Analytical method for chloropicrin in air from air sampling tubes

Reports:	ECM: EPA MRID No.: 49831301. Arndt, T., and C. Warling. 2016. Method Validation of an Analytical Method for Monitoring Chloropicrin in Air. PTRL Project No.: 2540W. Report prepared by PTRL West (a division of EAG Laboratories), Hercules, California, and sponsored and submitted by Chloropicrin Manufacturers' Task Force, Mojave, California, and Toxicology Consultants, Inc., Gibsonia, Pennsylvania; 92 pages. Final report issued February 17, 2015; Amended Study dated January 22, 2016. ILV: EPA MRID No. 50030701. Bendig, P., and C. Wabbel. 2016. Independent Laboratory Validation (ILV) of an Analytical Method for Monitoring Chloropicrin in Air. PTRL Europe ID: P 3822 G. Report prepared by PTRL Europe, Ulm, Germany, sponsored and submitted by Chloropicrin Manufacturers' Task Force, Mojave, California; 42 pages. Final report issued September 8, 2016.
Document No.:	MRIDs 49831301 & 50030701
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR, Part 160 p. 3 of MRID 49831301). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of authenticity was included with the QA statement. ILV: The study was conducted in accordance with German GLP standards, which are based on OECD GLP standards, which are accepted by European communities, the USA (FDA and EPA, FIFRA GLP standards, 40 CFR, Part 160) and Japan (p. 3; Appendix 1, p. 40 of MRID 50030701). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5; Appendix 1, p. 40).
Classification:	This analytical method is classified as acceptable. ECM is classified as supplemental due to the number of samples was insufficient at all fortification levels (less than five as required). However, the deficiency has been made up in ILV, which has used five samples for each replicate.
PC Code:	081501

EFED Final Reviewer:	James Lin Environmental Engineer	Signature: Date: 12/13/2018	
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S. Environmental Scientist	Signature: Date:	 08/17/2018
	Joan Gaidos, Ph.D., Environmental Scientist	Signature: Date:	 09/14/2018

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, PTRL West Project No. 2540W, is designed for the quantitative determination of chloropicrin in air from from air sampling tubes at the LOQ of 30.0 ng/air sample cartridge using GC/MS/MS. The LOQ is less than the lowest toxicological level of concern in air. The ECM and ILV test matrices were XAD-4 resin air sampling cartridges. Air was drawn through the air sampling apparatuses at *ca*. 100 mL/min., ca. 40% relative humidity, and 20-25°C for 48 hours before extraction. Although the specific number of trials was not reported in the ILV, the reviewer assumed that method was validated in the first trial with insignificant modifications of the analytical instrumentation. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for chloropicrin, except that the number of samples was insufficient at all fortification levels 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						
Chloropicrin	49831301	50030701		Air[1,2]	17/02/2015 (Final) 22/01/2016 (Amended)	Chloropicrin Manufacturers' Task Force	GC/MS/MS	30 ng/tube

1 In the EM, the air sampling apparatus was composed of triplicate foil-wrapped XAD-4 resin (1400/250 mg; 10 mm O.D. x 156 mm L) air sampling tubes (SKC Inc. Cat No. 092314-002) attached to the manifold ports with Tygon® tubing to a SKC 224-44XR or SKC 224-PPCXR4 sample pump (pp. 14-15, 18-19; Table 1, p. 33; Figure 1, p. 38 of MRID 49831301). After chloropicrin was introduced, air was drawn through the air sampling apparatuses at *ca*. 100 mL/min., 38.2-40.1% relative humidity, and 20.4-21.9°C for 48 hours before extraction.

2 In the ILV, the air sampling apparatus was composed of triplicate foil-wrapped XAD-4 resin (1400/250 mg; 10 mm O.D. x 156 mm L) air sampling tubes (pp. 12, 14-15, 17; Figure 1, p. 26; Appendix 3, p. 42 of MRID 50030701). After chloropicrin was introduced, air was drawn through the air sampling apparatuses at *ca*. 100 mL/min., 42-51% relative humidity, and *ca*. 25°C for 48 hours before extraction.

