Analytical method for pyrifluquinazon and its metabolites IV-01, IV-02, IV-15, IV-27, IV-28, and IV-203 in soil

Reports:	ECM: EPA MRID No.: 50331001. Chickering, C. 2017. Environmental Chemistry Method for the Determination of Pyrifluquinazon and Relevant Metabolites in Soil. EAG Study No. 85916. Report prepared by Analytical Bio-Chemistry Laboratories, Inc., a wholly owned subsidiary of EAG, Inc., Columbia, Missouri, and sponsored and submitted by Nichino America, Inc., Wilmington, Delaware; 151 pages. Final report issued July 19, 2017. ILV: EPA MRID No. 49928710. Grant, J. 2013. PYRIFLUQUINAZON AND METABOLITES - INDEPENDENT LABORATORY VALIDATION OF MORSE LABORATORIES' METHOD METH-203 ENTITLED "DETERMINATION OF PYRIFLUQUINAZON AND RELEVANT METBAOLITES IN SOIL". ABC Laboratories Study No. 69534. Report prepared by ABC Laboratories, Inc., Columbia, Missouri, sponsored by Nihon Nohyaku Co., Ltd., Tokyo, Japan, and submitted by Nichino America, Inc., Wilmington, Delaware; 116 pages. Final report issued June 6, 2013.
Document No.:	MRIDs 50331001 & 49928710
Guideline:	850.6100
Statements:	ECM: The report was a summary of the Terrestrial Field Dissipation (TFD) Study No. ML10-1625-NAI which was conducted in accordance with USEPA FIFRA (40 CFR Part 160) and OECD Good Laboratory Practices (GLP) standards (p. 3). Signed and dated No Data Confidentiality, GLP, and Authenticity statements were provided (pp. 2-4). A Quality Assurance statement was not provided. ILV: The study was conducted in accordance with USEPA FIFRA (40 CFR Part 160) and OECD GLP standards (p. 3). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-5).
Classification:	This analytical method is classified as unacceptable. For ECM, no samples were prepared at 10×LOQ. For ILV, it could not be determined if the ILV was performed independently because direct contact between the ILV study author of ABC Laboratories, Inc., and ECM study author of Morse Laboratories, LLC, occurred.
PC Code:	555555
Final EPA Reviewer:	James Lin Environmental Engineer **Signature:** **Date:** 12/04/2018
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist Signature: Date: 08/30/2018
	Joan Gaidos, Ph.D., Environmental Scientist Signature:

Date: 09/14/2018

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

This analytical method, Morse Laboratories Analytical Method No. Meth-203, is designed for the quantitative determination of pyrifluquinazon and its metabolites IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203 at the LOQ of 0.01 mg/kg (ppm) in soil matrices using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in soil for pyrifluquinazon, IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203. In the method, two or four ion transitions were used for identification, but only one ion transition was used for quantification in the ECM and ILV. The ECM validated the method using four uncharacterized soil matrices collected from locations in a terrestrial field dissipation study. The ILV validated the ECM method in the first trial using characterized loamy sand soil with insignificant modifications to standard preparation and analytical procedure. However, **it could not be determined if the ILV was performed independently** because direct contact between the ILV study author of ABC Laboratories, Inc., and ECM study author of Morse Laboratories, LLC, occurred. 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. All ILV data regarding precision, accuracy, linearity, and specificity was acceptable for all analytes. All ECM data regarding precision and accuracy at the LOQ was deemed acceptable for all analytes/matrices based on recovery ranges and overall statistics since statistics for each fortification were not available; no samples were prepared at 10×LOQ. All ECM data regarding linearity and specificity was acceptable. The LOD for the method was not reported in the ILV.

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						
Pyrifluquinazon	50331001[1,2]	49928710[3]		Soil	09/05/2014 (Original Report)[1] 19/07/2017 (Summary Report)	Nichino America, Inc.	LC/MS/MS	0.01 mg/kg
IV-01								
IV-02								
IV-15								
IV-27								
IV-28								
IV-203								

1 The ECM MRID 50331001 was a summary report of validation data generated in September 2010, from the study entitled Terrestrial Field Dissipation of Pyrifluquinazon and its Metabolites Following Three Applications of NNI-0101 to Bare Soil, Morse Laboratories Laboratory Project ID: ML10-1625-NAI; Morse Laboratories, LLC/ABC Laboratories, Inc. (Rees, S., May 9, 2014; p. 6 of MRID 50331001).

2 In the ECM, soil matrices were collected from four locations in a terrestrial field dissipation study: New York, Florida, California, and Washington (p. 6 of MRID 50331001). Soil descriptions and characterization were not included in the study report.

3 In the ILV, loamy sand soil (81% sand, 8% silt, 11% clay; pH 6.0 in 1:1 water:soil; organic matter 1.7%) was well characterized (p. 16 of MRID 49928710). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil was provided by the laboratory; source was not further specified.

