

Analytical method for propargite in water

Reports: ECM: EPA MRID No.: 50356002. Keenan, D. 2016. Development and Validation of a Method for the Determination of Propargite in Surface and Drinking Water. Report prepared by PTRL West (now doing business as EAG Laboratories), Hercules, California, sponsored and submitted by Arysta LifeScience, Waterbury, Connecticut; 80 pages. PTRL Study No.: 2823W. Arysta Project No.: 16176-X5-03. Final report issued August 19, 2016.

ILV: EPA MRID No.: 50356003. MacGregor, J.A., and E.S. Bodle. 2017. INDEPENDENT LABORATORY VALIDATION OF METHODS FOR THE DETERMINATION OF PROPARGITE IN SURFACE AND DRINKING WATER. Report prepared by Wildlife International (now doing business as EAG Laboratories), Easton, Maryland, sponsored and submitted by Arysta LifeScience, Waterbury, Connecticut; 140 pages. Wildlife International Project No: 443C-128. Arysta Study No.: 2016-006. Final report issued May 1, 2017.

Document No.: MRIDs 50356002 & 50356003

Guideline: 850.6100


Statements: ECM: The study was conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR 160; p. 3 of MRID 50356002). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). The statement of authenticity was included with the Quality Assurance statement.

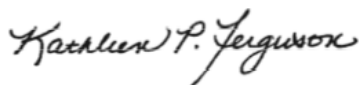
ILV: The study was conducted in compliance with USEPA FIFRA GLP standards, with the exception that periodic analyses of the test water for potential contaminants was not performed according to GLP standards (p. 3 of MRID 50356003). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). The statement of authenticity was not included.

Classification: This analytical method is classified as **Acceptable**. However, an LOD was not reported in the ILV report.

PC Code: 097601

EFED Final Reviewer: Lewis R. Brown, Env. Bio Signature: Lewis Ross Brown, III Env. Biologist
Date: 05/17/18

CDM/CSS-Dynamac JV Reviewers: Lisa Muto, Signature: 
Environmental Scientist Date: 11/13/17

Reviewers: Kathleen Ferguson, Ph.D., Signature: 
Environmental Scientist Date: 11/13/17

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

Executive Summary

The analytical method, PTRL Study No. 2823W (Arysta Project No. 16176-X5-03), is designed for the quantitative determination of propargite in water at the stated LOQ of 0.01 µg/L. The LOQ is less than the lowest toxicological level of concern in water (7 µg/L, the lowest aquatic life benchmark; DP 416479¹). Characterized surface and drinking water matrices were used for the ECM and ILV validations. Propargite was identified using two ion transitions in the ECM and ILV. The ECM method for propargite in water was validated by the ILV with insignificant modifications to the analytical equipment and parameters. The number of trials was not specified, but the reviewer assumed that the method was validated in the first trial based on the insignificant modifications to the method. Communication between the ILV and ECM was not reported or discussed. All ILV and ECM data regarding repeatability, accuracy, precision, linearity and specificity were satisfactory for propargite. The LOD was not reported in the ILV.

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						
Propargite	50356002 ¹	50356003 ²		Water	19/08/2016	Arysta LifeScience	LC/MS/MS	0.01 µg/L

1 In the ECM, the drinking water (2706W-055; pH 7.6, hardness 29 mg/L as CaCO₃, 58 ppm total dissolved solids) and surface water (2706W-049; pH 8.5, hardness 262 mg/L as CaCO₃, 204 ppm total dissolved solids) were characterized by Agvise Laboratories, Northwood, North Dakota (p. 13; Appendix C, pp. 65-66 of MRID 50356002). Water matrix sources were not reported.

2 In the ILV, the drinking (well) water (pH 8.1, hardness 142 mg/L as CaCO₃, 336 µS/cm specific conductance), obtained from a well at Easton Laboratories-Easton testing facility in Easton, Maryland, and surface (lake) water (pH 7.0, hardness 64.0 mg/L as CaCO₃, 188 µS/cm specific conductance), obtained from Tuckahoe Lake located in Tuckahoe State Park in Ridgely, Maryland, were characterized internally (pp. 11-12; Appendices III-IV, pp. 131-132 of MRID 50356003).

