 0.99968 (C1) r^2 = 0.99958 (C2)	r^2 = 0.99949 (Q) r^2 = 0.99973 (C1) r^2 = 0.99952 (C2)	r^2 = 0.99990 (Q) r^2 = 0.99997 (C1) r^2 = 0.99994 (C2)
		Clay	r^2 = 0.99972 (Q) r^2 = 0.99984 (C1) r^2 = 0.99973 (C2)	r^2 = 0.99958 (Q) r^2 = 0.99983 (C1) r^2 = 0.99978 (C2)	r^2 = 0.99955 (Q) r^2 = 0.99943 (C1) r^2 = 0.99935 (C2)	r^2 = 0.99937 (Q) r^2 = 0.99975 (C1) r^2 = 0.99938 (C2)
	ILV	Loamy Sand	r^2 = **0.99437** (Q) r^2 = **0.99355** (C1) r^2 = **0.99442** (C2)	r^2 = **0.99409** (Q) r^2 = **0.99188** (C1) r^2 = 0.99566 (C2)	r^2 = **0.99429** (Q) r^2 = **0.99052** (C1) r^2 = **0.99494** (C2)	r^2 = **0.99385** (Q) r^2 = **0.99355** (C1) r^2 = **0.99441** (C2)
		Clay[2]	r^2 = 0.99960 (Q) r^2 = 0.99958 (C1) r^2 = 0.99972 (C2)	r^2 = 0.99984 (Q) r^2 = 0.99960 (C1) r^2 = 0.99918 (C2)	r^2 = 0.99970 (Q) r^2 = 0.99812 (C1) r^2 = 0.99972 (C2)	r^2 = 0.99982 (Q) r^2 = 0.99966 (C1) r^2 = 0.99974 (C2)
	Concentration Range		0.045-2.0 µg/mL			
Repeatable	ECM[3]		Yes at LOQ and 10×LOQ (two characterized soil matrices).			
	ILV[4,5]					
Reproducible			Yes at LOQ and 10×LOQ.			
Specific	ECM	Sandy Loam	Yes, matrix interferences were <5% of the LOQ (based on quantified residues).	Yes, no matrix interferences were observed.		
		Clay				
	ILV	Loamy Sand	Yes, no matrix interferences were observed.	Yes, matrix interferences were <1% of the LOQ; C1 ion peak was small.	Yes, matrix interferences were <1% of the LOQ; C1 ion peak was very small.	Yes, matrix interferences were *ca.* 25% of the LOQ (based on peak area).[6]
		Clay				Yes, no matrix interferences were observed.

Data were obtained from p. 19; Tables 14-37, pp. 38-61 and Tables 38-49, pp. 62-65 (recovery data); Tables 50-53, pp. 66-69 (calculated LOD/LOQ); Figures 5-28, pp. 84-107 (calibration curves); Figures 34-65, pp. 111-142 (chromatograms) of MRID 50488801; pp. 13, 18; Tables 15-26, pp. 28-29 (correlation coefficients); Tables 27-50, pp. 30-41 and Tables 51-62, pp. 42-45 (recovery data); Tables 63-66, pp. 46-47 (calculated LOD/LOQ); Figures 5-16, pp. 56-67 (calibration curves); Figures 22-33, pp. 74-121 (chromatograms) of MRID 50488802. Q = Quantitation ion; C1 = Confirmatory ion 1; C2 = Confirmatory ion 2.

1 Ions and matrices combined.
2 Reported correlation coefficients (r^2) were reviewer-calculated for the MV02 set (Clay Soil) based on the reported r values in the study report (Tables 15-26, pp. 28-29 of MRID 50488802). Matrix-matched calibration standards were used.
3 In the ECM, sandy loam soil (Lufa Speyer 2.2; 76% sand 14% silt 10% clay, pH 6.0 in 1:1 soil:water ratio, 1.8% organic carbon) and clay soil (Lufa Speyer 6S; 22% sand 27% silt 51% clay, pH 7.2 in 1:1 soil:water ratio, 1.7% organic carbon) were obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture characterization; p. 16; Appendix 2, pp. 151-152 of MRID 50488801).
4 In the ILV, loamy sand soil (GTS-PF 0-6"; 17-4020; 87% sand 6% silt 7% clay, pH 7.0, 3.0% organic carbon) and clay soil (LAD-SCL-PF 0-6"; 17-4021; 33% sand 26% silt 41% clay, pH 8.4, 1.8% organic carbon) were obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture characterization; p. 14; Table 2, p. 23 of MRID 50488802).
5 In the ILV, the method for oxyfluorfen and its isomers was validated in the second trial with insignificant modifications of a few equipment substitutions in the sample processing procedure and the use of a different GC/MS system and GC liner (pp. 13-16, 18-20; Appendix II, pp. 130-138 of MRID 50488802). In the communications with the Sponsor, the reviewer noted that the first trial appeared to have failed due to not filtering matrix-matched standards and the required changing of the GC liner and accompanying O-ring and inlet septa between sets.
6 Based on Figure 24, p. 83, Figure 25, p. 87 and Figure 26, p. 91 of MRID 50488802.
Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. In the ILV, the linearity of the method for all analytes in loamy sand matrix was not supported by the linear regression correlation coefficients. Linear regression coefficients (r^2) for the quantitation ions ranged 0.99385-0.99437 for all four analytes; linear regression coefficients for the C1 and C2 ions ranged 0.99052-0.99494 for all analytes, except for the C2 ion of RH-34672 ($r^2 = 0.99566$). Linearity is satisfactory when $r^2 \geq 0.995$.

