

Analytical method for pyraclonil and its metabolites amidepyraclonil, M-1, and M-11 in soil and sediment

Reports: ECM: EPA MRID No. 50970989. Rodgers, C.A. 2019. Method Validation for the Determination of Residues of Pyraclonil and three metabolites (Amidepyraclonil, M-1 and M-11) in Soil, Soil-sediment, and Water by LC-MS/MS. EAG Study No.: 85901. Report prepared by Analytical Bio-Chemistry Laboratories, Inc. (a wholly owned subsidiary of EAG, Inc. is now Eurofins EAG Agrosience, LLC), Columbia, Missouri, and sponsored and submitted by Nichino America, Inc., Wilmington, Delaware; 263 pages. Final report issued January 23, 2019.

ILV: EPA MRID No. 50970990. Schoenau, E.A. 2019. Independent Laboratory Validation of the Residue Analytical Method for the Determination of Residues of Pyraclonil and Metabolites Amidepyraclonil, M-1, and M-11 in Soil, Sediment, and Water by LC-MS/MS (EAG Study Number: 85901). GPL Study No.: 180770. Report No.: PCL-W-1020. Report prepared by Golden Pacific Laboratories, LLC (GPL), Fresno, California, and sponsored and submitted by Nichino America, Inc., Wilmington, Delaware; 458 pages. Final report issued February 13, 2019.

Document No.: MRIDs 50970989 & 50970990

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practices (GLP; 40 CFR 160; p. 3 of MRID 50970989). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).
 ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 50970990). 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. Since the reported method LOQ were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest levels of method validation (LLMV) rather than LOQ. The ILV soil and sediment matrices were not characterized.

PC Code: 104502

EFED Final Reviewer: Taimei Harris, Ph.D. Signature:
 Chemist Date:

CDM/CSS-Dynamac JV Reviewers: Lisa Muto, M.S., Signature: *Lisa Muto*
 Environmental Scientist Date: 06/12/2020

Mary Samuel, M.S., Signature: *Mary Samuel*
 Environmental Scientist Date: 06/12/2020

EPA Reviewer: Katrina White, Ph.D.

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel. The CDM/CSS-Dynamac JV role does not include establishing Agency policies.

Executive Summary

This analytical method, EAG Study No. 85901, is designed for the quantitative determination of the pyraclonil and its metabolites amidepyraclonil, M-1, and M-11 in soil and sediment at the LOQ of 1.0 µg/kg using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in soil and sediment. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the reported method LOQ for pyraclonil, amidepyraclonil, M-1, and M-11 in soil and sediment. The ECM used two characterized soil/sediment matrices sourced from the California and Arkansas aquatic field dissipation study sites; the ILV used two uncharacterized soil/sediment matrices. The ILV validated the method in soil and sediment with a significant modification of the substitution of the filtration step with an additional centrifugation step and insignificant modifications of analytical instrumentation and equipment. However, the determinations of the LOQ and LOD in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. During the first analysis set in the ILV, a large chromatographic interference was observed for M-11 quantitation ion transition in all matrices which was determined as originating with the syringe filtration step. It was concluded that the filtration step was causing the low recoveries (ca. 25-90%) of each of the analytes at both fortification levels even when alternative filter types and sizes were evaluated. The validation of the method was successful with the second analysis set of the first trial samples incorporating the method modification. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for pyraclonil, amidepyraclonil, M-1, and M-11 in soil and sediment matrices after the change in the ILV method. In the ECM, three or four ion transitions were reported for monitoring and/or quantifying analytes; the ILV monitored ion transitions matched those of the ECM, except for the confirmation ion transition for pyraclonil in the California soil matrix. As the ILV had to change the method to achieve reliable recoveries, there are significant uncertainties with the reliability of the ECM.

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						
Pyraclonil	50970989 ^{1,2}	50970990 ^{2,3}	Taimei Harris	Soil and Sediment	23/01/2019	Nichino America, Inc.	LC/MS/MS	1.0 µg/kg
Amidepyraclonil								
M-1								
M-11								

¹ In the ECM, the soil/sediment matrices were obtained from Nichino aquatic field dissipation study sites at California (Study No. 85589; MRID 50970950) and Arkansas (Study No. 85590; MRID 50970949; p. 20 of MRID 50970989). The California sandy clay loam soil (Sample ID: 340221 SOIL 0-2^{''}; 53% sand, 26% silt, 21% clay; pH 7.1 in 1:1 soil:water ratio, 0.94% organic matter – Walkley-Black) and Arkansas clay soil-sediment (Sample ID: 340860 2-6^{''}; 14% sand, 38% silt, 48% clay; pH 6.8 in 1:1 soil:water ratio, 1.3% organic matter –

