Test Material: Cyclanilide

MRID: 49389501

Title: Independent Laboratory Validation (ILV) of Bayer Method CC-001-S14-01 for the Determination of Residues of Cyclanilide and its Metabolite 2,4-Dichloroaniline in Soil and Cyclanilide in Water by LC-MS/MS

EPA PC Code: 026201

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto

Signature: Lisa Muto

Date: 2/12/15

Secondary Reviewer: Lynne Binari

Signature: Lynne Binari

Date: 2/12/15

QC/QA Manager: Joan Gaidos

Signature:

Date: 2/12/15

For Review Considerations, Please refer to **ATTACHMENT**
Starting page 12

Analytical methods for cyclanilide and its metabolite, 2,4-dichloroaniline, in water and for cyclanilide in soil

Reports: ECM: EPA MRID No. 49389501 (Appendix 2, pp. 74-92). Netzband, D. 2014. Bayer Method CC-001-S14-01: An Analytical Method for the Determination of Residues of Cyclanilide and its Metabolite 2,4-Dichloroaniline in Soil and Cyclanilide in Water by LC-MS/MS. Bayer Method No.: CC-001-S14-01. Report prepared by Bayer CropScience, Environmental Safety, Research Triangle Park, North Carolina; sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 19 pages. Final report issued March 28, 2014.

ILV: EPA MRID No. 49389501. Perez, R., A. Caro. 2014. Independent Laboratory Validation (ILV) of Bayer Method CC-001-S14-01 for the Determination of Residues of Cyclanilide and its Metabolite 2,4-Dichloroaniline in Soil and Cyclanilide in Water by LC-MS/MS. ADPEN Study No.: 2K14-0331-RACCL011. Study ID: RACCL011. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida; sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 117 pages (including the ECM). Final report issued May 21, 2014.

Document No.: MRID 49389501

Guideline: 850.6100

Statements: ECM: The study was not conducted in accordance with U.S. EPA GLP (CFR Title 40, Part 160) since it was not an experimental study (Appendix 2, p. 76). Signed and dated No Data Confidentiality, GLP and Authenticity statements were provided (Appendix 2, pp. 75-77). A Quality Assurance statement was not included.

ILV: The study was conducted in accordance with U.S. EPA GLP (CFR Title 40, Part 160; p. 3). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). An authenticity statement was not included, but a signature page was included (p. 5).

Classification: This analytical method is classified as **supplemental** that may be upgradable by providing responses to reviewer comments pages 7-9. Would prefer resubmittal of the ECM and the ILV as two separate stand-alone studies. Only one set of performance data was submitted to validate the method; the ECM study report did not contain any performance data including chromatograms and recovery data. The spectra of 2,4-DCA contained a significant interfering peak which affected proper integration of the analyte peak. The determination of the LOQ was not based on scientifically acceptable procedures; the LOD was not reported. The number of samples was insufficient (n = 4) in the confirmation ion analysis for soil samples fortified at the LOQ with 2,4-DCA. ILV matrices were not characterized.

PC Code: 026201

Reviewer: Mohammed A. Ruhman, **Signature:**
Senior Agronomist, EFED, EPA **Date:** 08/05/2015

All page numbers refer to those in the uppermost right-hand corner of the MRID.

Executive Summary

The analytical method, Bayer Method CC-001-S14-01, is designed for the quantitative determination of cyclanilide and 2,4-dichloroaniline (2,4-DCA) in soil at the LOQ of 5.0 ng/g and for cyclanilide in water at the LOQ of 0.5 ng/g using LC/MS/MS. The LOQ is less than lowest toxicological level of concern in water and no toxicological data is available to compare with in soils/sediments. Only one set of performance data was submitted to validate the method; the ECM study report did not contain any performance data. The spectra of 2,4-DCA contained a significant interfering peak which interfered with proper integration of the analyte peak. The number of samples was sufficient for all analysis, except for confirmation ion analysis for soil samples fortified at the LOQ with 2,4-DCA where n = 4. The method was validated with the first trial with no modifications other than instrument optimization. ILV matrices were not characterized. The LODs were not reported in the ECM or ILV for either analyte in water or soil.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Cyclanilide & 2,4-Dichloroaniline	49389501*	49389501		Soil	03/28/2014	Bayer CropScience	LC/MS/MS	5 ng/g
Cyclanilide				Water				0.5 ng/g

* Appendix 2, pp. 74-92.

