

Test Material: Carbaryl

MRID: 49033301

Title: Carbaryl - Independent Laboratory Validation of Residue Method (CA-001-S12-01) for the Determination of Carbaryl and 1-Naphthol in Soil by LC-MS/MS – Final Report

EPA PC Code: 056801

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto

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Date: 3/28/16

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Date: 3/28/16

Analytical methods for carbaryl and its metabolite, 1-naphthol, in soil

Reports: ECM: EPA MRID No. 49033301 (Appendix 1, pp. 47-72). Netzband, D. 2014. Bayer Method CC-001-S14-01: An Analytical Method for the Determination of Residues of Carbaryl and 1-Naphthol in Soil and Sediment by LC/MS/MS. Bayer Method No.: CA-001-S12-01. Report prepared by Bayer CropScience, Environmental Safety, Research Triangle Park, North Carolina; sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 26 pages. Final report issued October 23, 2012.

ILV: EPA MRID No. 49033301. Perez, R., D. Patel, and J.L. Miska. 2012. Carbaryl - Independent Laboratory Validation of Residue Method (CA-001-S12-01) for the Determination of Carbaryl and 1-Naphthol in Soil by LC-MS/MS – Final Report. ADPEN Study No.: 2K12-1025-982. Bayer Study ID: RACAL001. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida; sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 98 pages (including the ECM). Final report issued December 13, 2012.

Document No.: MRID 49033301

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Statements: ECM: The study was not conducted in accordance with U.S. EPA GLP (CFR Title 40, Part 160) since it was not an experimental study (Appendix 1, p. 49). Signed and dated No Data Confidentiality and GLP statements were provided (Appendix 1, pp. 48-49). A Quality Assurance and Authenticity statements were not included.

ILV: The study was conducted in accordance with U.S. EPA GLP (CFR Title 40, Part 160; p. 3). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). An authenticity statement was not included.

Classification: This analytical method is classified as supplemental. Only one set of performance data was submitted to validate the method; the ECM study report did not contain any performance data. The determination of the LOQ and LOD was not based on scientifically acceptable procedures; the LOD was not reported in the ILV. ILV recoveries were corrected for residues found in the controls (<10% of the LOQ).

PC Code: 056801

Reviewer: James N. Carleton, Ph.D., Senior Scientist, U.S. EPA **Date:** 10/26/16

All page numbers refer to those in the uppermost right-hand corner of the MRID.

Executive Summary

The analytical method, Bayer Method CA-001-S12-01, is designed for the quantitative determination of carbaryl and its metabolite, 1-naphthol, in soil and sediment at the LOQ of 10 ng/g using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in soil or sediment suggested by a preliminary review of the available open literature. Only one set of performance data was submitted to validate the method; the ECM study report did not contain any performance data. The method was validated with the first trial with insignificant modifications. Sandy loam soil was used in the ILV; no sediment was used. ILV recoveries were corrected for residues found in the controls (<10% of the LOQ). The LOD was not reported in the ILV.

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						
Carbaryl	49033301 ¹	49033301 ²		Soil & Sediment	23/10/2012	Bayer CropScience	LC/MS/MS	10 ng/g
1-Naphthol								

1 The ECM (Appendix 1, pp. 47-72 of MRID 49033301) did not include any recovery data – method report only.

2 Sandy loam soil (57% sand, 33% silt, 10% clay; 3.2% organic matter) was used (p. 10; Appendix 4, p. 89).

I. Principle of the Method

Soil/sediment samples (5.0 ± 0.1 g) were fortified in disposable 50-mL conical tubes and extracted with 20 mL of acetone:water (1:1, v:v) with 0.2% formic acid (Appendix 1, p. 56; Appendix 1, Appendix 4, p. 71). After shaking for 10 minutes and centrifugation (*ca.* 2500 rpm for *ca.* 10 minutes), the aqueous layer was removed. For the second extraction, *ca.* 20 mL of acetonitrile:water (1:1, v:v) was added. After shaking for *ca.* 1 minute and centrifugation (*ca.* 2500 rpm for *ca.* 10 minutes), the aqueous layer was removed. The combined extracts were mixed with 40 μ L of the 10 μ g/mL isotopic internal standard solution then diluted to *ca.* 40 mL with acetone:water (1:1, v:v) and mixed. A *ca.* 5 mL aliquot of the extract was reduced to *ca.* 2.5 mL via turbo-evaporation at 40°C. The residue was mixed with *ca.* 2.5 mL of 20% acetonitrile in water. An aliquot was filtered (0.45 μ m Nylon filter) then transferred to a LC vial prior to analysis via LC/MS/MS.

