

**Analytical method for pyraclonil and its metabolites amidepyraclonil, M-1, and M-11 in water**

**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.


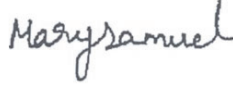
ILV: EPA MRID No. 50970990. Schoenau, E.A. 2019. Independent Laboratory Validation of the Residue Analytical Method for the Determination of Residues of Pyraclonil and Metabolites Amidepyraclonil, M-1, and M-11 in Soil, Sediment, and Water by LC-MS/MS (EAG Study Number: 85901). GPL Study No.: 180770. 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 Part 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. In the ILV analysis, when a polypropylene syringe filter was utilized there was an interfering ion for M-11 and recoveries were low (*ca.* 45-95%) for pyraclonile, amidepyraclonil, M-1, and M-11. When the lab substituted the polypropylene syringe filter step with centrifugation, the interference and recovery issues were no longer present. As the ILV could not confirm good recoveries and no interference utilizing the same method utilized in the ECM and Aquatic Field Dissipation study, there is uncertainty in the reliability of the ECM. 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.

<b>PC Code:</b>	104502		
<b>EFED Final Reviewer:</b>	Taimei Harris, Ph.D, Chemist	Signature:	TAIMEI HARRIS <small>Digitally signed by TAIMEI HARRIS Date: 2021.07.29 04:09:26 -04'00'</small>
	Lisa Muto, M.S., Environmental Scientist	Signature:	
<b>CDM/CSS-Dynamac JV Reviewers:</b>	Mary Samuel, M.S., Environmental Scientist	Signature:	
		Date:	06/12/2020
		Date:	06/12/2020
<b>EPA Reviewer:</b>	Katrina White, Ph.D.	White, Katrina	<small>Digitally signed by White, Katrina Date: 2021.08.13 12:08:25 -04'00'</small>

*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 water at the LOQ of 0.1 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water. 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 water. The ECM used two characterized water matrices sourced from the California and Arkansas aquatic field dissipation study sites; the ILV used one uncharacterized/unspecified water matrix. The ILV validated the method in water with the first trial as written, except for a significant modification of the substitution of the filtration step with an additional centrifugation step and insignificant modifications of analytical instrumentation and equipment. 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. It was concluded that the filtration step was causing the low recoveries (*ca.* 45-95%) 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 water matrices after the change in the ILV method. The same methods as used in the aquatic field dissipation (AFD) (MRID 50970949 and 50970950) studies were utilized. 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.

## 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 <sup>1,2</sup>	50970990 <sup>2,3</sup>	Taimei Harris	Water	23/01/2019	Nichino America, Inc.	LC/MS/MS	0.1 µg/L
Amidepyraclonil								
M-1								
M-11								

1 In the ECM, the water 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 paddy water (Sample ID: 340220 PADDY WATER; pH 8.0, hardness 488 mg equivalent CaCO<sub>3</sub>/L, conductivity 1.42 mmhos/cm) and Arkansas paddy water (Sample ID: 340858 PADDY WATER; pH 8.3, hardness 299 mg equivalent CaCO<sub>3</sub>/L, conductivity 0.51 mmhos/cm) were characterized by Agvise Laboratories (Northwood, North Dakota; Appendix VII, pp. 261, 263).

2 The ECM and ILV reported the method validation for soil, soil-sediment, and water matrices. This DER, designated with a file name of "water", evaluated the water portion, while the accompanying DER, designated with a file name of "soil", evaluated the soil/sediment portion.

3 In the ILV, the water matrix was not specified; however, it was reported that it was received from the EAG Laboratories, Inc. (a company related to the ECM) and sourced from California (p. 21 of MRID 50970990).