¹ <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk>

I. Principle of the Method

Water (100 mL) was fortified then extracted three times with 25 mL of hexane (pp. 14- 15; Figure 1, p. 31; Appendix D, pp. 72-73 of MRID 50356002). 10 g of sodium chloride was added to induce the separation of the aqueous and organic layers. After vigorously shaking by hand for 1 minute, the layers were allowed to separate for 10 minutes. The organic layer was transferred to a 125-mL concentration flask via a filter funnel containing glass wool and 5 g of sodium sulfate. The combined organic extracts were reduced to near dryness under increased pressure (230 mbar) in a water bath set at 40°C. The residue was reduced to dryness under a stream of nitrogen. The residue was reconstituted to 10 mL with acetonitrile via sonication. Approximately 500 µL of the final sample was transferred to a 0.45 µm microfilterfuge tube and centrifuged. The supernatant was transferred to an autosampler vial for analysis by LC/MS/MS.

Samples were analyzed for propargite using an Agilent 1260 Series HPLC coupled with an Applied Biosystems MDS/Sciex API 4000 Tandem Mass Spectrometer using a Turbo-Ion Spray source operated in the positive ion, multiple reaction monitoring (MRM) mode (300°C; pp. 12, 15; Appendix D, pp. 73-74 of MRID 50356002). The following LC conditions were used: Phenomenex Gemini 3 µ C18 110 column (2.0 mm x 50 mm, 3 µ; column temperature 55°C), mobile phase of (A) 10 mM ammonium acetate in HPLC grade water and (B) 10 mM ammonium acetate in HPLC grade methanol [mobile gradient phase of percent A:B (v:v) at 0.00 min. 70:30, 1.0-5.0 min. 5:95, 5.1-8.0 min. 70:30] and injection volume of 12 µL. Two ion pair transitions were monitored for propargite: m/z 368.1→231.6 (quantitation) and m/z 368.1→81.4 (confirmation). Reported retention times were *ca.* 5.0 minutes for propargite.

The ILV performed the ECM methods for each analyte as written, except insignificant modifications to the analytical equipment and parameters (pp. 13-14; Table 1, p. 20 of MRID 50356003). The LC/MS/MS instrument and parameters were similar to those of the ECM. Samples were analyzed for propargite using an Agilent Technologies 1200 Infinity Series HPLC coupled with an Applied Biosystems API 5000 Tandem Mass Spectrometer using Turbo-V Ion Spray Source, electrospray ionization (ESI) operated in the positive ion, and MRM. The other LC conditions were the same as those reported in the ECM. Two ion pair transitions were monitored for propargite: m/z 368.1→231.1 (quantitation) and m/z 368.1→81.1 (confirmation). Reported retention times were *ca.* 5.0 minutes for propargite. Other than the insignificant modifications to the analytical equipment, no other method modifications were reported.

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.01 µg/L for propargite in water (equivalent to 0.4 ng/mL using the current methodology; p. 11; Appendix D, pp. 68, 74 of MRID 50356002; p. 10 of MRID 50356003). In the ECM, the Limit of Detection (LOD) for propargite was determined to be 0.002 µg/L for drinking water and 0.003 µg/L for surface water. In the ILV, the LOD was not reported.

II. Recovery Findings

ECM (MRID 50356002): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of propargite at fortification levels of 0.01 $\mu\text{g/L}$ (LOQ) and 0.1 $\mu\text{g/L}$ (10 \times LOQ) in two water matrices (p.11; Tables III-IV, pp. 24-25). Two ion pair transitions were monitored for propargite using LC/MS/MS in positive ESI mode; the quantification and confirmation ion data was comparable. Drinking water (2706W-055; pH 7.6, hardness 29 mg/L as CaCO_3 , 58 ppm total dissolved solids) and surface water (2706W-049; pH 8.5, hardness 262 mg/L as CaCO_3 , 204 ppm total dissolved solids) were characterized by Agvise Laboratories, Northwood, North Dakota (p. 13; Appendix C, pp. 65-66). Water matrix sources were not reported.