I. Principle of the Method

Samples (10.0 g) of frozen soil in a 250-mL HDPE centrifuge bottle were fortified, if necessary, and kept in the frozen state in dry-ice until extraction (pp. 7, 10-12, 19-21; Appendix I, p. 26 of MRID 50331001). To the frozen sample, 120 mL of refrigerated neutral extraction solvent [acetonitrile:sodium ascorbate buffer (4:1, v:v)] was added. After capping the bottle, the mixture was shaken on a Wrist-Action shaker (at full speed) for 15 minutes. After centrifugation (*ca*. 30 minutes at *ca*. 2500 rpm), the supernatant was decanted through a glass funnel containing a loosely-packed plug of glass wool into a 250-mL mixing cylinder. The extraction was repeated with another 120 mL of refrigerated neutral extraction solvent. The volume of the combined extract was adjusted to 250 mL with the neutral extraction solvent. An aliquot (2.0 mL) of the combined extract was combined with 8.0 mL of de-ionized water in a 15-mL polypropylene centrifuge tube. The diluted extract was applied to an Oasis HLB solid phase extraction (SPE) cartridge (size 60 mg, 3 mL) which was pre-conditioned with 2 mL of acetonitrile and 3 x 2 mL deionized water (flow rate of *ca*. 2 mL/minute). The cartridge was not allowed to become dry between eluates. After the diluted extract was passed through the column under vacuum, the sample tube was rinsed with 2 mL of deionized water which was passed through the column to the level of the top frit under vacuum. The column was washed with 1 mL acetonitrile:water (5:95, v:v) and dried for 1 minute under vacuum. The analytes were eluted with 1.0 mL of HPLC acetonitrile; the column was allowed to go dry using vacuum. The volume of the eluate was adjusted to 2.0 mL with HPLC methanol. The final sample was mixed prior to LC/MS/MS analysis.

The quality control procedure for the Oasis HLB SPE cartridge was provided (Appendix II, p. 27 of MRID 50331001).

Samples were analyzed for pyrifluquinazon, IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203 using a Shimadzu LC-2AD HPLC coupled with an Applied Biosystems API4000 MS (Phenomenex Luna C(18)-HST, 2.0 mm x 100 mm, 2.5 µ column; column temperature 40°C) using a mobile phase of (A) 0.1% formic acid in HPLC grade water and (B) acetonitrile [percent A:B at 0.0-0.5 min. 90:10, 1.0 min. 75:25, 3.5 min. 55:45, 9.0 min. 40:60, 9.51-11.5 min. 0:100, 11.51-14.51 min. 90:10] and multiple reaction monitoring (MRM; pp. 10-12, 21-23 of MRID 50331001). Injection volume was 10 µL. MS/MS using Turbo Ion Spray (TIS, 650°C) detection in positive ion mode for pyrifluquinazon, IV-01, IV-02, IV-15, IV-27 and IV-28, and negative ion mode for IV-203 (switch to negative ion mode at *ca*. 7.5 minutes). Analytes, except for IV-203, were identified using two ion transitions; one for quantitation (Q) and one for confirmation (C). Ion transitions monitored were as follows: *m/z* 465.2→423.2 (Q) and *m/z* 465.2→92.3 (C) for pyrifluquinazon, *m/z* 423.2→106.9 (Q) and *m/z* 423.2→92.3 (C) for IV-01, *m/z* 421.0→104.9 (Q) and *m/z* 421.0→107.2 (C) for IV-02, *m/z* 437.0→93.1 (Q) and *m/z* 437.0→107.1 (C) for IV-15, *m/z* 438.6→421.0 (Q) and *m/z* 438.6→107.0 (C) for IV-27, and *m/z* 437.0→104.5 (Q) and *m/z* 437.0→92.2 (C) for IV-28. IV-203 was identified using four ion transitions: one for quantitation (Q) and three for confirmation (C). Ion transitions monitored were as follows: *m/z* 329.0→309.0 (Q), *m/z* 329.0→240.0 (C1), *m/z* 329.0→289.0 (C2) and *m/z* 329.0→268.9 (C3) for IV-203. Approximate retention times were 7.0, 6.0, 7.2, 5.7, 5.3, 6.8, and 8.0 minutes for pyrifluquinazon, IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203, respectively.

In the ILV, the ECM method was performed as written, except that the Wrist-Action shaker was substituted with a platform shaker run at high speed (pp. 17-19 of MRID 49928710). The ILV analytical instrument for pyrifluquinazon, IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203 was an Applied Biosystems/Sciex API 4000 MS with a mobile phase of (A) 0.1% formic acid in HPLC grade water and (B) acetonitrile [percent A:B at 0.0-0.5 min. 90:10, 1.0 min. 75:25, 3.0 min. 55:45, 8.5-9.0 min. 40:60, 9.1-11.0 min. 0:100, 11.01-16.0 min. 90:10] employing turbo ion spray (TIS) interface (switched from positive to negative ion mode at 10 min.). All other analytical parameters were the same as those in the ECM. Approximate retention times were 9.5, 8.4, 9.7, 8.0, 7.6, 9.3, and 10.4 minutes for pyrifluquinazon, IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203, respectively. Analytes were identified using two ion transitions; one for quantitation (Q) and one for confirmation (C). Ion transitions monitored were as follows: *m/z* 465→423 (Q) and *m/z* 465→92 (C) for pyrifluquinazon, *m/z* 423→107 (Q) and *m/z* 423→93 (C) for IV-01, *m/z* 421→104.8 (Q) and *m/z* 421→77.6 (C) for IV-02, *m/z* 437→93 (Q) and *m/z* 437→107 (C) for IV-15, *m/z* 439→421 (Q) and *m/z* 439→106.9 (C) for IV-27, *m/z* 437→104.8 (Q) and *m/z* 437→91.7 (C) for IV-28, and *m/z* 329→309 (Q) and *m/z* 329→240 (C) for IV-203.

