

Analytical method for hydramethylnon in soil

Reports: ECM: EPA MRID No.: 43345201. Fletcher, J.S. 1994. AMDRO® (CL 217,300): Validation of HPLC Method M 2266 for the Determination of CL 217,300 Residues in Soil. American Cyanamid Company Report No.: RES 93-142. Report prepared, sponsored, and submitted by American Cyanamid Company, Agricultural Research Division, Princeton, New Jersey; 33 pages (including an unpaginated cover page). Final report issued March 15, 1994.

ILV: EPA MRID No. 43345202. Fletcher, J.S. 1994. CL 217,300: Independent Laboratory Validation of HPLC Method M 2266 for the Determination of CL 217,300 Residues in Soil by Huntingdon Analytical Services, Inc. American Cyanamid Company Report No.: RES 94-071. Huntingdon Analytical Services Study No.: A011.066. Report prepared by Huntingdon Analytical Services, Division of Empire Soils Investigations, Inc., Middleport, New York, and sponsored and submitted by American Cyanamid Company, Agricultural Research Division, Princeton, New Jersey; 58 pages (including an unpaginated cover page). Final report issued August 15, 1994.

Document No.: MRIDs 43345201 & 43345202

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160; p. 3; Appendix D, p. 30 of MRID 43345201). Signed and dated No Data Confidentiality, GLP, and Quality Assurance were provided (pp. 2-3; Appendices D-E, pp. 29-32). An authenticity statement was not provided.

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3; Appendix A, p. 10; Appendix C, p. 55 of MRID 43345202). Signed and dated No Data Confidentiality, GLP, Quality Assurance, Authenticity statements were provided (pp. 2-3; Appendix A, pp. 10-12; Appendices C-D, pp. 54-57).

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

An insufficient number of samples were prepared for all soil/fortification level experiments in the ECM and ILV.


PC Code: 118401

EFED Final Reviewer: William Gardner, Ph.D.,
Environmental Scientist

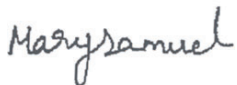
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Date: 2/26/19

William Gardner
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Date: 2019.03.21 10:07:51 -04'00'

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

Signature: 
Date: 01/31/2019

Mary Samuel, M.S.,
Environmental Scientist

Signature: 
Date: 1/31/2019

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, American Cyanamid Method M 2266, is designed for the quantitative determination of hydramethylnon in soil at the LOQ of 10 µg/kg using HPLC/UV. The LOQ is less than the lowest toxicological level of concern in soil for hydramethylnon (0.03 mg ai/kg; USEPA 2012b). The ECM and ILV validated the method using uncharacterized soil matrices. The ECM and ILV matrices appeared to be the same based on soil sample numbers. These matrices were reportedly related to hydramethylnon terrestrial field dissipation studies. ILV successfully validated the ECM in the first trial with only minor HPLC/UV instrument modifications. Hydramethylnon was identified using HPLC/UV; no confirmation method was used. All submitted ECM and ILV data pertaining to precision, repeatability, reproducibility, and specificity was acceptable at the LOQ, 2×LOQ, 5×LOQ, 10×LOQ, and 20×LOQ; however, insufficient number of samples were prepared for all soil/fortification level experiments. ILV linearity was acceptable; ECM linearity could not be validated. The LOD was not reported in the ECM and ILV

All referenced page numbers refer to those which are hand-written at the bottom of the pages in the MRIDs.

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						
Hydramethylnon	43345201 ¹	43345202 ²		Soil	15/03/1994	American Cyanamid Company	HPLC/UV	10 µg/kg

- 1 In ECM, the soil was not characterized or described; however, the method validation was an addendum to CL 217,300 terrestrial field dissipation studies (TFD) with MRIDs 43293101 & 43293102 (p. 1 of MRID 43345201). Based on the sample numbers of the soil, the reviewer believed that multiple (five) soils were used for the internal validation (Table I, p. 7). Additionally, the soil sample numbers were almost identical to those in the ILV, which were identified with study sites.
- 2 In the ILV, soils from Texas, Mississippi, Florida, and Georgia were used, but they were not characterized or described (Appendix A, p. 14 of MRID 43345202). The ILV was an addendum to CL 217,300 terrestrial field dissipation studies (TFD) with MRIDs 43293101 & 43293102 (p. 1).

