Analytical method for metamitron and its degradate desaminometamitron in soil

Reports: ECM: EPA MRID No. 51173631. Bacher, R. 2007. Validation of an Analytical Method for the Determination of Residues of Metamitron and Desaminometamitron in Soil. PTRL Europe Study/Report No.: P/B 1355 G. Report prepared by PTRL Europe, Ulm, Germany, sponsored by Quena Plant Protection, Curaçao, Netherlands Antilles, and submitted by Makhteshim Agan of North America, Inc. (d/b/a Adama), Raleigh, North Carolina (p. 2); 39 pages. Final report issued November 27, 2007.

ILV: EPA MRID No. 51173632. Persch, A. 2018. Independent Laboratory Validation for the Determination of Metamitron in Soil and Sediment. EAS Study Code: S17-07072; Sponsor Code: 90019568. Report prepared by Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany, sponsored by ADAMA Agan Ltd., Ashod, Israel, and submitted by Makhteshim Agan of North America, Inc. (d/b/a Adama), Raleigh, North Carolina (p. 2); 78 pages. Final report issued June 20, 2018.

Document No.: MRIDs 51173631 & 51173632

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with German Good Laboratory Practices (GLP; 2002), which are based on OECD GLP standards (1997/1998) and accepted by Regulatory Authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHW, MAFF and MITI; p. 3; Appendix 2, p. 38 of MRID 51173631). The study was conducted in compliance with Council Directive 91/414/EEC Annex II (Part A, Section 4.2.2), amended by Commission Directive 96/46/EC, and SANCO/825/00 rev. 7 (17/03/04; p. 5). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Compliance statements were provided (pp. 2-5; Appendix 2, p. 38). A statement of the authenticity of the study report was included with the Quality Assurance and Compliance statements (pp. 4-5).
ILV: The study was conducted in accordance with OECD GLP (1997) which are accepted by Regulatory Authorities throughout the European Community, the United States of America (FIFRA; 40 CFR Part 160) and Japan (Ministry of Agriculture, Forestry and Fisheries, 11 Nousan, Notification No. 6283; pp. 3, 5 of MRID 51173632). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-5). A statement of the authenticity of the study report was included with the Quality statement.

Classification: This analytical method is classified as **Supplemental**. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ. No ILV was submitted to validate PTRL Europe Study/Report No. P/B 1355 G for desamino-metamitron. The ILV reported an LOQ of 0.01 mg/kg for metamitron in soil; however, no ECM performance data was provided to validate that LOQ. The ILV

	validated PTRL Europe Study/Report No. P/B 1355 G for metamitron in sediment; however, no sediment matrix was included in the ECM. The ILV soil matrix [loamy sand] did not cover the range of soils used in the terrestrial field dissipation (TFD) studies since only one ILV test soil was included; however, the reviewer noted that the ILV test soil provided a difficult sample condition. The one submitted metamitron TFD study (MRID 51173794) contained four soil matrix types. The number of ILV trials was not reported.	
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EFED Final Reviewer:	A'ja Duncan, Ph.D. Chemist	Signature: AJA DUNCAN Digitally signed by AJA DUNCAN Date: 2024.09.24 08:23:11 -04'00' Date: 9/23/2024
PB&A/CSS JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature: Lisa Muto Date: 12/22/2022
	Mary Samuel, M.S., Environmental Scientist	Signature: Marysamuel Date: 1/5/2023

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by PB&A/CSS Joint Venture personnel. The PB&A/CSS JV role does not include establishing Agency policies.

The conclusions conveyed in this assessment were developed in full compliance with EPA Scientific Integrity Policy for Transparent and Objective Science, and EPA Scientific Integrity Program's Approaches for Expressing and Resolving Differing Scientific Opinions. The full text of EPA Scientific Integrity Policy for Transparent and Objective Science, as updated and approved by the Scientific Integrity Committee and EPA Science Advisor can be found here: https://www.epa.gov/system/files/documents/2023-12/scientific_integrity_policy_2012_accessible.pdf. The full text of the EPA Scientific Integrity Program's Approaches for Expressing and Resolving Differing Scientific Opinions can be found here: https://www.epa.gov/scientific-integrity/approaches-expressing-and-resolving-differing-scientific-opinions.

