

Analytical method for Prometon in soil and sediment

Reports: ECM 1: EPA MRID No.: 49599501. MacGregor, J.A., and E.S. Bodle. 2015. 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; 46 pages. Wildlife International Project No: 234C-115. Mana Study No.: 90017909. Final report issued February 3, 2015.

ECM 2: EPA MRID No.: 49599502. MacGregor, J.A., and E.S. Bodle. 2015. 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; 47 pages. Wildlife International Project No: 234C-116. Mana Study No.: 90017910. Final report issued January 7, 2015; amended report issued February 2, 2015.

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 49599501 & 49599502 & 49729001

Guideline: 850.6100

Statements: ECM 1: The study was conducted in compliance with USEPA FIFRA and OECD Good Laboratory Practice (GLP) standards, except for the test and reference substance characterizations and stability under storage conditions at the testing facility (p. 3 of MRID 49599501). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4 of MRID 49599501). The statement of authenticity was not included.

ECM 2: The study was conducted in compliance with USEPA FIFRA and OECD Good Laboratory Practice (GLP) standards, except for the test and reference substance characterizations and stability under storage conditions at the testing facility (p. 3 of MRID 49599502). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4 of MRID 49599502). The statement of authenticity was not included.

ILV: The study was conducted in compliance with USEPA FIFRA GLP standards, except for 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: These analytical methods are classified as **Acceptable**.

PC Code: 080804

EFED Final Lewis R. Brown, III

Signature: Lewis Ross Brown, III

Reviewer: Biologist

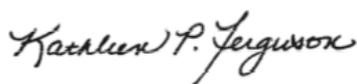
Date: 04/20/17

CDM/CSS- Lisa Muto,
Dynamac JV Environmental Scientist

Signature: 

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 is designed for the quantitative determination of prometon in sediment and soil at the stated LOQ of 0.050 mg/kg. The LOQ is greater than the lowest toxicological level of concern for terrestrial plants (0.0040 mg/kg soil)¹. Characterized silt loam sediment and sandy loam soil matrices were used for the ECM validations; the same sediment and soil matrices were used for the ILV validation. Matrix descriptions and characterizations were not reproduced in the ILV report. Prometon was identified using two ion transitions in the ECM 1, ECM 2 and ILV. The ECM methods for prometon in sediment and soil were validated by the ILV with insignificant modifications to the sample processing procedure and 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 ECMs. All ILV, ECM 1 and ECM 2 data regarding repeatability, accuracy, precision, linearity and specificity were satisfactory for prometon. In the ECMs 1 and 2, the linear regression curves were 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.

¹ The lowest toxicological level of concern for terrestrial plants in the 2013 "Registration Review Problem Formulation for Prometon" (DP 405083) is 0.0080 lbs ai/A, which is equivalent to 0.0040 mg/kg soil, assuming a 6-inch soil depth and 1.5 g/mL soil density.

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	49599501 ¹	49729001 ³		Sediment	03/02/2015	Agan Chemical Manufacturers, Ltd. ⁴	LC/MS/MS	0.050 mg/kg
	49599502 ²		Soil	07/01/2015 (Original) 02/02/2015 (Amended)				

1 In the ECM 1 (MRID 49599501), silt loam sediment (41% sand, 52% silt, 7% clay; pH 5.0; 11.5% organic matter; 6.7% organic carbon) was collected from West Bearskin Lake, Minnesota, and was characterized by Agvise Laboratories, Northwood, North Dakota (USDA textural classification; p. 13; Appendix 4, p. 45 of MRID 49599501).

2 In the ECM 2 (MRID 49599502), sandy loam soil (70% sand, 21% silt, 9% clay; pH 6.7; 5.6% organic matter) was obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA textural classification; p. 13; Appendix 4, p. 45 of MRID 49599502).