I. Principle of the Method

Soil samples (50 g) in 500-mL plastic, narrow-mouthed bottles were fortified (0.5 µg/mL fortification solution), as necessary (Appendix A, pp. 11, 13-17 of MRID 43345201). The samples were extracted with 15 mL of deionized water and 250 mL extraction solvent (333 mL methanol diluted to 1 L with methylene chloride) via shaking on a reciprocating shaker for one hour on “high” speed. The solution was separated from the soil by decanting into a 500-mL vacuum flask fitted with a Whatman 934-AH glass fiber filter. The extraction bottle was rinsed with 10 mL methylene chloride; the rinsate was passed through the filter. The extract was transferred to a 250-mL graduated mixing cylinder and allowed to come to room temperature. After the volume was adjusted to 250 mL with methylene chloride, a 100-mL aliquot was transferred to a 250-mL separatory funnel. A liquid-liquid partitioning was achieved using 50 mL of 0.05N HCl and shaking vigorously for 30 seconds. The methylene chloride layer was removed into a 200-mL pear-shaped flask. Another 25 mL of methylene chloride was added to the separatory funnel. After shaking vigorously for 30 seconds, the methylene chloride layer was transferred to the same 200-mL pear-shaped flask. After 1 mL of deionized water was added to the flask, the methylene chloride was completely on a flash evaporator with a water bath at *ca.* 30°C. The method noted that the residues should not be allowed to go to dryness. The residue was dissolved in 10 mL acetonitrile with sonication, then diluted with 10 mL of deionized water. The sample was passed through a Bond-Elut C18/OH solid phase extraction (SPE) cartridge (1000 mg; pre-conditioned with 5 mL of methanol, then 2 x 5 mL of deionized water) using vacuum at a rate of 2-3 drops per seconds. The column was washed with 15 mL of wash solvent (300 mL of deionized water diluted to 1 L with acetone), then the analyte was eluted with 15 mL of elution solvent (2.5 mL of triethylamine diluted to 1 L with acetonitrile) into a 30-mL beaker. The eluate was transferred to a 125-mL separatory funnel, and the beaker was rinsed with 25 mL methylene chloride which was added to the separatory funnel. The eluate was extracted with 10 mL 0.05N HCl and 25 mL deionized water via vigorous shaking for 30 seconds. The organic layer was removed, and the aqueous layer was extracted with another 25 mL methylene chloride via vigorous shaking for 30 seconds. The first organic layer was reduced to about 5 mL via flash evaporator with a water bath at *ca.* 30°C, then the second organic layer was added to the evaporation flask. The extract was reduced to dryness, then reconstituted with 4 mL HPLC dilution solvent (200 mL deionized water diluted to 1 L with acetonitrile). An aliquot of the final extract was transferred to an autosampler vial via a 10-mL Luer-Lok disposable syringe fitted with a Millex-SR 0.5 µm filter, then analyzed by HPLC/UV analysis. The method noted that the sample extracts should not be allowed to sit overnight prior to analysis.

Hydramethylnon was identified and quantified by HPLC/UV using an Applied Biosystems Spectroflow 400 HPLC coupled with an Applied Biosystems Spectroflow 783 UV detector (p. 14 of MRID 43345201). The following conditions were employed: REXCHROM S5-100-ODS analytical column (250 mm x 4.6 mm, particle size not reported; column temperature room temperature *ca.* 24°C) eluted with an isocratic mobile phase of acetonitrile:water:triethylamine (845:150:5, v:v:v) using UV detection (400 nm). The injection volume was 200 µL. Expected retention time was *ca.* 8.7 minutes for hydramethylnon. No confirmation method was reported.

In the ILV, the ECM was performed as written, except for insignificant HPLC/UV instrument modifications (Appendix A, pp. 14, 16 of MRID 43345202). Analytes were identified and

quantified by HPLC/UV using a Waters Model 590 HPLC coupled with a Dionex Variable Wavelength Model VDM-1 UV detector. The HPLC/UV conditions were the same as the ECM. Expected retention time was *ca.* 9.0 minutes for hydramethylnon. No confirmation method was reported.

