

Analytical method for prodiamine and its metabolite 6-amino-imizadole in soil

Reports: ECM: EPA MRID No. 50491101. Smith, K., and T. Bade. 1988. DETERMINATION OF PRODIAMINE AND ITS 6-AMINO-IMIDAZOLE METABOLITE IN SOIL - METHOD # AM-0817. Sandoz and Syngenta Crop Protection, LLC, Method/Study No. AM-0817. Report prepared by Sandoz Crop Protection Corporation, Des Plaines, Illinois, and sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 25 pages. Final report issued April 4, 1988.

ILV: EPA MRID No. 49385101. Smith, R. J. 2014. Prodiamine - Independent Laboratory Validation (ILV) of the Analytical Method - Determination of Prodiamine and its 6-Amino-Imidazole Metabolite in Soil (Method # AM-0817) – Final Report. Syngenta Task No. TK0124258. Report prepared by Smithers Viscient, Wareham Massachusetts, and sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 180 pages. Final report issued March 12, 2014.

Document No.: MRIDs 50491101 & 49385101

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with Sandoz Crop Protection Corporation Good Laboratory Practice (GLP) standards (unspecified; p. 10 of MRID 50491101). Signed and dated GLP and Authenticity statements were provided (p. 10).

ILV: The study was conducted in accordance with USEPA FIFRA (40 CFR 160) GLP standards (p. 3; Appendix 7, p. 180 of MRID 49385101). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4; Appendix 7, p. 180). An authenticity statement was included with the QA statement.

Classification: This analytical method is classified as unacceptable. However, according to the guidance when the field studies conducted as of April 18, 1996 or earlier do not require supporting ECM reports or ILV reports. Therefore, no new submission is required.

PC Code: 110201

**EFED Final
Reviewer:**

James Lin
Environmental Engineer

Signature: 
Date: 08/22/2017

**CDM/CSS-
Dynamac JV
Reviewers:** Lisa Muto, M.S.
Environmental Scientist

Signature: 

Date:

Joan Gaidos, Ph.D.,
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Date:

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

Executive Summary

The analytical method, Syngenta Crop Protection, LLC, Method No. AM-0817, is designed for the quantitative determination of prodiamine and its metabolite 6-amino-imizadole in soil at the LOQ of 0.01 mg/kg using GC/EC. GC/MS analysis was also performed for confirmation of identity, but not quantified. In the ILV, the GC/MS analysis was used for quantitation and designated the LOQ of 0.01 mg/kg. The LOQ is equal to the lowest toxicological level of concern in soil for the two analytes. The ECM validated the method using one uncharacterized soil matrix; the ILV validated the method using a characterized clay loam soil matrix. No terrestrial field dissipation (TFD) studies were cited or provided for review, so it could not be determined if the ILV soil matrix covered the range of soils used in the TFD studies. The ILV validated the ECM method for the analyses of prodiamine and 6-amino-imizadole in one soil matrix in the first trial for 10×LOQ fortifications and in the second trial for LOQ fortifications (the recoveries of the first trial were unacceptable). The ILV made several modifications to update the method for currently available equipment and reagents, as well as altering the oven programs of the GC/EC and GC/MS to ensure that both analytes were fully collected. The reviewer believed that an updated ECM should be submitted since it was out-dated for modern laboratory analysis and the analytical method was not reproducible. Additionally, the primary GC/EC method was not confirmed in the ECM, and the reproducibility of the GC/MS method could not be determined since there was only one set of performance data submitted. ILV performance data (10×LOQ) and linearity were not satisfactory for analysis of prodiamine via GC/MS. In the ECM, samples were not prepared at the LOQ, and chromatographic support was deficient. The LOD was not reported in the ILV and ECM.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide ¹	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Prodiamine	50491101	49385101		Soil ^{2,3}	04/04/1988	Syngenta Crop Protection, LLC	GC/EC	0.01 mg/kg
6-Amino-Imizadole							GC/MS	
Prodiamine	None submitted ¹							
6-Amino-Imizadole								

1 In the ECM MRID 50491101, samples were analyzed by GC/MS for confirmation of identity, but not quantified (p. 9; unpaginated page 1 of MRID 50491101). The GC/MS analysis was considered a confirmatory analysis, and the LOQ was not specified.