- Walkley-Black) were characterized by Agvise Laboratories (Northwood, North Dakota; Appendix VII, pp. 260, 262). Both soil/sediment classifications were USDA soil classification.
- 2 The ECM and ILV reported the method validation for soil, soil-sediment, and water matrices. This DER, designated with a file name of "soil", evaluated the soil/sediment portion, while the accompanying DER, designated with a file name of "water", evaluated the water portion.
 - 3 In the ILV, the soil and sediment matrices were not specified; however, it was reported that it was received from the EAG Laboratories, Inc. (a company related to the ECM) and sourced from Arkansas (soil) and California (sediment; p. 20 of MRID 50970990).

I. Principle of the Method

Soil/sediment samples (20.0 ± 0.10 mL) transferred into 250-mL polypropylene bottles and fortified with mixed fortification solutions, as necessary (pp. 19, 21 of MRID 50970989). Samples were extracted twice by shaking via mechanical shaker at low speed for *ca.* 20 minutes with 100 mL of acetonitrile:0.1M aqueous HCl (4:1, v:v), followed by centrifugation (*ca.* 3000 rpm for *ca.* 10 minutes), and transferred to a 250-mL graduated mixing cylinder. The volume of the combined extracts was adjusted to 200 mL using the extraction solvent. An aliquot (or diluted aliquot) was transferred to a 2-mL glass vial using a 0.2 μm , 25 mm polypropylene syringe filter then analyzed by UHPLC/MS/MS.

Samples were analyzed using a Shimadzu UPLC coupled to an Applied Biosystems/Sciex API 6500 Q-Trap MS (pp. 22-23 of MRID 50970989). The following LC conditions were used: Acquity UPLC HSS T3 column, 2.1 x 50 mm; 1.8 μm particle size; column temperature 40°C), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [percent A:B (v:v) at 0.50 min. 95:5, 5.00-6.00 min. 5:95, 6.01-7.00 min. 95:5], and injection volume of 10 μL . The following MS/MS conditions were used: positive mode (source temperature 600°C), Turbo Ion Spray (TIS) interface, and multiple reaction monitoring (MRM). Analytes were identified using three or four ion pair transitions (quantitation, confirmation 1, confirmation 2, and confirmation 3, respectively): m/z 315→169, m/z 315→241, and m/z 315→99 for pyraclonil, m/z 333→253, m/z 333→316, and m/z 333→288 for amidepyraclonil, m/z 277→250, m/z 277→214, m/z 277→182, and m/z 277→185 for M-1, and m/z 317→169, m/z 317→241, and m/z 317→275 for M-11. Expected retention time were *ca.* 3.43, 2.80, 3.21, and 3.62 minutes for pyraclonil, amidepyraclonil, M-1, and M-11, respectively.

The independent laboratory performed the ECM as written, except for a significant modification of the substitution of the filtration step with centrifugation (14000 rpm for 5 minutes) step and insignificant modifications of analytical instrumentation and equipment (pp. 15, 19-24; Appendix C, p. 83 of MRID 50970990). A Sciex Triple Quad 6500+ LC-MS/MS coupled with a Shimadzu LC-20AD HPLC was used. All LC and MS parameters were generally the same as the ECM, except that a Waters Acquity UPLC HSS T3 VanGuard TM column (2.1 x 5 mm; 1.8 μm particle size) was used and MS temperature was 500°C. Analytes were identified using only two ion pair transitions (quantitation and confirmation, respectively): m/z 315.2→169.2 and m/z 315.2→241.2 for pyraclonil, m/z 333.1→253.2 and m/z 333.1→316.1 for amidepyraclonil, m/z 277.2→250.2 and m/z 277.2→214.2 for M-1, and m/z 317.1→169.0 and m/z 317.1→241.1 for M-11. The monitored ion transitions were the same as the monitored quantitation and confirmation ions of the ECM, except for pyraclonil in the California matrix (for which the confirmation 2 ion was used for confirmation in the ECM). Expected retention time were *ca.* 3.8, 3.2, 3.6, and 3.9 minutes for pyraclonil, amidepyraclonil, M-1, and M-11, respectively.

