Analytical method for chlorothalonil in crops [apple, peach, grape, strawberry, orange (skin), orange (flesh), olive, banana (skin), banana (flesh), potato (tuber), carrot, onion, cabbage, cauliflower, leek, pea (fresh seed), pea (dried seed), French bean (fresh pod), tomato, melon (flesh, 1), cereal (grain), cereal (straw), cereal (forage), potato (foliage), peanut (nut), and melon (flesh, 2)]

Reports: ECM: EPA MRID No.: MRID 50826509. Chaggar, S. 2006. Chlorothalonil. Chlorothalonil (R44686) - Analytical Method for the Determination of Residues of Chlorothalonil and R182281 in Crops. Analytical Method. Syngenta Report No.: GRM005.01A and Task No.: TK0428370. Report prepared and sponsored by Syngenta Ltd, Berkshire, United Kingdom, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 67 pages. Final report issued October 31, 2006.

ILV: EPA MRID No. 50826510. Bluemink, S., and J. Mumford. 2019. Chlorothalonil. Chlorothalonil (R44686) - Independent Laboratory Validation of Analytical Method GRM005.01A for the Determination of Residues of Chlorothalonil in Crops. Final Report. Report and Study No.: 3202297. Task No.: TK0428828. Report prepared by Smithers Viscient (ESG) Ltd., North Yorkshire, United Kingdom, and sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 146 pages. Final report issued February 21, 2019.

Document No.: MRIDs 50826509 & 50826510

Guideline: 850.6100

Statements: ECM: The study was not conducted in accordance with the USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160; p. 3 of MRID 50826509). 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 the previous version was provided (p. 5).
ILV: The study was conducted in accordance with the UK GLP standards (1999) as amended by the GLP Regulations 2004 and OECD GLP (p. 3 of MRID 50826510). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-4, 6).

Classification: This analytical method is classified as SUPPLEMENTAL. Method validation reproducibility involves the comparison of similar matrices, of which there was only one crop (potato foliage/potato above-ground foliage); therefore, reproducibility was only assessed for potato foliage/potato above-ground foliage. For potato foliage/potato above-ground foliage, the reproducibility of the method could not be determined at 10×LOQ. Based on ILV conclusions from the organic ground almond matrix, the ECM should be updated to include a statement that the method was not suitable for high oil content matrices. ILV linearity was not satisfactory for chlorothalonil analysis in lemons. In the ECM, no samples were prepared at 10×LOQ for 13 of the 26 tested crop matrices. Insufficient ECM supporting data, including linear regression curves and representative chromatograms, was

	provided.	
PC Code:	081901	
EFED Final Reviewer:	Sheng Lin, Ph.D., Physical Scientist	Signature: SHENG LIN Digitally signed by SHENG LIN Date: 2020.12.29 12:06:28 -05'00' Date: 12/2/20
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature: Lisa Muto Date: 10/15/2019
	Mary Samuel, M.S., Environmental Scientist	Signature: Marysamuel Date: 10/15/2019

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, Syngenta Method No. GRM005.01A, is designed for the quantitative determination of chlorothalonil in crops at the LOQ of 0.01 mg/kg using GC/MS. The LOQ is less than the lowest toxicological level of concern in crops. The ECM was not a laboratory study, but summarized validation performance data for 27 crop matrices from at least two studies. The ILV was a laboratory study employing 5 crop matrices. Only one crop matrix of the ECM matched that of the ILV: potato foliage/potato above-ground foliage. The ILV validated the method as written, except for modification of the calibration range and insignificant modifications of the analytical instrumentation and equipment. The ILV validated the method in the first trial for peanut above-ground foliage, potato above-ground foliage, and organic lemons crop matrices. The ILV validated the method in the second trial for hybrid Bermuda grass clippings; the first trial failed due to poor matrix-matched calibration standards. The ILV originally included organic ground almonds as a crop matrix; however, the ILV study author determined that the method was not suitable for high oil content matrices. **The ECM should be updated to include a statement that the method was not suitable for high oil content matrices.** Method validation reproducibility involves the comparison of similar matrices, of which there was only one crop (potato foliage/potato above-ground foliage); therefore, reproducibility was only assessed for potato foliage/potato above-ground foliage. For potato foliage/potato above-ground foliage, the reproducibility of the method could not be determined at 10×LOQ since only ILV performance data was submitted. Performance data was acceptable for all matrices in the ECM and ILV at the tested fortification levels (LOQ and 10×LOQ/100×LOQ/1000×LOQ), except for analysis of almonds. In the ECM, no samples were prepared at 10×LOQ for 13 of the 26 tested crop matrices. All ILV data regarding linearity and specificity was satisfactory for chlorothalonil analysis in all crop matrices, except lemons; no supporting data for almonds was provided. Insufficient ECM supporting data, including linear regression curves and representative chromatograms, was provided for most crop matrices.

