Analytical method for chlorothalonil in soil

Reports: ECM: EPA MRID No.: 49659704. Lin, K. and S.-B. Huang. 2015. Chlorothalonil. Analytical Method (GRM005.08A) for Residue Determination of Chlorothalonil in Soil by LC-MS/MS. Analytical Method. Syngenta Report No. GRM005.08A and Task No. TK0225796. Report prepared by Syngenta Crop Protection, LLC, Greensboro, North Carolina; sponsored by Syngenta Crop Protection, LLC, Greensboro, North Carolina, Arysta LifeScience North America, LLC, Cary, North Carolina, Makhteshim Agan North America Inc., d/b/a ADAMA, Raleigh, North Carolina, and SipcamAdvan, Durham, North Carolina; and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 55 pages. Final report issued February 13, 2015.

ILV: EPA MRID No. 49659702. Guo, D. 2015. Chlorothalonil. Chlorothalonil - Independent Laboratory Validation (ILV) of Analytical Method (GRM005.08A) for the Residue Determination of Chlorothalonil in Soil by LC-MS/MS. Final Report Amendment 2. Report No.: PASC-REP-0528. PASC Project No.: 141-1072. Task No.: TK0225797. Report prepared by Primera Analytical Solutions Corp., Princeton, New Jersey; sponsored by Syngenta Crop Protection, LLC, Greensboro, North Carolina, Arysta LifeScience North America, LLC, Cary, North Carolina, Makhteshim Agan North America Inc., d/b/a ADAMA, Raleigh, North Carolina, and SipcamAdvan, Durham, North Carolina; and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 128 pages. Final report issued January 14, 2015, and Amendment 2 issued March 9, 2015.

Document No.: MRIDs 49659704 & 49659702

Guideline: 850.6100

Statements: ECM: The study was not conducted in accordance Good Laboratory Practice (GLP) standards (p. 3 of MRID 49659704). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity statements were not included. A signed and dated Summary of Revisions to Previous Versions was included (p. 4).
ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 49659702). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A certification of authenticity was not included. A change history of the study report was provided (p. 9).

Classification: This analytical method is classified as Supplemental. It could not be determined if the ILV was conducted independently of the ECM since the ILV study author communicated directly with Kaijun Lin of Syngenta who was the ECM study author, as well as the ILV Study Monitor. In the ECM, an insufficient number of samples was prepared for all fortifications/ matrices and no representative chromatograms of the validation were provided. It could not be determined that the ILV were provided with the most difficult matrices with which to validate the method and that ILV soil

	matrices covered the range of soils used in the terrestrial field dissipation studies. The LOD was not reported in the ILV.	
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CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature: Lisa Muto Date: 03/20/2019
	Mary Samuel, M.S., Environmental Scientist	Signature: Mary Samuel Date: 03/21/2019

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

This analytical method, Syngenta Residue Method GRM005.08A, is designed for the quantitative determination in soil at the LOQ of 0.005 mg/kg of chlorothalonil using LC/MS/MS. The LOQ of Syngenta Residue Method GRM005.08A is less than the lowest toxicological level of concern in soil. The ECM and ILV used two characterized soil matrices although USDA soil classification was not specified. The ILV soil sources and textures were the same as those of the ECM, but the characterization data differed. It could not be determined that the ILV were provided with the most difficult matrices with which to validate the method and that ILV soil matrices covered the range of soils used in the terrestrial field dissipation studies since ILV soils were not compared to or derived from terrestrial field dissipation studies. **It could not be determined if the ILV was conducted independently of the ECM** since the ILV study author communicated directly with the ECM study author. The ILV validated Syngenta Residue Method GRM005.08A in the first trial as written, except for insignificant modifications of the analytical instrumentation and equipment method. All ILV and ECM data regarding repeatability, accuracy, precision, and linearity were satisfactory; however, an insufficient number of samples was prepared for all fortifications/ matrices in the ECM. The specificity of the method was supported by ILV representative chromatograms, but no representative chromatograms of the validation 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						
Chlorothalonil	49659704 (GRM005.08A)	49659702		Soil[1,2]	13/02/2015	Syngenta Crop Protection, LLC	LC/MS/MS	0.005 mg/kg