The Limit of Quantification (LOQ) for hydramethylnon in soil was 10 µg/kg in the ECM and summary of the ILV (p. 4; Appendix A, p. 10 of MRID 43345201; p. 4 of MRID 43345202). The LOQ was not reported in the original ILV report. The Limit of Determination (LOD) of the method was not reported in the ECM or ILV.

II. Recovery Findings

ECM (MRID 43345201): Recoveries were within guideline requirements (between 70% and 120%) for analysis of hydramethylnon at fortification levels of 10 µg/kg (LOQ), 20 µg/kg (2×LOQ), 50 µg/kg (5×LOQ), 100 µg/kg (10×LOQ), and 200 µg/kg (20×LOQ) in five soil matrices (Table I, p. 7). An insufficient number of samples (n = 1) were prepared for all soil/fortification level experiments. Mean recoveries and relative standard deviations (RSDs) could not be determined. Hydramethylnon was identified using HPLC/UV; no confirmation method was used. A confirmation method is usually required when HPLC/MS and/or GC/MS is not used as the primary method to generate study data. Soil was not characterized or described; however, the method validation was an addendum to CL 217,300 terrestrial field dissipation studies (TFD) with MRIDs 43293101 & 43293102 (p. 1). Based on the sample numbers of the soil, the reviewer believed that multiple (five) soils were used for the internal validation (Table I, p. 7). Additionally, the soil sample numbers were almost identical to those in the ILV, which were identified with study sites. Soil AC 6794.91 was identified as obtained from Greenville, Mississippi (Appendix A, p. 19).

ILV (MRID 43345202): Recoveries were within guideline requirements (between 70% and 120%) for analysis of hydramethylnon at fortification levels of 10 µg/kg (LOQ), 20 µg/kg (2×LOQ), 50 µg/kg (5×LOQ), 100 µg/kg (10×LOQ), and 200 µg/kg (20×LOQ) in five soil matrices (Appendix A, p. 17). An insufficient number of samples (n = 1) were prepared for all soil/fortification level experiments. Mean recoveries and relative standard deviations (RSDs) could not be determined. Hydramethylnon was identified using HPLC/UV; no confirmation method was used. A confirmation method is usually required when HPLC/MS and/or GC/MS is not used as the primary method to generate study data. Soils from Texas, Mississippi, Florida, and Georgia were used, but they were not characterized or described (Appendix A, pp. 14-15). The ILV was an addendum to CL 217,300 terrestrial field dissipation studies (TFD) with MRIDs 43293101 & 43293102 (p. 1). The ILV successfully validated the ECM in the first trial with only minor HPLC/UV instrument modifications (p. 4; Appendix A, p. 16).

Table 2. Initial Validation Method Recoveries for Hydramethylnon in Soil

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
HPLC/UV¹						
Soil (7982.0102A)²						
Hydramethylnon	10 (LOQ)	1	99	-- ³	--	--
	50	1	88	--	--	--
Soil (7829.0105C)²						
Hydramethylnon	20	1	78	--	--	--
	200	1	75	--	--	--
Mississippi Soil (AC 6794.91)²						
Hydramethylnon	10 (LOQ)	1	99	--	--	--
	100	1	89	--	--	--
Soil (AC 5418.66B)²						
Hydramethylnon	20	1	90	--	--	--
	200	1	96	--	--	--
Soil (7970.0101C)²						
Hydramethylnon	50	1	85	--	--	--
	100	1	89	--	--	--

Data (uncorrected recovery results, p. 8; Appendix A, pp. 17-18) were obtained from Table I, p. 7 of MRID 43345201.

1 Soil was not characterized or described; however, the method validation was an addendum to CL 217,300 terrestrial field dissipation studies (TFD) with MRIDs 43293101 & 43293102 (p. 1). Based on the sample numbers of the soil, the reviewer believed that multiple (five) soils were used for the internal validation data presented above. Additionally, the soil sample numbers were almost identical to those in the ILV, which were identified with study sites. Soil AC 6794.91 was identified as obtained from Greenville, Mississippi (Appendix A, p. 19).