Executive Summary

This analytical method, PTRL Europe Study/Report No. P/B 1355 G, is designed for the quantitative determination of metamitron and its degradate desamino-metamitron at 0.05 mg/kg (50µg/kg) in soil using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in soil (35 µg/kg; MRID 51173881)for metamitron and its degradate desamino-metamitron. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for metamitron in soil (0.05 mg/kg).

The ILV validated the method using characterized loamy sand soil matrix and characterized sediment (artificial soil). Only metamitron was included as a test material in the ILV. The ECM validated the method using two different characterized sandy loam soil matrices. A sediment matrix was not included as a test matrix in the ECM.

The ILV validated the method, PTRL Europe Study/Report No. P/B 1355 G, for metamitron in soil at 0.05 mg/kg and 0.50 mg/kg with insignificant modifications to the analytical parameters and equipment. The number of ILV trials was not reported, but the reviewer assumed that the ILV validation occurred with the first trial based on the lack of method modifications and reported communications of method issues. The ILV included the additional test concentration of 0.01 mg/kg (as the ILV LOQ); however, no ECM performance data was submitted for this test concentration.

All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for metamitron in soil at 0.05 mg/kg and 0.50 mg/kg.

All ILV data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for metamitron in soil at 0.01 mg/kg and in sediment at 0.01 mg/kg, 0.05 mg/kg, and 0.50 mg/kg.

All ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for desaminometamitron in soil at 0.05 mg/kg and 0.50 mg/kg.

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						
Metamitron	51173631[1]	51173632[2]		Soil	27/11/2007	Makhteshim Agan of North America, Inc. (d/b/a Adama)	LC/MS/MS	0.05 mg/kg (ECM)
Desamino-metamitron		None submitted[3]						
Metamitron	None submitted[4]	51173632[2]			20/06/2018			0.01 mg/kg (ILV)
				Sediment				

1 In the ECM, the soil matrices were sandy loam 2.2 soil (LUFA Batch No. F220403; pH 5.6 ± 0.4 (0.01M $CaCl_2$); 75.3 ± 2.0% sand, 16.6 ± 1.4% silt, 8.1 ± 1.2% clay; 2.3 ± 0.2% organic carbon) and sandy loam 2.3 soil (LUFA Batch No. F230403; pH 6.3 ± 0.2 (0.01M $CaCl_2$); 58.2 ± 0.8% sand, 32.3 ± 1.3% silt, 9.6 ± 0.8% clay; 1.2 ± 0.2% organic carbon); matrices were obtained from Lufa Speyer, Germany, and characterized by the supplier (USDA

texture classification; Appendix 3, p. 39 of MRID 51173631). The soil texture of sandy loam 2.2 soil was determined by the reviewer using USDA-NRCS technical support tools; the USDA texture of this test soil was reported as loamy sand in the study. The soil texture of sandy loam 2.3 soil was verified by the reviewer using USDA-NRCS technical support tools.

2 In the ILV, the matrices were loamy sand 2.2 soil (LUFA 2.2; LUFA Batch ID: F2.2 0915; pH 5.84 (0.01M $CaCl_2$); 79.0% sand, 13.7% silt, 7.3% clay, 2.86% organic matter) and sediment [artificial soil: 4.5% (dry weight) sphagnum peat, 20% (dry weight) kaolin clay (kaolinite content ≥30 %), 75.28% (dry weight) quartz sand (grain size ≤2 mm, but >50% of particles in the range of 50-200 µm), and 0.22% lime (calcium carbonate; p. 17; Appendix G, p. 78 of MRID 51173632]. The soil matrix was obtained from Lufa Speyer, Germany, and characterized by the supplier (USDA texture classification). The soil texture was verified by the reviewer using USDA-NRCS technical support tools. The sediment was prepared in the laboratory prior to use via mixing all components with an electric mixer.