2 In the ECM, soil was obtained from Report No. 13, Project 480428 (Table I, p. 11 of MRID 50491101). Soil characterization was not provided. The soil was titled Soil Dissipation (0-10 R2&3) and Soil Dissipation (10-20 R2&3) in chromatograms (Figures 3-11, pp. 14-22).

3 In the ILV, clay loam soil (25% sand 43% silt 32% clay, pH 6.0 in 0.01 M CaCl₂ (1:1), 4.2% organic matter Walkley-Black) was obtained from Syngenta Study No. TK0002309 and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; pp. 14-15; Table 1, p. 28; Appendix 3, p. 119 of MRID 49385101). The study authors reported that a clay loam was used since that was the soil for which the method was designed.

I. Principle of the Method

Samples (20 g) were transferred to 8 oz. screw-cap bottles and fortified, as necessary, with mixed fortification solutions of prodiamine and 6-amino-imizadole (pp. 4-6 of MRID 50491101). After the solvent evaporated, the sample was extracted with 200 mL of methanol via shaking for 30 minutes on a platform shaker. After centrifugation (550 G for 30 minutes), the supernatant was isolated and stored in 8 oz amber bottles in the refrigerator. A 50-mL volume of the supernatant was combined with 250 mL of 5% NaCl solution and 25 mL of dichloromethane in a 500 mL separatory funnel. After shaking for 1 minute, the dichloromethane phase was removed through anhydrous sodium sulfate into a 250 mL round bottom flask. The extraction was repeated twice more with 25 mL dichloromethane. The sodium sulfate was washed three times with 5-10 mL of dichloromethane. The combined extracts and washes were reduced to near dryness on the rotary evaporator using a 40°C water bath. The remaining liquid was reduced under nitrogen, then the residue was reconstituted with 5 mL of 10% ethyl ester in pentane. A silica gel column was prepared with 20 g of 3% water deactivated silica gel and 70 mL of 10% ethyl ether in pentane. The column was washed with 10 mL of 10% ethyl ether in pentane. After 1.0 cm of granular Na₂SO₄ was added to the top of the column, the extracted sample was applied to the column. The sample flask was rinsed twice with 5 mL of 10% ethyl ether in pentane, and the rinsate was applied to the column. The column was washed with 70 mL of 10% ethyl ether in pentane. The prodiamine was eluted with 75 mL of 10% ethyl ether in pentane. The eluate was mixed with 1 mL hexane in a Kuderna Danish concentrator and reduced to approximately 1 mL using a 60°C water bath and Vigreux condenser. The remaining liquid was reduced under nitrogen, then the residue was reconstituted with 5.0 mL of toluene. This sample was covered in foil to protect it from light and stored in the refrigerator prior to analysis by GC/MS. The silica column was washed with 100 mL of 50% ethyl ether in pentane. The 6-amino-imizadole was eluted with 100 mL of ethyl ether. The eluate was reduced to near dryness on the rotary evaporator using a 40°C water bath. The remaining liquid was reduced under nitrogen, then the residue was reconstituted with 5.0 mL of toluene. This sample was covered in foil to protect it from light and stored in the refrigerator prior to analysis by GC/MS.

Samples were analyzed for both analytes using a Hewlett Packard 5880A gas chromatograph (HP-17 column, 0.53 mm x 10 m, 2.0 µm thickness) using a column temperature program (initial 185°C hold for 6 min. to post 225°C hold for 5 min.) and helium carrier gas coupled with an ⁶³Ni electron detector (EC; source temperature 350°C; pp. 6-7 of MRID 50491101). Retention times were 2.95 and 4.56 minutes for prodiamine and 6-amino-imizadole, respectively.

For confirmation of identification, samples were analyzed for both analytes using a Hewlett Packard 5880A gas chromatograph (HP-1 column, 0.2 mm x 25 m, 0.11 µm thickness) using a column temperature program (100°C hold for 0.5 min. to final 190°C at 30°C/min. hold for 5.5 min., post 250°C hold for 5 min.) and helium carrier gas coupled with a 5970 mass selective detector (source temperature 250°C) using electron ionization in Single Ion Monitoring (SIM) mode (p. 7 of MRID 50491101). Retention times were 7.25 and 8.11 minutes for prodiamine and 6-amino-imizadole, respectively. Three ions were monitored as follows (quantitation and confirmation, respectively): *m/z* 321, 279, and 333 for prodiamine and *m/z* 316, 239, and 228 for 6-amino-imizadole (p. 8 of MRID 50491101). Injection volume was 2 µL (splitless).