ILV (MRID 50356003): Mean recoveries and RSDs were within guidelines for analysis of propargite at fortification levels of 0.01 $\mu\text{g/L}$ (LOQ) and 0.1 $\mu\text{g/L}$ (10 \times LOQ) in two water matrices (Tables 2-5, pp. 21-24). Two ion pair transitions were monitored for propargite using LC/MS/MS in positive ESI mode; the quantification and confirmation ion data was comparable, except for the LOQ analysis in drinking water. For the LOQ quantitation and confirmation ion analyses in drinking water, the original result for one sample was deviant; therefore, the mean of duplicate re-analyses were reported for this sample. For the confirmation ion analysis, measured concentrations were corrected for extrapolated mean matrix blank contributions of 0.0024033 $\mu\text{g/L}$ (*ca.* 24% of the LOQ) in surface water and 0.0026306 $\mu\text{g/L}$ (*ca.* 26% of the LOQ) in drinking water. Drinking (well) water (pH 8.1, hardness 142 mg/L as CaCO_3 , 336 $\mu\text{S/cm}$ specific conductance), obtained from a well at Easton Laboratories-Easton testing facility in Easton, Maryland, and surface (lake) water (pH 7.0, hardness 64.0 mg/L as CaCO_3 , 188 $\mu\text{S/cm}$ specific conductance), obtained from Tuckahoe Lake located in Tuckahoe State Park in Ridgely, Maryland, were characterized internally (pp. 11-12; Appendices III-IV, pp. 131-132). The method for propargite was validated with insignificant modifications to the analytical equipment and parameters; the number of trials was not specified, but the reviewer assumed that the method was validated in the first trial (pp. 10, 13-14).

Table 2. Initial Validation Method Recoveries for Propargite in Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Drinking Water¹						
Quantitation ion ²						
Propargite	0.01 (LOQ)	5	98-109	104	4.3	4.2
	0.1	5	96-118	102	9.5	9.4
Confirmation ion ²						
Propargite	0.01 (LOQ)	5	101-110	106	3.4	3.2
	0.1	5	92-115	100	9.3	9.3
Surface Water¹						
Quantitation ion ²						
Propargite	0.01 (LOQ)	5	84-105	99	8.5	8.6
	0.1	5	82-102	93	9.6	10.4
Confirmation ion ²						
Propargite	0.01 (LOQ)	5	91-110	97	7.5	7.7
	0.1	5	80-101	92	9.5	10.4

Data (uncorrected recovery results; Tables III-IV, pp. 24-25) were obtained from p.11; Tables III-IV, pp. 24-25 of MRID 50356002.

1 Drinking water (2706W-055; pH 7.6, hardness 29 mg/L as CaCO₃, 58 ppm total dissolved solids) and surface water (2706W-049; pH 8.5, hardness 262 mg/L as CaCO₃, 204 ppm total dissolved solids) were characterized by Agvise Laboratories, Northwood, North Dakota (p. 13; Appendix C, pp. 65-66). Water matrix sources were not reported.

2 Two ion pair transitions were monitored for propargite: m/z 368.1→231.6 (quantitation) and m/z 368.1→81.4 (confirmation).

Table 3. Independent Validation Method Recoveries for Propargite in Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Drinking Water¹						
Quantitation ion ²						
Propargite	0.01 (LOQ)	5 ³	110-114	112	1.48	1.32
	0.1	5	104-108	106	1.52	1.43
Confirmation ion ^{2,4}						
Propargite	0.01 (LOQ)	5 ³	86.5-90.9	88.4	1.76	1.99
	0.1	5	101-103	102	1.00	0.980
Surface Water¹						
Quantitation ion ²						
Propargite	0.01 (LOQ)	5	96.2-117	105	7.88	7.50
	0.1	5	97.7-101	98.9	1.34	1.35
Confirmation ion ^{2,4}						
Propargite	0.01 (LOQ)	5	100-109	104	3.91	3.75
	0.1	5	98.1-101	99.5	1.06	1.07

Data (uncorrected recovery results, except where noted; Tables 2-5, pp. 21-24) were obtained from Tables 2-5, pp. 21-24 of MRID 50356003.

1 Drinking (well) water (pH 8.1, hardness 142 mg/L as CaCO₃, 336 µS/cm specific conductance), obtained from a well at Easton Laboratories-Easton testing facility in Easton, Maryland, and surface (lake) water (pH 7.0, hardness 64.0 mg/L as CaCO₃, 188 µS/cm specific conductance), obtained from Tuckahoe Lake located in Tuckahoe State Park in Ridgely, Maryland, were characterized internally (pp. 11-12; Appendices III-IV, pp. 131-132).

2 Two ion pair transitions were monitored for propargite: m/z 368.1→231.1 (quantitation) and m/z 368.1→81.1 (confirmation).

3 The original result for one sample was deviant; the mean of duplicate re-analyses were reported for this sample.

4 Measured concentrations were corrected for extrapolated mean matrix blank contributions of 0.0024033 µg/L (*ca.* 24% of the LOQ) in surface water and 0.0026306 µg/L (*ca.* 26% of the LOQ) in drinking water (Table 3, p. 22; Table 5, p. 24).