I. Principle of the Method

The air sampling apparatus was composed of a foil-wrapped XAD-4 resin (1400/250 mg; 10 mm O.D. x 156 mm L) air sampling tubes (SKC Inc. Cat No. 092314-002) attached to the manifold ports with Tygon® tubing to a SKC 224-44XR or SKC 224-PPCXR4 sample pump (pp. 14-15, 18-19; Figure 1, p. 38 of MRID 49831301). The air sampling apparatuses were set-up with triplicate tubes for the fortifications and duplicate tubes for the controls. After the airflow of each sample tube was adjusted to *ca*. 100 mL/min., the appropriate amount of chloropicrin in ethyl acetate was introduced to the apparatus, if necessary, via the trapping flask inlet with microliter syringe. Air was drawn through the air sampling apparatuses for 48 hours before extraction; temperature, humidity, and air flow was monitored throughout the sampling time. Sample tubes were extracted on the same day as the end of the trapping period.

For extraction, the front-end glass wool and front-side sorbent beads of the opened sorbent tube were transferred to chilled 10 mL ethyl acetate in an amber 16-mL vial (p. 21 of MRID 49831301). The back-side sorbent beads of the opened sorbent tube were transferred to chilled 5 mL ethyl acetate in an amber 10-mL vial. After vortexing, the vials were shaken for *ca*. 1 hour on a wrist-action shaker. The supernatants were transferred to 8 mL amber storage vials, diluted with ethyl acetate as necessary (10X for 10×LOQ and 500X for 500×LOQ), and analyzed by GC/MS/MS. Samples were stored in the freezer when not in use.

Samples were analyzed using an Agilent 7890A series gas chromatograph coupled to an Agilent 7000B series triple quad mass spectrometer (pp. 22-23 of MRID 49831301). The GC/MS/MS conditions consisted of a DB-624 (0.25 mm x 30 m, 1.40-µm), injector temperature 150°C, source temperature 230°C, temperature program [50°C for 1 min. then 15°C/min. to 140°C (no hold) then 30°C/min. to 200°C], carrier gas helium, 2 µL injection volume, and electron impact (+) ionization source. Chloropicrin was identified with the following two ion transitions (primary and confirmation, respectively): *m/z* 117→82 and *m/z* 119→84. Expected retention time was *ca*. 6.2 minutes.

The residues in the front and back extracts were quantified separately, then summed (Figure 22, p. 60 of MRID 49831301).

In the ILV, the ECM was performed as written, except for a different analytical instrumentation (pp. 12, 14-15, 17; Figure 1, p. 26 of MRID 50030701). A Thermo Trace 1310 GC coupled to a Thermo TSQ 8000 Evo Triple Quad MS was used. All other GC/MS/MS parameters were the same as those of the EM. Expected retention time was *ca*. 5 minutes.

The Limit of Quantification (LOQ) for chloropicrin in air was 30 ng chloropicrin per tube which was converted based on recorded air flow to 0.105 µg/m^3 in the ECM and *ca*. 0.1 µg/m^3 in the ILV (p. 25 of MRID 49831301; p. 11 of MRID 50030701). The Limit of Detection (LOD) was 0.5 ng/mL in the ECM and ILV.

II. Recovery Findings

EM (MRID 49831301): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of chloropicrin from air sampling tubes at fortification levels of 30 ng/tube (0.105 µg/m^3; LOQ), 300 ng/tube (1.00 µg/m^3; 10×LOQ), and 15,000 ng/tube (50.7 µg/m^3; 500×LOQ; Table 2, p. 34 and Table 4, p. 36; DER Attachment 2). Chloropicrin was identified using two ion transitions with GC/MS/MS. Performance data (recovery results) from primary and confirmatory analyses were comparable. The air sampling apparatus was composed of triplicate foil-wrapped XAD-4 resin (1400/250 mg; 10 mm O.D. x 156 mm L) air sampling tubes (pp. 14-15, 18-19; Table 1, p. 33; Figure 1, p. 38). After chloropicrin was introduced, air was drawn through the air sampling apparatuses at *ca*. 100 mL/min., 38.2-40.1% relative humidity, and 20.4-21.9°C for 48 hours before extraction. The number of samples was insufficient at all fortification levels (n = 3).