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	50826509[1]	50826510[2]		Crops	31/10/2006	Syngenta Crop Protection, LLC	GC/MS	0.01 mg/kg

1 In the ECM, the following crops were included: apple, peach, grape, strawberry, orange (skin), orange (flesh), olive, banana (skin), banana (flesh), potato (tuber), carrot, onion, cabbage, cauliflower, leek, pea (fresh seed), pea (dried seed), French bean (fresh pod), tomato, melon (flesh, 1), cereal (grain), cereal (straw), cereal (forage), potato (foliage), peanut (nut), and melon (flesh, 2; Appendix 3, Table 1, pp. 33-35 of MRID 50826509). The crop sources were not reported.

2 In the ILV, the following crops were included: hybrid Bermuda grass clippings, peanut above-ground foliage, potato above-ground foliage, organic lemons, and organic ground almonds (p. 21 of MRID 50826510). Hybrid Bermuda grass clippings, peanut above-ground foliage, and potato above-ground foliage were provided by the Sponsor and. The organic lemon and ground almonds were purchased by Sainbury's in Harrogate, United Kingdom. The only crop matrix of the ILV which matched a crop matrix of the ECM was the potato foliage; it could not be determined if the two potato foliage matrices were the same or different.

I. Principle of the Method

Crop samples were prepared acceptable SOPs, then further processed by one of the following methods: wet crops (e.g. tomatoes) were homogenized in the presence of 1M H_2SO_4 (10 mL acid to 100 g crop) and dry ice, dry crops (e.g. cereal straw) were prepared by chopping in a knife mill without acid, and dry seed crops (e.g. peanuts) were not prepared in any way (p. 12 of MRID 50826509). Crop samples (10 g for dry samples and 11 g for wet samples with acid) in screw-cap plastic centrifuge bottle (250 mL) or screw-cap glass blending jars (500 mL for larger grain size nuts and pulses) were fortified with the 1000 µg/mL fortification standard solution in toluene, if necessary (pp. 9-10, 12-14 of MRID 50826509). The crop samples in centrifuge bottles were extracted with 100 mL (minus the water content of the samples and a further 1 mL for crops treated with acid) of acetone:5M sulfuric acid solution (95:5, v:v) via homogenization at high speed for 3-5 minutes. For crops in screw-cap glass blending jars, the crop samples in centrifuge bottles were extracted with 100 mL (minus the water content of the samples) of acetone:5M sulfuric acid solution (95:5, v:v) via the standard blending blades for 3-5 minutes. The method cautioned against the use of detergents to clean blending containers as any residues remaining on glassware may cause instability problems with chlorothalonil. After homogenization/blending, the samples were centrifuged at 3500 rpm for 5 minutes (the speed should be adequate to see separation). Aliquots of the crop extract (equivalent to 0.2 g) were transferred to 10 mL disposable glass test tubes and mixed with 6 mL of water. The sample cleanup was accomplished using C8 (EC) solid phase extraction (100 mg, 3-mL) cartridge pre-conditioned as follows: 3 mL of methanol; then 3 mL of water. After the sample was loaded onto

the cartridge, the sample was washed with 1.5 mL of 70:30 (v:v) water:acetonitrile. The cartridge was dried under high vacuum (≤500 mbar) for *ca*. 15-20 minutes, then the analyte was eluted with 2 mL of toluene and collected into a clean pre-calibrated graduated collection tube at a rate of *ca*. 2 mL/min. The final volume of the eluant was adjusted to 4 mL with toluene, and a 1 mL aliquot was transferred to a vial for GC/MS analysis.

Samples were analyzed for chlorothalonil using an Agilent 6890 GC coupled to an Agilent 5973 series mass selective detector with negative chemical ionization mode using methane gas (pp. 16-17 of MRID 50826509). The GC/MS conditions consisted of a BPX-50 column (15.0 m x 0.25 mm x 0.25 µm), helium carrier gas, injector temperature 250°C, and temperature program of 120°C hold for 1 min., 20°C/min. to 300°C. Injection volume was 1 µL. Three ions were monitored as follows (quantitative, confirmatory 1, and confirmatory 2, respectively): *m/z* 266, 264, and 268. The ratio of the ions was *ca*. 100:80:45 for quantitative: confirmatory 1: confirmatory 2. Expected retention time was 6.07 minutes.