3 No ILV was submitted to validate PTRL Europe Study/Report No. P/B 1355 G for desaminometamitron.

4 A sediment matrix was not included as a test matrix in the ECM.

I. Principle of the Method

Soils (25 g) were transferred to 250-mL screw-capped PE bottles and fortified with 0.05 mL of 2.5 or 25 µg/mL mixed fortification solutions (metamitron and desaminometamitron), as necessary (pp. 12-14 of MRID 51173631). Samples were acidified with 1.0 mL of concentrated acetic (reviewer-corrected; reported as "acidic") acid then extracted three times with 50 mL each of methanol (3 x 50 mL) via shaking on a horizontal shaker at (270 rpm) for 30 minutes then centrifugation (2 minutes at 4000 rpm). The supernatant was filtered (folded filter paper; fluted cellulose filter paper) into a measuring cylinder. The volume of the combined extracts was adjusted to 200 mL using methanol. An aliquot (0.01 mL) of the combined extracts was transferred to an autosampler vial, diluted with 0.90 mL of purified water, then analyzed by HPLC.

Samples were analyzed for metamitron and desaminometamitron using an Agilent 1200 SL HPLC coupled to an Applied Biosystems MDS Sciex API 5000 triple quadrupole mass spectrometer equipped with positive TurboIonSpray (ESI) ionization interface, multiple reaction monitoring (MRM) mode (pp. 13, 15-16 of MRID 51173631). The following LC conditions were used: Phenomenex C_{18} pre-column (4 mm, i.d.: 3 mm), Thermo Aquasil C_{18} column (3.0 mm x 150 mm, 3 µm; column temperature not reported), 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. 85:15, 1.01-6.0 min. 35:65, 6.01-8.0 min. 0:100, 8.01-11.0 min. 85:15], MS temperature 550°C, and injection volume of 20 µL. Expected approximate retention times were 4.7 and 4.4 minutes for metamitron and desaminometamitron, respectively. Two ion pair transitions were monitored (primary and confirmatory, respectively): *m/z* 203→175 and *m/z* 203→104 for metamitron and *m/z* 188→160 and *m/z* 188→104 for desaminometamitron. Solvent-based calibration standards were used (p. 14).

The ILV performed the ECM method, PTRL Europe Study/Report No. P/B 1355 G, as written, except for the use of a sediment test matrix, the additional test concentration of 0.01 mg/kg (as the ILV LOQ), and insignificant modifications to the analytical parameters and equipment; however, only metamitron was included as a test material (pp. 17, 22; Appendix A, pp. 25-33 of MRID 51173632). Samples were analyzed for metamitron using Agilent 1290 Infinity Binary HPLC coupled with a Sciex TripleQuad 5000 MS equipped with positive TurboIonSpray (ESI) interface, MRM mode. The LC/MS/MS parameters were the same as those of the ECM, except

that the column temperature was specified as 30°C. Expected approximate retention time was 3.3 minutes for metamitron. Two ion pair transitions were monitored (primary and confirmatory, respectively): *m/z* 203→175 and *m/z* 203→104 for metamitron; the monitored ion transitions of the ILV were similar to those of the ECM. Matrix-match calibration standards were used (pp. 18-19). No ILV was submitted to validate PTRL Europe Study/Report No. P/B 1355 G for desaminometamitron.

The Limit of Quantification (LOQ) in soil in the ECM was reported as 0.05 mg/kg for metamitron and desaminometamitron (pp. 9, 19 of MRID 51173631). The Limit of Detection (LOD) in soil for the method was not specifically reported but indicated as 20% of the LOQ (0.01 mg/kg). The LOQ in soil in the ILV was reported as 0.01 mg/kg for metamitron (pp. 13, 17, 20 of MRID 51173632). The LOD in soil was reported as 20% of the LOQ (0.002 mg/kg). Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