I. Principle of the Method

Soil extraction:

Soil samples (20 ± 0.01 g) were fortified in glass jars and extracted twice with *ca*. 40 mL of methanol:water (1:1, v:v; Appendix 2, pp. 82-83). After shaking for *ca*. 10 minutes and centrifugation (*ca*. 3000 rpm for *ca*. 5 minutes), the aqueous layer was removed. During the second extraction, 80 µL of the 5 µg/mL isotopic internal standard solution was added prior to centrifugation. The combined extracts were diluted to *ca*. 80 mL with methanol and mixed. A 10 mL aliquot of the extract was reduced to *ca*. 5 mL via turboevaporation at 35°C. An aliquot (*ca*. 2 mL) was transferred to a LC vial prior to analysis via LC/MS/MS.

Water extraction:

Soil samples (20 ± 0.1 mL) were fortified in glass jars and mixed with 2 mL of methanol, 50 µL of formic acid and 50 µL of the 5 µg/mL isotopic internal standard solution (Appendix 2, p. 83). The mixture was diluted to 25 mL with deionized water. An aliquot (unreported volume) was transferred to a LC vial prior to analysis via LC/MS/MS.

Samples (water/soil matrices) were analyzed for cyclanilide and 2,4-dichloroaniline (2,4-DCA) using an AB Sciex API 5500 (LC/MS/MS) system employing a Phenomenex® Luna 2.5 µm C18(2)-HST (50 mm x 2.0 mm i.d., 2.5 µm) column with a gradient mobile phase of (A) 0.3% formic acid in water and (B) acetonitrile (time A:B; 0.0 min. 90:10, 5.0-6.0 min. 10:90, 6.1-9.0 min. 90:10) and equipped with an electrospray interface (Appendix 2, pp. 80, 83; Appendix 2, Appendix 1, pp. 86-87). Injection volume was 5 µL. Analyte ions were monitored using MS in either positive (2,4-dichloroaniline) or negative (cyclanilide) ESI mode. Two sets of MRM transitions were monitored for each analyte and the isotopic internal standard (quantitation and confirmation transitions, respectively): 272→160 m/z and 272→228 m/z for cyclanide; 162→126 m/z and 162→99 m/z for 2,4-DCA; and 168→133 m/z (only one transition) for 3,5-DCA-$^{13}C_6$. Retention times were 4.5 minutes for cyclanilide, 3.9 minutes for 2,4-DCA and 4.0 minutes for 3,5-DCA-$^{13}C_6$.

In the ILV, the sample processing procedure for the water and soil was the same as in the ECM, except that twice the volume of internal standard was added and the final volume was diluted as necessary to resolve matrix interferences (pp. 13-14; Appendix 1, pp. 71-72). LC/MS/MS analysis was performed using an Agilent 1290 UPLC coupled with an Agilent 6490 Triple Quad mass spectrometer. The analytical column and mobile phases were the same; however, the gradient of the mobile phase (time A:B; 0.0 min. 90:10, 4.0-5.0 min. 50:50, 6.0-7.0 min. 10:90, 7.1-9.0 min. 90:10) and injection volume (10 µL) were notably different. The two sets of monitored MRM transitions were the same for cyclanilide and the isotopic internal standard; however, for 2,4-DCA, monitored ion transitions were 162→99 m/z and 162→126 m/z for quantitation and confirmation transitions, respectively. Retention times ere the same for cyclanilide and the isotopic internal standard; however, for 2,4-DCA, the retention time was 4.1 minutes.

In the ECM and ILV, the Limit of Quantification (LOQ) value for cyclanilide and 2,4-dichloroaniline was 5.0 ng/g in soil; the LOQ for cyclanilide was 0.5 ng/g in water an (pp. 10, 15-17; Appendix 2, p. 80). The Limits of Detection (LODs) were not reported in the ECM or ILV for water or soil.

