

Analytical method for chlorothalonil in air from air sampling tubes

Reports: ECM: EPA MRID No.: 50826507. Mayer, L.C. 2015. Chlorothalonil. Chlorothalonil - Method GRM005.13A for the Determination of Chlorothalonil in OVS Silica Gel Air Sampling Tubes By GC-NICI-MS. Analytical Method. Report No.: GRM005.13A. Task No.: TK0225825. Report prepared, sponsored and submitted by Syngenta Crop Protection, LLC., Greensboro, North Carolina; 36 pages. Final report issued July 27, 2015.

ILV: EPA MRID No. 50826508. Guo, D. 2015. Chlorothalonil. Chlorothalonil - Independent Laboratory Validation of Analytical Method (GRM005.13A) for the Determination of Chlorothalonil in OVS Silica Gel Air Sampling Tubes by GC-NICI-MS. Final ILV Report. Report No.: PASC-REP-0645. PASC Project No.: 141-1171. Task No.: TK0261646. Report prepared by Primera Analytical Solutions Corp., Princeton, New Jersey, sponsored and submitted by Syngenta Crop Protection, LLC., Greensboro, North Carolina; 82 pages. Final report issued July 1, 2015.

Document No.: MRIDs 50826507 & 50826508

Guideline: 850.6100

Statements: ECM: The study was not conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards (p. 3 of MRID 50826507). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). A certification of authenticity and Quality Assurance statement were not included. A signed summary of revisions to previous method version 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 50826508). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). An authenticity statement was not included.

Classification: This analytical method is classified as Acceptable without any major deficiencies.

PC Code: 081901

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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 Method GRM005.13A, is designed for the quantitative determination of chlorothalonil in air from air sampling tubes at the LOQ of 0.01 µg/air sample tube using GC/MS. The LOQ is less than the lowest toxicological level of concern in air from air sampling tube. The ECM and ILV test matrices were OVS silica gel sorbent (520/260 mg) air sampling tube; the ECM noted that the method should not be used with OVS XAD-2 air sampling tubes as written. The ILV validated the method in the first trial without modifications; however, the ILV analytical instruments were not reported. **It could not be determined if the ILV was performed independently from the ECM since the ILV study author and ECM study author communicated directly with one another via email; however, no technical collusion was evident in the communication.** Chlorothalonil was identified via GC/MS using three ions; however, only the target ion recovery was quantified. A confirmatory method is not usually required when the primary analytical method is LC/MS/MS or GC/MS. All ILV and ECM data regarding repeatability, accuracy, and precision were satisfactory for chlorothalonil; however, no 10×LOQ representative chromatograms were provided in the ECM. No reagent blank was prepared in the ECM.

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	50826507	50826508		Air from air sampling tube ^{1,2}	27/07/2015	Syngenta Crop Protection, LLC	GC/MS	0.01 µg/air sample tube

1 OVS silica gel sorbent (520/260 mg) air sampling tube (SKC Inc. Cat No. 226-99) used in ECM and ILV (p. 9 of MRID 50826507; p. 10 of MRID 50826508).

2 In the ECM, it was reported that the method should not be used with OVS XAD-2 air sampling tubes as written, since low recoveries have been observed (p. 18 of MRID 50826507).

I. Principle of the Method

Air samples were collected from OVS silica gel sorbent (520/260 mg) air sampling tubes (SKC Inc. Cat No. 226-99; pp. 9-12; Appendix 3, p. 36 of MRID 50826507). After the sample tubes were fortified, if necessary, with the appropriate amount of chlorothalonil in toluene (10, 1.0 or 0.10 µg/mL fortification solutions, each sample stood for at least five minutes before extraction. For extraction, remove all contents of the tube into a 15 mL polypropylene tube; the glass tube is not included in the extraction. Extract sample using 10 mL of acetone via shaking for 2-3 minutes. An aliquot (1.0 mL) of the extract was transferred to a polypropylene tube, and the solvent was evaporated to dryness at ≤40°C under a gentle stream of nitrogen. The residue was reconstituted with 1.0 mL of toluene. After vortexing, the sample was analyzed by GC/NICI/MS directly or diluted, if necessary. The method noted that samples should be stored refrigerated in sealed containers where the analysis cannot be completed in a single day.