The Limit of Quantification (LOQ) for pyraclonil, amidepyraclonil, M-1, and M-11 was reported as 1.0 µg/kg in soil/sediment in the ECM and ILV (pp. 12, 28 of MRID 50970989; pp. 19, 33 of MRID 50970990). In the ECM, the Limits of Detection (LODs) were calculated as 0.0847, 0.361, 0.203, and 0.112 µg/kg for pyraclonil, amidepyraclonil, M-1, and M-11, respectively, for California Soil, and was 0.124, 0.0910, 0.201, and 0.124 µg/kg, respectively, for Arkansas Soil-sediment. In the ILV, the LOD was reported as 0.25 µg/kg for all analytes. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

II. Recovery Findings

ECM (MRID 50970989): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for analysis of pyraclonil, amidepyraclonil, M-1, and M-11 in two soil/sediment matrices at the LOQ (1.0 µg/kg) and 10×LOQ (10.0 µg/kg; pp. 13-14; Table 1, pp. 33-35; Table 3, pp. 40-43). Two ion transitions were quantified; recovery results of the quantitative and confirmatory ion transitions were comparable or fairly comparable. The soil/sediment matrices were obtained from Nichino aquatic field dissipation study sites at California (Study No. 85589; MRID 50970950) and Arkansas (Study No. 85590; MRID 50970949; p. 20). The California sandy clay loam soil (Sample ID: 340221 SOIL 0-2"; 53% sand, 26% silt, 21% clay; pH 7.1 in 1:1 soil:water ratio, 0.94% organic matter – Walkley-Black) and Arkansas clay soil-sediment (Sample ID: 340860 2-6"; 14% sand, 38% silt, 48% clay; pH 6.8 in 1:1 soil:water ratio, 1.3% organic matter – Walkley-Black) were characterized by Agvise Laboratories (Northwood, North Dakota; Appendix VII, pp. 260, 262). Both soil/sediment classifications were USDA soil classification.

ILV (MRID 50970990): Mean recoveries and RSDs met requirements for analysis of pyraclonil, amidepyraclonil, M-1, and M-11 in one soil/sediment matrix at the LOQ (1.0 µg/kg) and 10×LOQ (10.0 µg/kg; Tables 1-16, pp. 38-53). Two ion transitions were quantified; recovery results of the quantitative and confirmatory ion transitions were comparable or fairly comparable. The soil and sediment matrices were not specified; however, it was reported that it was received from the EAG Laboratories, Inc. (a company related to the ECM) and sourced from Arkansas (soil) and California (sediment; p. 20). The method was validated by the ILV in one soil/sediment matrix as written, except for a significant modification of the substitution of the filtration step with an additional centrifugation (14000 rpm for 5 minutes) step and insignificant modifications of analytical instrumentation and equipment (pp. 15, 19-24, 34-35). During the first analysis set, a large chromatographic interference was observed for M-11 quantitation ion transition in all matrices which was determined as originating with the syringe filtration step (pp. 25-27). Additionally, there were low recoveries observed for pyraclonil, amidepyraclonil, M-1, and M-11 near 50%. After running the method by replacing the polypropylene syringe filter with centrifugation, the interference and low recoveries were no longer an issue. It was concluded that the filtration step was causing the low recoveries (*ca.* 25-90%) of each of the analytes at both fortification levels even when alternative filter types and sizes were evaluated. The validation of the method was successful with the second analysis set of the first trial samples by replacing the polypropylene syringe filter with ultra-centrifugation. This analysis indicates that there are significant uncertainties with the submitted ECM.

Table 2. Initial Validation Method Recoveries for Pyraclonil, Amidepyraclonil, M-1, and M-11 in Soil and Sediment^{1,2}