Samples (water/soil matrices) were analyzed for carbaryl and 1-naphthol using Two Shimadzu LC-20AD XR (High Pressure Mixer) coupled with an ABSciex 5500 MS with Turbo Ion Spray (ESI; Appendix 1, Table 1, p. 59; Appendix 1, Appendix 1, pp. 61-62). The LC/MS/MS system parameters included a Phenomenex® Luna C18(2) (50 mm x 2 mm i.d., 2.5 μ m) column with a gradient mobile phase of (A) methanol:water (10:90, v:v) with 10 mM ammonium formate and 120 L per 1000 mL formic acid and (B) methanol:water (90:10, v:v) with 10 mM ammonium formate and 120 L per 1000 mL formic acid (time A:B; 0.02 min. 90:10, 6.50 min. 30:70, 6.60-9.00 min. 10:90, 9.01 min. 90:10) and injection volume 20 μ L. Analyte ions were monitored using MS in positive ESI mode. Two sets of MRM transitions were monitored for each analyte and (quantitation and confirmation transitions, respectively): 202→145 m/z and 202→127 m/z for carbaryl; and 145→127 m/z and 145→91 m/z for 1-naphthol. The isotopic internal standards were monitored with one transition: 209→152 m/z for d_1 -carbaryl; and 152→133 m/z for d_1 -1-naphthol. Retention times were 4.71-4.73 minutes for carbaryl and 1-naphthol and their internal standards (Appendix 1, Table 1, p. 59; Appendix 1, Appendix 3, p. 68).

In the ILV, the sample processing procedure for soil was the same as in the ECM, except an amber HPLC vial was specified for analysis (pp. 11-12; Table 1, p. 16). LC/MS/MS analysis was performed using an Agilent 1290 Infinity Series LC coupled with an Agilent 6490 Triple Quad mass spectrometer. The analytical column [Zorbax Eclipse (1.83 μ m, 50 mm x 2.10 mm)] and injection volume (10 μ L) were notably different from that in the ECM. Only the quantitation ion MRM transitions were monitored; these were the same as the ECM. Retention times were 4.660-4.664 min. for carbaryl and 1-naphthol (Figures 19-20, pp. 39-40).

In the ECM and ILV, the Limit of Quantification (LOQ) value for carbaryl and 1-naphthol was 10 ng/g in soil and sediment for both analytes (pp. 8, 13; Appendix 1, Table 2, p. 60). The Limit of Detection (LOD) was reported as 3.0 ng/g for both analytes in the ECM, but was not reported in the ILV.

II. Recovery Findings

ECM (MRID 49033301): No recovery data were included with the ECM report (Appendix 1, pp. 47-72).

ILV (MRID 49033301): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of carbaryl and 1-naphthol in soil at the LOQ (10 ng/g) and 10 \times LOQ (100 ng/g; p. 8; Tables 3-4, pp. 18-19). Only results for the quantification ion were reported. Recovery results were corrected for residues found in the controls (Table 3-4, pp. 18-19; Appendix 5, p. 90). The method was validated with the first trial with insignificant modifications (p. 13). The soil matrix was fully characterized by Agvise Laboratories, Northwood, North Dakota (USDA textural classification; Sample ID: 120913001-001; p. 10; Appendix 4, p. 89). Sandy loam (57% sand, 33% silt, 10% clay; 3.2% organic matter) was used.

Table 2. Initial Validation Method Recoveries for Carbaryl and 1-Naphthol in Soil

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil						
Carbaryl	No recovery data was included in ECM report					
1-Naphthol						

Table 3. Independent Validation Method Recoveries for Carbaryl and 1-Naphthol in Soil¹

Analyte	Fortification Level (ppb) ²	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam Soil						
Quantification ion						
Carbaryl	10 (LOQ)	5	90-96	93	2.3	2.4
	100	5	100-105	102	2.3	2.2
1-Naphthol	10 (LOQ)	5	97-101	99	1.6	1.6
	100	5	103-107	105	1.4	1.3
Confirmation ion						
Carbaryl	10 (LOQ)	5	Not evaluated			
	100	5				
1-Naphthol	10 (LOQ)	5				
	100	5				

Data were obtained from p. 8; Tables 3-4, pp. 18-19 of the study report.

¹ The soil matrix was fully characterized by Agvise Laboratories, Northwood, North Dakota (USDA textural classification; Sample ID: 120913001-001; p. 10; Appendix 4, p. 89). Sandy loam soil (57% sand, 33% silt, 10% clay; 3.2% organic matter) was used.