2. The matrix effects were found to be significant (>20%) for oxyfluorfen, RH-34672, RH-42382 and RH-50671 in the ECM and ILV (pp. 21-22; Tables 66-69, pp. 78-79 of MRID 50488801; p. 19; Tables 68-78, pp. 48-51 of MRID 50488802). Matrix-matched standards were used in the ECM and ILV.

3. In the ILV, the specificity of the method for RH-50671 in loamy sand matrix was not well-supported by representative chromatograms since matrix interferences were *ca*. 25% of the LOQ (*ca*. 51% of the LOD; based on peak area; Figure 24, p. 83, Figure 25, p. 87 and Figure 26, p. 91 of MRID 50488802).

4. The determinations of LOD and LOQ in the ILV were not based on procedures as defined in 40 CFR Part 136 (Tables 63-66, pp. 46-47 of MRID 50488802). The LOD and LOQ for determination of oxyfluorfen, RH-34672, RH-42382, and RH-50671 in soil were calculated using the standard deviation from the 0.010 mg/kg recovery results. The LOD was calculated as the standard deviation multiplied by the t-statistic (3.747) and the LOQ was calculated as three times the LOD result.

5. The ILV study author reported the communications between the ILV and Study Sponsor occurred (p. 19; Appendix II, pp. 130-138 of MRID 50488802). The ILV reported that

communication was necessary; however, the communication mainly involved the delay in receipt of the ECM, the delay in receipt of the GC liner, and the final ILV report compliance statements. The ILV critical steps of filtering the matrix-matched standards and replacing the GC liner were also discussed. The ILV did not require technical assistance other than clarifications and had not direct contact with ECM personnel. The reviewer determined that no collusion occurred.

6. It was reported for the ILV that one sample set (6 calibration samples, 1 reagent blank, 1 control, 11 fortified controls) required *ca*. 20.5 hours (*ca*. 1 business day) with GC/MS performed unattended overnight (p. 16 of MRID 50488802). Evaluation of the GC/MS results was performed the next day.

7. The ECM study author noted that matrix-matched calibration solutions should be prepared on the same day as sample extract to compensate for solvent evaporation which may occur after injection (p. 20 of MRID 50488801).

8. In the ECM, the final sample extracts were found to be stable after at least 8 days of frozen storage (pp. 19-21; Tables 54-69, pp. 70-79 of MRID 50488801). Fortification and stock solutions were found to be stable after at least 12 days of frozen storage.

V. References

Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* 1983, *55*, 2210-2218 (p. 17 of MRID 50488801).

U.S. Environmental Protection Agency (USEPA). 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.

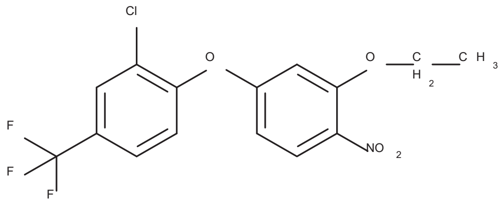
USEPA. 2014. Registration Review Problem Formulation for Oxyfluorfen. DP barcode 419419. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Environmental Fate and Effects Division. Memorandum to the Pesticide Re-Evaluation Division. Nov. 3, 2014.

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

Attachment 1: Chemical Names and Structures (Table 1, p. 25 of ECM)

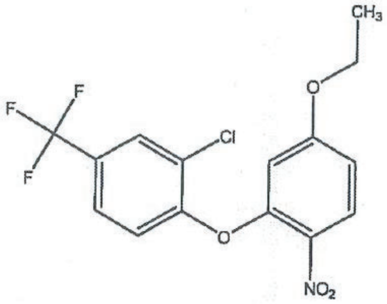
Oxyfluorfen (XDE-795)

IUPAC Name:	2-Chloro-α,α,α-trifluoro-p-tolyl 3-ethoxy-4-nitrophenyl ether
CAS Name:	2-Chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene
CAS Number:	42874-03-3
SMILES String:	Clc1cc(C(F)(F)F)ccc1Oc2cc(OCC)c(N(=O)(=O))cc2



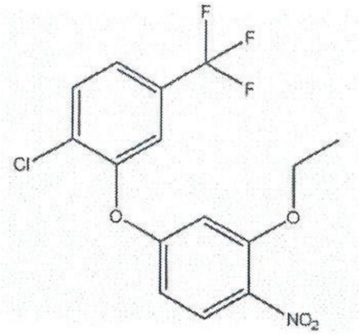
RH-34672

IUPAC Name:	2-Chloro-5’-ethoxy-2’-nitro-4-trifluoro-methyldiphenyl ether
CAS Name:	Not reported
CAS Number:	Not known
SMILES String:	Not found



RH-42382

IUPAC Name: 2-Chloro-1-(3-ethoxy-4-nitrophenoxy)-5-(trifluoromethyl)benzene
CAS Name: Not reported
CAS Number: Not known
SMILES String: Not found



RH-50671

IUPAC Name: 1-(2-Chloro-4(trifluoromethyl)phenoxy)-3-ethoxy-2-nitrobenzene
CAS Name: Not reported
CAS Number: Not known
SMILES String: Not found