In ECM and ILV, the Limit of Quantification (LOQ) for pyrifluquinazon, IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203 in soil matrices were reported as 0.10 mg/kg (ppm; pp. 7-8 of MRID 50331001; p. 10 of MRID 49928710). The Limit of Detection (LOD) was calculated as 0.00106-0.00369 mg/kg for all analytes in the ECM. The LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 50331001): ECM 50331001 was a summary of validation data generated in a terrestrial field dissipation study using Morse Laboratories Analytical Method No. Meth-203 (p. 6). Recovery ranges were within 70-115% for analysis of pyrifluquinazon and metabolites IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203 in four soil matrices at fortification levels of 0.01 mg/kg (LOQ) and 0.50 mg/kg (50×LOQ; pp. 6, 9-10). Mean recoveries and relative standard deviations (RSDs) for each fortification level could not be assessed for compliance with guideline requirements (mean 70-120%; RSD ≤20%) since individual recovery values were not reported. Overall statistics (0.01 and 0.5 mg/kg fortifications) for each analyte in each matrix were within guideline requirements. No samples were prepared at 10×LOQ. Pyrifluquinazon and its metabolites were identified via LC/MS/MS using at least two ion transitions; however, recovery results were only reported for the quantitation ion transition. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method. The soil matrices were collected from four locations in a terrestrial field dissipation study: New York, Florida, California, and Washington (p. 6). Soil descriptions and characterization were not included in the study report.

ILV (MRID 49928710): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of pyrifluquinazon and metabolites IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203 in loamy sand soil at fortification levels of 0.01 mg/kg (LOQ) and 0.10 mg/kg (10×LOQ; pp. 11, 23 and Table 1, pp. 26-27). Pyrifluquinazon and its metabolites were identified via LC/MS/MS using two ion transitions; however, recovery results were only reported for the quantitation ion transition. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method. The loamy sand soil (81% sand, 8% silt, 11% clay; pH 6.0 in 1:1 water:soil; organic matter 1.7%) was well characterized (p. 16). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil was provided by the laboratory; source was not further specified. The ILV validated the method in the first trial with insignificant modifications to standard preparation and analytical procedure (pp. 10, 18).

Table 2. Initial Validation Method Recoveries for Pyrifluquinazon and Its Metabolites IV-01, IV-02, IV-15, IV-27, IV-28, and IV-203 in Soil[1,2,3]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Soil (New York)					
	Quantitation ion[4]					
Pyrifluquinazon	0.01 (LOQ)	5	90-101	--[5]	--	--
	0.5	5	89-97	--	--	--
	Overall	10	89-101	94	4.0	4.3
IV-01	0.01 (LOQ)	5	87-96	--	--	--
	0.5	5	89-96	--	--	--
	Overall	10	87-96	91	3.2	3.5
IV-02	0.01 (LOQ)	5	81-91	--	--	--
	0.5	5	88-94	--	--	--
	Overall	10	81-94	88	3.9	4.4
IV-15	0.01 (LOQ)	5	89-98	--	--	--
	0.5	5	92-98	--	--	--
	Overall	10	89-98	94	3.0	3.2
IV-27	0.01 (LOQ)	5	70-79	--	--	--
	0.5	5	70-76	--	--	--
	Overall	10	70-79	75	3.8	5.1
IV-28	0.01 (LOQ)	5	74-82	--	--	--
	0.5	5	76-87	--	--	--
	Overall	10	74-87	80	4.4	5.5
IV-203	0.01 (LOQ)	5	86-93	--	--	--
	0.5	5	91-102	--	--	--
	Overall	10	86-102	92	5.2	5.7
	Soil (Florida)					
	Quantitation ion[4]					
Pyrifluquinazon	0.01 (LOQ)	5	93-100			
	0.5	5	85-106	--	--	--
	Overall	10	85-106	96	6.0	6.3
IV-01	0.01 (LOQ)	5	91-102	--	--	--
	0.5	5	79-107	--	--	--
	Overall	10	79-107	94	7.8	8.3
IV-02	0.01 (LOQ)	5	85-94	--	--	--
	0.5	5	75-99	--	--	--
	Overall	10	75-99	87	6.6	7.6
IV-15	0.01 (LOQ)	5	88-97	--	--	--
	0.5	5	77-99	--	--	--
	Overall	10	77-99	90	6.8	7.6