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
California Soil						
Quantitation ion transition						
Pyraclonil	1.00 (LOQ)	7	89-97	93	2.9	3.1
	10.0	5	99-100	99	0.55	0.55
Amidepyraclonil	1.00 (LOQ)	7	83-117	100	12	12
	10.0	5	95-107	103	4.8	4.7
M-1	1.00 (LOQ)	7	82-99	91	6.4	7.0
	10.0	5	86-91	89	2.1	2.3
M-11	1.00 (LOQ)	7	87-97	93	3.6	3.9
	10.0	5	92-98	95	2.4	2.6
Confirmation ion transition						
Pyraclonil	1.00 (LOQ)	7	84-131	102	16	15
	10.0	5	95-101	98	2.8	2.9
Amidepyraclonil	1.00 (LOQ)	7	99-119	110	8.1	7.4
	10.0	5	97-107	102	4.1	4.0
M-1	1.00 (LOQ)	7	80-99	89	6.8	7.6
	10.0	5	84-91	87	2.6	3.0
M-11	1.00 (LOQ)	7	80-106	91	9.1	10
	10.0	5	91-92	92	0.55	0.60
Arkansas Soil-sediment						
Quantitation ion transition						
Pyraclonil	1.00 (LOQ)	7	86-98	90	3.9	4.4
	10.0	5	90-94	92	1.6	1.7
Amidepyraclonil	1.00 (LOQ)	7	92-101	97	3.1	3.2
	10.0	5	82-101	90	8.5	9.5
M-1	1.00 (LOQ)	7	81-97	88	6.5	7.4
	10.0	5	85-88	87	1.4	1.6
M-11	1.00 (LOQ)	7	74-85	81	4.1	5.1
	10.0	5	87-91	89	1.6	1.8
Confirmation ion transition						
Pyraclonil	1.00 (LOQ)	7	73-94	80	6.9	8.6
	10.0	5	90-94	91	1.8	2.0
Amidepyraclonil	1.00 (LOQ)	7	76-101	92	8.7	9.4
	10.0	5	86-91	89	2.3	2.6
M-1	1.00 (LOQ)	7	81-96	90	4.9	5.4
	10.0	5	88-93	90	2.0	2.2
M-11	1.00 (LOQ)	7	72-109	88	15	17
	10.0	5	86-90	88	1.8	2.1

Data (uncorrected recovery results; pp. 24-26; Table 1, pp. 32-35; Table 3, pp. 40-43) were obtained from pp. 13-14; Table 1, pp. 32-35; Table 3, pp. 40-43 of MRID 50970989. Since the LOQ was not based on scientifically

acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

- 1 Analytes were identified using three or four ion pair transitions (quantitation, confirmation 1, confirmation 2, and confirmation 3, respectively): m/z 315→169, m/z 315→241 (AR), and m/z 315→99 (CA) for pyraclonil, m/z 333→253, m/z 333→316, and m/z 333→288 for amidepyraclonil, m/z 277→250, m/z 277→214, m/z 277→182, and m/z 277→185 for M-1, and m/z 317→169, m/z 317→241, and m/z 317→275 for M-11; however, analyte residues were only quantified using the **bolded** transitions.
- 2 The soil/sediment matrices were obtained from Nichino aquatic field dissipation study sites at California (Study No. 85589; MRID 50970950) and Arkansas (Study No. 85590; MRID 50970949; p. 20). The California sandy clay loam soil (Sample ID: 340221 SOIL 0-2"; 53% sand, 26% silt, 21% clay; pH 7.1 in 1:1 soil:water ratio, 0.94% organic matter – Walkley-Black) and Arkansas clay soil-sediment (Sample ID: 340860 2-6"; 14% sand, 38% silt, 48% clay; pH 6.8 in 1:1 soil:water ratio, 1.3% organic matter – Walkley-Black) were characterized by Agvise Laboratories (Northwood, North Dakota; Appendix VII, pp. 260, 262). Both soil/sediment classifications were USDA soil classification. The soil texture was verified by the reviewer using USDA-NRCS technical support tools.

Table 3. Independent Validation Method Recoveries for Pyraclonil, Amidepyraclonil, M-1, and M-11 in Soil and Sediment^{1,2}