## I. Principle of the Method

Water samples (10.0 ± 0.10 mL) transferred into 50-mL polypropylene tubes and fortified with mixed fortification solutions, as necessary (pp. 19, 21-22 of MRID 50970989). Samples were extracted with 10 mL of 0.2% formic acid in acetonitrile via vortexing for one minute. An aliquot (or diluted aliquot) was transferred to a 2-mL glass vial using a 0.2 µm, 25 mm polypropylene syringe filter (the exact source was not provided) 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 Ultra-Performance Liquid Chromatograph (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 (0.2 and 0.25 µm polypropylene syringe filter, VWR Catalog Number 28145-483) with a 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+ 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™ 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 pyraclozil,  $m/z$  333.1→253.2 and  $m/z$  333.1→316.1 for amidepyraclozil,  $m/z$  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 pyraclozil (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 pyraclozil, amidepyraclozil, M-1, and M-11, respectively.

The Limit of Quantification (LOQ) for pyraclozil, amidepyraclozil, M-1, and M-11 was reported as 0.1 µg/L in water 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 0.00974, 0.0192, 0.0334, and 0.0159 µg/L for pyraclozil, amidepyraclozil, M-1, and M-11, respectively, for California water, and was 0.0104, 0.0363, 0.0367, and 0.00770 µg/L, respectively, for Arkansas water. In the ILV, the LOD was reported as 0.05 µg/L 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 pyraclozil, amidepyraclozil, M-1, and M-11 in two water matrices at the LOQ (0.1 µg/L) and 10×LOQ (1.0 µg/L; pp. 13-14; Table 2, pp. 36-39; Table 4, pp. 44-47). Two ion transitions were quantified; recovery results of the quantitative and confirmatory ion transitions were comparable, except for the fact that the RSDs of the pyraclozil analyses at the LOQ were notably higher in the confirmation ion. The water 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 paddy water (Sample ID: 340220 PADDY WATER; pH 8.0, hardness 488 mg equivalent CaCO<sub>3</sub>/L, conductivity 1.42 mmhos/cm) and Arkansas paddy water (Sample ID: 340858 PADDY WATER; pH 8.3, hardness 299 mg equivalent CaCO<sub>3</sub>/L, conductivity 0.51 mmhos/cm) were characterized by Agvise Laboratories (Northwood, North Dakota; Appendix VII, pp. 261, 263).

ILV (MRID 50970990): Mean recoveries and RSDs met requirements for analysis of pyraclozil, amidepyraclozil, M-1, and M-11 in one water matrix at the LOQ (0.1 µg/L) and 10×LOQ (1.0 µg/L; Tables 17-24, pp. 54-61). Two ion transitions were quantified; recovery results of the quantitative and confirmatory ion transitions were comparable, except for the fact that the RSD of the M-11 analysis at the LOQ was notably higher in the confirmation ion. The water matrix was not specified; however, it was reported that it was received from the EAG Laboratories, Inc. (a company related to the ECM) and sourced from California (p. 21). The method was validated by the ILV with 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, 34-35). During the first analysis set with the use of the polypropylene syringe filter, 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.* 45-95%) 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 with replacement of the polypropylene syringe filter with centrifugation.