1 In the ECM, clay loam soil (25% sand, 43% silt, 32% clay, pH 6.5 in 1:1 soil:water, 4.2% organic carbon) from Underwood Farm 0-6" (TK0002309) and sandy loam soil (73% sand, 16% silt, 11% clay, pH 7.8 in 1:1 soil:water, 0.89% organic carbon) from Madera, California, 1-15-13 0-6" (TK0002309) were used in the study (USDA soil texture characterization not specified; Appendix 1, Table 1, p. 81 of MRID 49659702 – data found in ILV).

2 In the ILV, clay loam soil (21% sand, 42% silt, 37% clay, pH 7.6 in 0.01M $CaCl_2$, organic carbon not reported) from Underwood Farm 0-6" (TK0002309) and sandy loam soil (55% sand, 28% silt, 17% clay, pH 7.3 in 0.01M $CaCl_2$, organic carbon not reported) from Madera, California, 1-15-13 0-6" (TK0002309) were used in the study (USDA soil texture characterization not specified; pp. 12-13; Table 1, p. 23 of MRID 49659702). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

I. Principle of the Method

Syngenta Residue Method GRM005.08A

Soil samples (20 g) in a Nalgene plastic bottle (250 mL) was fortified with the fortification standard solution in methanol, if necessary (pp. 13-15 of MRID 49659704). After 5 minutes of equilibration, the soil was extracted with 100 mL of freshly prepared acidified acetone (freshly prepared by mixing 1,540 mL acetone with 60 mL of 50% H_2SO_4 in ultrapure water) via shaking on a mechanical shaker (*ca*. 300 cps) for 2 hours. After centrifugation at *ca*. 3500 rpm with refrigeration at 10°C for about 10 minutes, the supernatant was decanted into a second clean brown Nalgene plastic bottle (125 mL). The method noted that, with some soils, particularly those with high clay contents, the solution may still be visibly cloudy even after centrifugation. The supernatant was stored in a refrigerator if not processed immediately. The sample cleanup and concentration are accomplished using an Agilent Bond Elut C18 solid phase extraction (500 mg, 6-mL) cartridge. The SPE cartridge was conditioned as follows: 3 mL of methanol; then 3 mL of ultrapure water. After the sample (2 mL of the extract diluted with 10 mL of ultrapure water) was loaded onto the cartridge, the sample tube was washed with 3 mL of 50:50 (v:v) methanol:ultrapure water which was then transferred to the SPE cartridge. The cartridge was rinsed three times with 4 mL of 50:50 (v:v) methanol:ultrapure water. The analyte was eluted with 5 mL of acetonitrile and collected into a clean glass tube. The eluant was mixed with 1 mL of 0.1% of formic acid and evaporated to just less than 1 mL under a gentle stream of nitrogen at a bath temperature of *ca*. 40°C. The sample was reconstituted with 2 mL of 40:60 (v:v) methanol:0.1% of formic acid in ultrapure water. After sonication for 5 minutes, a *ca*. 1 mL of the aliquot was transferred into a HPLC injection vial for LCMS/MS analysis.

For optional SPE Clean-up Procedure for LC-MS/MS with APPI Ion Source, sample cleanup and concentration are accomplished using an Agilent Bond Elut C18 solid phase extraction (500 mg, 6-mL) cartridge (pp. 15-16 of MRID 49659704). The SPE cartridge was conditioned as follows: 3 mL of methanol; then 3 mL of ultrapure water. After the sample (2 mL of the extract diluted

with 10 mL of ultrapure water) was loaded onto the cartridge, the sample tube was washed with 1 mL of 20:80 (v:v) methanol:ultrapure water which was then transferred to the SPE cartridge. The cartridge was rinsed two times with 3 mL of 20:80 (v:v) methanol:ultrapure water. The analyte was eluted with 4 mL of methanol and collected into a clean glass tube. The eluant was mixed with 1 mL of 0.1% of formic acid and evaporated to just less than 1 mL under a gentle stream of nitrogen at a bath temperature of *ca*. 40°C. The sample was reconstituted with 2 mL of 0.1% formic acid in 50:50 (v:v) methanol:ultrapure water. After vortex mixing, the sample was transferred into a HPLC injection vial for LCMS/MS analysis with APPI Ion Source.