II. Recovery Findings

ECM (MRID 49389501): No recovery data was included with the ECM report (Appendix 2, pp. 74-92).

ILV (MRID 49389501): Mean recoveries and RSDs were within guideline requirements for analysis of cyclanilide and 2,4-dichloroaniline (2,4-DCA) in soil at the LOQ (5.0 ng/g) and 10×LOQ (50 ng/g) and for cyclanilide in water at the LOQ (0.5 ng/g) and 10×LOQ (5 ng/g; pp. 10, 15-16; Tables 3-8, pp. 22-27). Quantitation and confirmation results were comparable. The number of samples was five for all analysis, except for the confirmation ion of 2,4-DCA in soil where n = 4 (one value, 212% recovery, was excluded as an outlier as determined by the Grubbs Outlier Test at the 95% confidence level). The method was validated with the first trial. Both matrices, provided by Bayer, were not characterized (p. 12). The water was identified as pond water (Sample ID: 061113-W); the soil was not described in any detail (Sample ID: MESXN008CA-S001).

Table 2. Initial Validation Method Recoveries for Cyclanilide and 2,4-Dichloroaniline in Soil and for Cyclanilide in Water

Analyte	Fortification Level (ppt) or (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil						
Cyclanilide	No recovery data was included in ECM report					
2,4-Dichloroaniline						
Water						
Cyclanilide	No recovery data was included in ECM report					

Table 3. Independent Validation Method Recoveries for Cyclanilide and 2,4-Dichloroaniline in Soil and for Cyclanilide in Water [1]

Analyte	Fortification Level (ppt) or (ppb)[2]	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil						
Quantitation ion						
Cyclanilide	5.0 (LOQ)	5	100-111	109	5.0	4.6
	50	5	79-114	103	14.6	14.2
2,4-Dichloroaniline (2,4-DCA)	5.0 (LOQ)	5	75-99	83	10.9	13.2
	50	5	67-92	77	9.9	12.9
Confirmation ion						
Cyclanilide	5.0 (LOQ)	5	98-113	105	6.4	6.2
	50	5	78-114	102	14.8	14.5
2,4-Dichloroaniline (2,4-DCA)	5.0 (LOQ)	4[2]	71-91	84	10.9	13.0
	50	5	66-82	72	7.7	10.8
Pond Water						
Quantitation ion						
Cyclanilide	0.5 (LOQ)	5	101-106	102	2.3	2.2
	5	5	90-93	92	1.5	1.6
Confirmation ion						
Cyclanilide	0.5 (LOQ)	5	108-118	112	4.6	4.1
	5	5	88-94	92	2.5	2.7

Data were obtained from pp. 15-16; Tables 3-8, pp. 22-27 of the study report.

1 Both matrices, provided by Bayer, were not characterized (p. 12). The water was identified as pond water (Sample ID: 061113-W); the soil was not described in any detail (Sample ID: MESXN008CA-S001).

2 One value (212% recovery) was excluded as an outlier as determined by the Grubbs Outlier Test at the 95% confidence level (Table 6, p. 25).

III. Method Characteristics

In the ECM and ILV, the Limit of Quantification (LOQ) value for cyclanilide and 2,4-dichloroaniline was 5.0 ng/g in soil; the LOQ for cyclanilide was 0.5 ng/g in water an (pp. 10, 15-17; Appendix 2, p. 80). No justification or calculations were provided to support the LOQs. The Limits of Detection (LODs) were not reported in the ECM or ILV for water or soil.