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
IV-27	0.01 (LOQ)	5	72-95	--	--	--
	0.5	5	72-94	--	--	--
	Overall	10	72-94	84	7.7	9.2
IV-28	0.01 (LOQ)	5	85-94	--	--	--
	0.5	5	76-97	--	--	--
	Overall	10	76-97	87	6.6	7.6
IV-203	0.01 (LOQ)	5	91-105	--	--	--
	0.5	5	89-114	--	--	--
	Overall	10	89-114	99	8.4	8.5
	Soil (California)					
	Quantitation ion[4]					
Pyrifluquinazon	0.01 (LOQ)	5	86-100	--	--	--
	0.5	5	83-94	--	--	--
	Overall	10	83-100	91	5.1	5.6
IV-01	0.01 (LOQ)	5	85-97	--	--	--
	0.5	5	81-92	--	--	--
	Overall	10	81-97	90	4.5	5.0
IV-02	0.01 (LOQ)	5	78-93	--	--	--
	0.5	5	77-89	--	--	--
	Overall	10	77-93	85	4.8	5.6
IV-15	0.01 (LOQ)	5	87-95	--	--	--
	0.5	5	78-93	--	--	--
	Overall	10	78-95	89	4.8	5.4
IV-27	0.01 (LOQ)	5	70-89	--	--	--
	0.5	5	70-90	--	--	--
	Overall	10	70-90	81	7.4	9.1
IV-28	0.01 (LOQ)	5	73-98	--	--	--
	0.5	5	74-98	--	--	--
	Overall	10	73-98	86	8.7	10.1
IV-203	0.01 (LOQ)	5	84-98	--	--	--
	0.5	5	81-103	--	--	--
	Overall	10	81-103	92	6.5	7.1
	Soil (Washington)					
	Quantitation ion[4]					
Pyrifluquinazon	0.01 (LOQ)	5	102-112	--	--	--
	0.5	5	100-104	--	--	--
	Overall	10	100-112	104	4.0	3.8
IV-01	0.01 (LOQ)	5	106-115	--	--	--
	0.5	5	95-103	--	--	--
	Overall	10	95-115	105	5.5	5.2

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
IV-02	0.01 (LOQ)	5	93-104	--	--	--
	0.5	5	94-99	--	--	--
	Overall	10	93-104	97	3.8	3.9
IV-15	0.01 (LOQ)	5	90-105	--	--	--
	0.5	5	96-102	--	--	--
	Overall	10	90-105	98	4.0	4.1
IV-27	0.01 (LOQ)	5	96-110	--	--	--
	0.5	5	92-95	--	--	--
	Overall	10	92-110	98	5.8	5.9
IV-28	0.01 (LOQ)	5	82-93	--	--	--
	0.5	5	81-89	--	--	--
	Overall	10	81-93	86	4.1	4.8
IV-203	0.01 (LOQ)	5	104-115	--	--	--
	0.5	5	94-99	--	--	--
	Overall	10	94-115	103	7.3	7.1

Data (recoveries were corrected when residues were quantified in the controls, pp. 23-25) were obtained from pp. 6, 9-10 of MRID 50331001.

1 ECM 50331001 was a summary of validation data generated in Morse Laboratories Analytical Method No. Meth-203 (p. 6).

2 The soil matrices were collected from four locations in a terrestrial field dissipation study: New York, Florida, California, and Washington (p. 6). Soil descriptions and characterization were not included in the study report.

3 Analytes, except for IV-203, were identified using two ion transitions; one for quantitation (Q) and one for confirmation (C). Ion transitions monitored were as follows: *m/z* 465.2→423.2 (Q) and *m/z* 465.2→92.3 (C) for pyrifluquinazon, *m/z* 423.2→106.9 (Q) and *m/z* 423.2→92.3 (C) for IV-01, *m/z* 421.0→104.9 (Q) and *m/z* 421.0→107.2 (C) for IV-02, *m/z* 437.0→93.1 (Q) and *m/z* 437.0→107.1 (C) for IV-15, *m/z* 438.6→421.0 (Q) and *m/z* 438.6→107.0 (C) for IV-27, and *m/z* 437.0→104.5 (Q) and *m/z* 437.0→92.2 (C) for IV-28. IV-203 was identified using four ion transitions: one for quantitation (Q) and three for confirmation (C). Ion transitions monitored were as follows: *m/z* 329.0→309.0 (Q), *m/z* 329.0→240.0 (C1), *m/z* 329.0→289.0 (C2) and *m/z* 329.0→268.9 (C3) for IV-203.

4 Recovery results were only reported for the quantitation ion transition. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

5 Means, s.d., and RSDs for each fortification level were not reported in the study report. These values could not be reviewer-calculated since individual recovery values were not reported.

6 The study report provided overall statistics (0.01 and 0.5 mg/kg fortifications) for each analyte in each matrix. Overall statistics were included in the table to aid in data analysis.

Table 3. Independent Validation Method Recoveries for Pyrifluquinazon and Its Metabolites IV-01, IV-02, IV-15, IV-27, IV-28, and IV-203 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[4]					
Pyrifluquinazon	0.01 (LOQ)	5	85-97	91	4.16	4.58
	0.1	5	85-102	94	7.16	7.59
IV-01	0.01 (LOQ)	5	81-98	90	6.78	7.53
	0.1	5	86-103	96	6.66	6.92
IV-02	0.01 (LOQ)	5	79-94	87	5.43	6.24
	0.1	5	79-92	89	5.75	6.49
IV-15	0.01 (LOQ)	5	85-99	90	5.86	6.49
	0.1	5	83-99	92	6.55	7.08
IV-27	0.01 (LOQ)	5	87-94	90	3.07	3.40
	0.1	5	82-101	92	7.44	8.12
IV-28	0.01 (LOQ)	5	88-91	89	1.44	1.62
	0.1	5	80-93	88	5.54	6.27
IV-203	0.01 (LOQ)	5	93-108	100	7.01	6.97
	0.1	5	87-104	98	6.85	7.01

Data (uncorrected recovery results, pp. 20-21) were obtained from pp. 11, 23 and Table 1, pp. 26-27 of MRID 49928710.