Samples were analyzed for chlorothalonil using a Waters Acquity UPLC system (1 class) coupled to an Applied Biosystems Sciex API 5500 mass spectrometer (pp. 16-21 of MRID 49659704). The LC/MS conditions consisted of a Zorbax SB-Aq column (50 x 4.6 mm, 1.8 µm particle size; oven temperature ambient) with a mobile phase gradient of A) 0.1mM ammonium acetate in ultrapure water and B) methanol [percent A:B (v:v) at 0-0.5 min. 80:20, 8-12 min. 5/10:95/90, 12.1-15 min. 80:20] and TurboIonSpray (ESI) ionization interface MS detection in negative ion mode with MRM (TEM 650°C). Injection volume was 20 µL. Two ion transitions were monitored as follows (quantitative and confirmatory, respectively): *m/z* 244.8→181.9 and *m/z* 244.8→209.9. An alternative confirmatory ion transition was also provided: *m/z* 244.8→174.9. Expected retention time was *ca*. 7 minutes. Optional LC/MS/MS instruments and chromatography conditions were provided. The option of a Surveyor Plus LC system coupled with a Thermo Electron TSQ Quantum Ultra MS was suggested. The Optional LC/MS conditions consisted of a Zorbax SB-CN column (75 x 4.6 mm, 3.5 µm particle size; oven temperature 25°C) with a mobile phase gradient of A) 0.05% formic acid in ultrapure water and B) acetonitrile [percent A:B (v:v) at 0-0.5 min. 90:10, 3.0-6.0 min. 10:90, 6.1-7.0 min. 90:10] and HESI-II Probe (APPI) ionization interface MS detection in negative ion mode with MRM (vaporization temperature 400°C). Injection volume was 20 µL. Two ion transitions were monitored as follows (quantitative and confirmatory, respectively): *m/z* 244.9→182.0 and *m/z* 246.9→184.0. An alternative confirmatory ion transition was also provided: *m/z* 244.9→175.0. Expected retention time was *ca*. 3.0-5.5 minutes.

The method contained precautions for use of different SPE equipment, as well as non-HPLC grade solvents (p. 16 of MRID 49659704). Also, to minimize the chance of carry-over of high recovery samples, samples should be diluted and solvent blanks should be injected after high recovery samples. A Method Flow Chart was included (Appendix 4, p. 55).

ILV

The ILV reportedly performed Syngenta Residue Method GRM005.08A as written, except for insignificant modifications of the analytical instrumentation and equipment (pp. 11, 15-18 of MRID 49659702). Samples were analyzed for chlorothalonil using a Waters Acquity UPLC system coupled to an Applied Biosystems Sciex Triple Quad 6500 mass spectrometer. The LC/MS conditions consisted of a Zorbax SB-Aq column (50 x 4.6 mm, 1.8 mm; oven temperature ambient) with a mobile phase gradient of A) 0.1mM ammonium acetate in ultrapure water and B) methanol [percent A:B (v:v) at 0-0.5 min. 80:20, 8-12 min. 5:95, 12.1-15 min. 80:20] and TurboIonSpray ionization interface MS detection in negative ion mode with MRM (TEM 500°C). Injection volume was 20 µL. Three ion transitions were monitored as follows

(quantitative, confirmatory 1, and confirmatory 2, respectively): *m/z* 244.900→181.900, *m/z* 244.900→209.900, and *m/z* 244.900→174.900. Expected retention time was *ca*. 7 minutes. The optional SPE Clean-up Procedure for LC-MS/MS with APPI Ion Source was not performed.

In the ECM and ILV, the Limit of Quantification (LOQ) for chlorothalonil in Syngenta Residue Method GRM005.08A was reported as 0.005 mg/kg (ppm; pp. 24, 26 of MRID 49659704; pp. 10, 20 of MRID 49659702). The Limit of Detection (LOD) in the ECM was 4 pg injected on column, equivalent to 0.2 pg/µL, when using a 20 µL injection, for chlorothalonil. In the ILV, the LOD was not reported.