The ILV performed Syngenta Residue Method GRM005.01A as written, except for modification of the calibration range to 0.0001 to 0.01 µg/mL and insignificant modifications of the analytical instrumentation and equipment (pp. 13, 20-21, 24; Appendix 6, pp. 127-131; Appendix 7, p. 146 of MRID 50826510). Samples were analyzed for chlorothalonil using a Thermo TSQ8000 Evo GC/MS/MS in single MS (SIM) negative chemical ionization mode using methane gas. GC conditions were the same as those in the ECM, except that the injector temperature was not reported. The same three ions were monitored as in the ECM. The expected retention time was *ca*. 5.9 minutes. Crop matrices of hybrid Bermuda grass clippings, peanut above-ground foliage, and potato above-ground foliage were processed by the Sponsor prior to shipment, including addition of acid to potato above-ground foliage (p. 21 of MRID 50826510). The ground almonds were not processed further. The whole lemons were homogenized with dry ice according to local SOPs.

In the ECM and ILV, Limit of Quantification (LOQ) and Limit of Detection (LOD) in crops were 0.01 mg/kg and 0.002 mg/kg, respectively, for chlorothalonil (pp. 22-23 of MRID 50826509; p. 16 of MRID 50826510).

II. Recovery Findings

ECM (MRID 50826509): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of chlorothalonil in peach, orange (flesh), olive, banana (flesh), potato (tuber), carrot, onion, pea (fresh seed), pea (dried seed), melon (flesh, 1), cereal (grain), peanut (nut), and melon (flesh, 2) at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10×LOQ; Appendix 3, Table 1, pp. 33-35). Mean recoveries and RSDs were within guideline requirements for analysis of chlorothalonil in apple, grape, strawberry, orange (skin), banana (skin), cabbage, cauliflower, French bean (fresh pod), and tomato at fortification levels of 0.01 mg/kg (LOQ) and 1 mg/kg (100×LOQ); no samples were prepared at 10×LOQ. Mean recoveries and RSDs were within guideline requirements for analysis of chlorothalonil in leek, cereal (straw), cereal (forage), and potato (foliage) at fortification levels of 0.01 mg/kg (LOQ) and 10 mg/kg (1000×LOQ); no samples were prepared at 10×LOQ. Three ions were monitored using GC/MS; however, recovery results were only provided for the quantitation ion. A confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data. The crop sources were not reported.

ILV (MRID 50826510): Mean recoveries and RSDs were within guideline requirements for analysis of chlorothalonil in hybrid Bermuda grass clippings, peanut above-ground foliage, potato above-ground foliage, and organic lemons at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10×LOQ), except for the confirmatory ion 1 analysis of potato above-ground foliage at the LOQ (mean 69.8%; Tables 1-3, pp. 29-31). Mean recoveries and RSDs were not within guideline requirements for analysis of chlorothalonil in organic ground almonds at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10×LOQ) where means were 34.0-44.1% for both fortifications (p. 23). The study author concluded that the method was not suitable for high oil content matrices (pp. 18, 20, 23, 27). Analytes were identified and quantified using three ions and GC/MS analysis. Hybrid Bermuda grass clippings, peanut above-ground foliage, and potato above-ground foliage were provided by the Sponsor and. The organic lemon and ground almonds were purchased by Sainbury's in Harrogate, United Kingdom (p. 21). The only crop matrix of the ILV which matched a crop matrix of the ECM was the potato foliage; it could not be determined if the two potato foliage matrices were the same or different. The Syngenta Residue Method GRM005.01A was validated for chlorothalonil in the hybrid Bermuda grass clippings, peanut above-ground foliage, potato above-ground foliage, and organic lemons crop matrices at both fortification levels as written, except for modification of the calibration range and insignificant modifications of the analytical instrumentation and equipment (pp. 18, 27). The ILV validated the method in the first trial for peanut above-ground foliage, potato above-ground foliage, and organic lemons crop matrices (Appendix 4, p. 121). The ILV validated the method in the second trial for hybrid Bermuda grass clippings; the first trial failed due to poor matrix-matched calibration standards. **Based on the ILV conclusions, the ECM should be updated to include a statement that the method was not suitable for high oil content matrices.**