Table 4. Method Characteristics[1]

		Soil		**Pond Water**
		Cyclanilide	**2,4-DCA**	**Cyclanilide**
Limit of Quantitation (LOQ)		5.0 ng/g		0.5 ng/g
Limit of Detection (LOD)		Not reported		Not reported
Linearity[2]	calibration curve r^2	Cyclanilide $r^2 = 0.99848175$ (Q) $r^2 = 0.99802546$ (C) 2,4-DCA $r^2 = 0.99816531$ (Q) $r^2 = 0.99824503$ (C)		
	concentration range	(0.0025-0.2500 ng)		
Repeatable		Yes		
Reproducible		Could not be determined[3]		
Specific		Yes	No[4]	Yes

Data were obtained from pp. 10, 15-16; Tables 3-8, pp. 22-27; Figures 1-4, pp. 29-32 of the study report.

[1] The majority of the information in the table was based on the ILV, not the ECM. LOQ values were reported in the ECM report; however, no recovery or linearity data were provided. Chromatograms were only provided for one standard of each analyte.

[2] Linearity of the ILV calibration curves was verified by the reviewer, $r^2 = 0.9998$ for cyclanilide and $r^2 = 0.9993$-0.9999 for 2,4-DCA (Figures 1-4, pp. 29-32; DER Attachment 2).

[3] The ECM (Appendix 2, pp. 74-92 of MRID 49389501) did not include any recovery data – method report only.

[4] The reviewer noted a significant interfering peak near the retention time of 2,4-DCA in the soil samples (LOQ, Figures 27-28, pp. 55-56; 10×LOQ, Figures 31-32, pp. 59-60). The interfering peak was observed in both the confirmation ion and quantitation ion analysis; however, in the confirmation ion analysis, the interfering peak was larger than the analyte peak. This peak was also observed in the soil matrix blank (Figures 23-24, pp. 51-52).

IV. Method Deficiencies and Reviewer's Comments

1. The ECM report, which was provided in Appendix 2, pp. 74-92 of MRID 49389501, did not include sufficient performance data to provide support for the method. It appeared to be a method report only. LOQ values were reported; however, no recovery or linearity data was provided. Chromatograms were only provided for one standard of each analyte. OCSPP guidelines recommend that two sets of performance data are submitted, one for the initial or other internal validation and one for the ILV.

2. In the ECM, representative chromatograms were only provided for the analytes (MS spectra) and the 10 ng/mL calibration standard of each analyte/ion including the internal

2,4-DCA-$^{13}C_6$ standard (Appendix 2, Appendix 3, pp. 90-92). OCSPP guidelines recommend that representative chromatograms are provided for reagent blanks, matrix blanks, standard curves, and spiked samples at the LOQ and 10× LOQ for all analytes in each matrix

In the ILV, representative chromatograms of reagent blanks, matrix blanks, and fortifications at the LOQ and 10×LOQ were included for each analyte/ion/matrix (Figures 17-40, pp. 45-68). The reviewer noted a significant interfering peak near the retention time of 2,4-DCA in the soil samples (LOQ, Figures 27-28, pp. 55-56; 10×LOQ, Figures 31-32, pp. 59-60). The interfering peak was observed in both the confirmation ion and quantitation ion analyses; however, in the confirmation ion analysis, the interfering peak was larger than the analyte peak. The interfering peak affected proper integration of the analyte peak. This peak was also observed in the soil matrix blank (Figures 23-24, pp. 51-52). The ILV study author did not discuss the appearance of the interference. The reviewer also noted that an insignificant amount of cyclanilide (*ca*. 20% of the LOQ) was detected in the method blank (quantitation ion; Figure 17, p. 45).

3. The estimations of the LOQ for water and soil in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. No justification or calculations were provided to support the LOQs. OCSPP guidelines recommend that there are explanations of the LOD and LOQ were calculated.

   The LODs were not reported in the ECM or ILV for either analyte in water or soil.

   Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in water and soil were not reported. An LOQ above toxicological level of concern results in an unacceptable method classification.

4. The number of samples was insufficient (n = 4) in the 2,4-DCA confirmation ion analysis for soil samples fortified at the LOQ (Table 6, p. 25). One value (212% recovery) was excluded as an outlier as determined by the Grubbs Outlier Test at the 95% confidence level.

5. In the ILV, the matrices, provided by Bayer, were not characterized (p. 12). The water was identified as pond water (Sample ID: 061113-W); the soil was not described in any detail (Sample ID: MESXN008CA-S001).