**Table 2. Initial Validation Method Recoveries for Pyraclonil, Amidepyraclonil, M-1, and M-11 in Water<sup>1,2</sup>**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) <sup>3</sup>	Relative Standard Deviation (%)
<b>California Water</b>						
Quantitation ion transition						
Pyraclonil	0.10 (LOQ)	7	91-101	97	3.3	3.4
	1.0	5	91-95	93	1.8	2.0
Amidepyraclonil	0.10 (LOQ)	7	102-119	112	6.2	5.6
	1.0	5	93-100	97	2.7	2.8
M-1	0.10 (LOQ)	7	83-112	96	10	11
	1.0	5	100-106	104	2.3	2.2
M-11	0.10 (LOQ)	7	95-110	104	5.2	5.0
	1.0	5	101-106	103	2.6	2.5
Confirmation ion transition						
Pyraclonil	0.10 (LOQ)	7	78-114	99	13	13
	1.0	5	92-96	94	1.6	1.7
Amidepyraclonil	0.10 (LOQ)	7	86-116	103	9.5	9.2
	1.0	5	90-105	97	6.1	6.3
M-1	0.10 (LOQ)	7	87-97	93	4.2	4.5
	1.0	5	98-104	101	2.4	2.4
M-11	0.10 (LOQ)	7	96-116	110	7.3	6.6
	1.0	5	99-104	102	1.9	1.9
<b>Arkansas Water</b>						
Quantitation ion transition						
Pyraclonil	0.10 (LOQ)	7	87-96	92	3.5	3.8
	1.0	5	91-95	93	1.8	1.9
Amidepyraclonil	0.10 (LOQ)	7	86-120	105	11	11
	1.0	5	98-114	106	5.7	5.4
M-1	0.10 (LOQ)	7	95-126	106	12	11
	1.0	5	100-105	102	1.9	1.9
M-11	0.10 (LOQ)	7	98-106	101	2.6	2.6
	1.0	5	99-104	101	2.3	2.3
Confirmation ion transition						
Pyraclonil	0.10 (LOQ)	7	79-120	100	16	16
	1.0	5	83-94	91	4.5	5.0
Amidepyraclonil	0.10 (LOQ)	7	81-124	100	15	15
	1.0	5	95-106	102	4.8	4.7
M-1	0.10 (LOQ)	7	91-114	101	8.1	8.0
	1.0	5	97-105	101	3.4	3.4
M-11	0.10 (LOQ)	7	95-114	104	6.7	6.4
	1.0	5	96-104	99	3.4	3.4

Data (uncorrected recovery results; pp. 24-26; Table 2, pp. 36-39; Table 4, pp. 44-47) were obtained from pp. 13-14; Table 2, pp. 36-39; Table 4, pp. 44-47 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.

<sup>1</sup> 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; however, analyte residues were only quantified using the **bolded** transitions.

2 The water 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 paddy water (Sample ID: 340220 PADDY WATER; pH 8.0, hardness 488 mg equivalent CaCO<sub>3</sub>/L, conductivity 1.42 mmhos/cm) and Arkansas paddy water (Sample ID: 340858 PADDY WATER; pH 8.3, hardness 299 mg equivalent CaCO<sub>3</sub>/L, conductivity 0.51 mmhos/cm) were characterized by Agvise Laboratories (Northwood, North Dakota; Appendix VII, pp. 261, 263).

**Table 3. Independent Validation Method Recoveries for Pyraclonil, Amidepyraclonil, M-1, and M-11 in Water<sup>1,2</sup>**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) <sup>3</sup>	Relative Standard Deviation (%)
<b>Water</b>						
Quantitation ion transition						
Pyraclonil	0.10 (LOQ)	5	85.1-95.4	90.4	4.39	4.86
	1.0	5	78.3-90.3	86.8	4.84	5.58
Amidepyraclonil	0.10 (LOQ)	5	101-108	104	2.49	2.39
	1.0	5	99.1-105	102	2.37	2.32
M-1	0.10 (LOQ)	5	95.1-109	103	5.41	5.25
	1.0	5	100-110	105	3.58	3.41
M-11	0.10 (LOQ)	5	97.1-110	104	5.29	5.09
	1.0	5	89.0-108	102	7.50	7.35
Confirmation ion transition						
Pyraclonil	0.10 (LOQ)	5	74.7-90.8	85.1	6.36	7.47
	1.0	5	77.8-93.6	88.6	6.63	7.48
Amidepyraclonil	0.10 (LOQ)	5	95.3-108	101	6.20	6.14
	1.0	5	99.1-105	102	2.56	2.51
M-1	0.10 (LOQ)	5	114-121	118	3.08	2.61
	1.0	5	96.5-114	106	6.79	6.41
M-11	0.10 (LOQ)	5	85.4-126	114	16.9	14.8
	1.0	5	87.5-102	97.7	5.81	5.95

Data (uncorrected recovery results, pp. 28-29) were obtained from Tables 17-24, pp. 54-61 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 (for which the confirmation 2 ion was used for confirmation in the ECM).