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

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
Quantitation ion						
	Apple					
Chlorothalonil	0.01 (LOQ)	5	92-98	95	2	2
	1	5	75-81	78	3	4
	Peach					
Chlorothalonil	0.01 (LOQ)	5	103-109	105	2	2
	0.1	5	91-111	100	8	8
	Grape					
Chlorothalonil	0.01 (LOQ)	5	82-94	88	5	6
	1	5	96-103	100	3	3
	Strawberry					
Chlorothalonil	0.01 (LOQ)	5	88-100	93	5	5
	1	5	91-106	99	6	6
	Orange (skin)					
Chlorothalonil	0.01 (LOQ)	5	86-95	92	4	4
	1	5	83-91	88	3	4
	Orange (flesh)					
Chlorothalonil	0.01 (LOQ)	5	72-92	85	8	9
	0.1	5	92-98	94	2	3
	Olive					
Chlorothalonil	0.01 (LOQ)	5	77-85	81	3	4
	0.1	5	76-80	78	2	2
	Banana (skin)					
Chlorothalonil	0.01 (LOQ)	5	92-97	95	2	2
	1	5	96-105	101	4	3
	Banana (flesh)					
Chlorothalonil	0.01 (LOQ)	5	99-103	101	1	1
	0.1	5	99-110	105	4	4
	Potato (tuber)					
Chlorothalonil	0.01 (LOQ)	5	66-77	72	4	6
	0.1	5	92-101	96	3	4
	Carrot					
Chlorothalonil	0.01 (LOQ)	5	97-104	100	3	3
	0.1	5	90-104	99	5	5
	Onion					
Chlorothalonil	0.01 (LOQ)	5	94-100	96	3	3
	0.1	5	84-105	96	8	8
	Cabbage					
Chlorothalonil	0.01 (LOQ)	5	90-96	94	2	2
	1	5	87-96	93	4	4
	Cauliflower					
Chlorothalonil	0.01 (LOQ)	5	103-114	108	4	4
	1	5	97-107	101	4	4

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	Leek					
Chlorothalonil	0.01 (LOQ)	5	79-99	89	8	9
	10	5	88-97	93	4	4
	Pea (fresh seed)					
Chlorothalonil	0.01 (LOQ)	5	80-102	92	8	9
	0.1	5	77-91	86	6	6
	Pea (dried seed)					
Chlorothalonil	0.01 (LOQ)	5	90-102	96	4	4
	0.1	5	99-107	104	4	3
	French bean (fresh pod)					
Chlorothalonil	0.01 (LOQ)	5	69-87	79	9	11
	1	5	77-87	82	4	4
	Tomato					
Chlorothalonil	0.01 (LOQ)	5	77-82	79	2	3
	1	5	84-86	85	1	1
	Melon (flesh, 1)[4]					
Chlorothalonil	0.01 (LOQ)	5	90-124	100	14	14
	0.1	5	75-92	86	7	8
	Cereal (grain)					
Chlorothalonil	0.01 (LOQ)	5	79-94	86	7	8
	0.1	5	102-109	106	3	2
	Cereal (straw)					
Chlorothalonil	0.01 (LOQ)	5	85-94	90	4	4
	10	5	93-97	95	1	2
	Cereal (forage)					
Chlorothalonil	0.01 (LOQ)	5	95-104	101	4	4
	10	5	93-103	98	4	4
	Potato (foliage)					
Chlorothalonil	0.01 (LOQ)	5	88-110	95	9	9
	10	5	81-99	91	7	8
	Peanut (nut)					
Chlorothalonil	0.01 (LOQ)	5	84-92	88	4	4
	0.1	5	85-91	89	2	3
	Melon (flesh, 2)[4]					
Chlorothalonil	0.01 (LOQ)	5	91-113	100	9	9
	0.1	5	87-100	92	5	6

Data (uncorrected recovery results; pp. 20-21) were obtained from Appendix 3, Table 1, pp. 33-35 of MRID 50826509.

1 The following crops were included: apple, peach, grape, strawberry, orange (skin), orange (flesh), olive, banana (skin), banana (flesh), potato (tuber), carrot, onion, cabbage, cauliflower, leek, pea (fresh seed), pea (dried seed), French bean (fresh pod), tomato, melon (flesh), cereal (grain), cereal (straw), cereal (forage), potato (foliage), peanut (nut), and melon (flesh; Appendix 3, Table 1, pp. 33-35 of MRID 50826509). The crop sources were not reported.

2 Three ions were monitored as follows (quantitative, confirmatory 1, and confirmatory 2, respectively): *m/z* 266, 264, and 268; however, recovery results were only provided for the quantitation ion (p. 17 of MRID 50826509). A confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.

3 The standard deviations were reviewer-calculated from the recovery results since these values were not reported in

the study report (DER Attachment 2). Rules of significant figures were followed.
4 The two melon (flesh) matrices were presumed to be different; the results were not combined.

