

Analytical method for Prometon in water

Reports: ECM: EPA MRID No.: 49553105. MacGregor, J.A., and E.S. Bodle. 2014. VALIDATION OF A METHOD FOR THE DETERMINATION OF PROMETON IN FRESHWATER FOR SUPPORT OF AQUATIC FIELD DISSIPATION STUDIES. Report prepared by Wildlife International, Easton, Maryland, sponsored and submitted by Agan Chemical Manufacturers, Ltd., c/o Makhteshim-Agan of North America, Inc. (d/b/a ADAMA), Raleigh, North Carolina; 45 pages. Wildlife International Project No: 234C-114. Mana Study No.: 90017908. Final report issued December 17, 2014.

ILV: EPA MRID No.: 49729001. Keenan, D. 2015. Independent Laboratory Validation of an Analytical Method for the Determination of Prometon in Water, Soil, and Sediment. Report prepared by PTRL West (a division of EAG, Inc.), Hercules, California, sponsored and submitted by ADAMA Agan Ltd., c/o Makhteshim-Agan of North America, Inc. (d/b/a ADAMA), Raleigh, North Carolina; 94 pages. PTRL Study No: 2744W. Final report issued September 18, 2015.

Document No.: MRIDs 49553105 & 49729001

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with USEPA FIFRA and OECD Good Laboratory Practice (GLP) standards, with the exception of the test and reference substance characterizations and stability under storage conditions at the testing facility (p. 3 of MRID 49553105). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). The statement of authenticity was not included.

ILV: The study was conducted in compliance with USEPA FIFRA GLP standards, with the exception of the test and reference substance characterizations and stability under storage conditions at the testing facility (p. 3 of MRID 49729001). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3). The statement of Authenticity was included with the QA statement.

Classification: This analytical method is classified as **Acceptable**.

PC Code: 080804

EFED Final Lewis Ross Brown

Signature: Lewis Ross Brown, III

Reviewer: Biologist

Date: 04/18/17

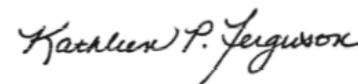
Lisa Muto,
Environmental Scientist

Signature: 

**CDM/CSS-
Dynamac JV**

Date: 3/28/17

Reviewers: Kathleen Ferguson, Ph.D.,
Environmental Scientist

Signature: 

Date: 3/28/17

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, Mana Study No. 90017908, is designed for the quantitative determination of prometon in water at the stated LOQ of 0.100 mg/L. The LOQ is similar to the lowest toxicological level of concern in water (98 µg/L). Characterized surface water (freshwater, lake) was used for the ECM validation; the same water matrix was used for the ILV validation. Matrix description and characterization were not reproduced in the ILV report. Prometon was identified using two ion transitions in the ECM and ILV. The ECM method for prometon 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 and no communication between the ILV and ECM. All ILV and ECM data regarding repeatability, accuracy, precision, linearity and specificity were satisfactory for prometon. In the ECM, the linear regression curve was only provided for the quantitation ion transition; 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.

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						
Prometon	49553105 ¹	49729001 ²		Water	17/12/2014	Agan Chemical Manufacturers, Ltd. ³	LC/MS/MS	0.100 mg/L

1 In the ECM, fresh (lake) water (surface water; pH 7.56, hardness 76.0 mg/L as CaCO₃, specific conductance 184 µS/cm) was obtained from Tuckahoe Lake, Ridgley, Maryland and was characterized by Wildlife International (p. 13; Appendix 4, p. 44 of MRID 49553105).

2 In the ILV, the same water matrix which was used in the ECM was used in the ILV; the matrix was provided by Wildlife International (Inventory No. 2744W-001; p. 18 of MRID 49729001). Matrix description and characterization were not reproduced in the ILV report.

3 c/o Makhteshim-Agan of North America, Inc. (d/b/a ADAMA).

I. Principle of the Method

Water (10.0 mL) was fortified then processed using a direct injection approach (pp. 12, 15; Figure 1, p. 23 of MRID 49553105). An aliquot (2.00 mL) of the sample was diluted volumetrically with 2.00 mL of methanol:0.2% formic acid (v:v) containing 0.005 µg/mL of prometryn IS (Dilution Solvent #1) in a disposable 15-mL glass culture tube. After vortexing, an aliquot was filtered through a 0.2 µm PTFE syringe filter assembly into a second tube. A 1.00 mL aliquot of the filtered dilution mixture was volumetrically diluted further to a 25.0 mL final volume using methanol:HPLC grade water:formic acid (50:50:0.1, v:v:v) containing 0.0025 µg/mL of prometryn IS (Dilution Solvent #2). The final solution was mixed by inversion and an aliquot was transferred to an autosampler vial for analysis by LC/MS/MS.