ILV (MRID 50030701): Mean recoveries and RSDs were within guideline requirements for analysis of chloropicrin from air sampling tubes at fortification levels of 30 ng/tube (0.101 µg/m^3; LOQ), 300 ng/tube (1.01 µg/m^3; 10×LOQ), and 15,000 ng/tube (50.5 µg/m^3; 500×LOQ; Table 1, p. 23; DER Attachment 2). Chloropicrin was identified using two ion transitions with GC/MS/MS. Performance data (recovery results) from primary and confirmatory analyses were comparable. The air sampling apparatus was composed of triplicate foil-wrapped XAD-4 resin (1400/250 mg; 10 mm O.D. x 156 mm L) air sampling tubes (pp. 12, 14-15, 17; Figure 1, p. 26; Appendix 3, p. 42). After chloropicrin was introduced, air was drawn through the air sampling apparatuses at *ca*. 100 mL/min., 42-51% relative humidity, and *ca*. 25°C for 48 hours before extraction. Although the specific number of trials was not reported in the ILV, the reviewer assumed that method was validated in the first trial with insignificant modifications of the analytical instrumentation (pp. 9-10, 12, 14-15, 20, 22).

Table 2. Initial Validation Method Recoveries for Chloropicrin in Air[1,2]

Analyte	Fortification Level [ng/tube (µg/m³)][3]	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[4]	Relative Standard Deviation (%)
	XAD-4 sorbent air sampling tube					
	Quantitation ion transition					
Chloropicrin	30 (0.105)	3	88-97	94	5	5.3
	300 (1.00)	3	72-90	81	9	11.1
	15,000 (50.7)	3	81-86	83	3	3.2
	Confirmation ion transition					
Chloropicrin	30 (0.105)	3	86-93	90	4	3.9
	300 (1.00)	3	76-91	81	8	10.3
	15,000 (50.7)	3	78-83	81	3	3.1

Data (uncorrected recovery results; pp. 23-24) were obtained from Table 2, p. 34 and Table 4, p. 36 of MRID 49831301 and DER Attachment 2.

1 The air sampling apparatus was composed of triplicate foil-wrapped XAD-4 resin (1400/250 mg; 10 mm O.D. x 156 mm L) air sampling tubes (SKC Inc. Cat No. 092314-002) attached to the manifold ports with Tygon® tubing to a SKC 224-44XR or SKC 224-PPCXR4 sample pump (pp. 14-15, 18-19; Table 1, p. 33; Figure 1, p. 38). After chloropicrin was introduced, air was drawn through the air sampling apparatuses at *ca*. 100 mL/min., 38.2-40.1% relative humidity, and 20.4-21.9°C for 48 hours before extraction; sample tubes were extracted on the same day as the end of the trapping period.

2 Chloropicrin was identified with the following two ion transitions (primary and confirmation, respectively): *m/z* 117→82 and *m/z* 119→84.

3 Reported µg/m³ were calculated in the study report based on measured air flow.

4 Standard deviations were reviewer-calculated using the data in the study report since the study author did not report these values (see DER Attachment 2). Rules of significant figures were followed.

Table 3. Independent Validation Method Recoveries for Chloropicrin in Air[1,2]

Analyte	Fortification Level [ng/tube (µg/m³)][3]	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[4]	Relative Standard Deviation (%)
	XAD-4 sorbent air sampling tube					
	Quantitation ion transition					
Chloropicrin	30 (0.101)	5[5]	79-94	88	5	6
	300 (1.01)	5[6]	77-92	82	6	8
	15,000 (50.5)	5[5]	94-109	99	6	6
	Confirmation ion transition					
Chloropicrin	30 (0.101)	5[5]	81-94	89	5	6
	300 (1.01)	5[6]	77-97	84	8	9
	15,000 (50.5)	5[5]	96-108	100	5	5

Data (uncorrected recovery results, p. 19) were obtained from Table 1, p. 23 of MRID 50030701.

1 The air sampling apparatus was composed of triplicate foil-wrapped XAD-4 resin (1400/250 mg; 10 mm O.D. x 156 mm L) air sampling tubes (SKC Inc. Cat No. 092314-002) attached to the manifold ports with Tygon® tubing to a SKC 224-44XR or SKC 224-PPCXR4 sample pump (pp. 12, 14-15, 17; Figure 1, p. 26; Appendix 3, p. 42). After chloropicrin was introduced, air was drawn through the air sampling apparatuses at *ca*. 100 mL/min., 42-51% relative humidity, and *ca*. 25°C for 48 hours before extraction; sample tubes were extracted on the same day as the end of the trapping period.