II. Recovery Findings

ECM (MRID 51173631): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for analysis of metamitron and desaminometamitron in two soil matrices at the LOQ (0.05 mg/kg) and 10×LOQ (0.50 mg/kg; Tables 1-2, pp. 21-22). Recovery results of the quantitative and confirmatory ion transitions were comparable. The result for the first sample of each set was the mean of two injections. The soil matrices were sandy loam 2.2 soil (LUFA Batch No. F220403; pH 5.6 ± 0.4 (0.01M $CaCl_2$); 75.3 ± 2.0% sand, 16.6 ± 1.4% silt, 8.1 ± 1.2% clay; 2.3 ± 0.2% organic carbon) and sandy loam 2.3 soil (LUFA Batch No. F230403; pH 6.3 ± 0.2 (0.01M $CaCl_2$); 58.2 ± 0.8% sand, 32.3 ± 1.3% silt, 9.6 ± 0.8% clay; 1.2 ± 0.2% organic carbon); matrices were obtained from Lufa Speyer, Germany, and characterized by the supplier (USDA texture classification; Appendix 3, p. 39). The soil texture of sandy loam 2.2 soil was determined by the reviewer using USDA-NRCS technical support tools; the USDA texture of this test soil was reported as loamy sand in the study. The soil texture of sandy loam 2.3 soil was verified by the reviewer using USDA-NRCS technical support tools.

ILV (MRID 51173632): Mean recoveries and RSDs met requirements for analysis of metamitron in one soil matrix and one sediment matrix at the LOQ (0.01 mg/kg), 5×LOQ (0.05 mg/kg), and 50×LOQ (0.50 mg/kg; p. 20). Recovery results of the quantitative and confirmatory ion transitions were comparable. The matrices were loamy sand 2.2 soil (LUFA 2.2; LUFA Batch ID: F2.2 0915; pH 5.84 (0.01M $CaCl_2$); 79.0% sand, 13.7% silt, 7.3% clay, 2.86% organic matter) and sediment [artificial soil: 4.5% (dry weight) sphagnum peat, 20% (dry weight) kaolin clay (kaolinite content ≥30 %), 75.28% (dry weight) quartz sand (grain size ≤2 mm, but >50% of particles in the range of 50-200 µm), and 0.22% lime (calcium carbonate; p. 17; Appendix G, p. 78]. The soil matrix was obtained from Lufa Speyer, Germany, and characterized by the supplier (USDA texture classification). The soil texture was verified by the reviewer using USDA-NRCS technical support tools. The sediment was prepared in the laboratory prior to use via mixing all components with an electric mixer.

The method, PTRL Europe Study/Report No. P/B 1355 G, was validated by the ILV for metamitron in soil at 0.05 mg/kg and 0.50 mg/kg with insignificant modifications to the

analytical parameters and equipment (p. 22 of MRID 51173632). The number of ILV trials was not reported, but the reviewer assumed that the ILV validation occurred with the first trial based on the lack of method modifications and reported communications of method issues. No ILV was submitted to validate PTRL Europe Study/Report No. P/B 1355 G for desaminometamitron. An ECM/ILV pair was not submitted for an analytical method for the determination of metamitron residues in sediment. An ECM/ILV pair was not submitted for an analytical method for the determination of metamitron residues in soil at an LOQ of 0.01 mg/kg.

Table 2. Initial Validation Method Recoveries for Metamitron and Its Metabolite Desaminometamitron in Soil[1,2,3,4]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam* 2.2 Soil						
Quantitation ion transition						
Metamitron	0.05 (LOQ)	5	97-101	99	2	2
	0.50	5	97-99	98	1	1
Desaminometamitron	0.05 (LOQ)	5	103-105	104	1	1
	0.50	5	91-100	96	3	4
Confirmation ion transition						
Metamitron	0.05 (LOQ)	5	96-103	98	3	3
	0.50	5	98-100	99	1	1
Desaminometamitron	0.05 (LOQ)	5	93-104	98	5	5
	0.50	5	89-96	94	3	3
Sandy Loam 2.3 Soil						
Quantitation ion transition						
Metamitron	0.05 (LOQ)	5	98-102	100	2	2
	0.50	5	98-104	101	2	2
Desaminometamitron	0.05 (LOQ)	5	101-105	104	2	2
	0.50	5	100-104	102	2	2
Confirmation ion transition						
Metamitron	0.05 (LOQ)	5	98-105	101	3	3
	0.50	5	97-103	100	2	2
Desaminometamitron	0.05 (LOQ)	5	96-103	99	3	3
	0.50	5	96-103	99	3	3