In the ILV, the ECM was performed as written with several modifications, including: 1) use of a new silica gel (silica gel 60, 70-200 mesh) since the one in the method was no longer available; 2) shaker table speed reported as 150 rpm; 3) reduced water bath temperature to 56°C since it was the maximum; 4) increasing the 100 mL of 50% ethyl ether in pentane to 200 mL during column elution and combining this eluate with the 100% ethyl ether eluate; 5) modifying the GC/EC oven program to initial 185°C hold for 12 min.; and 6) modifying the GC/MS oven program to 100°C hold for 0.5 min. to 190°C at 30°C/min. hold for 5.5 min. to 250°C at 30°C/min. hold for 5 min.; pp. 15, 17-22 of MRID 49385101). The modifications of the GC/EC and GC/MS temperature programs were done to ensure that both analytes were fully collected. For GC/EC, a Hewlett Packard Series 7890A gas chromatograph coupled with a Hewlett Packard Series 7890A micro electron capture detector (μ EC) was used. Retention times were *ca.* 5.6 and 8.7 minutes for prodiamine and 6-amino-imizadole, respectively. For GC/MS, a Hewlett Packard Series 6890 gas chromatograph coupled with a mass selective detector (MSD) Series 5973 was used. Retention times were *ca.* 9.6 and 10.4 minutes for prodiamine and 6-amino-imizadole, respectively. Three ions were monitored as follows (quantitation, confirmation 1 and confirmation 2, respectively): *m/z* 321, 279, and 333 for prodiamine and 316, 228, and 239 for 6-amino-imizadole.

The Limit of Quantification (LOQ) was 0.01 mg/kg for prodiamine and 6-amino-imizadole in soil via GC/EC analysis in the ECM (p. 9 of MRID 50491101; pp. 22, 25 of MRID 49385101). The LOQ was 0.01 mg/kg for prodiamine and 6-amino-imizadole in soil via GC/EC and GC/MS analysis in the ILV. The Limit of Detection (LOD) was not reported in the ECM or ILV.

II. Recovery Findings

ECM (MRID 50491101): Mean recoveries and relative standard deviations (RSDs) from GC/EC analysis were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of prodiamine and its metabolite 6-amino-imizadole in one soil matrix at the fortification level of 0.1 mg/kg (10 \times LOQ; Table I, p. 11; DER Attachment 2). Samples were not prepared at 0.01 mg/kg (LOQ). Several means, standard deviations, relative standard deviations were reviewer-calculated based on all values reported in the study report since these values were either not reported or not reported for *n* = 6. Samples were analyzed by GC/MS for confirmation of identity, but not quantified (p. 9; unpaginated page 1). The soil was obtained from Report No. 13, Project 480428 (Table I, p. 11). Soil characterization was not provided. The soil was titled Soil Dissipation (0-10 R2&3) and Soil Dissipation (10-20 R2&3) in chromatograms (Figures 3-11, pp. 14-22).

ILV (MRID 49385101): Mean recoveries and RSDs from GC/EC analysis were within guideline requirements for analysis of prodiamine and its metabolite 6-amino-imizadole in one soil matrix at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 \times LOQ; p. 23 and Tables 3-10, pp. 30-37). Mean recoveries and RSDs from GC/MS analysis were within guideline requirements for analysis of prodiamine and its metabolite 6-amino-imizadole in one soil matrix at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 \times LOQ), except for all 10 \times LOQ analysis of prodiamine (RSDs 22.7-23.5%). For GC/MS, performance data (recovery results) from primary and confirmatory analyses were comparable. The clay loam soil (25% sand 43% silt 32% clay,

pH 6.0 in 0.01 M CaCl₂ (1:1), 4.2% organic matter Walkley-Black) was obtained from Syngenta Study No. TK0002309 and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; pp. 14-15; Table 1, p. 28; Appendix 3, p. 119). The study authors reported that a clay loam was used since that was the soil for which the method was designed. The ECM method for the analyses of prodiamine and 6-amino-imizadole in one soil matrix was validated in the first trial for 10×LOQ fortifications and in the second trial for LOQ fortifications (the recoveries of the first trial were unacceptable; p. 22). The ILV made several modifications to update the method for currently available equipment and reagents, as well as altering the oven programs of the GC/EC and GC/MS to ensure that both analytes were fully collected (pp. 15, 17-22). The reviewer believed that an updated ECM should be submitted since it was out-dated for modern laboratory analysis and the analytical method was not reproducible.