2 Chloropicrin was identified with the following two ion transitions (primary and confirmation, respectively): *m/z* 117→82 and *m/z* 119→84.

3 Reported µg/m³ were calculated in the study report based on measured air flow.

4 Standard deviations were reviewer-calculated using the data in the study report since the study author did not report these values (see DER Attachment 2). Rules of significant figures were followed.

5 The reported value for the first sample was the means of two injections. This was done to demonstrate repeatability of injection (p. 21).

6 The reported value for the first and last samples was the means of two injections. This was done to demonstrate repeatability of injection (p. 21).

III. Method Characteristics

The LOQ for chloropicrin in air was 30 ng chloropicrin per tube which was converted based on recorded air flow to 0.105 µg/m³ in the ECM and *ca*. 0.1 µg/m³ in the ILV (p. 25 of MRID 49831301; p. 11 of MRID 50030701). In the ECM and ILV, the LOQ was determined by the rate of air flow (*ca*. 100 mL/min.) when trapping for 48 hours at a 30-ng injection amount. The LOD was 0.5 ng/mL in the ECM and ILV. In the ECM, the LOD was calculated as 3xs the standard deviation of the peak area multiplied by the standard concentration (1 ng/mL) divided by the average peak area. In the ILV, the LOD was determined based on the 10 mL of ethyl acetate which was used to extract the air sampling samples. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV, or the LOD in the ILV.

Table 4. Method Characteristics

Analyte[1]		**Chloropicrin**
Limit of Quantitation (LOQ)	ECM	30 ng/tube (0.105 µg/m^3)
	ILV	30 ng/tube (*ca*. 0.10 µg/m^3)
Limit of Detection (LOD)	ECM	0.05 µg/m^3
	ILV	
Linearity (calibration curve r^2 and concentration range)[1]	ECM	r^2 = 0.99711841 (Q) r^2 = 0.99668969 (C) (0.5-20 ng/mL)
	ILV	r^2 = 0.9983 (Q) r^2 = 0.9988 (C) (0.50-30 ng/mL)
Repeatable	ECM[2]	Yes at LOQ, 10×LOQ and 500×LOQ, but n = **3** (air sampling tubes)
	ILV[3,4]	Yes at LOQ, 10×LOQ and 500×LOQ (air sampling tubes)
Reproducible		Yes at LOQ, 10×LOQ and 500×LOQ
Specific	ECM	Yes, matrix interferences were *ca*. 9% of the LOQ (based on peak area).
	ILV	Yes, matrix interferences were <3% of the LOQ (based on peak area).

Data were obtained from p. 25 (LOQ/LOD); Table 2, p. 34 and Table 4, p. 36 (recovery data); Figure 2, pp. 39-40 (calibration curve); Figures 3-21, pp. 41-59 (chromatograms) of MRID 49831301; p. 11 (LOQ/LOD); Table 1, p. 23 (recovery data); Figures 2-3, pp. 27-28 (calibration curves); Figures 4-13, pp. 29-38 (chromatograms) of MRID 50030701.

1 Quadratic equations were used in the ECM and ILV.

2 In the EM, the air sampling apparatus was composed of triplicate foil-wrapped XAD-4 resin (1400/250 mg; 10 mm O.D. x 156 mm L) air sampling tubes (SKC Inc. Cat No. 092314-002) attached to the manifold ports with Tygon® tubing to a SKC 224-44XR or SKC 224-PPCXR4 sample pump (pp. 14-15, 18-19; Table 1, p. 33; Figure 1, p. 38 of MRID 49831301). After chloropicrin was introduced, air was drawn through the air sampling apparatuses at *ca*. 100 mL/min., 38.2-40.1% relative humidity, and 20.4-21.9°C for 48 hours before extraction; sample tubes were extracted on the same day as the end of the trapping period.

