

Analytical method for pyridate and CL-9673 in soil

Reports: ECM: EPA MRID No.: MRID 50234902. Chambers, J.G., and G. Tsui. 2011. Pyridate and CL-9673: Determination of Residues of Pyridate and the metabolite CL-9673 in Soil – Method Validation. Report prepared by Battelle UK Ltd., Essex, United Kingdom, sponsored by Belchim Crop Protection S.A., Londerzeel, Belgium, and submitted by Belchim Crop Protection NV/SA, Londerzeel, Belgium; 47 pages. Belchim Crop Protection NV/SA Method, Report, and Study No.: OZ/10/012. Final report issued October 4, 2011.

ILV: EPA MRID No.: MRID 50234905. Wooler, G. 2018. Independent Laboratory Validation of Belchim Method OZ/10/012- Pyridate and CL-9673: Determination of Residues of Pyridate and the Metabolite CL-9673 in Soil. Report prepared by knoell USA, LLC (kUSA), Garnet Valley, Pennsylvania, sponsored, and submitted by Belchim Crop Protection NV/SA, Londerzeel, Belgium; 201 pages. kUSA Study No.: 109409. Final report issued August 29, 2018.

Document No.: MRID 50234902 & 50234905

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with OCED and UK Good Laboratory Practice (GLP) standards, which are compatible with GLP regulations throughout Europe, US and Japan (p. 3 of MRID 50234902). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). The statement of authenticity was included with the Quality Assurance statement.


ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 50234905). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5). The statement of authenticity was not included.

Classification: This analytical method is classified as unacceptable. The specificity of the shaking and homogenization extraction methods was not supported by the ILV representative chromatograms. For the Solvent Shake extraction sample processing portion of the ECM method, an insufficient number of samples was prepared, and no representative chromatograms were provided. The ILV soil matrix was not representative of a range of soils. Reasons for the failed ILV trials were not reported.

PC Code: 128834

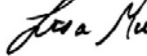
EFED Final Reviewer:

James Lin,
Environmental Engineer

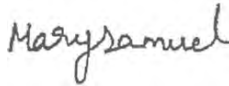
Signature: 
Date: 9/18/2019

**CDM/CSS-
Dynamac JV
Reviewers:**

Lisa Muto,
Environmental Scientist

Signature: 
Date: 02/08/2019

Mary Samuel, M.S.,
Environmental Scientist

Signature: 

Date: 02/08/2019

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

Executive Summary

The analytical method, Belchim Method OZ/10/012, is designed for the quantitative determination of pyridate and CL-9673 in soil at the stated LOQ of 0.01 mg/kg. The LOQ is less than the lowest toxicological level of concern in soil. Belchim Method OZ/10/012 contained two sample processing methods: Solvent Shake Extraction (shaking) and Ultra-Turrax Extraction (homogenization). The ILV used characterized clay loam soil, while the ECM used loamy sand soil; the ILV soil matrix did not cover the range of soils used in the terrestrial field dissipation studies. The shaking and homogenization extraction methods were successfully validated in the second trial (pyridate by shaking method only and CL-9673 by both methods) or third trial (pyridate by homogenization method). Both extraction methods were validated by the ILV with minor modifications, including evaporation of the fortification solvent before extraction, the use of a Wrist Action® Shaker, specification of the homogenization instrument speed and the centrifugation settings, as well as insignificant modifications to the analytical equipment and parameters. Reasons for the failed ILV trials were not reported; it is preferable to have the causes of failed ILV trials discussed or explained to determine the importance of the ILV modifications. All submitted ECM and ILV data pertaining to precision, repeatability, reproducibility, and linearity was acceptable at the LOQ and 10×LOQ; however, an insufficient number of samples was prepared for all fortifications/analytes in the ECM. The specificity of the shaking and homogenization extraction methods was not supported by the ILV representative quantitation ion transition chromatograms because baseline noise height was > LOQ peak height. The method should have been modified to include sample clean-up steps, such as solid phase extraction (SPE) column clean-up. ECM specificity was acceptable for the Ultra-Turrax Extraction method, but the specificity of the Solvent Shake Extraction method could not be assessed because no representative chromatograms were provided.

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						
Pyridate	MRID 50234902 ¹	MRID 50234905 ²		Soil	04/10/2011	Belchim Crop Protection NV/SA	LC/MS/MS	0.01 mg/kg
CL-9673								

¹ In the ECM, the loamy sand soil [Lufa 2.2 (10/008); pH 5.7 (in 1:1 soil:water), 82% sand, 11% silt, 7% clay, 3.2% organic matter] was characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 12; Appendix 3, p. 37 of MRID 50234902). Soil matrix source was not reported.