Table 2. Initial Validation Method Recoveries for Prodiamine and 6-Amino-Imizadole in Soil¹

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil						
GC/EC						
Prodiamine	0.01 (LOQ)	Not reported				
	0.1	6	89-98	94.2	3.5	4 ²
6-Amino-Imizadole	0.01 (LOQ)	Not reported				
	0.1 ²	6	58-81	74	8	11
GC/MS						
Prodiamine	0.01 (LOQ)	Not quantified				
	0.1					
6-Amino-Imizadole	0.01 (LOQ)					
	0.1					

Data (uncorrected recovery results, Tables I, p. 11) were obtained from Tables I, p. 11 of MRID 50491101 and DER Attachment 2.

¹ The soil was obtained from Report No. 13, Project 480428 (Table I, p. 11). Soil characterization was not provided. The soil was titled Soil Dissipation (0-10 R2&3) and Soil Dissipation (10-20 R2&3) in chromatograms (Figures 3-11, pp. 14-22).

² Means, standard deviations, relative standard deviations were reviewer-calculated based on all values reported in the study report since these values were either not reported or not reported for n = 6 (DER Attachment 2).

Table 3. Independent Validation Method Recoveries for Prodiamine and 6-Amino-Imizadole in Soil¹

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Clay Loam Soil						
GC/μEC						
Prodiamine	0.01 (LOQ)	5	71.0-84.4	76.6	5.25	6.85
	0.1	5	49.4-86.7	72.8	14.5	19.9
6-Amino-Imizadole	0.01 (LOQ)	5	70.2-83.5	77.7	5.13	6.60
	0.1	5	79.8-94.3	89.0	5.89	6.61
GC/MS²						
Quantitation ion						
Prodiamine	0.01 (LOQ)	5	59.7-79.1	71.7	7.69	10.7
	0.1	5	53.0-102	81.2	18.4	22.7
6-Amino-Imizadole	0.01 (LOQ)	5	65.2-87.0	79.0	10.3	13.0
	0.1	5	84.1-106	97.8	9.01	9.22
Confirmation ion 1						
Prodiamine	0.01 (LOQ)	5	68.5-87.7	79.6	7.27	9.14
	0.1	5	48.6-96.0	76.8	17.9	23.2
6-Amino-Imizadole	0.01 (LOQ)	5	79.8-91.5	84.5	4.91	5.81
	0.1	5	79.5-111	101	12.9	12.7
Confirmation ion 2						
Prodiamine	0.01 (LOQ)	5	62.2-82.8	73.5	9.18	12.5
	0.1	5	46.0-92.1	75.0	17.6	23.5
6-Amino-Imizadole	0.01 (LOQ)	5	76.8-86.5	80.3	3.82	4.75
	0.1	5	84.8-98.5	90.1	5.66	6.28

Data (uncorrected recovery results, Tables 3-10, pp. 30-37) were obtained from p. 23 and Tables 3-10, pp. 30-37 of MRID 49385101.

- 1 The clay loam soil (25% sand 43% silt 32% clay, pH 6.0 in 0.01 M CaCl₂ (1:1), 4.2% organic matter Walkley-Black) was obtained from Syngenta Study No. TK0002309 and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; pp. 14-15; Table 1, p. 28; Appendix 3, p. 119). The study authors reported that a clay loam was used since that was the soil for which the method was designed.
- 2 For GC/MS, three ions were monitored as follows (quantitation, confirmation 1 and confirmation 2, respectively): *m/z* 321, 279, and 333 for prodiamine and 316, 228, and 239 for 6-amino-imizadole.