1 The loamy sand soil (81% sand, 8% silt, 11% clay; pH 6.0 in 1:1 water:soil; organic matter 1.7%) was well characterized (p. 16 of MRID 49928710). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil was provided by the laboratory; source was not further specified.

2 Ion transitions monitored were as follows [quantitation (Q) and one for confirmation (C)]: *m/z* 465→423 (Q) and *m/z* 465→92 (C) for pyrifluquinazon, *m/z* 423→107 (Q) and *m/z* 423→93 (C) for IV-01, *m/z* 421→104.8 (Q) and *m/z* 421→77.6 (C) for IV-02, *m/z* 437→93 (Q) and *m/z* 437→107 (C) for IV-15, *m/z* 439→421 (Q) and *m/z* 439→106.9 (C) for IV-27, *m/z* 437→104.8 (Q) and *m/z* 437→91.7 (C) for IV-28, and *m/z* 329→309 (Q) and *m/z* 329→240 (C) for IV-203 (p. 19).

3 Results were reported from the first trial (p. 10).

4 Recovery results were only reported for the quantitation ion transition. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

III. Method Characteristics

In ECM and ILV, the LOQ for or pyrifluquinazon, IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203 in soil matrices were reported as 0.10 mg/kg (ppm; pp. 7-8 of MRID 50331001; p. 10 of MRID 49928710). In the ECM, the LOQ was defined as the lowest concurrent fortification successfully recovered; no justification was provided in the ILV. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV**.** In the ECM, the LOD was calculated for each matrix using the following equation:

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

Where, $t_{0.99}$ is the one-tailed t-test at 99% confidence for n-1 degrees of freedom (n = 5, the number of replicates) and SD is the standard deviation of the analyte recovery measurements at the target LOQ. The LOD was calculated as 0.00106-0.00369 mg/kg for all analytes in the ECM (see below for details). The LOD was not reported in the ILV.

Table 4. Method Characteristics

Analyte		Pyrifluquinazon	IV-01	IV-02	IV-15	IV-27	IV-28	IV-203
Limit of Quantitation (LOQ)	ECM	0.01 mg/kg						
	ILV							
Limit of Detection (LOD)	ECM	0.00125-0.00213 mg/kg	0.00131-0.00175 mg/kg	0.00138-0.00205 mg/kg	0.00141-0.00209 mg/kg	0.00144-0.00270 mg/kg	0.00140-0.00369 mg/kg	0.00106-0.00217 mg/kg
	ILV	**Not** reported						
Linearity (calibration curve r^2 and concentration range)[1,2]	ECM	r^2 = 1.0000 (Q)	r^2 = 0.9998 (Q)	r^2 = 0.9998 (Q)	r^2 = 1.0000 (Q)	r^2 = 0.9994 (Q)	r^2 = 1.0000 (Q)	r^2 = 1.0000 (Q)
	ILV	r^2 = 0.9986 (Q)	r^2 = 0.9986 (Q)	r^2 = 0.9994 (Q)	r^2 = 0.9990 (Q)	r^2 = 0.9990 (Q)	r^2 = 0.9998 (Q)	r^2 = 0.9994 (Q)
	Range	0.2-10 ng/mL						
Repeatable[1]	ECM[3,4,5]	Recovery ranges were within 70-115% at LOQ and 50×LOQ. Yes for Overall Statistics at LOQ and 50×LOQ for each analyte/matrix. **No** samples were prepared at 10×LOQ.						
	ILV[6,7]	Yes at LOQ and 10×LOQ.						
Reproducible		Yes at LOQ. **Could not be determined at 10×LOQ.**						
Specific	ECM	Yes, no matrix interferences were noted.						Yes, matrix interferences were *ca*. 2% of the LOQ (based on peak area).
	ILV	Yes, no matrix interferences were noted. Minor baseline noise was observed in some chromatograms.						

Data were obtained from pp. 7-8 (LOD/LOQ); pp. 6, 9-10 (recovery data); Figure 1, pp. 29-35 (calibration curves); Figures 3-14, pp. 43-126 (chromatograms) of MRID 50331001 (ECM); pp. 10-11, 23; Table 1, pp. 26-27 (recovery data); Figure 4-10, pp. 40-46 (calibration curves); Figures 11-13, pp. 47-58 (chromatograms) of MRID 49928710 (ILV); DER Attachment 2. Q = quantitation ion.

1 Two or four ion transitions were monitored for each analyte; however, recovery results were only reported for the quantitation ion transition. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

2 Calibration coefficients (r^2) were reviewer-calculated from r values provided in the study report (DER Attachment 2).

3 ECM MRID 50331001 was a summary report of validation data generated in a terrestrial field dissipation study using Morse Laboratories Analytical Method No. Meth-203 (p. 6 of MRID 50331001).