III. Method Characteristics

In the ECM and ILV, the method LOQ was 0.01 µg/L for propargite in water (equivalent to 0.4 ng/mL using the current methodology; pp. 11, 18-19; Table VIII, p. 29; Appendix D, pp. 68, 74 of MRID 50356002; p. 10 of MRID 50356003). In the ECM, the LOQ was defined as the lowest concentration validated for each matrix, and the LOQ was reported from the ECM in the ILV. No calculation was provided for the method LOQ in the ECM or ILV. In the ECM, the LOD for propargite was calculated to be 0.002 µg/L for drinking water and 0.003 µg/L for surface water. The LOD was calculated using the following equation: $LOD = t_{0.99} \times S$, where $t_{0.99}$ equaled 3.747 (the one-tailed t-statistic at the 99% confidence level for n-1 replicates) and S equaled the standard deviation of n samples fortified at the LOQ. In the ILV, the LOD was not reported.

Table 4. Method Characteristics for Propargite in Water

		Propargite	
Limit of Quantitation (LOQ)	ECM	0.01 µg/L (equivalent to 0.4 ng/mL using the current methodology)	
	ILV	0.01 µg/L	
Limit of Detection (LOD)	ECM	0.002 µg/L for drinking water 0.003 µg/L for surface water	
	ILV	Not reported	
Linearity (calibration curve r^2 and concentration range)	ECM	Drinking	$r^2 = 0.9998$ (Q) $r^2 = 0.9992$ (C)
		Surface	$r^2 = 0.9986$ (Q) $r^2 = 0.9978$ (C)
	ILV ¹	Drinking	$r^2 = 0.9997$ (Q) $r^2 = 0.9995$ (C)
		Surface	$r^2 = 0.9994$ (Q) $r^2 = 0.9995$ (C)
	Concentration range		0.04-5.0 ng/mL
Repeatable	ECM ²	Yes at LOQ and 10×LOQ.	
	ILV ^{3,4}	Drinking ⁵	Yes at LOQ ⁶ and 10×LOQ.
		Surface	Yes at LOQ and 10×LOQ.
Reproducible		Yes at LOQ and 10×LOQ.	
Specific	ECM		Yes, matrix interferences were <5% of the LOQ (based on peak area). Some minor baseline interference was observed in the C chromatogram. ⁷
	ILV	Q	Yes, matrix interferences were less than <i>ca.</i> 14% of the LOQ (based on measured concentration).
		C	Matrix interferences were <i>ca.</i> 24-26% of the LOQ (based on measured concentration). ⁷

Data were obtained from p.11; Tables I-II, pp. 22-23 (calibration coefficients); Tables III-IV, pp. 24-25 (recovery results); Figure 2, pp. 32-33 and Figure 11, pp. 42-43 (calibration curve); Figures 3-10, pp. 34-41 and Figures 12-19, pp. 44-51 (chromatograms) of MRID 50356002; pp. 13; Tables 2-5, pp. 21-24 (recovery results); Figures 1-2, pp. 25-26 (calibration curves); Figures 5-8, pp. 29-32 (chromatograms) of MRID 50356003. Q = quantitation ion; C = confirmation ion. All results reported for Q and C ions unless specified otherwise.

1 Correlation coefficients (r^2) were reviewer-calculated based on r values (1/x weighted linear regression analysis) reported in the study report (Figures 1-2, pp. 25-26 of MRID 50356003; DER Attachment 2).

2 In the ECM, the drinking water (2706W-055; pH 7.6, hardness 29 mg/L as CaCO₃, 58 ppm total dissolved solids) and surface water (2706W-049; pH 8.5, hardness 262 mg/L as CaCO₃, 204 ppm total dissolved solids) were characterized by Agvise Laboratories, Northwood, North Dakota (p. 13; Appendix C, pp. 65-66 of MRID 50356002). Water matrix sources were not reported.

3 In the ILV, the drinking (well) water (pH 8.1, hardness 142 mg/L as CaCO₃, 336 µS/cm specific conductance), obtained from a well at Easton Laboratories-Easton testing facility in Easton, Maryland, and surface (lake) water (pH 7.0, hardness 64.0 mg/L as CaCO₃, 188 µS/cm specific conductance), obtained from Tuckahoe Lake located in Tuckahoe State Park in Ridgely, Maryland, were characterized internally (pp. 11-12; Appendices III-IV, pp. 131-132 of MRID 50356003).