II. Recovery Findings

ECM (MRID 49659704): For Syngenta Residue Method GRM005.08A, mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of chlorothalonil at the LOQ (0.005 mg/kg) and 10×LOQ (0.05 mg/kg) in two soil matrices; however, an insufficient number of samples was prepared for all fortifications/matrices, n = 3 (Appendix 1, Tables 2-3, p. 82 of MRID 49659702 – data found in ILV). Two ion transitions were monitored for chlorothalonil; performance data (results) of the quantitation and confirmation analyses were comparable. Clay loam soil (25% sand, 43% silt, 32% clay, pH 6.5 in 1:1 soil:water, 4.2% organic carbon) from Underwood Farm 0-6" (TK0002309) and sandy loam soil (73% sand, 16% silt, 11% clay, pH 7.8 in 1:1 soil:water, 0.89% organic carbon) from Madera, California, 1-15-13 0-6" (TK0002309) were used in the study (USDA soil texture characterization not specified; Appendix 1, Table 1, p. 81 of MRID 49659702).

ILV (MRID 49659702): For Syngenta Residue Method GRM005.08A, mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of chlorothalonil at the LOQ (0.005 mg/kg) and 10×LOQ (0.05 mg/kg) in two soil matrices (Tables 3-5, pp. 24-26). Two ion transitions were monitored for chlorothalonil; performance data (results) of the quantitation and confirmation analyses were comparable. Different confirmatory ion transitions were used for each soil. Clay loam soil (21% sand, 42% silt, 37% clay, pH 7.6 in 0.01M $CaCl_2$, organic carbon not reported) from Underwood Farm 0-6" (TK0002309) and sandy loam soil (55% sand, 28% silt, 17% clay, pH 7.3 in 0.01M $CaCl_2$, organic carbon not reported) from Madera, California, 1-15-13 0-6" (TK0002309) were used in the study (USDA soil texture characterization not specified; pp. 12-13; Table 1, p. 23). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil sources and textures were the same as those of the ECM, but the characterization data differed. The ILV validated Syngenta Residue Method GRM005.08A in the first trial as written, except for insignificant modifications of the analytical instrumentation and equipment method (pp. 11, 15-18 of MRID 49659702).

Table 2. Initial Validation Method Recoveries for Chlorothalonil in Soil[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	Sandy Loam Soil					
	Quantitation ion transition					
Chlorothalonil	0.005	3	78-85	82	4	4.3
	0.05	3	71-76	73	3	3.3
	Confirmation ion transition					
Chlorothalonil	0.005	3	73-100	87	14	15
	0.05	3	70-76	74	3	4.2
	Clay Loam Soil					
	Quantitation ion transition					
Chlorothalonil	0.005	3	106-117	113	6	5.0
	0.05	3	99-104	101	3	2.6
	Confirmation ion transition					
Chlorothalonil	0.005	3	107-131	120	12	10
	0.05	3	96-101	99	3	2.7

Data (uncorrected recovery results; pp. 21-23 of MRID 49659704) were obtained from Appendix 1, Tables 2-3, p. 82 of MRID 49659702 and DER Attachment 2.

1 The clay loam soil (25% sand, 43% silt, 32% clay, pH 6.5 in 1:1 soil:water, 4.2% organic carbon) from Underwood Farm 0-6" (TK0002309) and sandy loam soil (73% sand, 16% silt, 11% clay, pH 7.8 in 1:1 soil:water, 0.89% organic carbon) from Madera, California, 1-15-13 0-6" (TK0002309) were used in the study (USDA soil texture characterization not specified; Appendix 1, Table 1, p. 81 of MRID 49659702).

2 Two ion transitions were monitored as follows (quantitative and confirmatory, respectively): *m/z* 244.8→181.9 and *m/z* 244.8→209.9.

3 Standard deviations were reviewer-calculated since these values were not provided in the study report. Rules of significant figures were followed.