2 In the ILV, the clay loam soil [Sample # GS-18-47-1; 43% sand, 26% silt, 31% clay, pH 5.2 (in saturated paste), 6.5% organic matter, 3.8% organic carbon] was provided by and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 14; Appendix 2, p. 91 of MRID 50234905).

I. Principle of the Method

Ultra-Turrax Extraction

Soil (10 g) was fortified in plastic bottles then extracted with 100 mL of methanol:acetic acid (100:0.5, v:v) by blending with an Ultra-Turrax macerator (p. 12; Figure 3, p. 26 of MRID 50234902). After centrifugation (conditions not reported), the supernatant was transferred to glass vials and analyzed by LC/MS/MS.

Solvent Shake Extraction

Soil (10 g) was fortified in plastic bottles then extracted with 100 mL of methanol:acetic acid (100:0.5, v:v) by shaking for 20 minutes (p. 12; Figure 3, p. 26 of MRID 50234902). After centrifugation (conditions not reported), the supernatant was transferred to glass vials and analyzed by LC/MS/MS.

LC/MS/MS Analysis

Samples were analyzed for pyridate and CL-9673 using an Agilent 1100 Series HPLC coupled with an API 5000 Mass Spectrometer with Q Jet Ion Guide using a Turbo-Ion Spray source operated in the positive ion, multiple reaction monitoring (MRM) mode (600°C; pp. 12-13 of MRID 50234902). The following LC conditions were used: Phenomenex Aqua C18 column (2.0 mm x 50 mm, 5 μ , 125 Å; column temperature not reported), mobile phase of (A) water:acetic acid (99:1, v:v) and (B) acetonitrile:acetic acid (99:1, v:v) [mobile gradient phase of percent A:B (v:v) at 0.0-0.2 min. 90:10, 1.0-2.0 min. 10:90, 2.65-3.5 min. 90:10] and injection volume of 10 μ L. Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 379.05 \rightarrow 77.20 and m/z 379.05 \rightarrow 68.10 for pyridate and m/z 207.07 \rightarrow 103.97 and m/z 207.07 \rightarrow 67.97 for CL-9673. Observed retention times were *ca.* 2.0 and 1.25 minutes for pyridate and CL-9673, respectively (Figures 4-7, pp. 27-30).

ILV

The ILV performed the ECM method using the solvent shake extraction as written, except for the evaporation of the fortification solvent before extraction, the use of a Wrist Action® Shaker, that the homogenization with the Ultra-Turrax was specified as at high speed for 5 minutes, that the centrifugation settings were specified as 10 minutes at 3600 rpm, and insignificant modifications to the analytical equipment and parameters (pp. 15-19 of MRID 50234905). The LC/MS/MS instrument and parameters were similar to those of the ECM. Samples were analyzed for pyridate and CL-9673 using a Shimadzu 1200 LC-30AD HPLC coupled with an AB Sciex API 6500 LC/MS/MS Tandem Mass Spectrometer (600°C). A Phenomenex Aqua C18 column (2.0 mm x 50 mm, 5 μ m; column temperature ambient) was used. Injection volume was 20.0 μ L. The other LC conditions were the same as those reported in the ECM. Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 379.0 \rightarrow 77.0 and m/z 379.0 \rightarrow 68.0 for pyridate and m/z 207.1 \rightarrow 68.0 and m/z 207.1 \rightarrow 104.0 for CL-9673; quantitation and confirmation ion transitions were switched for CL-9673. Reported retention times were *ca.* 1.74 and 0.93 minutes for

pyridate and CL-9673, respectively. The ILV reported that the 20.0 ng/mL calibration standard was replaced with a 15.0 ng/mL calibration standard; no other method modifications were reported (p. 23).

LOQ/LOD

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.01 mg/kg for pyridate and CL-9673 in soil (pp. 10, 15 of MRID 50234902; pp. 21, 23 of MRID 50234905). In the ECM, the Limit of Detection (LOD) was determined to be 0.0021 mg/kg for pyridate and 0.0002 mg/kg CL-9673. In the ILV, the LOD was calculated to be 0.00257-0.00387 mg/kg for pyridate and 0.00126-0.00155 mg/kg for CL-9673.