Data (uncorrected results, pp. 16-17) were obtained from Tables 1-2, pp. 21-22 and Appendix 3, p. 39 of MRID 51173631. The result for the first sample of each set was the mean of two injections. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

1 A sediment matrix was not included as a test matrix in the study.

2 Standard deviations were reviewer-calculated based on recovery values reported in the study since standard deviations were not calculated in the study.

3 In the ECM, the soil matrices were sandy loam 2.2 soil (LUFA Batch No. F220403; pH 5.6 ± 0.4 (0.01M $CaCl_2$); 75.3 ± 2.0% sand, 16.6 ± 1.4% silt, 8.1 ± 1.2% clay; 2.3 ± 0.2% organic carbon) and sandy loam 2.3 soil (LUFA Batch No. F230403; pH 6.3 ± 0.2 (0.01M $CaCl_2$); 58.2 ± 0.8% sand, 32.3 ± 1.3% silt, 9.6 ± 0.8% clay; 1.2 ± 0.2% organic carbon); matrices were obtained from Lufa Speyer, Germany, and characterized by the supplier (USDA texture classification; Appendix 3, p. 39).

4 Two ion pair transitions were monitored (primary and confirmatory, respectively): *m/z* 203→175 and *m/z* 203→104 for metamitron and *m/z* 188→160 and *m/z* 188→104 for desaminometamitron.

* The soil texture was determined by the reviewer using USDA-NRCS technical support tools. The USDA soil texture was reported as loamy sand in Appendix 3, p. 39 of MRID 51173631.

Table 3. Independent Validation Method Recoveries for Metamitron in Soil and Sediment[1,2,3,4]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Loamy Sand 2.2 Soil						
Quantitation ion transition						
Metamitron	0.01 (LOQ)	5	78-93	87	7	8
	0.05	5	80-89	85	3	4
	0.50	5	75-89	85	6	7
Confirmation ion transition						
Metamitron	0.01 (LOQ)	5	79-94	87	7	8
	0.05	5	80-88	84	3	4
	0.50	5	76-89	85	5	6
Sediment (Artificial Soil)						
Quantitation ion transition						
Metamitron	0.01 (LOQ)	5	85-99	95	6	6
	0.05	5	82-88	86	2	3
	0.50	5	85-90	87	2	3
Confirmation ion transition						
Metamitron	0.01 (LOQ)	5	85-101	95	6	7
	0.05	5	83-88	86	2	2
	0.50	5	84-89	86	2	2

Data (uncorrected results, Appendix A, pp. 31-32) were obtained from pp. 17, 20 and Appendix G, p. 78 of MRID 51173632. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

1 Desaminometamitron was not included in the study.

2 Standard deviations were reviewer-calculated based on recovery values reported in the study since standard deviations were not calculated in the study.

3 In the ILV, the matrices were loamy sand 2.2 soil (LUFA 2.2; LUFA Batch ID: F2.2 0915; pH 5.84 (0.01M $CaCl_2$); 79.0% sand, 13.7% silt, 7.3% clay, 2.86% organic matter) and sediment [artificial soil: 4.5% (dry weight) sphagnum peat, 20% (dry weight) kaolin clay (kaolinite content ≥30 %), 75.28% (dry weight) quartz sand (grain size ≤2 mm, but >50% of particles in the range of 50-200 µm), and 0.22% lime (calcium carbonate; p. 17; Appendix G, p. 78]. The soil matrix was obtained from Lufa Speyer, Germany, and characterized by the supplier (USDA texture classification). The sediment was prepared in the laboratory prior to use via mixing all components with an electric mixer.

4 Two ion pair transitions were monitored (primary and confirmatory, respectively): *m/z* 203→175 and *m/z* 203→104 for metamitron; the monitored ion transitions of the ILV were similar to those of the ECM.