Samples were analyzed for chlorothalonil by Hewlett Packard 6890 GC coupled with a Hewlett Packard 5973 detector (pp. 13-14 of MRID 50826507). The following conditions were used: HP-5MS column (30.0 x 0.25 mm i.d.), injector temperature 250°C, oven temperature gradient (120°C for 1 min. to 300°C at 20°C/min. and hold for 2 min.), helium carrier gas (1.0 mL/min.), and negative polarity chemical ionization mode (ion source temperature 230°C). Injection volume was 2 µL. Chlorothalonil was identified with the following ions: *m/z* 266 (target ion), *m/z* 264 (qualifier 1), and *m/z* 268 (qualifier 2). Ion ratio was 100:65:55. Retention time was *ca.* 7.1 minutes for chlorothalonil.

In the ILV, the ECM was performed as written, also using the OVS silica gel sorbent (520/260 mg) air sampling tubes (SKC Inc. Cat No. 226-99; pp. 10-13 of MRID 50826508). The analytical instrument and parameters were not reported, but it was reported that the ILV used the instrument parameters described in the method. Retention time was *ca.* 7.0 minutes for chlorothalonil (Figures 1-5, pp. 20-24). The same MS ions were used. No other modifications of the ECM were reported.

The Limit of Quantification (LOQ) for chlorothalonil was reported as 0.01 µg/air sample tube in the ECM and the ILV (pp. 9, 17 of MRID 50826507; pp. 8, 11 of MRID 50826508). The Limit of Detection (LOD) for chlorothalonil was 0.10 pg/µL, equivalent to 0.2 pg injected on column when using a 2 µL injection volume by GC/NICI/MS in the ECM and ILV.

II. Recovery Findings

ECM (MRID 50826507): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of chlorothalonil from air sampling tubes at fortification levels of 0.01 µg/tube (LOQ) and 0.10 µg/tube (10×LOQ; n = 4 for both fortifications; Table 1, p. 21). Chlorothalonil was identified with three ions using GC/MS; however, only the target ion recovery was quantified. A confirmatory method is not usually required when the primary analytical method is LC/MS/MS or GC/MS. The matrix was OVS silica gel sorbent (140/260 mg) air sampling tube (SKC Inc. Cat No. 226-99; p. 9).

ILV (MRID 50826508): Mean recoveries and RSDs were within guideline requirements for analysis of chlorothalonil from air sampling tubes at fortification levels of 0.01 µg/tube (LOQ) and 0.10 µg/tube (10×LOQ; Table 3, p. 18). Chlorothalonil was identified with three ions using GC/MS; however, only the target ion recovery was quantified. A confirmatory method is not usually required when the primary analytical method is LC/MS/MS or GC/MS. The matrix was OVS silica gel sorbent (520/260 mg) air sampling tube (SKC Inc. Cat No. 226-99; p. 10). The method was validated in the first trial without modifications; however, the ILV analytical instruments were not reported (pp. 11-12). It could not be determined if the ILV was performed independently from the ECM since the ILV study author and ECM study author communicated directly with one another via email; however, no technical collusion was evident in the communication (p. 23; Appendix 4, pp. 81-82). The brief communication included method clarification (typographical error correction) and exchange of ILV draft report and raw data.

Table 2. Initial Validation Method Recoveries for Chlorothalonil in Air^{1,2}

Analyte	Fortification Level (µg/tube)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
OVS Silica Gel Sorbent						
Target Ion						
Chlorothalonil	0.01 (LOQ)	4	95-118	104	--	10
	0.10	4	101-110	106	--	4

Data (recovery results were corrected for residues quantified in the controls, pp. 14-16) were obtained from Table 1, p. 21 of MRID 50826507.