II. Recovery Findings

ECM (MRID 50234902): Using the Ultra-Turrax Extraction sample processing, mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of pyridate and CL-9673 at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 \times LOQ) in one soil matrix (Table 1, pp. 16-17; Table 3, p. 19). Two ion pair transitions were monitored for pyridate and CL-9673 using LC/MS/MS in positive mode; the quantification and confirmation ion data was comparable. Using the Solvent Shake Extraction sample processing, mean recoveries and RSDs were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of pyridate and CL-9673 at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 \times LOQ) in one soil matrix; however, an insufficient number of samples was prepared for all fortifications/analytes (n = 2 or 3; Table 3, p. 19). For 10 \times LOQ, where n = 2, the study authors calculated means, but not standard deviations and relative standard deviations. The reviewer did not calculate standard deviations and relative standard deviations since n = 2. Two ion pair transitions were monitored for pyridate and CL-9673 using LC/MS/MS in positive mode; the quantification and confirmation ion data were not comparable. Loamy sand soil [Lufa 2.2 (10/008); pH 5.7 (in 1:1 soil:water), 82% sand, 11% silt, 7% clay, 3.2% organic matter] was characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 12; Appendix 3, p. 37). Soil matrix source was not reported.

ILV (MRID 50234905): Using the Solvent Shake Extraction sample processing, mean recoveries and RSDs were within guidelines for analysis of pyridate and CL-9673 at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 \times LOQ) in one soil matrix (p. 22; Tables 1-4, pp. 26-29). Using the Ultra-Turrax Extraction sample processing, mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of pyridate and CL-9673 at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 \times LOQ) in one soil matrix. Two ion pair transitions were monitored for pyridate and CL-9673 using LC/MS/MS in positive mode; the quantification and confirmation ion data was comparable. Clay loam soil [Sample # GS-18-47-1; 43% sand, 26% silt, 31% clay, pH 5.2 (in saturated paste), 6.5% organic matter, 3.8% organic carbon] was provided by and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 14; Appendix 2, p. 91). The shaking and homogenization extraction methods were successfully validated in the second trial (pyridate by shaking method only and CL-9673 by both methods) or third trial (pyridate by homogenization method; p. 14). Both methods were validated with minor modifications, including evaporation of the fortification solvent before extraction, the use of a Wrist Action® Shaker, specification of the homogenization

instrument speed and the centrifugation settings, as well as insignificant modifications to the analytical equipment and parameters (pp. 15-19). No reason for the trial failures was reported (Appendix 8, p. 201).

Table 2. Initial Validation Method Recoveries for Pyridate and CL-9673 in Soil

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ultra-Turrax Extraction						
Loamy Sand Soil¹						
Quantitation ion ²						
Pyridate	0.01 (LOQ)	6	75-98	86	10.6	12.3
	0.1	6	71-96	83	10.5	12.7
CL-9673	0.01 (LOQ)	6	84-94	87	3.7	4.3
	0.1	6	87-97	92	4.4	4.7
Confirmation ion ²						
Pyridate	0.01 (LOQ)	6	72-89	82	6.5	7.9
	0.1	6	75-101	86	10.8	12.5
CL-9673	0.01 (LOQ)	6	87-99	91	4.5	4.9
	0.1	6	87-96	92	3.9	4.2
Solvent Shake Extraction						
Loamy Sand Soil¹						
Quantitation ion ²						
Pyridate	0.01 (LOQ)	3	80-88	83	4.3	5.1
	0.1	2³	83, 96	90	--	--
CL-9673	0.01 (LOQ)	3	73-83	79	5.1	6.4
	0.1	2³	75, 77	76	--	--
Confirmation ion ²						
Pyridate	0.01 (LOQ)	3	88-93	90	2.5	2.7
	0.1	2³	73, 76	74	--	--
CL-9673	0.01 (LOQ)	3	79-85	83	3.6	4.3
	0.1	2³	74, 82	78	--	--

Data (uncorrected recovery results; Appendix 4, p. 38) were obtained from Table 1, pp. 16-17; Table 3, p. 19 of MRID 50234902.

1 Loamy sand soil [Lufa 2.2 (10/008); pH 5.7 (in 1:1 soil:water), 82% sand, 11% silt, 7% clay, 3.2% organic matter] was characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 12; Appendix 3, p. 37). Soil matrix source was not reported.

2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 379.05→77.20 and m/z 379.05→68.10 for pyridate and m/z 207.07→103.97 and m/z 207.07→67.97 for CL-9673.