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

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
Quantitation ion						
	Hybrid Bermuda grass clippings					
Chlorothalonil	0.01 (LOQ)	5	100-125	112	10	8.46
	0.1	5	90.0-111	102	8	7.27
	Peanut above-ground foliage					
Chlorothalonil	0.01 (LOQ)	5	74.6-95.2	84.9	7.8	9.15
	0.1	5	86.5-98.8	94.8	4.9	5.19
	Potato above-ground foliage					
Chlorothalonil	0.01 (LOQ)	5	63.2-86.3	73.4	8.6	11.7
	0.1	5	90.1-98.2	94.0	3.7	3.97
	Organic lemons					
Chlorothalonil	0.01 (LOQ)	5	79.3-115	102	14	13.4
	0.1	5	95.0-116	106	9	8.41
	Organic ground almonds[4]					
Chlorothalonil	0.01 (LOQ)	5	--	**39.5**	--	7.52
	0.1	5	--	**34.3**	--	14.5
Confirmatory ion 1						
	Hybrid Bermuda grass clippings					
Chlorothalonil	0.01 (LOQ)	5	81.1-114	97.1	15	15.1
	0.1	5	92.7-109	103	7	6.39
	Peanut above-ground foliage					
Chlorothalonil	0.01 (LOQ)	5	82.8-97.4	88.2	5.6	6.36
	0.1	5	88.5-103	96.9	6	5.71
	Potato above-ground foliage					
Chlorothalonil	0.01 (LOQ)	5	52.9-79.5	**69.8**	12.0	17.2
	0.1	5	87.8-100	93.7	5	5.26
	Organic lemons					
Chlorothalonil	0.01 (LOQ)	5	80.0-109	92.0	11	11.7
	0.1	5	92.2-110	103	8	7.67
	Organic ground almonds[4]					
Chlorothalonil	0.01 (LOQ)	5	--	**44.1**	--	10.2
	0.1	5	--	**34.3**	--	14.2
Confirmatory ion 2						
	Hybrid Bermuda grass clippings					
Chlorothalonil	0.01 (LOQ)	5	78.3-109	94.6	15	15.4
	0.1	5	87.8-110	102	9	8.52
	Peanut above-ground foliage					
Chlorothalonil	0.01 (LOQ)	5	61.2-95.2	79.0	13.0	16.5
	0.1	5	90.7-102	96.4	5	5.14
	Potato above-ground foliage					
Chlorothalonil	0.01 (LOQ)	5	67.1-87.9	78.3	7.9	10.1
	0.1	5	86.6-97.5	91.9	4.0	4.35

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	Organic lemons					
Chlorothalonil	0.01 (LOQ)	5	81.4-126	96.5	19	19.5
	0.1	5	94.2-110	104	7	6.69
	Organic ground almonds[4]					
Chlorothalonil	0.01 (LOQ)	5	--	**39.5**	--	12.3
	0.1	5	--	**34.0**	--	16.3

Data (uncorrected recovery results; Appendix 6, p. 131) were obtained from p. 23; Tables 1-3, pp. 29-31 of MRID 50826510.

1 The following crops were included: hybrid Bermuda grass clippings, peanut above-ground foliage, potato above-ground foliage, organic lemons, and organic ground almonds (p. 21 of MRID 50826510). Hybrid Bermuda grass clippings, peanut above-ground foliage, and potato above-ground foliage were provided by the Sponsor and. The organic lemon and ground almonds were purchased by Sainbury's in Harrogate, United Kingdom. The only crop matrix of the ILV which matched a crop matrix of the ECM was the potato foliage; it could not be determined if the two potato foliage matrices were the same or different.

2 Three ions were monitored as follows (quantitative, confirmatory 1, and confirmatory 2, respectively): *m/z* 266, 264, and 268; these were the same as those of the ECM (Appendix 6, p. 131 of MRID 50826510).

3 The standard deviations were reviewer-calculated from the recovery results since these values were not reported in the study report (DER Attachment 2). Rules of significant figures were followed.

4 Raw data, recovery range, and standard deviations were not reported (p. 23 of MRID 50826510).

III. Method Characteristics

In the ECM and ILV, the LOQ and LOD in crops were 0.01 mg/kg and 0.002 mg/kg, respectively, for chlorothalonil (pp. 22-23 of MRID 50826509; pp. 16, 22 of MRID 50826510). In the ECM and ILV, 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%. In the ECM, it was stated that the LOQ for accurate quantitation should yield a response which is no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. 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 author noted that the LOD may vary between runs and from instrument to instrument. In the ILV, the LOD was defined as the sample concentration equivalent to the lowest calibration standard (0.0001 µg/mL), which is also equivalent to the method detection limit (MDL). No calculations supporting the method LOQ and LOD were provided in the ECM and ILV.