2 The water matrix was not specified; however, it was reported that it was received from the EAG Laboratories, Inc. (a company related to the ECM) and sourced from California (p. 21).

### III. Method Characteristics

The LOQ for pyraclonil, amidepyraclonil, M-1, and M-11 was reported as 0.1 µg/L in water 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 0.00974, 0.0192, 0.0334, and 0.0159 µg/L for pyraclonil, amidepyraclonil, M-1, and M-11, respectively, for California water, and was 0.0104, 0.0363, 0.0367, and 0.00770 µg/L, respectively, for Arkansas water. The reviewer noted that the calculated ECM LODs were equivalent to Method Detection Limits (MDLs). In the ILV, the LOD was reported as 0.05 µg/L 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 - Water**

		Pyraclonil	Amidepyraclonil	M-1	M-11
Limit of Quantitation (LOQ)*	ECM	0.1 µg/L			
	ILV				
Limit of Detection (LOD)	ECM (calc)	0.00974 µg/L (CA) 0.0104 µg/L (AR)	0.0192 µg/L (CA) 0.0363 µg/L (AR) <sup>1</sup>	0.0334 µg/L (CA) <sup>1</sup> 0.0367 µg/L (AR) <sup>1</sup>	0.0159 µg/L (CA) 0.00770 µg/L (AR)
	ILV (method)	0.05 µg/L <sup>2</sup>			
Linearity (calibration curve r and concentration range) <sup>3</sup>	ECM <sup>4</sup>	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 <sup>4</sup>	Yes for LOQ and 10×LOQ in two characterized water matrices			
	ILV <sup>5,6</sup>	Yes for LOQ and 10×LOQ in one uncharacterized water matrix, with replacement of the polypropylene syringe filter step with centrifugation instead.			
Reproducible		Yes for 0.1 µg/L (LLMV)* and 1.0 µg/L			
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. <sup>7</sup>	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed; however, LOQ peak was small compared to baseline noise. <sup>7</sup>	Yes, no matrix interferences were observed; however, LOQ peak was very small compared to baseline noise. <sup>7</sup>

Data were obtained from pp. 12, 28 (LOQ/LOD); pp. 13-14; Table 2, pp. 36-39; Table 4, pp. 44-47 (recovery data); p. 27; Figures 1-8, pp. 109-116 (calibration curves); Figures 9-32, pp. 117-140; Figures 45-56, pp. 153-164 (chromatograms) of MRID 50970989; pp. 19, 33 (LOQ/LOD); Tables 17-24, pp. 54-61 (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 water 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 paddy water (Sample ID: 340220 PADDY WATER; pH 8.0, hardness 488 mg equivalent CaCO<sub>3</sub>/L, conductivity 1.42 mmhos/cm) and Arkansas paddy water (Sample ID: 340858 PADDY WATER; pH 8.3, hardness 299 mg equivalent CaCO<sub>3</sub>/L, conductivity 0.51 mmhos/cm) were characterized by Agvise Laboratories (Northwood, North Dakota; Appendix VII, pp. 261, 263).

5 In the ILV, the water matrix was not specified; however, it was reported that it was received from the EAG Laboratories, Inc. (a company related to the ECM) and sourced from California (p. 21 of MRID 50970990).

6 The ILV validated the method in one water matrix with 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). When the polypropylene syringe filter was used, 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.* 45-95%) of each of the analytes at both fortification levels even when alternative filter types and sizes were evaluated. Recovery issues and interferences were no longer present when the polypropylene filter was not utilized.