III. Method Characteristics

The LOQ in soil in the ECM was reported as 0.05 mg/kg for metamitron and desaminometamitron (pp. 9, 19 of MRID 51173631). The LOD in soil for the method was not specifically reported but indicated as 20% of the LOQ (0.01 mg/kg). No justifications or calculations for the LOQ or LOD were reported in the ECM.

The LOQ in soil in the ILV was reported as 0.01 mg/kg for metamitron (pp. 13, 17, 20 of MRID 51173632). The LOQ was defined as the lowest analyte concentration at which the methodology had been successfully validated. The LOD in soil was reported as 20% of the LOQ (0.002 mg/kg). The ILV report noted that chromatographic peaks at the LOD were equivalent to three times or more than the background noise. No calculations for the LOQ or LOD were reported in the ILV.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

Table 4. Method Characteristics

		Metamitron	Desaminometamitron
Limit of Quantitation (LOQ)*	ECM	0.05 mg/kg	
	ILV	0.01 mg/kg	**Not performed**
Limit of Detection (LOD)	ECM	0.01 mg/kg (20% of the LOQ)	
	ILV	0.005 mg/kg (20% of the LOQ)	**Not performed**
Linearity (calibration curve r and concentration range)	ECM	r = 0.9999 (Q & C)	r = 0.9988 (Q) r = 0.9994 (C)
		0.10-10 ng/mL	
	ILV	r = 0.999 (Soil, Q & C) r = 0.999 (Sediment, Q & C)	**Not performed**
		0.025-10 ng/mL	
Repeatable	ECM[1,2]	Yes for LOQ (0.05 mg/kg) and 10×LOQ (0.50 mg/kg) in two characterized soil matrices (two sandy loam)	
	ILV[3,4,5]	Yes for LOQ (0.01 mg/kg), 5×LOQ (0.05 mg/kg), and 50×LOQ (0.50 mg/kg) in one characterized soil matrix (loamy sand) and one characterized sediment matrix (artificial soil)	**Not performed**
Reproducible[6]	Soil	Yes for 0.05 mg/kg (LLMV)* and 0.50 mg/kg in soil matrices.	**No**; only one set of performance data was submitted.
	Sediment	**No**; only one set of performance data was submitted.[7]	**Not performed**
Specific	ECM	Yes, matrix interferences were <12% of the LOQ (based on peak areas).[8]	Yes, no matrix interferences were observed at the RT of desaminometamitron. Minor baseline noise was noted.
	ILV	Yes, no matrix interferences were observed at the RT of metamitron in soil or sediment. Baseline noise was noted at 0.01 mg/kg fortification.[9]	**Not performed**

Data were obtained from pp. 9, 19 (LOQ/LOD); Tables 1-2, pp. 21-22 (recovery data); p. 18; Figures 1-2, pp. 23-26 (calibration curves); Figures 3-11, pp. 27-35 (chromatograms) of MRID 51173631; pp. 13, 17, 20 (LOQ/LOD); p. 20 (recovery data); p. 19; Appendix C, Figures 2-5, pp. 39-42 (calibration curves); Appendix D, Figures 6-22, pp. 44-60 (chromatograms) of MRID 51173632; DER Excel Attachment. Q = quantitative ion transition; C = confirmatory ion transition.

* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 In the ECM, the soil matrices were sandy loam 2.2 soil (LUFA Batch No. F220403; pH 5.6 ± 0.4 (0.01M $CaCl_2$); 75.3 ± 2.0% sand, 16.6 ± 1.4% silt, 8.1 ± 1.2% clay; 2.3 ± 0.2% organic carbon) and sandy loam 2.3 soil (LUFA Batch No. F230403; pH 6.3 ± 0.2 (0.01M $CaCl_2$); 58.2 ± 0.8% sand, 32.3 ± 1.3% silt, 9.6 ± 0.8% clay; 1.2 ± 0.2% organic carbon); matrices were obtained from Lufa Speyer, Germany, and characterized by the supplier (USDA texture classification; Appendix 3, p. 39 of MRID 51173631). The soil texture of sandy loam 2.2 soil was determined by the reviewer using USDA-NRCS technical support tools; the USDA texture of this test soil was reported as loamy sand in the study. The soil texture of sandy loam 2.3 soil was verified by the reviewer using USDA-NRCS technical support tools.