Table 4. Method Characteristics for Chlorothalonil in Crops

Analyte		**Chlorothalonil**				
MRID		**ECM Crop Matrices**			**ILV Crop Matrices**	
Crop Matrices		**Peach, Orange (flesh), Olive, Banana (flesh), Potato (tuber), Carrot, Onion, Pea (fresh seed), Pea (dried seed), Melon (flesh, 1), Cereal (grain), Peanut (nut), and Melon (flesh, 2)**	**Apple, Grape, Strawberry, Orange (skin), Banana (skin), Cabbage, Cauliflower, French Bean (fresh pod), and Tomato**	**Leek, Cereal (straw), Cereal (forage), and Potato (foliage)**	**Hybrid Bermuda grass clippings, Peanut above-ground foliage, Potato above-ground foliage, and Organic lemons**	**Organic ground almonds**
Limit of Quantitation (LOQ)		0.01 mg/kg				
Limit of Detection (LOD)	ECM	0.002 mg/kg				
	ILV					
Linearity (calibration curve r^2 and concentration range)	ECM[1]	r^2 = 1.000 (Q, barley grain)	None reported		Not applicable	
		0.4-100 ng/mL				
	ILV[2]	Not applicable			r^2 = 0.9962 (Q, grass) r^2 = 0.9972 (C1, grass) r^2 = 0.9950 (C2, grass) r^2 = 0.9992 (Q, peanut) r^2 = **0.9941** (C1, peanut) r^2 = **0.9926** (C2, peanut) r^2 = 0.9983 (Q, potato) r^2 = 0.9984 (C1, potato) r^2 = 0.9975 (C2, potato) r^2 = **0.9883** (Q, lemon) r^2 = **0.9911** (C1, lemon) r^2 = **0.9863** (C2, lemon)	
					0.1-100 ng/mL	
Repeatable	ECM[3]	Yes at LOQ and 10×LOQ	Yes at LOQ and 100×LOQ; **no** samples prepared at 10×LOQ.	Yes at LOQ and 1000× LOQ; **no** samples prepared at 10×LOQ.	Not applicable	

<table>
<tr><td colspan="2">Analyte</td><td colspan="4">Chlorothalonil</td></tr>
<tr><td colspan="2">MRID</td><td colspan="3">ECM Crop Matrices</td><td colspan="2">ILV Crop Matrices</td></tr>
<tr><td colspan="2">Crop Matrices</td><td>Peach, Orange (flesh), Olive, Banana (flesh), Potato (tuber), Carrot, Onion, Pea (fresh seed), Pea (dried seed), Melon (flesh, 1), Cereal (grain), Peanut (nut), and Melon (flesh, 2)</td><td>Apple, Grape, Strawberry, Orange (skin), Banana (skin), Cabbage, Cauliflower, French Bean (fresh pod), and Tomato</td><td>Leek, Cereal (straw), Cereal (forage), and Potato (foliage)</td><td>Hybrid Bermuda grass clippings, Peanut above-ground foliage, Potato above-ground foliage, and Organic lemons</td><td>Organic ground almonds</td></tr>
<tr><td></td><td>ILV[4,5]</td><td colspan="3">Not applicable</td><td>Yes at LOQ and 10×LOQ (C1 mean at LOQ was 69.8% for potato above-ground foliage)[6]</td><td>No at LOQ and 10×LOQ[7]</td></tr>
<tr><td colspan="2">Reproducible</td><td colspan="2">Could not be determined; crop matrices differed.</td><td>Potato foliage: Yes at LOQ. Could not be determined at 10×LOQ; only one set of performance data was submitted. Other matrices: Could not be determined; crop matrices differed.</td><td>Could not be determined; crop matrices differed.</td></tr>
<tr><td rowspan="3">Specific</td><td rowspan="2">ECM</td><td colspan="2">Only LOQ and control representative chromatograms provided. Minor contaminants observed near RT of analyte.</td><td colspan="2" rowspan="2">Not applicable</td></tr>
<tr><td>Olive/Cereal (grain)/ Strawberry/Cauliflower: Yes, matrix interferences were <5% of the LOQ (based on peak area). No chromatograms provided for all other matrices.</td><td>No chromatograms provided.</td></tr>
<tr><td>ILV</td><td colspan="3">Not applicable</td><td>Yes, matrix interferences were <10% of the LOQ (based on peak area) for all matrices but potato foliage where matrix interferences were <20% of the LOQ (based on peak area). Peak shouldering observed in lemons.</td><td>None provided</td></tr>
</table>