Samples were analyzed for prometon using an Agilent Technologies 1260 Infinity Series HPLC coupled with an AB Sciex 5500 Triple Quad Mass Spectrometer using a Turbo-Ion Spray source operated in the positive ion, multiple reaction monitoring (MRM) mode (pp. 15-16; Table 1, p. 20 of MRID 49553105). The following LC conditions were used: THERMO EC Betasil C-18 column (2.1 mm x 50 mm, 5 µm; column temperature 40°C), THERMO EC Javelin Betasil C-18 guard

column (10 mm x 2.1 mm), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.00-1.00 min. 90.0:10.0, 4.00-5.00 min. 10.0:90.0, 5.01-9.00 min. 90.0:10.0] and injection volume of 5.0 μ L. Two ion pair transitions were monitored for prometon: m/z 226 \rightarrow 142 (quantitation) and m/z 226 \rightarrow 184 (confirmation). One ion transition was monitored for the prometryn IS: m/z 242 \rightarrow 158. Reported retention times were *ca.* 4.32 minutes for prometon and *ca.* 5.26 minutes for prometryn IS.

The ILV performed the ECM methods for each analyte as written, except insignificant modifications to the analytical equipment and parameters (pp. 18, 20-23; Figure 1, p. 31 of MRID 49729001). The LC/MS/MS instrument and parameters were similar to those of the ECM. Samples were analyzed for prometon using an Agilent Technologies 1200 HPLC coupled with an Applied Biosystems API 5500 Tandem Mass Spectrometer using electrospray ionization (ESI) operated in the positive ion, multiple reaction monitoring (MRM) mode (pp. 22-24). The following LC conditions were used: Thermo-Scientific Hypersil Gold column (2.1 mm x 50 mm, 1.9 μ ; column temperature not reported), Thermo-Scientific Hypersil Gold guard column (10 mm x 2.1 mm, 3 μ), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.0-1.0 min. 90:10, 4.0-5.0 min. 10:90, 5.1-9.0 min. 90:10] and injection volume of 1 μ L. The same two ion pair transitions were monitored for prometon and prometryn IS as were monitored in the ECM. Observed retention times were *ca.* 4.3 minutes for prometon and *ca.* 4.7 minutes for prometryn IS (Figures 41-42, pp. 71-72; Figures 47-48, pp. 77-78). Other than the insignificant modifications to the analytical equipment and parameters, no other method modifications were reported (p. 28).

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.100 mg/L (0.1 ppm) for prometon in water (method LOQ; p. 16 of MRID 49553105; p. 26 of MRID 49729001). In the ECM, the theoretical and actual LOQ were determined to be 0.0250 mg/L and 0.000441 mg/L, respectively. In the ECM, the Limit of Detection (LOD) for prometon was calculated to be 0.0000026 mg/L. In the ILV, the LOD was not reported, but it was defined to be *ca.* 20% of the LOQ.

II. Recovery Findings

ECM (MRID 49553105): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of prometon at fortification levels of 0.100 mg/L (0.1 ppm; LOQ) and 1.00 mg/L (1.0 ppm; 10 \times LOQ) in the surface (lake) water matrix (Tables 2-3, pp. 21-22). Two ion pair transitions were monitored for prometon using LC/MS/MS in positive ESI mode; the quantification and confirmation ion data was comparable. Fresh (lake) water (surface water; pH 7.56, hardness 76.0 mg/L as CaCO₃, specific conductance 184 μ S/cm) was obtained from Tuckahoe Lake, Ridgley, Maryland and was characterized by Wildlife International (p. 13; Appendix 4, p. 44).

ILV (MRID 49729001): Mean recoveries and RSDs were within guidelines for analysis of prometon at fortification levels of 0.100 mg/L (0.1 ppm; LOQ) and 1.00 mg/L (1.0 ppm; 10 \times LOQ) in the surface (lake) water matrix (Table I, p. 30). Two ion pair transitions were monitored for prometon using LC/MS/MS in positive ESI mode; the quantification and confirmation ion data was comparable. Fresh (lake) water was the same water matrix which was used in the ECM was used in the ILV; the matrix was provided by Wildlife International (Inventory No. 2744W-001; p. 18). Matrix description and characterization were not reproduced in the ILV report. The method for

prometon 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. 13-14, 18, 21-23, 28).