III. Method Characteristics

The LOQ was 0.01 mg/kg for prodiamine and 6-amino-imizadole in soil via GC/EC analysis in the ECM (p. 9; unpaginated page 1 of MRID 50491101; pp. 22, 25 of MRID 49385101). In the ECM, the LOQ was termed the limit of detection and determined by the sample dilution (5 g equiv./5 mL) and the lowest standard used (0.00001 μ g/ μ L). The GC/MS analysis was considered a confirmatory analysis, and the LOQ was not specified. The LOQ was 0.01 mg/kg for prodiamine and 6-amino-imizadole in soil via GC/EC and GC/MS analysis in the ILV. No justification or calculation of the LOQ was reported in the ECM or ILV. The LOD was not reported in the ECM or ILV.

Table 4. Method Characteristics

Analyte ¹		Prodiamine		6-Amino-Imizadole		
Limit of Quantitation (LOQ)	ECM	0.01 mg/kg (GC/EC) ¹				
	ILV	0.01 mg/kg (GC/EC and GC/MS)				
Limit of Detection (LOD)	ECM	Not reported				
	ILV					
Linearity (calibration curve r^2 and concentration range)	ECM ²	GC/EC	$r^2 = 0.9982$	$r^2 = 0.9988$		
		GC/MS	Not quantified			
	ILV ³	GC/EC	$r^2 = 0.99955$ (LOQ) $r^2 = 0.99987$ (10×LOQ)	$r^2 = 0.99960$ (LOQ) $r^2 = 0.99984$ (10×LOQ)		
		GC/MS	$r^2 = 0.99489$ (Q, LOQ) $r^2 = 0.99648$ (C1, LOQ) $r^2 = 0.99705$ (C2, LOQ) $r^2 = 0.99768$ (Q, 10×LOQ) $r^2 = 0.99665$ (C1, 10×LOQ) $r^2 = 0.99546$ (C2, 10×LOQ)	$r^2 = 0.99600$ (Q, LOQ) $r^2 = 0.99651$ (C1, LOQ) $r^2 = 0.99712$ (C2, LOQ) $r^2 = 0.99681$ (Q, 10×LOQ) $r^2 = 0.99703$ (C1, 10×LOQ) $r^2 = 0.99565$ (C2, 10×LOQ)		
			Range	0.005-1.00 ng/μL		
Repeatable	ECM ⁴	GC/EC	Yes at 10×LOQ, but No at LOQ - no samples prepared (one uncharacterized soil matrix).			
		GC/MS	Not quantified			
	ILV ^{5,6}	GC/EC	Yes at LOQ and 10×LOQ (one characterized soil matrix).			
		GC/MS	Yes at LOQ, but No at 10×LOQ - RSDs 22.7-23.5% (one characterized soil matrix).	Yes at LOQ and 10×LOQ (one characterized soil matrix).		
Reproducible	GC/EC	Yes at 10×LOQ, but No at LOQ - Only one set of performance data was provided.				
	GC/MS	Could not be determined – Only one set of performance data was provided.				
Specific	ECM	GC/EC	Yes, matrix interferences were <i>ca.</i> 7% of the LOQ (extrapolated based on peak area of 10×LOQ peak). Some nearby contaminants interfered with peak attenuation and integration.	Yes, matrix interferences were <i>ca.</i> 9% of the LOQ (extrapolated based on peak area of 10×LOQ peak).		
			No LOQ chromatograms were provided.			
		GC/MS	No chromatograms were provided.			
	ILV	GC/EC	Yes, no matrix interferences were observed at analyte peak retention time.	Yes, no matrix interferences were observed at analyte peak retention time, but multiple significant contaminants (peak height ≥ LOQ peak height) were observed on the baseline.		
		GC/MS	Yes, no matrix interferences were observed at analyte peak retention time.	Yes, no matrix interferences were observed at analyte peak retention time, but peaks of confirmation ions were poorly resolved.		

Data were obtained from p. 9; unpaginated page 1 (LOQ); Table I, p. 11 (recovery data); Figures 2 and 7, pp. 13, 18 (calibration curves); Figures 3-11, pp. 14-22 (chromatograms) of MRID 50491101; pp. 22, 25 (LOQ); p. 23 and

Tables 3-10, pp. 30-37 (recovery data); Figures 5-6, pp. 45-46; Figures 20-25, pp. 63-68; Appendix 5, pp. 122-137 (calibration curves); Figures 1-37, pp. 41-83 (chromatograms) of MRID 49385101; and DER Attachment 2. Q = Quantitation ion; C1 = Confirmation ion 1; C2 = Confirmation ion 2.