3 In the ILV, the same sediment and soil matrices which were used in the ECM were used in the ILV; the matrices were provided by Wildlife International (Inventory Nos. 2744W-002 and 2744W-003 for sediment and soil, respectively; p. 18 of MRID 49729001). Matrix descriptions and characterizations were not reproduced in the ILV report.

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

I. Principle of the Method

Sediment or soil (5.00 g) was weighed into 50-mL plastic graduated centrifuge tubes and fortified (pp. 12, 15-16; Figure 1, p. 24 of MRID 49599501; pp. 14-15; Figure 1, p. 23 of MRID 49599502). The sample was extracted with 20 mL of methanol:0.2% formic acid (v:v) containing 0.005 µg/mL of prometryn IS via hand shaking and vortexing then sonicated for approximately one minute using a Branson ultrasonic disruption sample processor at an amplitude setting of approximately 45%. The samples were capped and shaken on a gyratory shaker table at a setting of approximately 250 excursions per minute for approximately 15 minutes. After centrifugation at approximately 4500 rpm for approximately 10 minutes, the supernatant was transferred to a 100-mL graduated cylinder. The pellet was extracted a second time in the same manner as before, except the sonication step was omitted. The volume of the combined supernatants was adjusted to 50.0 mL using extraction solvent. The final extract was transferred to a 100-mL beaker and diluted 1:1 (v:v) by combining 2.00 mL of the extract and 2.00 mL of the dilution solvent #1 (HPLC grade bottled water) in 20-mL scintillation vials to achieve a final extract solvent composition of methanol:water:formic acid (50:50:0.1, v:v:v) containing 0.00250 µg/mL of prometryn IS. The diluted extract aliquot was filtered using an assembly of a 5-mL BD disposable plastic syringe connected to a 0.2 m Whatman Puradisk 25 TF syringe filter into a 20-mL glass scintillation vial. A 1.00 mL aliquot of the filtered extract was transferred to a 15-mL plastic graduated centrifuge tube and adjusted to 5.00 mL using methanol:water:formic acid (50:50:0.1, v:v:v) containing 0.0025 µg/mL of prometryn IS (Dilution Solvent #2). 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 (p. 16; Table 1, p. 21 of

MRID 49599501; pp. 15-16; Table 1, p. 20 of MRID 49599502). The following LC conditions were used: THERMO EC Betasil C-18 column (2.1 mm x 50 mm, 5 μ ; 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 for sediment sample analysis were *ca.* 4.37 minutes for prometon and *ca.* 5.28 minutes for prometryn IS; reported retention times for soil sample analysis 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 for the exclusion of the sonication step in the sample processing and insignificant modifications to the analytical equipment and parameters (pp. 18, 20, 22-23; Figure 2, p. 32 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 sample processing procedure and analytical equipment and parameters, no other method modifications were reported (pp. 22, 28).

In the ECM 1, ECM 2 and ILV, the Limit of Quantification (LOQ) was 0.050 mg/kg (0.05 ppm) for prometon in sediment and soil (method LOQ; pp. 16-17 of MRID 49599501; p. 16 of MRID 49599502; p. 26 of MRID 49729001). In the ECM 1, the theoretical and actual LOQ for sediment were determined to be 0.0100 mg/kg and 0.000787 mg/kg, respectively (see Reviewer's Comment #4). In the ECM 2, the theoretical and actual LOQ for soil were determined to be 0.0100 mg/kg and 0.00055 mg/kg, respectively. In the ECMs 1 and 2, the Limit of Detection (LOD) for prometon was calculated to be 0.0000024 mg/L for sediment and 0.0000016 mg/L for soil. In the ILV, the LOD was not reported, but it was defined to be *ca.* 20% of the LOQ.

II. Recovery Findings

ECM 1 (MRID 49599501): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of prometon at fortification levels of 0.050 mg/kg (0.05 ppm; LOQ) and 0.500 mg/L (0.5 ppm; 10 \times LOQ) in the silt loam sediment matrix (Tables 2-3, pp. 22-23). Two ion pair transitions were monitored for prometon using LC/MS/MS in positive ESI mode; the quantification and confirmation ion data was comparable. The silt loam sediment (41% sand, 52% silt, 7% clay; pH 5.0; 11.5% organic matter; 6.7% organic carbon) was collected from West Bearskin Lake, Minnesota, and was characterized by Agvise Laboratories, Northwood, North Dakota (USDA textural classification; p. 13; Appendix 4, p. 45).