Table 2. Initial Validation Method Recoveries for Prometon in Water

Analyte ¹	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Fresh (Lake) Water¹						
Quantitation ion ²						
Prometon	0.100 (LOQ)	5	97.1-101	99.4	1.71	1.72
	1.00	5	102-104	103	0.837	0.813
Confirmation ion ²						
Prometon	0.100 (LOQ)	5	96.9-100	98.7	1.56	1.58
	1.00	5	100-102	101	1.00	0.990

Data (uncorrected recovery results; pp. 17-18) were obtained from Tables 2-3, pp. 21-22 of MRID 49553105.

1 Fresh (lake) water (surface water; pH 7.56, hardness 76.0 mg/L as CaCO₃, specific conductance 184 µS/cm) was obtained from Tuckahoe Lake, Ridgley, Maryland and was characterized by Wildlife International (p. 13; Appendix 4, p. 44).

2 Two ion pair transitions were monitored for prometon: m/z 226→142 (quantitation) and m/z 226→184 (confirmation).

Table 3. Independent Validation Method Recoveries for Prometon in Water

Analyte ¹	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Fresh (Lake) Water¹						
Quantitation ion ²						
Prometon	0.100 (LOQ)	5	97-103	100	2.9	2.9
	1.00	5	100-109.5	103	3.8	3.7
Confirmation ion ²						
Prometon	0.100 (LOQ)	5	98-101	100	2.1	2.1
	1.00	5	99.5-108	102	3.7	3.6

Data (uncorrected recovery results; pp. 24-26) were obtained from Table I, p. 30 of MRID 49729001.

1 The same water matrix which was used in the ECM was used in the ILV; the matrix was provided by Wildlife International (Inventory No. 2744W-001; p. 18). Matrix description and characterization were not reproduced in the ILV report.

2 Two ion pair transitions were monitored for prometon: m/z 226→142 (quantitation) and m/z 226→184 (confirmation).

III. Method Characteristics

In the ECM and ILV, the method LOQ was 0.100 mg/L (0.1 ppm) for prometon in water (p. 16 of MRID 49553105; p. 26 of MRID 49729001). No justification was provided for the method LOQ in the ECM or ILV. In the ECM, the theoretical and actual LOQ were determined to be 0.0250 mg/L and 0.000441 mg/L, respectively. The theoretical LOQ was calculated as the product of the lowest calibration standard (0.000500 µg/mL) and the dilution factor of the matrix blank samples (50.0). The actual LOQ was calculated using the following equation:

Actual LOQ = lowest calibration standard / [(average signal to noise ratio) x 10 x (dilution factor of the matrix blank samples)],

Where the lowest calibration standard is 0.000500 µg/mL, the average signal to noise ratio is 567.4, and the dilution factor of the matrix blank samples is 50.0. No other justification for the LOQ was provided. In the ECM, the instrumental LOD for prometon was calculated to be 0.0000026 mg/L. The LOD was calculated using the following equation:

Instrumental LOD = lowest calibration standard / [(average signal to noise ratio) x 3 x (dilution factor of the matrix blank samples)],

Where the lowest calibration standard is 0.000500 µg/mL, the average signal to noise ratio is 567.4, and the dilution factor of the matrix blank samples is 1.00. No other justification for the LOQ was provided. In the ILV, the LOD was not reported, but it was defined to be *ca.* 20% of the LOQ. This would be equivalent to 0.02 mg/L.

Table 4. Method Characteristics for Prometon in Water

		Prometon
Limit of Quantitation (LOQ)	ECM	0.100 mg/L (0.1 ppm; method LOQ) 0.0250 mg/L (theoretical LOQ) 0.000441 mg/L (actual LOQ)
	ILV	0.100 mg/L (0.1 ppm)
Limit of Detection (LOD)	ECM	0.0000026 mg/L (instrumental LOD)
	ILV	<i>ca.</i> 20% of the LOQ (equivalent to 0.02 mg/L)
Linearity (calibration curve r^2 and concentration range)	ECM ¹	$r^2 = 0.9985$ (Q) ²
		Range: 0.000500-0.0500 µg/mL
	ILV ³	$r^2 = 0.9996$ (Q)
		Range: 0.1-50 ng/mL
Repeatable	ECM ⁴	Yes at LOQ and 10×LOQ.
	ILV ⁵	Yes at LOQ and 10×LOQ. ⁶
Reproducible		Yes at LOQ and 10×LOQ.
Specific	ECM	Yes, no matrix interferences were observed.
	ILV	Yes, no matrix interferences were observed.