III. Method Characteristics

In the ECM and ILV, the Limit of Quantification (LOQ) value for carbaryl and 1-naphthol was 10 ng/g in soil and sediment for both analytes (pp. 8, 13; Appendix 1, Table 2, p. 60). The Limit of Detection (LOD) was reported as 3.0 ng/g for both analytes in the ECM, but was not reported in the ILV. No justification or calculations were provided to support the LOQ or LOD.

Table 4. Method Characteristics

		Carbaryl	1-Naphthol
Limit of Quantitation (LOQ)		10 ng/g	
Limit of Detection (LOD)		3.0 ng/g	
Linearity (calibration curve r^2 and concentration range)	ECM	Not reported ¹	
	ILV	$r^2 = 0.99999604$ (2.5-1000 pg)	$r^2 = 0.99999815$ (2.5-1000 pg)
Repeatable		Yes ²	
Reproducible		Could not be determined ¹	
Specific		Yes, matrix interferences were <10% of the LOQ. ²	

Data were obtained from pp. 8; Tables 3-4, pp. 18-19; Figures 15-24, pp. 35-44 of the study report.

1 The ECM (Appendix 1, pp. 47-72 of MRID 49033301) did not include any recovery data – method report only.

2 Only the quantification ion data and chromatograms were reported in the ILV. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

IV. Method Deficiencies and Reviewer's Comments

1. The ECM report, which was provided in Appendix 2, pp. 47-72 of MRID 49033301, did not include sufficient performance data to provide support for the method. It appeared to be a method report only. LOQ and LOD values were reported; however, no recovery or linearity data were provided. OCSPP guidelines recommend that two sets of performance data be submitted, one for the initial or other internal validation and one for the ILV.
2. In the ECM, representative chromatograms were only provided for the analytes (MS spectra), the 10.0 ng/mL calibration standard, the 100.0 ng/mL spiking solution, and the control solution of each analyte (Appendix 1, Appendix 3, pp. 65-70 of MRID 49033301). These chromatograms may have been taken from a published study since the ECM referenced Analytical Method R013916 (1985) for “additional recovery data” (Appendix 1, pp. 53, 58). OCSPP guidelines recommend that representative chromatograms be provided for reagent blanks, matrix blanks, standard curves, and spiked samples at the LOQ and 10×LOQ for all analytes in each matrix

In the ILV, representative chromatograms of reagent blanks, matrix blanks, and fortifications at the LOQ and 10×LOQ were included for each analyte/ion/matrix (Figures 15-24, pp. 35-44 of MRID 49033301).

3. The estimations of the LOQ and LOD for soil and sediment in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. No justification or calculations were provided to support the LOQ or LOD. OCSPP guidelines recommend that there are explanations of the LOD and LOQ were calculated.

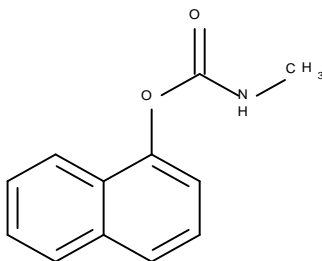
The LOD was not reported in the ILV for either analyte.

Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in soil and sediment were not reported. An LOQ above toxicological level of concern results in an unacceptable method classification.

4. The ILV recoveries were corrected for residue found in the control samples (Table 3-4, pp. 18-19; Appendix 5, p. 90). Residues in the controls were reported as 0.7737-0.8707 ppb in the carbaryl controls and 0.6078-0.6166 ppb in the 1-naphthol controls.
5. In the ILV, the one laboratory equipment modification and analytical instrumental and parameter modifications were considered insignificant modifications of the ECM.
6. Although the ECM provided chromatograms of the standards for the quantification and confirmation ion, only the quantification ion chromatograms and data were provided in the ILV. A confirmatory method is not usually required when LC/MS or GC/MS is the primary method.
7. Communication between the ECM personnel and ILV study author was reported (p. 13; Appendix 7, p. 98). This communication was limited to notification of trial success.
8. The time requirement for the sample sets of the ILV experiment was reported as one working day for a set of 13 soil samples, not including LC/MS/MS analysis which was performed overnight (p. 13).

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**Carbaryl****IUPAC name** 1-Naphthyl methylcarbamate**CAS Name** 1-Naphthalenyl N-methylcarbamate**CAS #** 63-25-2**SMILES** O=C(Oc1c2ccccc2c1)NC**1-Naphthol****IUPAC name:** 1-Naphthalenol**CAS Name** 1-Naphthalenol**CAS #** Not reported**SMILES** 90-15-3Oc1c2ccccc2c1