- 1 Samples were analyzed by GC/MS for confirmation of identity, but not quantified (p. 9; unpaginated page 1 of MRID 50491101). The GC/MS analysis was considered a confirmatory analysis, and the LOQ was not specified.
- 2 Correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report (Figures 2 and 7, pp. 13, 18 of MRID 50491101; DER Attachment 2).
- 3 Calibration curves were titled as those of the LOQ Attempt II set. No calibration curves were provided for the 10×LOQ Attempt I set.
- 4 In the ECM, soil was obtained from Report No. 13, Project 480428 (Table I, p. 11 of MRID 50491101). Soil characterization was not provided. The soil was titled Soil Dissipation (0-10 R2&3) and Soil Dissipation (10-20 R2&3) in chromatograms (Figures 3-11, pp. 14-22).
- 5 In the ILV, clay loam soil (25% sand 43% silt 32% clay, pH 6.0 in 0.01 M CaCl_2 (1:1), 4.2% organic matter Walkley-Black) was obtained from Syngenta Study No. TK0002309 and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; pp. 14-15; Table 1, p. 28; Appendix 3, p. 119 of MRID 49385101). The study authors reported that a clay loam was used since that was the soil for which the method was designed.
- 6 The ILV validated the ECM method for the analyses of prodiamine and 6-amino-imizadole in one soil matrix in the first trial for 10×LOQ fortifications and in the second trial for LOQ fortifications (the recoveries of the first trial were unacceptable; p. 22 of MRID 49385101). The ILV made several modifications to update the method for currently available equipment and reagents, as well as altering the oven programs of the GC/EC and GC/MS to ensure that both analytes were fully collected (pp. 15, 17-22). The reviewer believed that an updated ECM should be submitted since it was out-dated for modern laboratory analysis and the analytical method was not reproducible.

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. ECM No Data Confidentiality and Quality Assurance statements were not provided.
2. An updated ECM should be submitted since it was out-dated for modern laboratory analysis and the analytical method was not reproducible. The ILV made several modifications, including: 1) use of a new silica gel (silica gel 60, 70-200 mesh) since the one in the method was no longer available; 2) shaker table speed reported as 150 rpm; 3) reduced water bath temperature to 56°C since it was the maximum; 4) increasing the 100 mL of 50% ethyl ether in pentane to 200 mL during column elution and combining this eluate with the 100% ethyl ether eluate; 5) modifying the GC/EC oven program to initial 185°C hold for 12 min.; and 6) modifying the GC/MS oven program to 100°C hold for 0.5 min. to 190°C at 30°C/min. hold for 5.5 min. to 250°C at 30°C/min. hold for 5 min.; pp. 15, 17-22 of MRID 49385101). The modifications of the GC/EC and GC/MS temperature programs were done to ensure that both analytes were fully collected. All ILV modifications were necessary for the successful validation of the ECM.

Additionally, the ECM did not provide performance data for the confirmatory GC/MS analysis. A confirmation method is generally required when LC/MS or GC/MS are not the primary methods for generating study data. Therefore, not only was the primary GC/EC method not confirmed in the ECM, but the reproducibility of the GC/MS method could not be determined since there was only one set of performance data submitted.

3. ILV performance data from GC/MS analysis was not satisfactory for all 10×LOQ

analysis of prodiamine (RSDs 22.7-23.5%; p. 23 and Tables 3-10, pp. 30-37 of MRID 49385101). OCSPP guideline requirements state that the mean recovery is 70-120% and the RSD is $\leq 20\%$.

4. In the ECM, samples were not prepared at the LOQ. OCSPP guideline requirements state that a minimum of five spiked replicates should be analyzed at each concentration (*i.e.*, minimally, the LOQ and 10 \times LOQ) for each analyte.
5. In the ILV, the linearity was not satisfactory for the quantitation ion analysis in the GC/MS method for prodiamine, $r^2 = 0.99489$ (Figures 20-25, pp. 63-68 of MRID 49385101). Linearity is satisfactory when $r^2 \geq 0.995$.
6. The specificity of the GC/EC and GC/MS methods of the ECM was not supported since no LOQ chromatograms were provided for the GC/EC method and no chromatograms were provided for the GC/MS method.
7. The ECM soil matrix was not characterized; soil was obtained from Report No. 13, Project 480428 (Table I, p. 11). The soil was titled Soil Dissipation (0-10 R2&3) and Soil Dissipation (10-20 R2&3) in chromatograms (Figures 3-11, pp. 14-22 of MRID 50491101).