4 The ECM method for propargite was validated by the ILV with insignificant modifications to the analytical equipment and parameters; the number of trials was not specified, but the reviewer assumed that the method was validated in the first trial (pp. 10, 13-14 of MRID 50356003).

5 For the ILV confirmation ion analysis only, measured concentrations were corrected for extrapolated mean matrix blank contributions of 0.0024033 µg/L (*ca.* 24% of the LOQ) in surface water and 0.0026306 µg/L (*ca.* 26% of the LOQ) in drinking water (Table 3, p. 22; Table 5, p. 24 of MRID 50356003).

6 The original result for one ILV LOQ sample in drinking water was deviant; the mean of duplicate re-analyses were reported for this sample (Tables 4-5, pp. 23-24 of MRID 50356003).

7 A confirmatory method is not typically required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data.

IV. Method Deficiencies and Reviewer's Comments

1. The communications between the ILV staff and study developers and sponsors was not reported or addressed in the ILV. The ECM laboratory (PTRL West) and ILV laboratory (Wildlife International) are both now doing business as EAG Laboratories; however, the ECM and ILV laboratories were located at different facilities and all laboratory personnel and equipment differed between the two laboratories. The registrant, Arysta, sent an email to Wilhelmena Livingston, EPA, dated 05/16/18, with the list of communications between the method development staff and ILV staff. This email indicated that there was no collusion between the ILV staff and the study developers and sponsors.
2. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 11, 18-19; Table VIII, p. 29; Appendix D, pp. 68, 74 of MRID 50356002; p. 10 of MRID 50356003). In the ECM, the LOQ was defined as the lowest concentration validated for each matrix, and the LOQ was reported from the ECM in the ILV. No calculation was provided for the method LOQ in the ECM or ILV. In the ECM, the LOD for propargite was calculated using the following equation: $LOD = t_{0.99} \times S$, where $t_{0.99}$ equaled 3.747 (the one-tailed t-statistic at the 99% confidence level for n-1 replicates) and S equaled the standard deviation of n samples fortified at the LOQ. In the ILV, the LOD was not reported.
3. The number of trials required by the ILV to validate the ECM method was not specified; however, the reviewer assumed that the method was validated in the first trial based on the insignificant modifications to the method (pp. 10, 13-14 of MRID 50356003).
4. For the ILV confirmation ion analysis only, measured concentrations were corrected for extrapolated mean matrix blank contributions of 0.0024033 µg/L (*ca.* 24% of the LOQ) in surface water and 0.0026306 µg/L (*ca.* 26% of the LOQ) in drinking water (Table 3, p. 22; Table 5, p. 24 of MRID 50356003). The ILV study author discussed that the matrix interference was less in the quantitation ion transition because of differences in ionization efficiency of the transitions by the LC/MS/MS instrument (pp. 17-18). These interferences and recovery corrections did not have an effect on the validity of the study since a confirmation method is not required when the primary method is GC/MS or LC/MS/MS.
5. The reviewer noted the following significant typographical error in the ECM: the ionization mode was reported as “negative positive mode” in the analytical method (Appendix D, p. 73 of MRID 50356002). The reviewer assumed that the ionization mode was positive, based on the ILV, despite the fact that “Negative Ion Spray Voltage (IS):” was listed as one of the LC/MS/MS settings (Appendix D, p. 74).
6. In the ECM, matrix effects were assessed and determined to be insignificant in the surface and drinking water matrices (<20% difference; p. 18; Table V, p. 26 of MRID 50356002). Solvent-based calibration standards were used for the recovery results. In the ECM, the stability of the calibration standards and final extracts were assessed and determined to be up to 21 days and up to 18-21 days, respectively, when stored under frozen conditions (p. 18; Tables VI-VII, pp. 27-28).

7. In the ECM, the time required to complete the extraction of one set of 12 samples (two matrix controls and ten fortified samples) was reported as 6 hours for one chemist, followed by ca. 10 hours for LC/MS/MS analysis (unattended), and then *ca.* 2 hours of data processing for one chemist (Appendix D, p. 75 of MRID 50356002). The time requirements of the method were 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**Propargite****IUPAC Name:** 2-(4-Tert-butylphenoxy)cyclohexyl prop-2-ynyl sulfite**CAS Name:** 2-[4-(1,1-Dimethylethyl)phenoxy]cyclohexyl 2-propyn-1-yl sulfite**CAS Number:** 2312-35-8**SMILES String:** Not provided