Data were obtained from pp. 22-23 (LOQ/LOD); Appendix 3, Table 1, pp. 33-35 (recovery results); Appendix 4, Figures 4-15, pp. 39-50 (chromatograms); p. 23; Appendix 5, Figures 42-43, p. 63 (calibration curves) of MRID 50826509; pp. 16, 22 (LOQ/LOD); p. 23; Tables 1-3, pp. 29-31 (recovery results); Figures 1-15, pp. 45-59; Figures 19-33, pp. 63-77; Figures 37-51, pp. 81-95; Figures 55-69, pp. 99-113 (chromatograms); p. 27; Figures 16-18, pp. 60-62; Figures 34-36, pp.

78-80; Figures 52-54, pp. 96-98; Figures 70-72, pp. 114-116 (calibration curves) of MRID 50826510; DER Attachment 2. Q = Quantitation ion; C1 = Confirmatory ion 1; C2 = Confirmatory ion 2.

1 Solvent-based standards were used (p. 23 of MRID 50826509). Only one calibration curve was provided for review.

2 Matrix-matched calibration standards were used (p. 27 of MRID 50826510).

3 In the ECM, the following crops were included: apple, peach, grape, strawberry, orange (skin), orange (flesh), olive, banana (skin), banana (flesh), potato (tuber), carrot, onion, cabbage, cauliflower, leek, pea (fresh seed), pea (dried seed), French bean (fresh pod), tomato, melon (flesh), cereal (grain), cereal (straw), cereal (forage), potato (foliage), peanut (nut), and melon (flesh; Appendix 3, Table 1, pp. 33-35 of MRID 50826509). The crop sources were not reported.

4 In the ILV, the following crops were included: hybrid Bermuda grass clippings, peanut above-ground foliage, potato above-ground foliage, organic lemons, and organic ground almonds (p. 21 of MRID 50826510). Hybrid Bermuda grass clippings, peanut above-ground foliage, and potato above-ground foliage were provided by the Sponsor and. The organic lemon and ground almonds were purchased by Sainbury's in Harrogate, United Kingdom. The only crop matrix of the ILV which matched a crop matrix of the ECM was the potato foliage; it could not be determined if the two potato foliage matrices were the same or different.

5 The ILV validated Syngenta Residue Method GRM005.01A for chlorothalonil in the hybrid Bermuda grass clippings, peanut above-ground foliage, potato above-ground foliage, and organic lemons crop matrices at both fortification levels as written, except for modification of the calibration range and insignificant modifications of the analytical instrumentation and equipment (pp. 18, 27 of MRID 50826510). The ILV validated the method in the first trial for peanut above-ground foliage, potato above-ground foliage, and organic lemons crop matrices (Appendix 4, p. 121 of MRID 50826510). The ILV validated the method in the second trial for hybrid Bermuda grass clippings; the first trial failed due to poor matrix-matched calibration standards. These ILV modifications did not warrant an updated ECM.

6 Performance data deviations in the confirmation ion transition did not affect the specificity of the method since a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.

7 The ILV originally included organic ground almonds as a crop matrix; however, the ILV study author determined that the method was not suitable for high oil content matrices (pp. 18, 20, 23, 27 of MRID 50826510). **The ECM should be updated to include a statement that the method was not suitable for high oil content matrices.**

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. ECM MRID 50826509 and ILV MRID 50826510 were submitted for the method validation of chlorothalonil in crops. The ECM was not a laboratory study, but summarized validation performance data for 26 crop matrices from at least two studies (Hill, S.E., 2002, and Chaggar, S., 2006; References 9 and 10; p. 25 of MRID 50826509). The ILV was a laboratory study employing 5 crop matrices. Only one crop matrix of the ECM matched that of the ILV: potato foliage/potato above-ground foliage.

2. Typically, method validation reproducibility involves the comparison of similar matrices. The similarity of the matrices in this method validation was defined as the same crop, of which there was only one crop (potato foliage/potato above-ground foliage); therefore, reproducibility was only assessed for potato foliage/potato above-ground foliage. However, the reviewer noted that performance data was acceptable for all matrices in the ECM and ILV at the tested fortification levels, except for analysis of almonds.

3. For potato foliage/potato above-ground foliage, the reproducibility of the method could not be determined at 10×LOQ; only one set of performance data was submitted.