2 A sediment matrix was not included as a test matrix in the study.

3 In the ILV, the matrices were loamy sand 2.2 soil (LUFA 2.2; LUFA Batch ID: F2.2 0915; pH 5.84 (0.01M $CaCl_2$); 79.0% sand, 13.7% silt, 7.3% clay, 2.86% organic matter) and sediment [artificial soil: 4.5% (dry weight) sphagnum peat, 20% (dry weight) kaolin clay (kaolinite content ≥30 %), 75.28% (dry weight) quartz sand (grain size ≤2 mm, but >50% of particles in the range of 50-200 µm), and 0.22% lime (calcium carbonate; p. 17; Appendix G, p. 78 of MRID 51173632]. The soil matrix was obtained from Lufa Speyer, Germany, and characterized by the supplier (USDA texture classification). The soil texture was verified by the reviewer using USDA-NRCS technical support tools. The sediment was prepared in the laboratory prior to use via mixing all components with an electric mixer.

4 The ILV validated the method, PTRL Europe Study/Report No. P/B 1355 G, for metamitron in soil at 0.05 mg/kg and 0.50 mg/kg with insignificant modifications to the analytical parameters and equipment (p. 22 of MRID 51173632). The number of ILV trials was not reported, but the reviewer assumed that the ILV validation occurred with the first trial based on the lack of method modifications and reported communications of method issues.

5 No ILV was submitted to validate PTRL Europe Study/Report No. P/B 1355 G for desaminometamitron.

6 An ECM/ILV pair was not submitted for an analytical method for the determination of metamitron residues in soil at an LOQ of 0.01 mg/kg (the reported ILV LOQ).

7 An ECM/ILV pair was not submitted for an analytical method for the determination of metamitron residues in sediment; only ILV performance data was reported.

8 Based on Figures 7-8, pp. 31-32, and Figures 10-11, pp. 34-35, of MRID 51173631.

9 The reviewer observed a nearby peak (RT *ca*. 3 min.; *ca*. 20% of analyte peak; reviewer-estimated) in both confirmation chromatograms of the 0.01 mg/kg fortification in soil and sediment; however, this contaminant did not affect the validity of the method since a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data (Appendix D, Figures 15-16, pp. 53-54 of MRID 51173632).

IV. Method Deficiencies and Reviewer's Comments

1. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ (pp. 9, 19 of MRID 51173631; pp. 13, 17, 20 of MRID 51173632). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for metamitron in soil (0.05 mg/kg).

2. No ILV was submitted to validate PTRL Europe Study/Report No. P/B 1355 G for desaminometamitron.

3. An ECM/ILV pair was not submitted for an analytical method for the determination of metamitron residues in soil at an LOQ of 0.01 mg/kg (the reported ILV LOQ); only ILV performance data was reported.

4. An ECM/ILV pair was not submitted for an analytical method for the determination of metamitron residues in sediment; only ILV performance data was reported.

5. The ILV soil matrix [loamy sand 2.2 soil (LUFA 2.2; LUFA Batch ID: F2.2 0915; pH 5.84 (0.01M $CaCl_2$); 79.0% sand, 13.7% silt, 7.3% clay, 2.86% organic matter); Appendix G, p. 78 of MRID 51173632] did not cover the range of soils used in the terrestrial field dissipation (TFD) studies since only one ILV test soil was included; however, the reviewer noted that the ILV test soil provided a difficult sample condition.

OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs.

Even though a certain number of soil matrices is not specified in the OCSPP guidelines, more than one soil/soil matrix would need to be included in an ILV in order to cover the range of soils used in the terrestrial field dissipation studies.