ECM 2 (MRID 49599502): Mean recoveries and RSDs were within guidelines for analysis of prometon at fortification levels at fortification levels 0.050 mg/kg (0.05 ppm; LOQ) and 0.500 mg/L (0.5 ppm; 10×LOQ) in the sandy loam soil 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. The sandy loam soil (70% sand, 21% silt, 9% clay; pH 6.7; 5.6% organic matter) was obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA textural classification; p. 13; Appendix 4, p. 45).

ILV (MRID 49729001): Mean recoveries and RSDs were within guidelines for analysis of prometon at fortification levels of 0.050 mg/kg (0.05 ppm; LOQ) and 0.500 mg/L (0.5 ppm; 10×LOQ) in the silt loam sediment and sandy loam soil matrices (Tables II-III, 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. The same sediment and soil matrices which were used in the ECM were used in the ILV; the matrices were provided by Wildlife International (Inventory Nos. 2744W-002 and 2744W-003 for sediment and soil, respectively; p. 18). Matrix descriptions and characterizations were not reproduced in the ILV report. The methods for prometon were validated with insignificant modifications to the sample processing procedure and analytical equipment and parameters, no other method modifications were reported; the number of trials was not specified, but the reviewer assumed that the methods were validated in the first trial (pp. 13-14, 18, 21-23, 28).

Table 2. Initial Validation Method Recoveries for Prometon in Sediment/Soil

Analyte ¹	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Silt Loam Sediment¹						
Quantitation ion ²						
Prometon	0.050 (LOQ)	5	95.1-102	99.0	2.51	2.54
	0.500	5	98.1-108	105	4.04	3.85
Confirmation ion ²						
Prometon	0.050 (LOQ)	5	92.1-99.3	97.2	3.00	3.09
	0.500	5	97.3-108	104	4.27	4.11
Sandy Loam Soil³						
Quantitation ion ²						
Prometon	0.050 (LOQ)	5	94.5-102	98.5	2.66	2.70
	0.500	5	104-108	106	1.41	1.33
Confirmation ion ²						
Prometon	0.050 (LOQ)	5	94.2-101	97.5	2.50	2.56
	0.500	5	104-109	107	1.92	1.79

Data (uncorrected recovery results; p. 18 of MRID 49599501; p. 18 of MRID 49599502) were obtained from Tables 2-3, pp. 22-23 of MRID 49599501; Tables 2-3, pp. 21-22 of MRID 49599502.

1 The silt loam sediment (41% sand, 52% silt, 7% clay; pH 5.0; 11.5% organic matter; 6.7% organic carbon) was collected from West Bearskin Lake, Minnesota, and was characterized by Agvise Laboratories, Northwood, North Dakota (USDA textural classification; p. 13; Appendix 4, p. 45 of MRID 49599501).

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

3 The sandy loam soil (70% sand, 21% silt, 9% clay; pH 6.7; 5.6% organic matter) was obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA textural classification; p. 13; Appendix 4, p. 45 of MRID 49599502).

Table 3. Independent Validation Method Recoveries for Prometon in Sediment/Soil

Analyte ¹	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Silt Loam Sediment¹						
Quantitation ion ²						
Prometon	0.050 (LOQ)	5	80.8-99.8	93	7.4	8.0
	0.500	5	87.4-96.6	95	4.0	4.2
Confirmation ion ²						
Prometon	0.050 (LOQ)	5	80.2-98.2	93	7.4	8.0
	0.500	5	90.2-98	96	3.2	3.3
Sandy Loam Soil¹						
Quantitation ion ²						
Prometon	0.050 (LOQ)	5	89-103.6	97	5.3	5.5
	0.500	5	84.6-95.6	91	4.8	5.3
Confirmation ion ²						
Prometon	0.050 (LOQ)	5	87.2-103.6	95	5.8	6.1
	0.500	5	85.8-95.2	90	4.3	4.8

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

1 The same sediment and soil matrices which were used in the ECM were used in the ILV; the matrices were provided by Wildlife International (Inventory Nos. 2744W-002 and 2744W-003 for sediment and soil, respectively; p. 18).