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
Soil						
Quantitation ion transition						
Pyraclonil	1.00 (LOQ)	5	89.1-92.7	90.9	1.61	1.77
	10.0	5	87.2-93.6	91.8	2.67	2.91
Amidepyraclonil	1.00 (LOQ)	5	84.8-86.2	85.4	0.596	0.698
	10.0	5	83.5-89.1	86.6	2.01	2.32
M-1	1.00 (LOQ)	5	83.0-97.8	90.3	6.96	7.71
	10.0	5	84.1-98.0	92.2	5.29	5.74
M-11	1.00 (LOQ)	5	92.3-97.1	94.2	1.94	2.06
	10.0	5	94.8-102	97.4	2.72	2.79
Confirmation ion transition						
Pyraclonil	1.00 (LOQ)	5	87.1-100	93.0	5.79	6.23
	10.0	5	83.9-94.5	89.1	4.62	5.19
Amidepyraclonil	1.00 (LOQ)	5	76.4-94.3	83.6	7.09	8.48
	10.0	5	81.2-91.9	86.4	4.11	4.76
M-1	1.00 (LOQ)	5	71.1-101	91.8	11.9	13.0
	10.0	5	88.1-97.8	92.4	3.57	3.86
M-11	1.00 (LOQ)	5	83.4-116	103	14.0	13.6
	10.0	5	93.7-104	99.0	4.08	4.12
Sediment						
Quantitation ion transition						
Pyraclonil	1.00 (LOQ)	5	89.9-95.4	92.7	2.18	2.35
	10.0	5	89.6-93.6	91.0	1.95	2.14
Amidepyraclonil	1.00 (LOQ)	5	77.8-83.4	81.4	2.30	2.83
	10.0	5	77.7-85.1	81.4	2.65	3.26
M-1	1.00 (LOQ)	5	86.2-99.0	92.1	4.60	4.99
	10.0	5	88.5-94.3	91.1	2.31	2.54
M-11	1.00 (LOQ)	5	88.1-92.0	90.0	1.41	1.57
	10.0	5	90.5-98.1	94.1	2.87	3.05
Confirmation ion transition						
Pyraclonil	1.00 (LOQ)	5	76.5-118	95.6	15.6	16.3
	10.0	5	86.4-95.4	89.7	3.45	3.85
Amidepyraclonil	1.00 (LOQ)	5	79.1-86.0	83.2	2.75	3.31
	10.0	5	78.2-87.7	84.2	3.55	4.22
M-1	1.00 (LOQ)	5	82.5-100	91.4	6.95	7.60
	10.0	5	84.9-95.2	90.6	3.91	4.32
M-11	1.00 (LOQ)	5	89.0-106	97.2	7.09	7.29
	10.0	5	91.4-100	94.5	3.51	3.71

Data (uncorrected recovery results, pp. 28-29) were obtained from Tables 1-16, pp. 38-53 of MRID 50970990. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

¹ Analytes were identified using only two ion pair transitions (quantitation and confirmation, respectively): m/z 315.2→169.2 and m/z 315.2→241.2 for pyraclonil, m/z 333.1→253.2 and m/z 333.1→316.1 for amidepyraclonil, m/z 277.2→250.2 and m/z 277.2→214.2 for M-1, and m/z 317.1→169.0 and m/z 317.1→241.1 for M-11. The

monitored ion transitions were the same as the monitored quantitation and confirmation ions of the ECM, except for pyraclonil in the California matrix (for which the confirmation 2 ion was used for confirmation in the ECM).
2 The soil and sediment matrices were not specified; however, it was reported that it was received from the EAG Laboratories, Inc. (a company related to the ECM) and sourced from Arkansas (soil) and California (sediment; p. 20).

III. Method Characteristics

The LOQ for pyraclonil, amidepyraclonil, M-1, and M-11 was reported as 1.0 µg/kg in soil/sediment in the ECM and ILV (pp. 12, 28; Appendix D, Appendix II, p. 173 of MRID 50970989; pp. 19, 33 of MRID 50970990). In the ECM, the LOQ was defined as the lowest fortification tested with consistent recovery between 70-120%. No calculations to support the LOQ were reported in the ECM; no justifications or calculations to support the LOQ were reported in the ILV. In the ECM, the LODs were calculated as the one-tailed t-statistic at the 99% confidence level for n-1 replicates ($t_{0.99}$) multiplied by the standard deviation of the measured concentrations of the replicates fortified at the LOQ which equated to 0.0847, 0.361, 0.203, and 0.112 µg/kg for pyraclonil, amidepyraclonil, M-1, and M-11, respectively, for California Soil, and was 0.124, 0.0910, 0.201, and 0.124 µg/kg, respectively, for Arkansas Soil-sediment. The reviewer noted that the calculated ECM LODs were equivalent to Method Detection Limits (MDLs). In the ILV, the LOD was reported as 0.25 µg/kg for all analytes without justification.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