4 In the ECM, mean recoveries and relative standard deviations (RSDs) for each fortification level could not be assessed for compliance with guideline requirements (mean 70-120%; RSD ≤20%) since individual recovery values were not reported.

5 In the ECM, soil matrices were collected from four locations in a terrestrial field dissipation study: New York, Florida, California, and Washington (p. 6 of MRID 50331001). Soil descriptions and characterization were not included in the study report.

6 In the ILV, loamy sand soil (81% sand, 8% silt, 11% clay; pH 6.0 in 1:1 water:soil; organic matter 1.7%) was well characterized (p. 16 of MRID 49928710). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil was provided by the laboratory; source was not further specified. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method.

7 Results were reported from the first trial with insignificant modifications to standard preparation and analytical procedure (pp. 10, 18 of MRID 49928710).

IV. Method Deficiencies and Reviewer's Comments

1. A ECM/ILV method validation set was previously submitted and reviewed for pyrifluquinazon and its metabolites in soil; however, the ECM which was validated by ILV MRID 49928710 was only provided in Appendix I of ECM MRID 49083717 (a terrestrial field dissipation study, TFD) and Appendix 1 of ILV MRID 49928710 (Appendix I, pp. 1125-1147 of MRID 49083717; Appendix 1, pp. 59-80 of MRID 49928710). However, both presentations of Morse Laboratories Analytical Method No. Meth-203 were only a method. No performance data for Morse Laboratories Analytical Method No. Meth-203 was provided. No soil matrices were specified or characterized. Only Volume 3 of 4 of MRID 49083717 was provided; no Table of Contents for the ECM submission was included in the MRID. The DER for MRIDs 49083717 & 49928710 was written by CDM Smith/CSS JV Primary Reviewer Lisa Muto, with CDM Smith/CSS JV Secondary Reviewer Kathleen Ferguson. The reviewer determined that an updated, complete ECM/ILV method validation set should be submitted for pyrifluquinazon or pyrifluquinazon and its metabolites in soil.

   For this review, ECM data was replaced, and ILV was verified and edited, as needed.

2. The ECM MRID 50331001 was a summary report of validation data generated in September 2010, from the study entitled Terrestrial Field Dissipation of Pyrifluquinazon and its Metabolites Following Three Applications of NNI-0101 to Bare Soil, Morse Laboratories Laboratory Project ID: ML10-1625-NAI; Morse Laboratories, LLC/ABC Laboratories, Inc. (Rees, S., May 9, 2014; p. 6 of MRID 50331001).

3. It could not be determined if the ILV was performed independently due to the fact that direct contact between the ILV study author and ECM study author occurred. In the ILV, communications between the ILV and Sponsor Representative (Ken Chisholm of Nichino) were summarized and detailed in the study (pp. 10, 22; Appendix 4, pp. 102-115 of MRID 49928710). Communication regarded chromatography issues, clarification of the optimization procedures for the LC/MS/MS as they pertain to the HPLC column and analyte retention, and recovery updates between the Sponsor Representative and Study Director. Direct contact between the ILV study author (J. Grant) and the ECM study author of Morse Laboratories, LLC, (S. Rees) occurred via email and phone to discuss ILV difficulties with the LC/MS/MS analysis and clarify the ECM analytical procedure. More details of this communication are necessary to determine if collusion occurred. The reviewer noted that Sponsor Representative told the ECM study author that three contacts to the ECM were allowed and that some portion of the communication between the ECM and ILV appeared to be missing in the email dated March 18, 2013 from J. Grant to S. Brown (Rees; pp. 106, 108-109).

   The reviewer noted that the ECM study author for MRID 50331001 was from the same laboratory as the ILV study author of MRID 49928710, ABC Laboratories, Inc., Columbia, Missouri; however, ECM MRID 50331001 was a summary of an ECM performed by Morse Laboratories, LLC (pp. 6, 25 of MRID 50331001; Appendix 1, p. 59 of MRID 49928710).

4. In the ECM, no samples were prepared at 10×LOQ (pp. 6, 9-10 of MRID 50331001). OCSPP guidelines state that a minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10×LOQ) for each analyte. The reproducibility of the method could not be determined at 10×LOQ since only one set of performance data was submitted for this fortification level.

5. 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. The ILV validated the method with one characterized soil (loamy sand soil) while the ECM validated the method with four uncharacterized soils from terrestrial field dissipation sites: New York, Florida, California, and Washington (p. 6 of MRID 50331001; p. 16 of MRID 49928710). The ILV soil could not be compared to the ECM soils due to lack of characterization data, and the ILV should have been performed using more than one soil with a range of compositions to validate the method for the soil which is most difficult to extract. Guideline 850.6100 says "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.

6. The estimation of LOQ in ECM and ILV was not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 7-8 of MRID 50331001; p. 10 of MRID 49928710). In the ECM, the LOQ was defined as the lowest fortification level where an acceptable mean recovery is obtained. In the ECM, the LOD was calculated for each analyte and matrix using the following equation: LOD = ($t_{0.99}$ x SD), where, $t_{0.99}$ is the one-tailed t-test at 99% confidence for n-1 degrees of freedom (n = 5, the number of replicates) and SD is the standard deviation of the analyte recovery measurements at the target LOQ. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV. Detection limits should not be based on arbitrary values. The LOD was not reported in the ILV.