3 For $n = 2$, the study authors calculated means, but not standard deviations and relative standard deviations. The reviewer did not calculate standard deviations and relative standard deviations since $n = 2$.

Table 3. Independent Validation Method Recoveries for Pyridate and CL-9673 in Soil

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Solvent Shake Extraction – Primary Method						
Clay Loam Soil¹						
Quantitation ion ²						
Pyridate	0.01 (LOQ)	7	74.7-97.1	81.2	8.2	10
	0.1	5	76.4-110	88.5	13	15
CL-9673	0.01 (LOQ)	7	86.5-99.5	94.0	4.2	4.5
	0.1	5	80.7-95.1	88.9	6.6	7.4
Confirmation ion ²						
Pyridate	0.01 (LOQ)	7	68.7-101	86.1	12	14
	0.1	5	79.1-114	92.4	13	14
CL-9673	0.01 (LOQ)	7	85.9-99.6	93.2	4.6	4.9
	0.1	5	79.4-94.7	87.9	6.1	6.9
Ultra-Turrax Extraction						
Clay Loam Soil¹						
Quantitation ion ²						
Pyridate	0.01 (LOQ)	7	92.9-112	96.1	8.8	9.2
	0.1	5	79.3-90.4	84.4	4.8	5.7
CL-9673	0.01 (LOQ)	7	81.2-93.5	87.7	4.0	4.6
	0.1	5	84.0-94.0	88.3	3.6	4.1
Confirmation ion ²						
Pyridate	0.01 (LOQ)	7	79.8-107	95.8	9.4	10
	0.1	5	77.5-98.5	85.7	8.2	9.6
CL-9673	0.01 (LOQ)	7	78.8-93.0	86.1	4.9	5.7
	0.1	5	85.3-92.9	88.3	2.9	3.3

Data (uncorrected recovery results; pp. 19-20; Appendix 3, p. 92) were obtained from p. 22; Tables 1-4, pp. 26-29 of MRID 50234905.

1 Clay loam soil [Sample # GS-18-47-1; 43% sand, 26% silt, 31% clay, pH 5.2 (in saturated paste), 6.5% organic matter, 3.8% organic carbon] was provided by and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 14; Appendix 2, p. 91).

2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 379.0→77.0 and m/z 379.0→68.0 for pyridate and m/z 207.1→68.0 and m/z 207.1→104.0 for CL-9673; quantitation and confirmation ion transitions were switched for CL-9673.

III. Method Characteristics

In the ECM and ILV, the LOQ was 0.01 mg/kg for pyridate and CL-9673 in soil (pp. 10, 15 of MRID 50234902; pp. 21, 23 of MRID 50234905). In the ECM, the LOQ was reported as the lowest quantifiable level. No calculations were provided for the LOQ in the ECM or ILV. In the ECM, the LOD was determined to be 0.0021 mg/kg for pyridate and 0.0002 mg/kg CL-9673. The LOD was estimated based on three times the baseline noise; the calculations were not provided. In the ILV, the LOD was calculated to be 0.00257-0.00387 mg/kg for pyridate and 0.00126-0.00155 mg/kg for CL-9673. The LOD was calculated for each mixing method using the following equation:

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

Where, $t_{0.99}$ is the one-tailed t-statistic value of 3.143 at the 99% confidence level and SD is the standard deviation of the analyte recovery measurements at the target LOQ.