Table 3. Independent Validation Method Recoveries for Chlorothalonil in Soil[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Sandy Loam Soil					
	Quantitation ion transition					
Chlorothalonil	0.005	5	82-97	88	6	7
	0.05	5	78-94	83	7	8
	Confirmation ion transition 1					
Chlorothalonil	0.005	5	76-98	89	10	11
	0.05	5	78-96	84	7	9
	Confirmation ion transition 2					
Chlorothalonil	0.005	5	Not quantified			
	0.05	5				
	Clay Loam Soil					
	Quantitation ion transition					
Chlorothalonil	0.005	5	61-83	73	8	11
	0.05	5	82-99	90	6	7
	Confirmation ion transition					
Chlorothalonil	0.005	5	Not quantified			
	0.05	5				
	Confirmation ion transition 2					
Chlorothalonil	0.005	5	71-83	76	5	7
	0.05	5	82-98	89	6	6

Data (uncorrected recovery results; Appendix 3, p. 120) were obtained from Tables 3-5, pp. 24-26 of MRID 49659702.

1 The clay loam soil (21% sand, 42% silt, 37% clay, pH 7.6 in 0.01M $CaCl_2$, organic carbon not reported) from Underwood Farm 0-6" (TK0002309) and sandy loam soil (55% sand, 28% silt, 17% clay, pH 7.3 in 0.01M $CaCl_2$, organic carbon not reported) from Madera, California, 1-15-13 0-6" (TK0002309) were used in the study (USDA soil texture characterization not specified; pp. 12-13; Table 1, p. 23). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil sources and textures were the same as those of the ECM, but the characterization data differed.

2 Three ion transitions were monitored as follows (quantitative, confirmatory 1, and confirmatory 2, respectively): *m/z* 244.900→181.900, *m/z* 244.900→209.900, and *m/z* 244.900→174.900.; however, only two ion transitions were quantified for each soil. Ion transitions were similar to those of the ECM.

III. Method Characteristics

In the ECM and ILV, the LOQ for chlorothalonil in Syngenta Residue Method GRM005.08A was reported as 0.005 mg/kg (ppm; pp. 24, 26 of MRID 49659704; pp. 10, 20 of MRID 49659702). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated, i.e. which yielded a mean recovery of 70-110% and relative standard deviation of ≤20%. No justifications were reported in the ILV. The LOD in the ECM was 4 pg injected on column, equivalent to 0.2 pg/µL, when using a 20 µL injection, for chlorothalonil. In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as three times the mean amplitude of the background noise. The ECM study authors noted that the LOD may vary between runs and from instrument to instrument. In the ILV, the LOD was not reported. No calculations for LOQ and LOD were reported in the ECM; no calculations for LOQ were reported in the ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

Table 4. Method Characteristics in Soil

Analyte		**Chlorothalonil**
Analysis[1]		LC/MS/MS
Limit of Quantitation (LOQ)	ECM	0.005 mg/kg
	ILV	
Limit of Detection (LOD)	ECM	4 pg injected on column, equivalent to 0.2 pg/µL, when using a 20 µL injection
	ILV	**Not reported**
Linearity (calibration curve r^2 and concentration range)	ECM[2]	r^2 = 0.9990 (Q) r^2 = 0.9980 (C)
	ILV[3]	r^2 = 0.9958 (Q) r^2 = 0.9956 (C1) r^2 = 0.9980 (C2)
	Range	0.2-20 ng/mL (solvent-based)
Repeatable	ECM[4]	Yes at LOQ and 10×LOQ, but n = **3** (two characterized soil matrices).
	ILV[5,6]	Yes at LOQ and 10×LOQ (two characterized soil matrices).
Reproducible		Yes at LOQ and 10×LOQ
Specific	ECM[2]	**No** representative chromatograms for validation provided.
	ILV	Yes, matrix interferences were <7% (Q) and <11% (C) of the LOQ (based on peak area). Minor baseline noise interfered with LOQ peak integration in the sandy loam soil.