1 The matrix was OVS silica gel sorbent (520/260 mg) air sampling tube (SKC Inc. Cat No. 226-99; p. 9).

2 Chlorothalonil was identified with the following ions: *m/z* 266 (target ion), *m/z* 264 (qualifier 1), and *m/z* 268 (qualifier 2); however, only the target ion recovery was quantified. A confirmatory method is not usually required when the primary analytical method is LC/MS/MS or GC/MS.

3 Standard deviations were not reported since the study author did not report these values and raw data was not provided in the study report.

Table 3. Independent Validation Method Recoveries for Chlorothalonil in Air^{1,2}

Analyte	Fortification Level (µg/tube)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
OVS Silica Gel Sorbent						
Target Ion						
Chlorothalonil	0.01 (LOQ)	5	105-111	108	3	2
	0.10	5	98-105	101	3	3

Data (uncorrected recovery results,) were obtained from Table 3, p. 18 of MRID 50826508.

1 The matrix was OVS silica gel sorbent (520/260 mg) air sampling tube (SKC Inc. Cat No. 226-99; p. 10). This was the same matrix as the ECM.

2 Chlorothalonil was identified with the following ions: *m/z* 266 (target ion), *m/z* 264 (qualifier 1), and *m/z* 268 (qualifier 2); however, only the target ion recovery was quantified. A confirmatory method is not usually required when the primary analytical method is LC/MS/MS or GC/MS.

III. Method Characteristics

The LOQ for chlorothalonil was reported as 0.01 µg/air sample tube in the ECM and the ILV (pp. 9, 17 of MRID 50826507; pp. 8, 11 of MRID 50826508). In the ECM and ILV, the LOQ was defined as the lowest analyte concentration which was demonstrated to have acceptable mean recovery (70 to 120%) and precision (relative standard deviation of ≤20%). The ECM also stated that the response of the LOQ analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. The LOD for chlorothalonil in the ECM was 0.10 pg/µL, equivalent to 0.2 pg injected on column when using a 2 µL injection volume by GC/NICI/MS in the ECM and ILV. In the ECM and ILV, 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. The ECM also stated that an estimate of the LOD can be taken as three times background noise.

Table 4. Method Characteristics

Analyte		Chlorothalonil ¹
Matrix		OVS Silica Gel Sorbent
Limit of Quantitation (LOQ)	ECM	0.01 µg/air sample tube
	ILV	
Limit of Detection (LOD)	ECM	0.10 pg/µL (0.2 pg injected on column when using a 2 µL injection volume)
	ILV	
Linearity (calibration curve r ² and concentration range)	ECM ²	r ² = 0.998 (0.1 pg/µL to 50 pg/µL, equivalent to 0.2 pg to 100 pg on column)
	ILV	r ² = 1.00 (0.1 pg/µL to 50.72 pg/µL)
Repeatable	ECM ³	Yes at LOQ and 10×LOQ.
	ILV ^{4,5}	
Reproducible		Yes at LOQ and 10×LOQ.
Specific	ECM	Yes, matrix interferences were <5% of the LOQ (based on peak height estimation). Some minor peak tailing was observed. No 10×LOQ representative chromatograms were provided. ⁶
	ILV	Yes, matrix interferences were <5% of the LOQ (based on peak height estimation). Some minor peak tailing was observed.

Data were obtained from pp. 9, 17 (LOQ/LOD); Table 1, p. 21 (recovery data); Figures 2-8, pp. 25-31 (chromatograms); Figure 9, p. 32 (calibration curve) of MRID 50826507; pp. 8, 11 (LOQ/LOD); Table 3, p. 18 (recovery data); Figures 1-10, pp. 20-29 (chromatograms); Figure 11, p. 30 (calibration curve) of MRID 50826508; and DER Attachment 2.