Matrix descriptions and characterizations 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 1, ECM 2 and ILV, the method LOQ was 0.050 mg/kg (0.05 ppm) for prometon in sediment and soil (pp. 16-17 of MRID 49599501; p. 16 of MRID 49599502; p. 26 of MRID 49729001). No justification was provided for the method LOQ in the ECM 1, ECM 2 or ILV. In the ECM 1, the theoretical and actual LOQ for sediment were determined to be 0.0100 mg/kg and 0.000787 mg/kg, respectively (see Reviewer's Comment #4). In the ECM 2, the theoretical and actual LOQ for soil were determined to be 0.0100 mg/kg and 0.00055 mg/kg, respectively. The theoretical LOQ was calculated as the product of the lowest calibration standard (0.000100 µg/mL) and the dilution factor of the matrix blank samples (100). 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 0.000100 µg/mL, the average signal to noise ratio is 131.8 for sediment and 189.05 for soil, and the dilution factor of the matrix blank samples is 100. No other justification for the LOQ was provided. In the ECMs 1 and 2, the instrumental LOD for prometon was calculated to be 0.0000024 mg/L for sediment and 0.0000016 mg/L for soil. 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 0.000100 µg/mL, the average signal to noise ratio is 131.8 for sediment and 189.05 for soil, 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 0.01 mg/kg.

Table 4. Method Characteristics for Prometon in Sediment/Soil

		Prometon	
		Sediment	Soil
Limit of Quantitation (LOQ)	ECM 1 & 2	0.050 mg/kg (0.05 ppm; method LOQ)	
		0.0100 mg/kg and (theoretical LOQ)	
	ILV	0.000787 mg/kg (actual LOQ)	0.00055 mg/kg (actual LOQ)
Limit of Detection (LOD)	ECM 1 & 2	0.0000024 mg/L (instrumental LOD)	0.0000016 mg/L (instrumental LOD)
	ILV	<i>ca.</i> 20% of the LOQ (equivalent to 0.01 mg/kg)	
Linearity (calibration curve r^2 and concentration range)	ECM 1 & 2 ¹	$r^2 = 0.9997$ (Q) ²	$r^2 = 0.9995$ (Q) ²
		Range: 0.000100-0.0100 µg/mL	
	ILV ³	$r^2 = 0.9995$ (Q) $r^2 = 0.9999$ (C)	$r^2 = 0.9995$ (Q) $r^2 = 0.9998$ (C)
Repeatable	ECM 1 & 2 ^{4,5}	Yes at LOQ and 10×LOQ.	
	ILV ⁶	Yes at LOQ and 10×LOQ. ⁷	
Reproducible		Yes at LOQ and 10×LOQ.	
Specific	ECM 1 & 2	Yes, no matrix interferences were observed.	
	ILV	Yes, no matrix interferences were observed.	

Data were obtained from pp. 16-17; Tables 2-3, pp. 22-23 (recovery results); Figure 2, p. 25 (calibration curve); Figures 5-10, pp. 28-33 (chromatograms) of MRID 49599501; p. 16; Tables 2-3, pp. 21-22 (recovery results); Figure 2, p. 24 (calibration curve); Figures 5-10, pp. 27-32 (chromatograms) of MRID 49599502; pp. 21; 26 Table I, p. 30 (recovery results); Figures 3-6, pp. 33-36 (reagent blank chromatograms); Figures 13-16, pp. 43-46 (control chromatograms); Figures 37-40, pp. 67-70 (calibration curves); Figures 43-46, pp. 73-76; Figures 49-52, pp. 79-82 (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. 25 of MRID 49599501; p. 14; Figure 2, p. 24 of MRID 49599502; DER Attachment 2).