4. The ILV originally included organic ground almonds as a crop matrix; however, low individual and average recoveries were observed (pp. 18, 20, 23, 27 of MRID 50826510). The ILV study author determined that the method was not suitable for high oil content matrices. However, the reviewer noted that the ECM included peanut (nut) as a crop matrix and produced acceptable results at the LOQ and 10×LOQ (Appendix 3, Table 1, pp. 33-35 of MRID 50826509). The ILV was planning to attempt another validation for almonds using a higher sorbent mass SPE cartridge but did not perform this attempt due to delays in the SPE cartridge delivery (Appendix 5, pp. 123-124 of MRID 50826510). **The ECM should be updated to include a statement that the method was not suitable for high oil content matrices.**

5. ILV linearity was not satisfactory for chlorothalonil analysis in lemons [r^2 = 0.9883 (Q) 0.9911 (C1), and 0.9863 (C2); p. 27; Figures 16-18, pp. 60-62; Figures 34-36, pp. 78-80; Figures 52-54, pp. 96-98; Figures 70-72, pp. 114-116 of MRID 50826510]. Linearity is satisfactory when $r^2 \geq 0.995$. The reviewer noted that the linearity of the confirmatory ion analyses was unacceptable for peanut above-ground foliage [r^2 = 0.9941 (C1) and 0.9926 (C2)]. The reviewer noted that a confirmatory method is not usually required when GC/MS or LC/MS are used as the primary methods for generating data; therefore, the linearity of the confirmation ion transition does not affect the validity of the method.

6. In the ECM, no samples were prepared at 10×LOQ for the following crop matrices: Apple, Grape, Strawberry, Orange (skin), Banana (skin), Cabbage, Cauliflower, French Bean (fresh pod), Tomato, Leek, Cereal (straw), Cereal (forage), and Potato (foliage; Appendix 3, Table 1, pp. 33-35 of MRID 50826509). 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.

7. In the ECM, representative chromatograms were only provided for 4 of the 27 crop matrices, and only LOQ and control representative chromatograms provided. Linearity results were only provided for 1 of the 27 crop matrices. Supporting data, including linear regression curves and representative chromatograms, should be provided for all matrices/fortifications which are tested in order to assess the method.

8. ILV performance data was not acceptable for the confirmation ion analysis of chlorothalonil in potato above-ground foliage (C1 mean at LOQ was 69.8%; Tables 1-3, pp. 29-31 of MRID 50826510). The reviewer noted that data deviations in the confirmation ion transition did not affect the specificity of the method since a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.

9. The reviewer noted that the ILV stated that the communications with the Study Sponsor included discussion of water content of crop matrices, GC/MS injection temperature, trial outcomes, new LOD definition, and elimination of almond matrix (Appendix 5, pp. 122-124 of MRID 50826510). The Sponsor Representative was not named in the Communications but named as Chad E. Wujick in the GLP statement (p. 3). Louis Mayer was named as the Syngenta Study Monitor for the ILV (p. 5). Neither of these individuals was involved in the ECM; therefore, no collusion between the ECM and ILV occurred.

10. The matrix effects were determined to be significant (±20%) in the ILV, and matrix-matched calibration standards were used for quantification (p. 26; Tables 5-7, pp. 33-38 of MRID 50826510). Minor matrix effects were observed for lemons, but matrix-matched standards were used, as well.

11. In the ILV, the study author determined that LOQ and 10×LOQ chlorothalonil extracts of hybrid Bermuda grass clippings, peanut above ground foliage, potato above ground foliage and lemon were stable when stored under refrigerated conditions (2-8°C) for a period of 7 days (pp. 26-27; Tables 10-12, pp. 41-43 of MRID 50826510).

12. Two melon (flesh) matrices were included in the ECM (Appendix 3, Table 1, pp. 33-35 of MRID 50826509). The reviewer presumed the two melon (flesh) matrices to be different; the results were not combined.

13. No reagent blank was present in the ECM.

14. 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. 22-23 of MRID 50826509; pp. 16, 22 of MRID 50826510). In the ECM and ILV, 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%. In the ECM, it was stated that the LOQ for accurate quantitation should yield a response which is no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. 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 author noted that the LOD may vary between runs and from instrument to instrument. In the ILV, the LOD was defined as the sample concentration equivalent to the lowest calibration standard (0.0001 µg/mL), which is also equivalent to the method detection limit (MDL). No calculations supporting the method LOQ and LOD were provided in the ECM and ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

15. It was reported for the ILV that each set of samples required 7.5 hours including start of extraction to completion of instrumental analysis (p. 21 of MRID 50826510).

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