6. The ILV study author reported that the Agilent 6490 mass spectrometer coupled with the Agilent 1290 UHPLC "can be 10 times more sensitive than an ABSciex API 5500" (p. 15). The study author reported that optimization was performed on the analytical instrument and the final volume of the extracts was diluted as necessary to resolve matrix interferences (pp. 14-15). These are not considered significant modifications of the ECM.

7. The ILV data tables indicated that the recoveries were corrected for residue found in the control samples; however, only one sample set contained residues in the control samples

(cyclanilide, quantitation ion analysis, soil), and no correction was indicated (Table 3-8, pp. 22-27).

8. Communication between the ECM personnel and ILV study author was reported (p. 16; Appendix 6, p. 117). This communication was limited to notification of trial success.

9. The time requirement for the sample sets of the ILV experiment was reported as less than 2 hours for a set of 13 water samples and *ca*. 8 hours for a set of 13 soil samples, not including LC/MS/MS analysis which was performed overnight (p. 16).

V. References

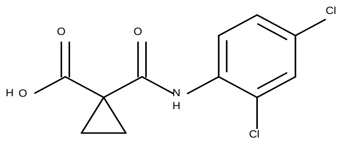
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

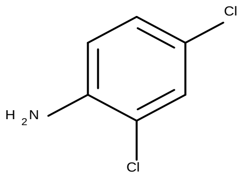
Cyclanilide (AE 0195157)

IUPAC name	1-(2,4-Dichloroanilinocarbonyl)cyclopropanecarboxylic acid
CAS Name	1-[[(2,4-Dichlorophenyl)amino]carbonyl]cyclopropanecarboxylic acid
CAS #	113136-77-9
SMILES	c1cc(Cl)cc(Cl)c1NC(=O)C2CC2C(=O)O



2,4-Dichloroaniline

IUPAC name	2,4-Dichloroaniline
CAS Name	Not reported
CAS #	554-00-7
SMILES	Not found



ATTACHMENT

Section III. Environmental Chemistry Method Report Review Considerations

This list of considerations is provided to help reviewers assess the acceptability of environmental chemistry method reports and their associated independent laboratory validation reports. This list may be used as a screen or a checklist but is not meant to be attached to the method report reviews. Listed considerations carry unequal weight. Evaluate them using best professional judgment. Consider all information from the method reports and from reports for similar methods to determine whether any deficiencies affect the method report classification.

ECM Report (Appendix 2, pp. 74-92 of MRID 49389501)

» The required instrumentation, glassware, and chemicals were identified in the report and are commercially available.

In the ECM, chemicals and equipment along with recommended suppliers were listed in 2. Apparatus and 3. Reagents (Appendix 2, p. 80) and Appendices 1-2 (Appendix 2, Appendices 1-2, pp. 86, 88-89).

In the ILV (pp. 11-14), chemicals and equipment were listed in 3.0 Materials and Methods along with some recommended suppliers.

» The matrix/matrices was/were well characterized. (For example, for soil, pH and percentages of organic carbon, moisture, sand, silt, and clay, *etc.* were reported.)

The ECM report did not include sufficient performance data. No matrices were reportedly tested.

In the ILV, the matrices, provided by Bayer, were not characterized (p. 12). The water was identified as pond water (Sample ID: 061113-W); the soil was not described in any detail (Sample ID: MESXN008CA-S001).

» All steps in the ECM are scientifically sound. Mass spectrometry or another technique was used to confirm the identity of the analyte(s).

Cyclanilide and 2,4-dichloroaniline (2,4-DCA) were identified by LC/MS/MS using MS in either positive (2,4-dichloroaniline) or negative (cyclanilide) ESI mode. Two sets of MRM transitions were monitored for each analyte and the isotopic internal standard.

» Any encountered interferences, problem areas, or critical steps were described and/or explained.

None reported.

» The matrix blank was free of interference(s).

The ECM report did not include sufficient performance data. Chromatograms of the matrix blanks were not included.