2 Only the quantitation calibration curve was provided in the ECM 1 and ECM 2. 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 37-40, pp. 67-70 of MRID 49729001; DER Attachment 2).

4 In the ECM 1 (MRID 49599501), silt loam sediment (41% sand, 52% silt, 7% clay; pH 5.0; 11.5% organic matter; 6.7% organic carbon) was collected from West Bearskin Lake, Minnesota, and was characterized by Agvise Laboratories, Northwood, North Dakota (USDA textural classification; p. 13; Appendix 4, p. 45 of MRID 49599501).

5 In the ECM 2 (MRID 49599502), sandy loam soil (70% sand, 21% silt, 9% clay; pH 6.7; 5.6% organic matter) was obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA textural classification; p. 13; Appendix 4, p. 45 of MRID 49599502).

6 In the ILV, the same sediment and soil matrices which were used in the ECM were used in the ILV; the matrices were provided by Wildlife International (Inventory Nos. 2744W-002 and 2744W-003 for sediment and soil, respectively; p. 18 of MRID 49729001). Matrix descriptions and characterizations were not reproduced in the ILV report.

7 The ECM methods for prometon were 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 methods were 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 sediment and soil matrices which were used in the ECM were used in the ILV; the matrices were provided by Wildlife International (Inventory Nos. 2744W-002 and 2744W-003 for sediment and soil, respectively; p. 13; Appendix 4, p. 45 of MRID 49599501; p. 13; Appendix 4, p. 45 of MRID 49599502; p. 18 of MRID 49729001). Matrix description and characterization were not reproduced in the ILV report; the characteristics and constitution of the sediment/soil matrix/matrices should be clear in the method validations. Additionally, it could not be determined if the ILV was provided with the most difficult matrices with which to validate the method.
2. The determinations of the LOD and LOQ in the ECM 1, ECM 2 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 ECMs 1 and 2 (pp. 16-17 of MRID 49599501; p. 16 of MRID 49599502; p. 26 of MRID 49729001). In the ECM 1, the method, theoretical and actual LOQ for sediment were determined to be 0.050 mg/kg, 0.0100 mg/kg and 0.000787 mg/kg, respectively (see Reviewer's Comment #4). In the ECM 2, the method, theoretical and actual LOQ for soil were determined to be 0.050 mg/kg, 0.0100 mg/kg and 0.00055 mg/kg, respectively. The theoretical LOQ was calculated as the product of the lowest calibration standard (0.000100 µg/mL) and the dilution factor of the matrix blank samples (100). 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 0.000100 µg/mL, the average signal to noise ratio is 131.8 for sediment and 189.05 for soil, and the dilution factor of the matrix blank samples is 100. In the ECMs 1 and 2, the instrumental LOD for prometon was calculated to be 0.0000024 mg/L for sediment and 0.0000016 mg/L for soil. 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 0.000100 µg/mL, the average signal to noise ratio is 131.8 for sediment and 189.05 for soil, 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 methods were 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 reviewer assumed that the theoretical LOQ for sediment reported in ECM 1 was erroneously reported as 0.0150 mg/kg, instead of 0.0100 mg/kg, based on the theoretical LOQ equation reported (p. 16 of MRID 49599501). Additionally, the reviewer noted that Dilution Solvent #1 was erroneously reported in ILV as water in Figure 2 instead of methanol:0.2% formic acid (v:v) containing 0.005 µg/mL of prometryn IS (Figure 2, p. 32 of MRID 49729001).

5. The ILV reported that communications between the ILV and study developers and sponsors did not occur (p. 28 of MRID 49729001).
6. 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: Oc(nc(nc1NC(C)C)NC(C)C)n1C