7. ECM recoveries were corrected when residues were quantified in the controls (pp. 23-25 of MRID 50331001). Residues were only observed in the IV-203 chromatograms (ca. 2% of the LOQ, based on peak area; Figures 3-14, pp. 43-126).

8. The reviewer noted that the purity of the IV-27 standard was >90%, but significantly lower than the other standards, 91.5% (p. 16 of MRID 50331001; p. 15 of MRID 49928710).

9. The citation for the previously-submitted and reviewed ECM is provided below, taken from the previous DER:

   EPA MRID No.: 49083717 (Appendix I, pp. 1125-1147). Wyatt, D.R. (TFD) 2013. Terrestrial Field Dissipation of Pyrifluquinazon and its Metabolites Following Three Applications of NNI-0101 to Bare Soil. TFD Study No.: TCI-10-260. Morse Laboratories

Laboratory Project ID: ML10-1625-NAI. Morse Laboratories Analytical Method No.: Meth-203 (Original). TFD Testing Facility: The Carringers, Inc., Apex, North Carolina. Analytical Phase Report prepared by Morse Laboratories, LLC, Sacramento, California, sponsored by Nihon Nohyaku Co., Ltd., Tokyo, Japan, and submitted by Nichino America, Inc., Wilmington, Delaware; 428 pages (TFD Vol. 3 of 4), 22 pages (Analytical Method). TFD final report issued March 1, 2013; Analytical Method dated September 7, 2010 (p. 1126).

10. In the ILV, the matrix effects of the soil sample were evaluated to determine the soil control suitability; only control samples free of any interferences in the area of analyte elution (corresponding to analyte residue levels of <30% of the LOQ) were chosen for use in the study (pp. 21-22 of MRID 49928710).

11. It was reported for the ILV that one batch of thirteen samples required one workday (8 hours) with LC/MS/MS performed overnight (pp. 11, 24 of MRID 49928710).

12. The reviewer noted that Table 2 of the DER deviates from the DER template by the inclusion of Overall Statistics. Overall statistics were included only in this table of this DER to aid in data analysis.

V. References

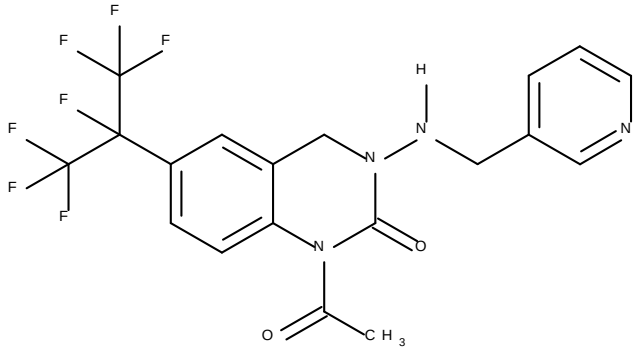
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

Attachment 1: Chemical Names and Structures

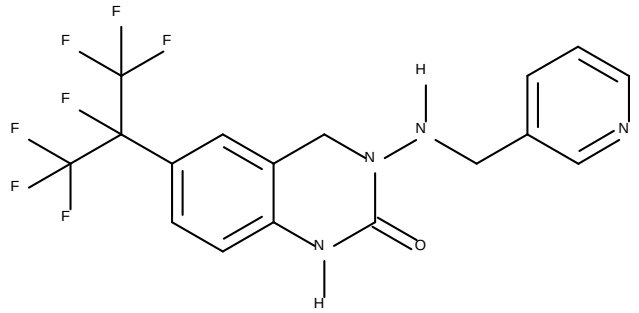
Pyrifluquinazon (NNI-0101)

IUPAC Name: 1-Acetyl-1,2,3,4-tetrahydro-3-[(3-pyridylmethyl)amino]-6-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]quinazolin-2-one
CAS Name: 1-Acetyl-3,4-dihydro-3-[(3-pyridinylmethyl)amino]-6-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]-2(1H)-quinazolinone
CAS Number: 337458-27-2
SMILES String: [H]N(Cc1cccnc1)N2Cc3cc(ccc3N(C2=O)C(=O)C)C(C(F)(F)F)(C(F)(F)F)F



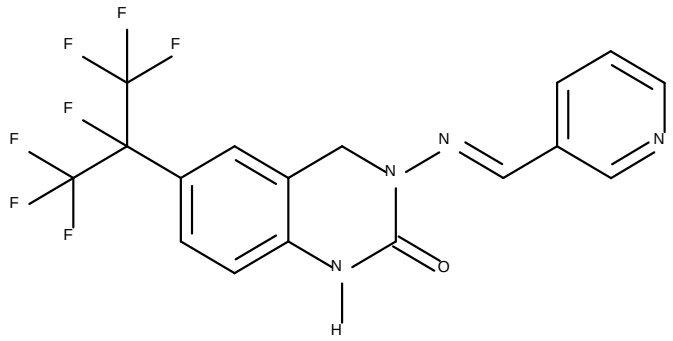
IV-01 (NNI-0101-1H)