The ILV soil matrix was characterized, but the study from which it was obtained, Syngenta Study No. TK0002309 was not referenced (pp. 14-15; Table 1, p. 28; Appendix 3, p. 119 of MRID 49385101). Since no terrestrial field dissipation (TFD) studies were provided for review, it could not be determined if the ILV soil matrix covered the range of soils used in the TFD studies.

8. In the ECM and ILV, the LOD was not reported.
9. The specificity of the GC/EC and GC/MS methods for 6-amino-imidazole was not well-supported by the ILV chromatograms since multiple significant contaminants (peak height \geq LOQ peak height) were observed on the baseline (GC/EC) and analyte peaks of confirmation ions were poorly resolved (GC/MS; Figures 1-37, pp. 41-83 of MRID 49385101).
10. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 9 of MRID 50491101; pp. 22, 25 of MRID 49385101). In the ECM, the LOQ was termed the limit of detection and determined by the sample dilution (5 g equiv./5 mL) and the lowest standard used (0.00001 $\mu\text{g}/\mu\text{L}$). the GC/MS analysis was considered a confirmatory analysis, and the LOQ was not specified. The LOQ was 0.01 mg/kg for prodiamine and 6-amino-imidazole in soil via GC/EC and GC/MS analysis in the ILV. No justification or calculation of the LOQ was reported in the ECM or ILV. Detection limits should not be based on arbitrary values.
11. Matrix effects for GC/ μEC were studied in the ILV (pp. 24-25; Tables 11-12, pp. 38-39

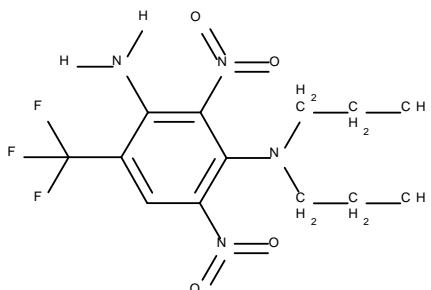
- of MRID 49385101). No significant matrix effects were seen, so solvent standards were used. Matrix effects for GC/MS were not studied.
12. The communications between the ILV study author and the Syngenta Sponsor Monitor (Myra E. Manuli) were included and discussed (p. 24; Appendix 6, pp. 141-179 of MRID 49385101). The communication involved the clarification of the protocol and method, acquisition of analytical standard and control sample, questions regarding preparation of reagents, and pre-validation evaluation and method establishment including calibration curve linearity. A good portion of the communications regarded ILV issues with the specified equipment and reagents in the ECM.
 13. The ECM reported that the oral LD₅₀ of prodiamine in rats is greater than 5000 mg/kg (unpaginated page 1 of MRID 50491101).
 14. It was reported for the ILV that one sample set of 8 samples required *ca.* 2 working days with GC/EC and GC/MS analysis performed overnight (p. 24 of MRID 49385101).

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 {Struct. File; p.12 of ECM}**Prodiamine (SAN745H, CSCA086715)**

IUPAC Name: 5-Dipropylamino- α,α,α -trifluoro-4,6-dinitro-o-toluidine
CAS Name: 2,6-Dinitro-N¹,N¹-dipropyl-6-(trifluoromethyl)-1,3-benzenediamine
CAS Number: 29091-21-2
SMILES String: O=N(=O)c(c(N(CCC)CCC)c(N(=O)=O)c(N)c1C(F)(F)F)c1

**6-Amino-Imizadole (SYN530120, CSCC235132)**

IUPAC Name: 2-Ethyl-4-nitro-3-propyl-6-trifluoromethyl-3H-benzimidazol-5-ylamine
CAS Name: Not reported
CAS Number: Not reported
SMILES String: CCCN1c(nc2c1c(c(c2)C(F)(F)F)N)N(=O)=O)CC