» Representative chromatograms were provided for reagent blanks, matrix blanks, standard curves, and spiked samples at the LOQ and 10× LOQ for all analytes in each matrix.

The ECM report did not include sufficient performance data. Representative chromatograms were only provided for the analytes (MS spectra) and the 10 ng/mL calibration standard of each analyte/ion including the internal 2,4-DCA-$^{13}C_6$ standard (Appendix 2, Appendix 3, pp. 90-92). Chromatograms for reagent blanks, matrix blanks, standard curves, and spiked samples at the LOQ and 10× LOQ for cyclanilide and 2,4-DCA were not included. Standard curves were not included.

In the ILV, representative chromatograms of reagent blanks, matrix blanks, and fortifications at the LOQ and 10×LOQ were included for each analyte/ion/matrix (Figures 17-40, pp. 45-68). Standard curves with solvent-based calibrants were also included (Figures 1-4, pp. 29-32).

» The chromatograms of the lowest spiking level are attenuated to where one can measure the peak accurately (accounting for the noise on the baseline).

The ECM report did not include sufficient performance data. No chromatograms of the LOQ samples were included.

» There are explanations of how the LOD and LOQ were calculated. The procedures are scientifically acceptable. A best effort was demonstrated to achieve a low LOQ. (LOD and LOQ are often calculated as the mean matrix blank value plus 3 times the standard deviation and 10 times the standard deviation, respectively. 40 CFR Part 136, Appendix B lists some scientifically accepted procedures for estimating detection limits. Actual detection limits are not based on the arbitrarily selected lowest concentration in the spiked samples.)

In the ECM and ILV, no justification or calculations were provided to support the LOQs. The LODs were not reported in the ECM or ILV for water or soil.

» The LOQ(s) is/are less than toxicological levels of concern. (Concentrations in soil with units of mass/area (*e.g.*, lbs/acre) are converted to units of mass/mass (*e.g.*, mg/kg) using a soil depth of six inches and the soil density. The 6-inch soil depth is a default to use unless there is a reason to use an alternative depth.)

No toxicological levels of concern for cyclanilide or 2,4-DCA were reported.

» For ECMs used in submitted field studies, the LOQ(s) is/are less than 10% of the expected or actual peak concentration of the test compound in the field.

Not applicable to this review.

ILV Report (MRID 49389501)

» An ILV was performed and documented in a report separate from the ECM report.

Yes.

» The ILV was independent. (If the laboratory that conducted the validation belonged to the same organization as the originating laboratory, the analysts, study director, equipment, instruments, and supplies of the two laboratories must have been distinct and operated separately and without collusion. The analysts and study director of the ILV must have been unfamiliar with the method both in its development and subsequent use in field studies.)

The ILV was performed by ADPEN Laboratories, Inc., which is separate from the ECM authors, Bayer CropScience.

- All communication prior to running the samples between the independent laboratory and the developers or previous users of the ECM was documented.

  Communication between the ECM personnel and ILV study author was reported (p. 16; Appendix 6, p. 117). This communication was limited to notification of trial success.

- A maximum of three sample sets were used to validate the ECM (*i.e.*, produce recoveries with acceptable precision and accuracy). A minimally complete sample set includes a reagent blank, two matrix blanks, five samples spiked at the LOQ, and five samples spiked at 10× LOQ for each matrix.

  The method was validated with the first trial (pp. 10, 15-16; Tables 3-8, pp. 22-27). The sample set included a reagent blank, two matrix blanks, five samples spiked at the LOQ, and five samples spiked at 10× LOQ for water and soil. Sampling sets consisted of cyclanilide and 2,4-dichloroaniline (2,4-DCA) in soil at the LOQ (5.0 ng/g) and 10×LOQ (50 ng/g) and of cyclanilide in water at the LOQ (0.5 ng/g) and 10×LOQ (5 ng/g). The number of samples was five for all analyses, except for the confirmation ion of 2,4-DCA in soil where n = 4 (one value, 212% recovery, was excluded as an outlier as determined by the Grubbs Outlier Test at the 95% confidence level).