Data were obtained from pp. 16-17; Tables 2-3, pp. 21-22 (recovery results); Figure 2, p. 24 (calibration curve); Figures 5-10, pp. 27-32 (chromatograms) of MRID 49553105; pp. 21; 26 Table I, p. 30 (recovery results); Figures 3-6, pp. 33-36 (reagent blank chromatograms); Figures 11-12, pp. 41-42 (control chromatograms); Figures 35-36, pp. 65-66 (calibration curves); Figures 41-42, pp. 71-72; Figures 47-48, pp. 77-78 (LOQ and 10×LOQ chromatograms) of MRID 49729001. Q = quantitation ion; C = confirmation ion. All results reported for Q and C ions unless specified otherwise.

1 Correlation coefficient (r^2) was reviewer-calculated based on r value (1/x weighted linear regression analysis) reported in the study report; solvent standards were used (pp. 14-15; Figure 2, p. 24 of MRID 49553105; DER Attachment 2).

2 Only the quantitation calibration curve was provided in the ECM. The reviewer noted that 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.

3 Correlation coefficients (r^2) were reviewer-calculated based on r values (1/x weighted linear regression analysis) reported in the study report; (Figures 35-36, pp. 65-66 of MRID 49729001; DER Attachment 2).

4 In the ECM, fresh (lake) water (surface water; pH 7.56, hardness 76.0 mg/L as CaCO₃, specific conductance 184 µS/cm) was obtained from Tuckahoe Lake, Ridgley, Maryland and was characterized by Wildlife International (p. 13; Appendix 4, p. 44 of MRID 49553105).

5 In the ILV, the same water matrix which was used in the ECM was used in the ILV; the matrix was provided by Wildlife International (Inventory No. 2744W-001; p. 18 of MRID 49729001). Matrix description and characterization were not reproduced in the ILV report.

6 The ECM method for prometon 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. 13-14, 18, 21-23, 28).

IV. Method Deficiencies and Reviewer's Comments

1. The ILV reported that the same water matrix which was used in the ECM was used in the ILV; the matrix was provided by Wildlife International (p. 13; Appendix 4, p. 44 of MRID 49553105; p. 18 of MRID 49729001). Matrix description and characterization were not reproduced in the ILV report; the characteristics and constitution of the water matrix/matrices should be clear in the method validations. Additionally, it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method.
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. The LOQ and LOD were not adequately supported by calculations or comparison to background levels in the ECM (p. 16 of MRID 49553105; pp. 26 of MRID 49729001). In the ECM, the method, theoretical and actual LOQ were determined to be 0.100 mg/L, 0.0250 mg/L and 0.000441 mg/L, respectively. No justification was provided for the method LOQ. The theoretical LOQ was calculated as the product of the lowest calibration standard (0.000500 µg/mL) and the dilution factor of the matrix blank samples (50.0). The actual LOQ was calculated using the following equation: $\text{actual LOQ} = \text{lowest calibration standard} / [(\text{average signal to noise ratio}) \times 10 \times (\text{dilution factor of the matrix blank samples})]$, where the lowest calibration standard is 0.000500 µg/mL, the average signal to noise ratio is 567.4, and the dilution factor of the matrix blank samples is 50.0. In the ECM, the instrumental LOD for prometon was calculated to be 0.0000026 mg/L. The LOD was calculated using the following equation: $\text{instrumental LOD} = \text{lowest calibration standard} / [(\text{average signal to noise ratio}) \times 3 \times (\text{dilution factor of the matrix blank samples})]$, where the lowest calibration standard is 0.000500 µg/mL, the average signal to noise ratio is 567.4, and the dilution factor of the matrix blank samples is 1.00. In the ILV, the LOD was not reported, but it was defined to be *ca.* 20% of the LOQ.
3. The number of trials required by the ILV to validate the ECM method was not specified; however, but the reviewer assumed that the method was validated in the first trial based on the insignificant modifications to the method and no communication between the ILV and ECM (pp. 13-14, 18, 21-23, 28).
4. The ILV reported that communications between the ILV and study developers and sponsors did not occur (p. 28 of MRID 49729001).
5. In the ILV, the total time required to complete one set of 13 samples (one reagent blank, two matrix controls and ten fortified samples) was reported as *ca.* 15 hours to complete, where solution preparation required *ca.* 6 hours, sample processing required *ca.* 4 hours and LC/MS/MS analysis and data processing required *ca.* 5 hours (p. 26 of MRID 49729001).

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

Prometon

IUPAC Name: N²,N⁴-diisopropyl-6-methoxy-1,3,5-triazine-2,4-diamine

CAS Name: 6-Methoxy-N,N'-bis(1-methylethyl)-1,3,5-triazine-2,4-diamine

CAS Number: 1610-18-0

SMILES String: O(c(nc(nc1NC(C)C)NC(C)C)n1)C