Table 4. Method Characteristics for Pyridate and CL-9673 in Soil

		Pyridate		CL-9673	
Limit of Quantitation (LOQ)	ECM	0.01 mg/kg			
	ILV				
Limit of Detection (LOD)	ECM	0.0021 mg/kg		0.0002 mg/kg	
	ILV	0.00257-0.00387 mg/kg		0.00126-0.00155 mg/kg	
Linearity (calibration curve r^2 and concentration range) ¹	ECM	$r^2 = 0.9992$ (Q) $r^2 = 0.9998$ (C)		$r^2 = 0.9980$ (Q) $r^2 = 0.9994$ (C)	
		0.200-20.0 ng/mL			
	ILV	$r^2 = 0.9958$ (Q) $r^2 = 0.9954$ (C)		$r^2 = 0.9980$ (Q & C)	
		0.200-15.0 ng/mL			
Repeatable	ECM ²	Ultra-Turrax Extraction	Yes at LOQ and 10×LOQ. (characterized loamy sand soil used)		
		Solvent Shake Extraction	Yes at LOQ and 10×LOQ; however, n = 2-3 . (characterized loamy sand soil used)		
	ILV ^{3,4}	Ultra-Turrax Extraction	Yes at LOQ and 10×LOQ. (characterized clay loam soil used)		
		Solvent Shake Extraction			
Reproducible	Ultra-Turrax Extraction		Yes at LOQ and 10×LOQ.		
	Solvent Shake Extraction				
Specific	ECM	Ultra-Turrax Extraction	Q	Yes, matrix interferences were <3% of the LOQ (based on peak area), but some minor baseline noise was observed in the control.	Yes, no matrix interferences were observed, but some minor baseline noise was observed in the control.
			C ⁵	Yes, matrix interferences were <7% of the LOQ (based on peak area), but some baseline noise was observed in the control.	Analyte peak was barely resolved above the baseline.
		Solvent Shake Extraction	No representative chromatograms provided. ⁶		
	ILV	Ultra-Turrax Extraction	Q	No , no matrix interferences were observed, but baseline noise height was > LOQ peak height. ⁷	Yes, no matrix interferences were observed, but some minor baseline noise was observed in the control.
			Solvent Shake Extraction		
		Ultra-Turrax Extraction	C	Yes, no matrix interferences were observed, but some minor baseline noise interfered with LOQ peak attenuation and integration.	
Solvent Shake Extraction					

Data were obtained from pp. 10, 15 (LOQ/LOD); Table 1, pp. 16-17; Table 3, p. 19 (recovery results); p. 14; Figure 8, pp. 31-32 (calibration curve); Figures 4-7, pp. 27-30 (chromatograms) of MRID 50234902; pp. 21, 23 (LOQ/LOD); p. 22; Tables 1-4, pp. 26-29 (recovery results); p. 21; Figures 2-5, pp. 32-35 (calibration curve); Figures 12-15, pp. 42-45; Figures 18-21, pp. 48-51; Figures 24-27, pp. 54-57; Figures 30-33, pp. 60-63; Figures 36-39, pp. 66-69; Figures 42-45, pp. 72-75; Figures 48-51, pp. 78-81; Figures 54-57, pp. 84-87 (chromatograms) of MRID 50234905. Q = quantitation ion; C = confirmation ion. All results reported for Q and C ions unless specified otherwise.

¹ Correlation coefficients (r^2) were reviewer-calculated based on r values (1/x weighted linear regression analysis) reported in the study report (p. 14; Figure 8, pp. 31-32 of MRID 50234902; p. 21; Figures 2-5, pp. 32-35 of MRID

- 50234905; DER Attachment 2).
- 2 In the ECM, the loamy sand soil [Lufa 2.2 (10/008); pH 5.7 (in 1:1 soil:water), 82% sand, 11% silt, 7% clay, 3.2% organic matter] was characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 12; Appendix 3, p. 37 of MRID 50234902). Soil matrix source was not reported.
 - 3 In the ILV, the clay loam soil [Sample # GS-18-47-1; 43% sand, 26% silt, 31% clay, pH 5.2 (in saturated paste), 6.5% organic matter, 3.8% organic carbon] was provided by and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 14; Appendix 2, p. 91 of MRID 50234905).
 - 4 The shaking and homogenization extraction methods were successfully validated in the second trial (pyridate by shaking method only and CL-9673 by both methods) or third trial (pyridate by homogenization method; p. 14 of MRID 50234905). Both methods were validated with minor modifications, including evaporation of the fortification solvent before extraction, the use of a Wrist Action® Shaker, specification of the homogenization instrument speed and the centrifugation settings, as well as insignificant modifications to the analytical equipment and parameters (pp. 15-19). No reason for the trial failures was reported (Appendix 8, p. 201).
 - 5 A confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method with which to generate study data.
 - 6 The reviewer ascertained that the representative chromatograms correlated to the Ultra-Turrax Extraction samples by comparing recoveries for the first samples of the LOQ and 10×LOQ analyses (REC 1 and REC 7).
 - 7 Based on Figures 14-15, pp. 44-45 and Figures 38-39, pp. 68-69 of MRID 50234905.