IUPAC Name: 3-[(Pyridin-3-ylmethyl)amino]-6-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]-3,4-dihydro-1H-quinazolin-2-one
CAS Name: Not reported
CAS Number: Not reported
SMILES String: [H]N1c2ccc(cc2CN(C1=O)N([H])Cc3cccnc3)C(C(F)(F)F)(C(F)(F)F)F



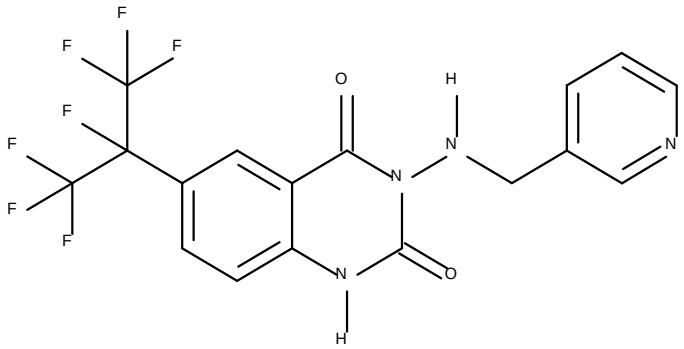
IV-02 (NNI-0101-1H-imino)

IUPAC Name: 3-[(Pyridin-3-ylmethylene)amino]-6-[l,2,2,2-tetrafluoro-l-(trifluoromethyl)ethyl]-3,4-dihydro-1H-quinazolin-2-one
CAS Name: Not reported
CAS Number: Not reported
SMILES String: [H]N1c2ccc(cc2CN(C1=O)/N=C/c3cccnc3)C(C(F)(F)F)(C(F)(F)F)F



IV-15 (NNI-0101-1H-4-oxo)

IUPAC Name: 3-[(Pyridin-3-ylmethyl)amino]-6-[l,2,2,2-tetrafluoro-l-(trifluoromethyl)ethyl]-1H-quinazolin-2,4-dione
CAS Name: Not reported
CAS Number: Not reported
SMILES String: [H]n1c2ccc(cc2c(=O)n(c1=O)N([H])Cc3cccnc3)C(C(F)(F)F)(C(F)(F)F)F



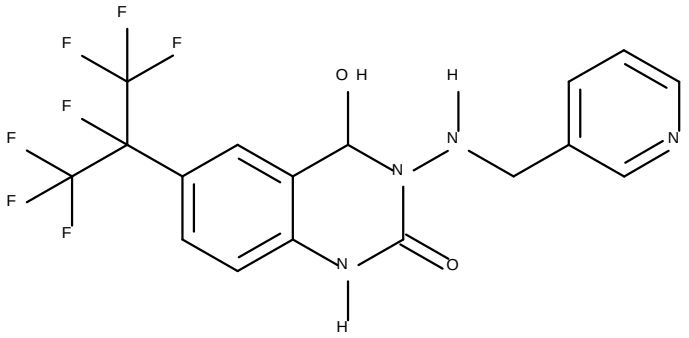
IV-27 (NNI-0101-1H-4-OH)

IUPAC Name: l,2,3,4-Tetrahydro-4-hydroxy-3-[(3-pyridylmethyl)amino]-6-[l,2,2,2-tetrafluoro-l-(trifluoromethyl)ethyl]quinazolin-2-one

CAS Name: Not reported

CAS Number: Not reported

SMILES String: [H]N1c2ccc(cc2C(N(C1=O)N([H])Cc3cccnc3)O)C(C(F)(F)F)(C(F)(F)F)F



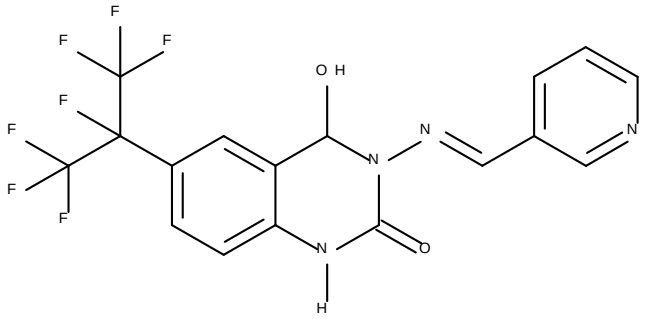
IV-28 (NNI-0101-1H-imino-4-OH)

IUPAC Name: 4-Hydroxy-3-[(3-pyridin-3-ylmethylene)amino]-6-[l,2,2,2-tetrafluoro-l-(trifluoromethyl)ethyl]-3,4-dihydro-lH-quinazolin-2-one

CAS Name: Not reported

CAS Number: Not reported

SMILES String: [H]N1c2ccc(cc2C(N(C1=O)/N=C/c3cccnc3)O)C(C(F)(F)F)(C(F)(F)F)F



IV-203 (NNI-0101-quinazolinedione)

IUPAC Name: 6-[1,2,2,2-Tetrafluoro-1-trifluoromethyl)ethyl]-1H-quinazolin-2,4-dione
CAS Name: Not reported
CAS Number: Not reported
SMILES String: [H]n1c2ccc(cc2c(=O)n(c1=O)[H])C(C(F)(F)F)(C(F)(F)F)F