One metamitron TFD study (MRID 51173794) was submitted for screening, but not reviewing, by *PB&A/CSS Joint Venture personnel*. The following soils were included in the metamitron TFD study: loamy sand (0-60 cm: 3% clay, 0.06-0.66% organic matter) and sand (60-90 cm: 1-3% clay, 0.06-0.11% organic matter) [California site]; loamy sand (0-15 cm: 3-5% clay, 2.00-2.30% organic matter) and sand (15-90 cm: 1-3% clay, 0.04-1.30% organic matter) [New York site]; sandy loam (0-45, 60-90 cm: 5-19% clay, 0.17-1.6% organic matter) and sandy clay loam (45-60 cm: 21% clay, 0.25% organic matter) [North Carolina site]; and sand (0-75 cm: 1-2% clay, 0.26-0.65% organic matter) and loamy sand (75-90 cm: 3% clay, 0.26% organic matter) [Washington site; Tables 19-22, pp. 63-66 of MRID 51173794].

6. The number of ILV trials was not reported, but the reviewer assumed that the ILV validation occurred with the first trial based on the lack of method modifications and reported communications of method issues.

7. The ILV reported that no communication with the method developers or others familiar with the method occurred (p. 22 of MRID 51173632).

8. In the ECM, the soil texture of sandy loam 2.2 soil was determined by the reviewer using USDA-NRCS technical support tools; the USDA texture of this test soil was reported as loamy sand in the study (Appendix 3, p. 39 of MRID 51173631).

9. 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. 9, 19 of MRID 51173631; pp. 13, 17, 20 of MRID 51173632). In the ECM, no justifications or calculations for the LOQ or LOD were reported. In the ILV, the LOQ was defined as the lowest analyte concentration at which the methodology had been successfully validated. The ILV report noted that chromatographic peaks at the LOD were equivalent to three times or more than the background noise. No calculations for the LOQ or LOD were reported in the ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

   Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

10. In the ILV, the storage stability of the metamitron stock, fortification, and solvent calibration solutions was determined to be up to 7 days under storage at 1-10°C in the dark (pp. 20-22 of MRID 51173632). The stability of the final extract solutions from the 0.50 mg/kg fortification was determined to be up to 7 days under storage at 1-10°C in the dark.

11. Matrix effects were studied in the ILV and determined to be insignificant (<20%); however, matrix-match calibration standards were used in the ILV (pp. 18-19 of MRID 51173632). Solvent-based calibration standards were used in the ECM (p. 14 of MRID 51173631).

12. No time requirement for the method was reported in the ILV. In the ECM, the time requirement for the method was reported as *ca*. 1.5 calendar days (8 hours) for a batch of 12 samples (p. 19 of MRID 51173631). Sets of 12 soil specimens were processed by one person during one workday (8 hours). This was followed by sets of LC/MS/MS injections (*ca*. 11 minutes per LC/MS run, i.e., *ca*. 4 instrument hours including an appropriate number of standard injections) and evaluation of results (*ca*. 1 hour).

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.

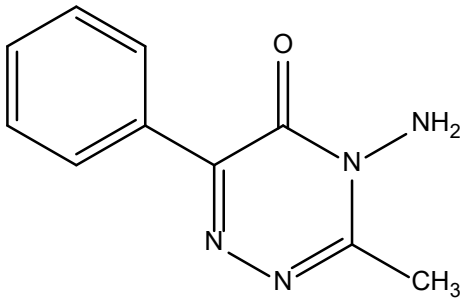
USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 344-347, and Revision 2; 2015 and 2016.

Attachment 1: Chemical Names and Structures

Metamitron

IUPAC Name: 4-Amino-4,5-dihydro-3-methyl-6-phenyl-1,2,4-triazin-5-one
CAS Name: 4-Amino-3-methyl-6-phenyl-1,2,4-triazin-5(4H)-one
CAS Number: 41394-05-2
SMILES String: c1ccccc1C2=NN=C(C)N(N)C2(=O)



Desaminometamitron

IUPAC Name: 3-Methyl-6-phenyl-1,2,4-triazin-5(4H)-one
CAS Name: Not reported
CAS Number: 36993-94-9
SMILES String: O=C1NC(C)=NN=C1C2=CC=CC=C2