Table 4. Method Characteristics – Soil and Sediment

		Pyraclonil	Amidepyraclonil	M-1	M-11
Limit of Quantitation (LOQ)*	ECM	1.0 µg/kg			
	ILV				
Limit of Detection (LOD)	ECM (calc)	0.0847 µg/kg (CA) 0.124 µg/kg (AR)	0.361 µg/kg (CA) ¹ 0.0910 µg/kg (AR)	0.203 µg/kg (CA) 0.201 µg/kg (AR)	0.112 µg/kg (CA) 0.124 µg/kg (AR)
	ILV (method)	0.25 µg/kg			
Linearity (calibration curve r and concentration range) ³	ECM ⁴	r = 0.9998 (Q) r = 0.9995 (C)	r = 0.9974 (Q) r = 0.9990 (C)	r = 0.9997 (Q) r = 0.9994 (C)	r = 1.0000 (Q) r = 0.9998 (C)
	ILV	r = 0.9995 (Q) r = 0.9992 (C)	r = 0.9995 (Q) r = 0.9997 (C)	r = 0.9978 (Q) r = 0.9965 (C)	r = 0.9997 (Q) r = 0.9991 (C)
	Range	0.0250-2.00 ng/mL			
Repeatable	ECM ⁴	Yes, for LOQ and 10×LOQ in one characterized soil matrix and one characterized soil-sediment matrix, with replacement of the polypropylene syringe filter step with centrifugation instead			
	ILV ^{5,6}	Yes, for LOQ and 10×LOQ in one uncharacterized soil matrix and one uncharacterized sediment matrix			
Reproducible		Yes for 1.0 µg/kg (LLMV)* and 10.0 µg/kg			
Specific	ECM	Yes, no matrix interferences were observed. Some minor baseline noise interfered with LOQ peak attenuation and integration.			
	ILV, with centrifugation step in place of polypropylene syringe filter.	Yes, no matrix interferences were observed; however, LOQ C peak was very small compared to baseline noise. ⁷	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed; however, LOQ C peak was very small compared to baseline noise. ⁷	

Data were obtained from pp. 12, 28 (LOQ/LOD); pp. 13-14; Table 1, pp. 32-35; Table 3, pp. 40-43 (recovery data); p. 27; Figures 1-8, pp. 109-116 (calibration curves); Figures 33-44, pp. 141-152; Figures 57-68, pp. 165-176 (chromatograms) of MRID 50970989; pp. 19, 33 (LOQ/LOD); Tables 1-16, pp. 38-53 (recovery data); p. 18; Appendix F, Figure 8, p. 393, Figure 26, p. 411, Figure 44, p. 429, Figure 62, p. 447 (calibration curves); Appendix F, Figures 1-73, pp. 386-458 (chromatograms) of MRID 50970990; DER Attachment 2. Q = quantitative ion transition; C = confirmatory ion transition. CA = California; AR = Arkansas.

* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 Calculated LOD was >30% of the LOQ.

2 Method LOD was >30% of the LOQ.

3 Solvent-based calibrations were used in the ECM and ILV (pp. 19-20 of MRID 50970989; p. 18 of MRID 50970990)

4 In the ECM, the soil/sediment matrices were obtained from Nichino aquatic field dissipation study sites at California (Study No. 85589; MRID 50970950) and Arkansas (Study No. 85590; MRID 50970949; p. 20 of MRID 50970989). The California sandy clay loam soil (Sample ID: 340221 SOIL 0-2"; 53% sand, 26% silt, 21% clay; pH 7.1 in 1:1 soil:water ratio, 0.94% organic matter – Walkley-Black) and Arkansas clay soil-sediment (Sample ID: 340860 2-6"; 14% sand, 38% silt, 48% clay; pH 6.8 in 1:1 soil:water ratio, 1.3% organic matter – Walkley-Black) were characterized by Agvise Laboratories (Northwood, North Dakota; Appendix VII, pp. 260, 262). Both soil/sediment classifications were USDA soil classification.

5 In the ILV, the soil and sediment matrices were not specified; however, it was reported that it was received from the EAG Laboratories, Inc. (a company related to the ECM) and sourced from Arkansas (soil) and California (sediment; p. 20 of MRID 50970990).

- 6 The ILV validated the method in one soil/sediment matrix as written in the ECM, except for a significant modification of the substitution of the filtration step with an additional centrifugation (14000 rpm for 5 minutes) step and insignificant modifications of analytical instrumentation and equipment (pp. 15, 19-24, 34-35 of MRID 50970990). During the first analysis set where a polypropylene syringe filter was utilized, a large chromatographic interference was observed for M-11 quantitation ion transition in all matrices which was determined as originating with the syringe filtration step (pp. 25-27). It was concluded that the filtration step was causing the low recoveries (*ca.* 25-90%) of each of the analytes at both fortification levels even when alternative filter types and sizes were evaluated. The validation of the method was successful with the second analysis set of the first trial samples incorporating the method modification.
- 7 Based on Appendix F, Figure 11, p. 396, Figure 14, p. 399, Figure 47, p. 432, Figure 50, p. 435, Figure 65, p. 450, and Figure 68, p. 453, of MRID 50970990. The confirmation ion LOQ peaks were extremely small compared to baseline noise and relied on RT to identify. Specificity deviations in the confirmation ion do not affect the validity of the method since a confirmatory method is not usually required when LC/MS/MS or GC/MS is used as the primary method to generate study data.