IV. Method Deficiencies and Reviewer's Comments

1. The specificity of the shaking and homogenization extraction methods was not supported by the ILV representative quantitation ion transition chromatograms because baseline noise height was > LOQ peak height (Figures 14-15, pp. 44-45; Figures 38-39, pp. 68-69 of MRID 50234905). The analyte peak was differentiated from the baseline noise by retention time. The method should have been modified to include sample clean-up steps, such as solid phase extraction (SPE) column clean-up. The reviewer also noted that the representative confirmation ion transition chromatograms were acceptable.
2. For the Solvent Shake extraction sample processing, the ECM contained the following deficiencies:

An insufficient number of samples was prepared for all fortifications/analytes (n = 2 or 3; Table 3, p. 19 of MRID 50234902). OCSPP guidelines state that a minimum of five spiked replicates should be analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.

The specificity of the method could not be assessed because no representative chromatograms provided. The reviewer ascertained that the representative chromatograms correlated to the Ultra-Turrax Extraction samples by comparing recoveries for the first samples of the LOQ and 10×LOQ analyses (REC 1 and REC 7).
3. The ILV matrix was a clay loam soil, while the ECM matrix was a loamy sand soil. In the TFD studies MRIDs 50164489, 50164490, 50164496 and 50164497, the test soils were characterized as sandy loam, loamy sand, loam and silt loam (Table 11, p. 40 of MRID 50164489; Table 3, p. 33 of MRID 50164490; Appendix 8, p. 72 of MRID 50164496; Appendix 8, p. 74 of MRID 50164497). While it appeared that the ILV was provided with the most difficult matrix with which to validate the method, it could not be determined if the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. No

minimum number of ILV test soils is specified in OCSPP guidelines; however, it is assumed that more than one ILV test soil would need to be included in order to cover the range of soils used in the terrestrial field dissipation studies.

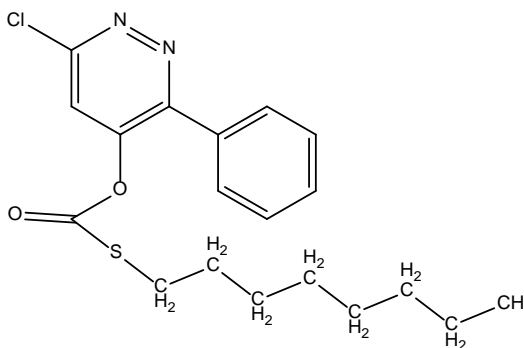
4. The communications between the ECM and ILV were reportedly limited to email exchange regarding study progress and trial results (p. 23; Appendix 8, p. 201 of MRID 50234905). Detailed communication records were not provided. Reasons for the failed ILV trials were not reported as discussed or reported in the ILV report. It is preferable to have the causes of failed ILV trials discussed or explained to determine the importance of the ILV modifications.
5. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 10, 15 of MRID 50234902; pp. 21, 23 of MRID 50234905). In the ECM, the LOQ was reported as the lowest quantifiable level. No calculations were provided for the LOQ in the ECM or ILV. In the ECM, the LOD was estimated based on three times the baseline noise; the calculations were not provided. In the ILV, the LOD was calculated for each mixing method using the following equation: $LOD = (t_{0.99} \times SD)$, where, $t_{0.99}$ is the one-tailed t-statistic value of 3.143 at the 99% confidence level and SD is the standard deviation of the analyte recovery measurements at the target LOQ. Detection limits should not be based on arbitrary values.
6. The reviewer noted that the GLP statement of the ECM contained a contradictory statement at the end: "Data is presented for typical soil analysis, but no claim is made of GLP compliance" (p. 3 of MRID 50234902).
7. In the ECM, the stability of the final extracts was assessed and determined to be up to 5 days when stored under frozen conditions (nominally -18°C; Table 2, p. 18 of MRID 50234902).
8. In the ILV, the time required to complete the extraction of one set of 13 samples (one reagent blank, two matrix controls and ten fortified samples) was reported as *ca.* 4 hours, followed by *ca.* 4 hours for LC/MS/MS analysis (p. 23 of MRID 50234905). The total time requirement of the method was reported as *ca.* one working day in the ILV.

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**Pyridate**

IUPAC Name: *O*-6-chloro-3-phenylpyridazin-4-yl *S*-octyl thiocarbonate
CAS Name: *O*-6-chloro-3-phenylpyridazin-4-yl *S*-octyl carbonothioate
CAS Number: 55512-33-9
SMILES String: c1ccccc1c2nnc(Cl)cc2OC(=O)SCCCCCCCC

**CL-9673 (Pyridafol)**

IUPAC Name: 6-Chloro-3-phenylpyridazin-4-ol
CAS Name: Not reported
CAS Number: 40020-01-7
SMILES String: ClC1=CC(O)=C(N=N1)C2=CC=CC=C2