Data were obtained from pp. 24, 26 (ECM LOQ/LOD) of MRID 49659704; pp. 10, 20 (ILV LOQ/LOD); Tables 3-5, pp. 24-26 (ILV recovery results); Figures 25-27, pp. 41-42 (ILV calibration curves); Figures 1-24, pp. 29-40 (ILV chromatograms) of MRID 49659702; Appendix 1, Tables 2-3, p. 82 (ECM recovery data); Appendix 1, Figures 6-7, pp. 108-111 (ECM calibration curves); Appendix 1, Figures 2-5, pp. 88-106 (ECM chromatograms) of MRID 49659702; DER Attachment 2. Q = Quantitation ion transition; C = Confirmation ion transition; C1 = Confirmation ion transition 1; C2 = Confirmation ion transition 2.

1 Two ion transitions were monitored for R182281, R611965, R611968, R417888, SYN546669 and SYN510573 using LC/MS/MS. R611966 and R613636 were identified using three ions via GC/MS; however, only the primary and confirmatory 1 were quantified.

2 ECM correlation coefficients (r^2) values for LC/MS/MS analyses were reviewer-calculated from r values provided in the ILV MRID (Appendix 1, Figures 6-7, pp. 108-111 of MRID 49659702; DER Attachment 2). The correlation coefficients (r^2) values for the Optional LC/MS/MS (APPI) were also reported [(r^2 = 0.9984 (Q) and 0.9999 (C)].

3 ILV correlation coefficients (r^2) values for LC/MS/MS analyses were reviewer-calculated from r values provided in the study report (Figures 25-27, pp. 41-42 of MRID 49659702; DER Attachment 2).

4 In the ECM, clay loam soil (25% sand, 43% silt, 32% clay, pH 6.5 in 1:1 soil:water, 4.2% organic carbon) from Underwood Farm 0-6" (TK0002309) and sandy loam soil (73% sand, 16% silt, 11% clay, pH 7.8 in 1:1 soil:water, 0.89% organic carbon) from Madera, California, 1-15-13 0-6" (TK0002309) were used in the study (USDA soil texture characterization not specified; Appendix 1, Table 1, p. 81 of MRID 49659702 – data found in ILV).

5 In the ILV, clay loam soil (21% sand, 42% silt, 37% clay, pH 7.6 in 0.01M $CaCl_2$, organic carbon not reported) from Underwood Farm 0-6" (TK0002309) and sandy loam soil (55% sand, 28% silt, 17% clay, pH 7.3 in 0.01M $CaCl_2$, organic carbon not reported) from Madera, California, 1-15-13 0-6" (TK0002309) were used in the study (USDA soil texture characterization not specified; pp. 12-13; Table 1, p. 23 of MRID 49659702). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil sources and textures were the same as those of the ECM, but the characterization data differed.

6 The ILV validated Syngenta Residue Method GRM005.08A in the first trial as written, except for insignificant modifications of the analytical instrumentation and equipment method (pp. 11, 15-18 of MRID 49659702 of MRID 49659702).

IV. Method Deficiencies and Reviewer's Comments

1. It could not be determined if the ILV was conducted independently of the ECM since the ILV study author (Dan Guo) communicated directly with Kaijun Lin of Syngenta who was the ECM study author, as well as the ILV Study Monitor (pp. 5, 19; Appendix 5, p. 128 of MRID 49659702). These communications included exchange of protocols, acquisition of analytical standard and control sample, questions regarding preparation of reagents, and pre-validation evaluation and method establishment including calibration curve linearity. Details reports of the communication were not included in the ILV since the ILV reported that there were no client communications requiring inclusion in the report. OCSPP guidelines state that ILV validations are performed without collusion with the ECM personnel.

2. In the ECM, an insufficient number of samples was prepared for all fortifications/ matrices, n = 3 (Appendix 1, Tables 2-3, p. 82 of MRID 49659702 – data found in ILV). OCSPP guidelines state that each set of performance data should contain a minimum of five spiked replicates which were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.

3. The specificity of the method was not supported in the ECM since no representative chromatograms of the validation were provided, only those of calibration standards (Appendix 1, Figures 2-5, pp. 88-106 of MRID 49659702).