IV. Method Deficiencies and Reviewer's Comments

1. During the first ILV analysis set, a large chromatographic interference was observed for M-11 quantitation ion transition in all matrices which was determined as originating with the syringe filtration step. In the ILV, it was concluded that the filtration step was causing the low recoveries (*ca.* 25-90%) of each of the analytes at both fortification levels even when alternative filter types and sizes were evaluated. Therefore, there is uncertainty in the reliability of the ECM, as it could not be verified by a different laboratory.
2. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ (pp. 12, 28 of MRID 50970989; pp. 19, 33 of MRID 50970990). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the reported method LOQ for pyraclonil, amidepyraclonil, M-1, and M-11 in soil and sediment.
3. The ECM and ILV reported the method validation for soil, soil-sediment, and water matrices. This DER, designated with a file name of "soil", evaluated the soil/sediment portion, while the accompanying DER, designated with a file name of "water", evaluated the water portion.
4. The ILV soil and sediment matrices were not specified; however, it was reported that it was received from the EAG Laboratories, Inc. (a company related to the ECM) and sourced from Arkansas (soil) and California (sediment; p. 20 of MRID 50970990).
5. The specificity of the method for pyraclonil, M-1, and M-11 was not well-supported by ILV representative chromatograms of the confirmation ion since LOQ peaks were extremely small compared to baseline noise and relied on RT to identify (Appendix F, Appendix F, Figure 11, p. 396, Figure 14, p. 399, Figure 47, p. 432, Figure 50, p. 435, Figure 65, p. 450, and Figure 68, p. 453, of MRID 50970990).

In the ECM, the pyraclozil confirmation ion LOQ peak in the representative chromatograms was small compared to baseline noise (Figure 37, p. 145; Figure 61, p. 169 of MRID 50970989).

6. For soil/sediment analysis, the ILV monitored ion transitions were the same as the monitored quantitation and confirmation ions of the ECM, except for pyraclozil in California soil (for which the confirmation 2 ion was used for confirmation in the ECM; pp. 13-14, 23 of MRID 50970989; p. 23 of MRID 50970990).

The reviewer noted that the monitored confirmation ion transition for pyraclozil in the Arkansas system was m/z 315→241 for the soil-sediment matrix and m/z 315→99 for the water matrix (pp. 13-14 of MRID 50970989). The monitored confirmation ion transition for pyraclozil in the California system was m/z 315→99 for all matrices.

7. Based on the information provided in the ECM and ILV, it was determined that the ILV was performed independently of the ECM. The communications between the ILV laboratory and Study Monitor (Mark Lenz of Exponent) were only summarized, and raw communication data was not provided (pp. 34-35 of MRID 50970990). Communications involved ILV Study Author (Elisabeth A. Schoenau) relaying ILV trial results and ILV modification. The provided lists of ILV and ECM study personnel were distinct, but Lydia Cox served as the Sponsor Representative for the ECM and ILV (pp. 1, 10 of MRID 50970989; pp. 1, 7 of MRID 50970990).
8. The determinations of the LOQ and LOD in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 12, 28; Appendix D, Appendix II, p. 173 of MRID 50970989; pp. 19, 33 of MRID 50970990). In the ECM, the LOQ was defined as the lowest fortification tested with consistent recovery between 70-120%. No calculations to support the LOQ were reported in the ECM; no justifications or calculations to support the LOQ were reported in the ILV. In the ECM, the LODs were calculated as the one-tailed t-statistic at the 99% confidence level for n-1 replicates ($t_{0.99}$) multiplied by the standard deviation of the measured concentrations of the replicates fortified at the LOQ. The reviewer noted that the calculated ECM LODs were equivalent to Method Detection Limits (MDLs). In the ILV, the LOD was reported as 0.25 µg/kg for all analytes without justification. Detection Limit should not be based on the arbitrarily selected lowest concentration in the spiked samples.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

The reviewer noted that the ECM calculated LODs were >30% of the LOQ for amidepyraclozil in AR soil-sediment and M-1 in CA and AR soil/sediment (pp. 12, 28 of MRID 50970989; pp. 19, 33 of MRID 50970990). The method ILV LOD for all analytes was also >30% of the LOQ.