3 In the ILV, the air sampling apparatus was composed of triplicate foil-wrapped XAD-4 resin (1400/250 mg; 10 mm O.D. x 156 mm L) air sampling tubes (pp. 12, 14-15, 17; Figure 1, p. 26; Appendix 3, p. 42 of MRID 50030701). After chloropicrin was introduced, air was drawn through the air sampling apparatuses at *ca*. 100 mL/min., 42-51% relative humidity, and *ca*. 25°C for 48 hours before extraction; sample tubes were extracted on the same day as the end of the trapping period.

4 Although the specific number of trials was not reported in the ILV, the reviewer assumed that method was validated in the first trial with insignificant modifications of the analytical instrumentation (pp. 9-10, 12, 14-15, 20, 22 of MRID 50030701).

5 The reviewer noted that the residues in the back portion extract of the control sample was quantified as 14 ct (0.175 ng/mL), while the residues in the back portion extract of the LOQ sample was 0 ct (0.142 ng/mL; Figure 11, p. 49; Figure 13, p. 51; Figure 22, p. 60 of MRID 49831301.

IV. Method Deficiencies and Reviewer's Comments

1. Communications between the ECM and ILV were not addressed in the ECM or ILV.

2. In the ECM, the number of samples was insufficient at all fortification levels (n = 3; Table 2, p. 34 of MRID 49831301; DER Attachment 2). OCSPP guideline state that a minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.

3. The number of trials required by the ILV to validate the ECM was not reported. Although the specific number of trials was not reported in the ILV, the reviewer assumed that method was validated in the first trial with insignificant modifications of the analytical instrumentation (pp. 9-10, 12, 14-15, 20, 22 of MRID 50030701).

4. Concurrent recoveries (set forts) were prepared in the ECM to assess extraction efficiency (p. 28, Table 3, p. 35 of MRID 49831301). Recoveries [mean (RSD)] were 95% (2.8%), 101% (1.0%), and 91% (1.9%) for the quantitation analysis of the LOQ, 10×LOQ and 500×LOQ fortifications, respectively, and 97% (2.8%), 97% (1.0%), and 90% (2.2%) for the confirmation analysis of the LOQ, 10×LOQ and 500×LOQ fortifications, respectively.

5. The ILV analyzed the back-portion of the sampling tubes to study possible breakthrough of the analyte (pp. 16, 21; Figure 9, p. 34; Figure 11, p. 36; Figure 13, p. 38 of MRID 50030701). It was determined that no breakthrough occurred since breakthrough was less than the LOD.

6. The reviewer noted that the chromatogram of the back portion of the LOQ sample showed a peak with a peak height of *ca*. 80 count which was very close to the expected analyte peak retention time, but it did not appear that this peak was quantified as chloropicrin (Figure 13, p. 51 of MRID 49831301). It was quantified as 0 count peak area (0.142 ng/mL), while the residues in the control were quantified as 14 count peak area (0.175 ng/mL) when that peak height was only *ca*. 30 count (Figure 11, p. 49).

7. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 25 of MRID 49831301; p. 11 of MRID 50030701). In the ECM and ILV, the LOQ was determined by the rate of air flow (*ca*. 100 mL/min.) when trapping for 48 hours at a 30-ng injection amount. In the ECM, the LOD was calculated as 3xs the standard deviation of the peak area multiplied by the standard concentration (1 ng/mL) divided by the average peak area. In the ILV, the LOD was determined based on the 10 mL of ethyl acetate which was used to extract the air sampling samples. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV, or the LOD in the ILV. Detection limits should not be based on arbitrary values.

8. In the ECM, the storage stability of the sorbent tube and final sample extracts was determined to be 28 and 66 days, respectively, when stored in the freezer (n = 2; p. 30; Table 5, p. 37 of MRID 49831301).

9. The matrix effects were determined to be insignificant in the ECM and ILV (p. 29; Table 3, p. 35 of MRID 49831301; p. 21 of MRID 50030701).

10. The ECM reported the Amendments made to the final report in Appendix D, p. 92 of MRID 49831301.

11. It was reported for the ILV that one sample set required *ca*. 48 hours of air sampling, *ca*. 3 hours for extraction and dilution, and *ca*. 16 hours of GC/MS/MS analysis (p. 22 of MRID 50030701). The total time was reported as *ca*. 3 working days.

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

Chloropicrin

IUPAC Name: Trichloronitromethane
CAS Name: Not reported
CAS Number: 76-06-2
SMILES String: Not found