2 No confirmation method was used.

3 Could not be determined since n = 1.

Table 3. Independent Validation Method Recoveries for Hydramethylnon in Soil

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
HPLC/UV¹						
Texas Soil (7982.0102B)²						
Hydramethylnon	10 (LOQ)	1	89	-- ³	--	--
	50	1	93	--	--	--
Texas Soil (7829.0101A)²						
Hydramethylnon	20	1	110	--	--	--
	200	1	119	--	--	--
Mississippi Soil (AC 6794.91)²						
Hydramethylnon	10 (LOQ)	1	87	--	--	--
	100	1	111	--	--	--
Florida Soil (AC 5418.66B)²						
Hydramethylnon	20	1	102	--	--	--
	200	1	95	--	--	--
Georgia Soil (7970.0101C)²						
Hydramethylnon	50	1	95	--	--	--
	100	1	87	--	--	--

Data (uncorrected recovery results, pp. 15-16) were obtained from Appendix A, p. 17 of MRID 43345202.

1 Soils from Texas, Mississippi, Florida, and Georgia were used, but they were not characterized or described (Appendix A, pp. 14-15). The method validation was an addendum to CL 217,300 terrestrial field dissipation studies (TFD) with MRIDs 43293101 & 43293102 (p. 1).

2 No confirmation method was used.

3 Could not be determined since n = 1.

III. Method Characteristics

The LOQ for hydramethylnon in soil was 10 µg/kg in the ECM and summary of the ILV (p. 4; Appendix A, p. 10 of MRID 43345201; p. 4 of MRID 43345202). The LOQ was not reported in the original ILV report. No calculations or justifications for the LOQ were provided in the ECM and ILV. The LOD of the method was not reported in the ECM or ILV.

Table 4. Method Characteristics

Analyte	Hydramethylnon	
Limit of Quantitation (LOQ)	10 µg/kg	
Limit of Detection (LOD)	Not reported	
Linearity (calibration curve r ² and concentration range)	ECM	Not reported¹
	ILV	r ² = 0.99998 ²
	Range	0.025-0.1 µg/mL
Repeatable	ECM ³ ILV ^{4,5}	Yes at LOQ, 2×LOQ, 5×LOQ, 10×LOQ, and 20×LOQ; however, n = 1 . (five uncharacterized soils).
Reproducible	Yes at LOQ, 2×LOQ, 5×LOQ, 10×LOQ, and 20×LOQ; however, n = 1 .	
Specificity	No confirmation method was performed.	
	ECM	Yes, no matrix interferences were observed; however, a significant

	ILV	contaminant was observed in the control and sample chromatogram.
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Data were obtained from p. 4; Appendix A, p. 10 (LOQ/LOD); Table I, p. 7 (recovery data); Appendix A, pp. 13-14 (calibration data); Appendix A, Figure 1, p. 19 (chromatograms) of MRID 43345201; p. 4 (LOQ/LOD); Appendix A, p. 17 (recovery data); Appendix A, Figures 1-6, pp. 20-25 (chromatograms); Appendix A, Figure 7, p. 26 (calibration curves); of MRID 43345202.

- 1 Linearity of instrument response was checked; however, calculation of hydramethylnon recovery was based on peak height of sample and peak of height of a working standard, not based on the linear regression equation (p. 8; Appendix A, pp. 17-18 of MRID 43345201).
- 2 Based on hand-written note below calibration curve; it could not be determined if this value was r or r^2 (Appendix A, p. 26 of MRID 43345202).
- 3 In ECM, the soil was not characterized or described; however, the method validation was an addendum to CL 217,300 terrestrial field dissipation studies (TFD) with MRIDs 43293101 & 43293102 (p. 1 of MRID 43345201). Based on the sample numbers of the soil, the reviewer believed that multiple soils were used for the internal validation data presented above (Table I, p. 7). Additionally, the soil sample numbers were almost identical to those in the ILV, which were identified with study sites.
- 4 In the ILV, soils from Texas, Mississippi, Florida, and Georgia were used, but they were not characterized or described (Appendix A, pp. 14-15 of MRID 43345202). The ILV was an addendum to CL 217,300 terrestrial field dissipation studies (TFD) with MRIDs 43293101 & 43293102 (p. 1).
- 5 The ILV successfully validated the ECM in the first trial with only minor HPLC/UV instrument modifications (p. 4; Appendix A, p. 16 of MRID 43345202).