- 1 Chlorothalonil was identified using GC/MS with the following ions: *m/z* 266 (target ion), *m/z* 264 (qualifier 1), and *m/z* 268 (qualifier 2); however, only the target ion recovery was quantified. A confirmatory method is not usually required when the primary analytical method is LC/MS/MS or GC/MS.
- 2 For the ECM, the correlation coefficient was reviewer-calculated from *r* value provided in the study report (Figure 9, p. 32 of MRID 50826507; DER Attachment 2).
- 3 The ECM matrix was OVS silica gel sorbent (520/260 mg) air sampling tube (SKC Inc. Cat No. 226-99; p. 9 of MRID 50826507).
- 4 The ILV matrix was OVS silica gel sorbent (520/260 mg) air sampling tube (SKC Inc. Cat No. 226-99; p. 10 of MRID 50826508). This was the same as that used in the ECM.
- 5 The ILV validated the method in the first trial without modifications; however, the ILV analytical instruments were not reported (pp. 11-12 of MRID 50826508).

6 The only representative chromatograms of the OVS filter were the control, 0.01 µg (LOQ), and 50 µg (Figures 6-8, pp. 29-31 of MRID 50826507). The 10×LOQ representative chromatogram would be titled with 0.10 µg.

IV. Method Deficiencies and Reviewer's Comments

1. In the ECM, it was noted that the method should not be used with OVS XAD-2 air sampling tubes as written, since low recoveries have been observed (p. 18 of MRID 50826507).
2. It could not be determined if the ILV was performed independently from the ECM since the ILV study author and ECM study author (Louis Mayer) communicated directly with one another via email; however, no technical collusion was evident in the communication (p. 1 of MRID 50826507; p. 23; Appendix 4, pp. 81-82 of MRID 50826508). Communications between the ILV study author and ECM study author was provided for review. The brief communication included method clarification (typographical error correction) and exchange of ILV draft report and raw data. OCSPP guidelines state that the analysts, study director, equipment, instruments, and supplies of the two laboratories must be distinct and operated separately and without collusion.
3. In the ECM, no 10×LOQ representative chromatograms were provided. The only representative chromatograms of the OVS filter were the control, 0.01 µg (LOQ), and 50 µg (Figures 6-8, pp. 29-31 of MRID 50826507). The 10×LOQ representative chromatogram would be titled with 0.10 µg. The reviewer noted that the titling of Figure 8 may have been a typographical error.
4. In the ECM, no reagent blank was prepared. A validation sample set should consist of, at a minimum, a reagent blank, two unspiked matrix control samples, five matrix control samples spike at the LOQ, and five matrix control samples spiked at 10×LOQ for each analyte and matrix. The reviewer also noted that the number of samples was four (n = 4) for both fortifications, which was less than the recommended number, but still adequate for statistics (Table 1, p. 21 of MRID 50826507).
5. In the ILV, the analytical instrument and parameters were not reported, but it was reported that the ILV used the instrument parameters described in the method (pp. 10-13 of MRID 50826508).
6. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 9, 17 of MRID 50826507; pp. 8, 11 of MRID 50826508). In the ECM and ILV, the LOQ was defined as the lowest analyte concentration which was demonstrated to have acceptable mean recovery (70 to 120%) and precision (relative standard deviation of ≤20%). The ECM also stated that the response of the LOQ analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. In the ECM and ILV, 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. The ECM also stated that an estimate of the LOD can be

taken as three times background noise. Detection limits should not be based on arbitrary values.

7. In the ECM, no significant matrix effects have been observed for the air sample sorbent type tested (p. 17; Table 2, p. 21 of MRID 50826507). Non-matrix-matched standards were recommended.
8. In the ECM, the final chlorothalonil extracts in toluene were determined to stable up to 7 days of storage at 4°C (p. 18; Table 3, p. 22 of MRID 50826507).
9. It was reported for the ECM that one batch of 25 samples required one working day (8 hours) for one skilled analyst (p. 12 of MRID 50826507). The time requirement was not reported in the ILV.

V. References

- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures**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(c1C#N)Cl)Cl)Cl)c1Cl