4. It could not be determined that the ILV was provided with the most difficult matrices with which to validate the method. OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs. Soil characterization data was provided for the ILV soils, but USDA soil texture characterization not specified (pp. 12-13; Table 1, p. 23 of MRID 49659702). The reviewer noted that soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Additionally, since no terrestrial field dissipation studies were submitted, it not be determined if the ILV soil matrices covered the range of soils used in the terrestrial field dissipation studies. The ILV test soils were reportedly from Syngenta Study # TK0002309, but the title, reference, and description for this study was not reported. The reviewer noted that a certain number of soil matrices is not specified in the OCSPP guidelines in order to cover the range of soils used in the terrestrial field dissipation studies.

   The reviewer noted that the ECM test soils were clay loam soil (25% sand, 43% silt, 32% clay, pH 6.5 in 1:1 soil:water, 4.2% organic carbon) from Underwood Farm 0-6" (TK0002309) and sandy loam soil (73% sand, 16% silt, 11% clay, pH 7.8 in 1:1 soil:water, 0.89% organic carbon) from Madera, California, 1-15-13 0-6" (TK0002309; USDA soil texture characterization not specified; Appendix 1, Table 1, p. 81 of MRID 49659702 – data found in ILV). The soil sources and textures were the same as those of the ILV, but the characterization data differed.

The reviewer also noted that the ILV soil matrices reported in this ILV study (MRID 49659702) matched the soil descriptions and soil characteristics reported in another soil ILV performed by the same ILV for chlorothalonil degradates (MRID 49659703); however, the soil sources (sites) differed. The reviewer believed that a typographical error occurred within the two ILVs.

5. The estimations of the LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 24, 26 of MRID 49659704; pp. 10, 20 of MRID 49659702). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated, i.e. which yielded a mean recovery of 70-110% and relative standard deviation of ≤20%. No justifications were reported in the ILV. In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as three times the mean amplitude of the background noise. The ECM study authors noted that the LOD may vary between runs and from instrument to instrument. In the ILV, the LOD was not reported. No calculations for LOQ and LOD were reported in the ECM; no calculations for LOQ were reported in the ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

6. The reviewer noted the following typographical error in the ECM regarding the HPLC mobile phase gradient: percent A:B (v:v) at 8 min. 5:95 and 12 min. **10:95** (p. 17 of MRID 49659704). In the ILV, the HPLC mobile phase gradient was **8-12 min. 5:**95 (pp. 11, 15-18 of MRID 49659702). The reviewer assumed that this was the correct mobile phase gradient. The ECM should be corrected.

   The reviewer also noted the following in the ILV: the specifications of the Zorbax SB-Aq column were reported as 50 x 4.6 mm, 1.8 **mm** (p. 17 of MRID 49659702). The reviewer believed that the specifications may have had a typographical error in them and that they should have been reported as 50 x 4.6 mm, 1.8 **µm** to match those of the ECM.

7. In the ILV, a change history of the study report was provided (p. 9 of MRID 49659702). The statement “The control samples are free of interference” was added to the Executive Summary Results and Discussion, and Conclusion. Elaboration of this statement was added to the Discussion. Additionally, the Syngenta Task Number was changed from "TK0225796” to “TK0225797”.

8. In the ILV and ECM, the matrix effects were determined to be insignificant (<±20%) for both soil types (p. 27; Table 7, p. 27; Appendix 1, p. 83 of MRID 49659702). The reviewer noted that matrix effect values were -22% for the Madera soil, which was described as sandy clay loam soil (instead of sandy loam soil).

9. In the ILV and ECM, the final soil extracts of chlorothalonil were found to be stable for up to *ca*. 7 days at *ca*. 4°C (Table 6, p. 27; Appendix 1, p. 84 of MRID 49659702).

10. The ILV reported that 1 sample set of 13 samples each can be completed in 1 day with LC/MS/MS performed overnight (p. 19 of MRID 49659702).

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

Chlorothalonil (R44686; SDS2787)

IUPAC Name:	Tetrachloroisophthalonitrile
CAS Name:	2,4,5,6-Tetrachloro-1,3-benzenedicarbonitrile
CAS Number:	1897-45-6
SMILES String:	N#Cc(c(c(c(c1C#N)Cl)Cl)Cl)c1Cl