- Interferences with peak areas were less than 50% at the LOD.

  Yes for cyclanilide in water and soil (Figures 17-40, pp. 45-68). The reviewer noted that an insignificant amount of cyclanilide (*ca*. 20% of the LOQ) was detected in the method blank (quantitation ion; Figure 17, p. 45).

  No for 2,4-DCA (Figures 17-40, pp. 45-68). The reviewer noted a significant interfering peak near the retention time of 2,4-DCA in the soil samples (LOQ, Figures 27-28, pp. 55-56; 10×LOQ, Figures 31-32, pp. 59-60). The interfering peak was observed in both the confirmation ion and quantitation ion analysis; however, in the confirmation ion analysis, the interfering peak was larger than the analyte peak. The interfering peak affected proper integration of the analyte peak. This peak was also observed in the soil matrix blank (Figures 23-24, pp. 51-52). The ILV study author did not discuss the appearance of the interference.

ECM and ILV Reports

- Sample recoveries were not corrected for reagent blanks, matrix blanks, or other recoveries.

  ILV data tables indicated that the recoveries were corrected for residue found in the control samples; however, only one sample set contained residues in the control samples (cyclanilide, quantitation ion analysis, soil), and no correction was indicated (Table 3-8, pp. 22-27).

  The ECM report did not include sufficient performance data. No recovery data was reported.

- A minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte. (For the initial method validation, the number of spiked samples analyzed at each concentration is at least equal to that of the independent method validation and is preferably seven.)

  The ECM report did not include sufficient performance data. No recovery data was reported. No sample sets were prepared.

  For the ILV, the sample set included a reagent blank, two matrix blanks, five samples spiked at the LOQ, and five samples spiked at 10× LOQ for water and soil. Sampling sets consisted of cyclanilide and 2,4-dichloroaniline (2,4-DCA) in soil at the LOQ (5.0 ng/g) and 10×LOQ (50 ng/g) and of cyclanilide in water at the LOQ (0.5 ng/g) and 10×LOQ (5 ng/g). The number of samples was five for all analyses, except for the confirmation ion of 2,4-DCA in soil where n = 4 (one value, 212% recovery, was excluded as an outlier as determined by the Grubbs Outlier Test at the 95% confidence level).

» The method recoveries met OCSPP Guideline 850.6100 criteria for precision and accuracy (mean recoveries for replicates at each spiking level between 70% and 120% and relative standard deviations (RSD) ≤20%) at the stated LOQ and at higher concentrations.

The ECM report did not include sufficient performance data. No recovery data was reported. No sample sets were prepared.

In the ILV, mean recoveries and RSDs were within guideline requirements for analysis of cyclanilide and 2,4-dichloroaniline (2,4-DCA) in soil at the LOQ (5.0 ng/g) and 10×LOQ (50 ng/g) and for cyclanilide in water at the LOQ (0.5 ng/g) and 10×LOQ (5 ng/g; pp. 10, 15-16; Tables 3-8, pp. 22-27).

» Two sets of performance data were submitted, one for the initial or other internal validation and one for the ILV, with the following exception. (If the initial validation was performed by a governmental agency, a reference to the agency's documentation of the ECM will serve as the ECM report. In this case, the applicant submitted an ILV report and documentation of the agency's ECM if not the full initial validation report for the ECM.

Only one set of performance data was submitted, the ILV. The ECM report did not include sufficient performance data. No recovery data was reported. No sample sets were prepared.
No chromatograms were provided aside from those of the test materials and one standard of each analyte.

» Any modifications to the method recommended by the independent laboratory were implemented in the ECM report. If substantial changes to the ECM were recommended, an internal validation was conducted for the updated ECM report.

The ILV study author reported that the Agilent 6490 mass spectrometer coupled with the Agilent 1290 UHPLC "can be 10 times more sensitive than an ABSciex API 5500" (p. 15). The study author reported that optimization was performed on the analytical instrument and the final volume of the extracts was diluted as necessary to resolve matrix interferences (pp. 14-15). These are not considered significant modifications of the ECM.