7 Based on Appendix F, Figure 17, p. 402, Figure 53, p. 438, and Figure 71, p. 456, 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 also concluded that the filtration step was causing the low recoveries (*ca.* 45-95%) of each of the analytes at both fortification levels even when alternative filter types and sizes were evaluated. The recovery issues were not observed when the polypropylene syringe filter step was replaced with centrifugation.
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 water.
3. The ECM and ILV reported the method validation for soil, soil-sediment, and water matrices. This DER, designated with a file name of "water", evaluated the water portion,

while the accompanying DER, designated with a file name of “soil”, evaluated the soil/sediment portion.

4. The ILV water matrix was not characterized or specified; however, it was reported that it was received from the EAG Laboratories, Inc. (a company related to the ECM) and sourced from California (p. 21 of MRID 50970990). The reviewer noted that it was possible that the ILV CA water matrix was the same as the ECM CA water matrix.
5. The specificity of the method for M-1 and M-11 was not well-supported by ILV representative chromatograms since the quantitation ion LOQ peaks were small compared to baseline noise and the confirmation ion LOQ peaks were very small compared to baseline noise and relied on RT to identify (Appendix F, Figure 53, p. 438, and Figure 71, p. 456, of MRID 50970990). The reviewer also noted that the RSD of the M-11 analysis at the LOQ was notably higher in the confirmation ion (Tables 17-24, pp. 54-61). For pyraclozil, the confirmation ion LOQ peak in the representative chromatogram was very small compared to baseline noise, as well (Appendix F, Figure 17, p. 402).
6. For water analysis, the ILV monitored ion transitions were the same as the monitored quantitation and confirmation ions of the ECM, except for pyraclozil (for which the confirmation 2 ion was used for confirmation for both matrices 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 0.05 µg/L 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 amidepyraclonil in AR water and M-1 in CA and AR water (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/L fortification of pyraclonil 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 pyraclonil, amidepyraclonil, 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.* 30 minutes 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.

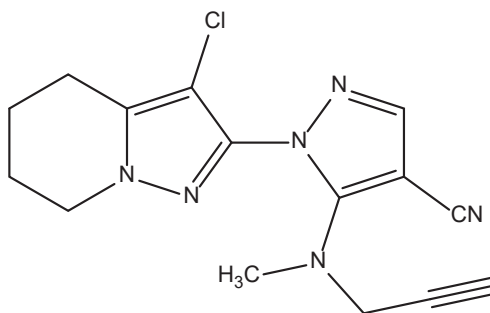
**Attachment 1: Chemical Names and Structures****Pyraclozil**

**IUPAC Name:** 1-(3-Chloro-4,5,6,7-tetrahydropyrazolo[1,5- $\alpha$ ]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

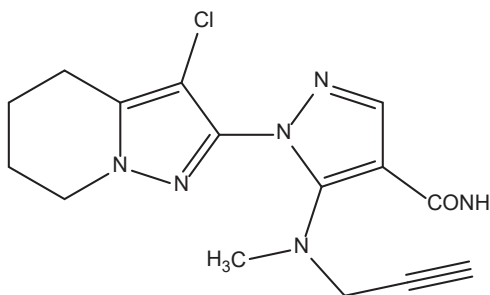
**Amidepyraclozil**

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

**CAS Name:** Not reported

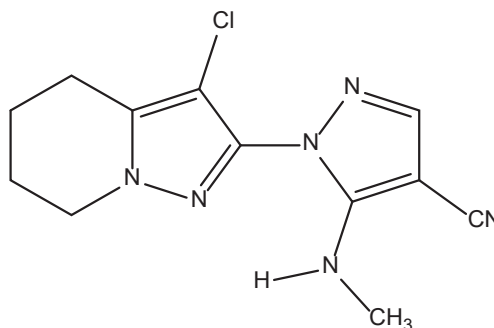
**CAS Number:** Not reported

**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- $\alpha$ ]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- $\alpha$ ]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