IV. Method Deficiencies and Reviewer's Comments

1. An insufficient number of samples ($n = 1$) were prepared for all soil/fortification level experiments in the ECM and ILV (Table I, p. 7 of MRID 43345201; Appendix A, p. 17 of MRID 43345202). Mean recoveries and relative standard deviations (RSDs) could not be determined. OCSPP Guideline 850.6100 states that a minimally complete sample set includes a reagent blank, two matrix blanks, five samples spiked at the LOQ, and five samples spiked at $10\times$ LOQ for each matrix.
2. The ECM linearity could not be validated since was not reported; no calibration curve was provided. The ECM linearity of instrument response was reportedly checked; however, calculation of hydramethylnon recovery was based on peak height of sample and peak of height of a working standard, not based on the linear regression equation (p. 8; Appendix A, pp. 17-18 of MRID 43345201).
3. ECM and ILV soil matrices were not characterized (p. 1; Table I, p. 7 of MRID 43345201; p. 1; Appendix A, pp. 14-15 of MRID 43345202). The ECM and ILV matrices appeared to be the same based on soil sample numbers. These matrices were reportedly related to hydramethylnon terrestrial field dissipation studies.
4. In the ECM and ILV, hydramethylnon was identified using HPLC/UV; no confirmation method was used. A confirmation method is usually required when HPLC/MS and/or GC/MS is not used as the primary method to generate study data.
5. No calculations for the LOQ were provided in the ECM and ILV. The LOD was not reported in the ECM and ILV. The reported limit of quantitation (LOQ) was determined

as the lowest level of method validation (LLMV). Further work could have been done to explore the actual LOQ.

6. The ILV specifically reported that no communication between the ILV and American Cyanamid Company occurred during validation, and only notification of the successful trial was communicated to the American Cyanamid Study Director for the ILV (J. Shahn Fletcher) who was also the ECM study author (Appendix C, p. 28 of MRID 43345201; Appendix A, p. 17; Appendix B, p. 53 of MRID 43345202). The reviewer noted that the ILV MRID had the same author as the ECM MRID (J. Shahn Fletcher of American Cyanamid Company); however, the ILV MRID contained the ILV study report (authored by Torren A. Bixler of Huntingdon Analytical Services) and the ILV summary report (authored by J. Shahn Fletcher of American Cyanamid Company; p. 1 of 43345201; p. 1; Appendix A, p. 8 of MRID 43345202). Therefore, the reviewer concluded that the ECM and ILV were conducted by distinct laboratories and laboratory personnel without collusion.
7. The time required to complete the method was not reported in the ECM or ILV; however, the ECM noted that method noted that the sample extracts should not be allowed to sit overnight prior to analysis (Appendix A, p. 15 of MRID 43345201).

V. References

- U.S. Environmental Protection Agency. 2012a. 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.
- U.S. Environmental Protection Agency. 2012b. Registration Review: Preliminary Problem Formulation for Environmental Fate, Ecological Risk, Endangered Species, and Drinking Water Exposure Assessments for Hydramethylnon. DP 403712.
- 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**Hydramethylnon**

IUPAC Name: 5,5-Dimethylperhydropyrimidin-2-one 4-(trifluoromethylstyryl)cinnamylidenehydrazone
CAS Name: Tetrahydro-5,5-dimethyl-2(1H)-pyrimidinone [3-[4-(trifluoromethyl)phenyl]-1-[2-[4-(trifluoromethyl)phenyl]ethenyl]-2-propen-1-ylidene]hydrazone
CAS Number: 67485-29-4
SMILES String: N1CC(C)(C)CNC1=NN=C(C=Cc2ccc(C(F)(F)F)cc2)C=Cc3ccc(C(F)(F)F)cc3