9. The reviewer noted the following typographical error in the title of Figure 29, p. 137, of MRID 50970989, which designated the 1.00 µg/kg fortification of pyraclozil as the LOQ instead of the 10×LOQ.
10. In the ECM, no significant matrix effects were observed (<20%; pp. 19-20 of MRID 50970989). Solvent-based calibration standards were used in the ECM and ILV (pp. 19-20 of MRID 50970989; p. 18 of MRID 50970990).
11. The ECM stock, fortification, and calibration stability results indicate that pyraclozil, amidepyraclozil, M-1 and M-11 stock standard solutions in acetonitrile were stable for at least 95 days when stored refrigerated (p. 28; Table 7, pp. 52-55 of MRID 50970989). Mixed fortification solutions in acetonitrile and mixed calibration standards were stable for at least 69 days when stored refrigerated. Extract stability was demonstrated (storage interval and conditions not reported; p. 22; Tables 8-12, pp. 56-73). Analyte stability was determined as up to 31 days when stored refrigerated (p. 28; Table 13, pp. 74-77).
12. In the ILV, the time requirement for the method was reported as up to 2 days to complete one sample set, with *ca.* 4 hours for preparation, *ca.* 3-4 hours for LC/MS/MS analysis, and *ca.* 2 hours for data calculation (p. 25 of MRID 50970990).

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, and Revision 2; 1994 and 2016.

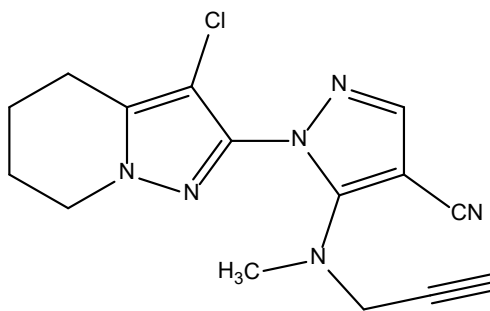
Attachment 1: Chemical Names and Structures**Pyraclozil**

IUPAC Name: 1-(3-Chloro-4,5,6,7-tetrahydropyrazolo[1,5- α]pyridin-2-yl)-5-[methyl(prop-2-ynyl)amino]-1H-pyrazole-4-carbonitrile

CAS Name: 1-(3-Chloro-4,5,6,7-tetrahydropyrazolo[1,5-a]pyridin-2-yl)-5-(methyl-2-propyn-1-ylamino)-1H-pyrazole-4-carbonitrile

CAS Number: 158353-15-2

SMILES String: CN(CC#C)C1=C(C#N)C=NN1C2=NN3CCCCC3=C2Cl

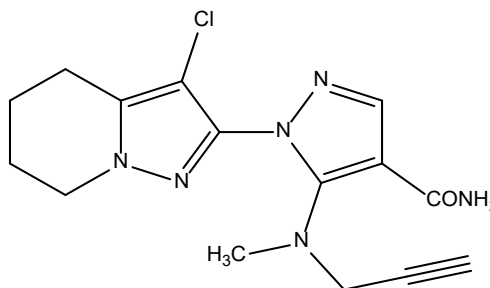
**Amidepyraclozil**

IUPAC Name: 1-(3-Chloro-4,5,6,7-tetrahydropyrazolo[1,5- α]pyridin-2-yl)-5-(methyl(prop-2-yn-1-yl)amino)-1H-pyrazole-4-carboxamide

CAS Name: Not reported

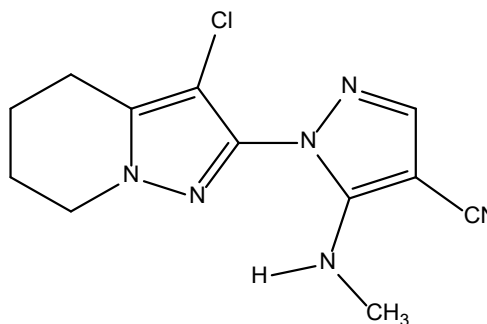
CAS Number: Not reported

SMILES String: CN(CC#C)C1=C(C(N)=O)C=NN1C2=NN3CCCCC3=C2Cl



M-1

IUPAC Name: 1-(3-Chloro-4,5,6,7-tetrahydropyrazolo[1,5- α]pyridin-2-yl)-5-(methylamino)-1H-pyrazole-4-carbonitrile
CAS Name: Not reported
CAS Number: Not reported
SMILES String: CN([H])C1=C(C#N)C=NN1C2=NN3CCCCC3=C2Cl

**M-11**

IUPAC Name: 1-(3-Chloro-4,5,6,7-tetrahydropyrazolo[1,5- α]pyridin-2-yl)-5-(methyl(prop-2-enyl)amino)-1H-pyrazole-4-carbonitrile
CAS Name: Not reported
CAS Number: Not reported
SMILES String: CN(CC=C)C1=C(C#N)C=NN1C2=NN3CCCCC3=C2Cl

