

**Test Material:** Nitrapyrin

**MRID:** 49567304

**Title:** Validation Report for Methods GRM 07.13 – Determination of Residues of Nitrapyrin and its 2-Chloro-6-(dichloromethyl)pyridine Metabolite in Soil by Gas Chromatography with Positive-Ion Electron-Impact Ionization Mass Spectrometry Detection and GRM 07.14 - Determination of Residues of 6-Chloropyridine-2-carboxylic Acid in Soil by Gas Chromatography with Negative-Ion Chemical Ionization Mass Spectrometry Detection

**MRID:** 49567303

**Title:** Independent Laboratory Validation of two analytical methods for the determination of Nitrapyrin and its metabolites, 2-chloro-6-(dichloromethyl)pyridine and 6-chloropyridine-2-carboxylic acid in Soil

**EPA PC Code:** 069203

**OCSPP Guideline:** 850.6100

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**Date:** 8/4/15

## **Analytical method for nitrapyrin and its transformation products, 2-chloro-6-(dichloromethyl)pyridine and 6-chloropyridine-2-carboxylic acid, in soil**

- Reports:** ECM: EPA MRID No.: 49567304. Olberding, E.L. and G.E. Dial, Jr. 2015. Validation Report for Methods GRM 07.13 – Determination of Residues of Nitrapyrin and its 2-Chloro-6-(dichloromethyl)pyridine Metabolite in Soil by Gas Chromatography with Positive-Ion Electron-Impact Ionization Mass Spectrometry Detection and GRM 07.14 - Determination of Residues of 6-Chloropyridine-2-carboxylic Acid in Soil by Gas Chromatography with Negative-Ion Chemical Ionization Mass Spectrometry Detection. Laboratory Study ID: 071125. Report prepared, sponsored and submitted by Regulatory Sciences and Regulatory Affairs, Dow AgroSciences LLC, Indianapolis, Indiana; 121 pages. Final report issued January 6, 2015.  
ILV: EPA MRID No. 49567303. Devine, T. 2015. Independent Laboratory Validation of two analytical methods for the determination of Nitrapyrin and its metabolites, 2-chloro-6-(dichloromethyl)pyridine and 6-chloropyridine-2-carboxylic acid in Soil. DAS PTR Study Reference No.: 10002280-001-40402-001. DAS Study No.: 140733. CEMAS Study No.: CEMS-6868 and Report No.: CEMR-6868. Report prepared by CEM Analytical Services Ltd (CEMAS), Berkshire, United Kingdom, sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 127 pages. Final report issued January 19, 2015.
- Document No.:** MRIDs 49567304 & 49567303
- Guideline:** 850.6100
- Statements:** ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards, with the exception of the lack of signature and date on one sample weight sheet (p. 3 of MRID 49567304). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).  
ILV: The study was conducted in accordance with USEPA and OECD GLP standards (1998; p. 3 of MRID 49567303). The test facility was compliant with UK Department of Health GLP (Appendix 3, p. 127). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).
- Classification:** This analytical method is classified as **SUPPLEMENTAL**. For all analytes in the ILV, performance data to validate the methods at 10×LOQ were not reported. ILV representative chromatograms were indecipherable, and the specificity of the methods could not be determined for 2-Cl-6-DCMP and 6-CPA based on matrix control recovery data. In the ECM, the number of samples was insufficient for all analyses in both methods. The ECM soil matrices were only partially characterized; it could not be determined if the ILV was provided with the most difficult soil type with which to validate the methods. In the ILV, the mean recovery and RSD were unsatisfactory for the secondary confirmation ion results of 2-Cl-6-DCMP. Sample recoveries

were corrected in the ECM. In the ECM, no chromatograms were provided for two of the four soil matrices or any 10×LOQ samples.

**PC Code:** 069203

**Reviewer:** He Zhong, Ph.D.  
Biologist, EFED

**Signature:**

**Date:** 9/29/2015

**All page numbers refer to those listed in the upper right-hand corner of the MRIDs.**

## Executive Summary

The analytical method, GRM 07.13, is designed for the quantitative determination of nitrapyrin and its transformation product 2-chloro-6-(dichloromethyl)pyridine (2-Cl-6-DCMP) in soil at the LOQ of 0.010 µg/g using GC/MS in positive-ion electron-impact ionization mode. The analytical method, GRM 07.14, is designed for the quantitative determination of the nitrapyrin transformation product 6-chloropyridine-2-carboxylic acid (6-CPA) in soil at the LOQ of 0.010 µg/g using GC/MS in negative-ion chemical ionization mode. The LOQ is less than 5 µg/g-soil resulting in a 10% reduction in plant growth, the lowest toxicological level of concern in soil for all analytes. Although it was not specifically reported, the reviewer assumed that Methods GRM 07.13 and 07.14 were validated in the first trial with only one minor modification; however, samples were not fortified at 10×LOQ, only the LOQ and 1000×LOQ. The soil matrices of the ECM were only partially characterized; USDA classifications were reported, but particle percentages were not reported. Therefore, it could not be determined if the ILV was provided with the most difficult soil type with which to validate the methods. The ILV validated the methods with loam soil (26.2% clay). Additionally, all of the chromatograms provided in the ILV were indecipherable.

**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						
Nitrapyrin	49567304	49567303		Soil <sup>2</sup>	06/01/2015	Dow AgroSciences LLC	GC/MS <sup>4</sup>	0.010 µg/g
2-Cl-6- DCMP <sup>1</sup>								
6-CPA <sup>1</sup>								

1 2-Cl-6-DCMP = 2-chloro-6-(dichloromethyl)pyridine; 6-CPA = 6-chloropyridine-2-carboxylic acid

2 For ECM Method GRM 07.13, loam soil (00-06"; 1.6% organic carbon) and silty clay loam (00-06"; 2.3% organic carbon) were used; the soil particle distribution was not reported. The soil matrix of the ILV was loam (26.2% clay, 2.21% organic carbon).

3 For ECM Method GRM 07.14, sandy loam soil (06-12"; 1.7% organic carbon) and silty clay loam (06-12"; 1.7% organic carbon) were used; the soil particle distribution was not reported. The soil matrix of the ILV was loam (26.2% clay, 2.21% organic carbon).

4 Method GRM 07.13 with the use of positive-ion electron-impact ionization mass spectrometry detection.

5 Method GRM 07.14 with the use of negative-ion chemical ionization mass spectrometry detection.

## I. Principle of the Method

### ECM Method GRM 07.13

Samples (5.0 g) of soil were fortified, as necessary, then extracted twice with acetone:0.1N hydrochloric acid (90:10, v:v; 25 mL then 14 mL) via sonication for *ca.* 10 minutes then shaking via reciprocating shaker for 30 minutes at *ca.* 180 excursions/minute (pp. 14-15; Appendix A, pp. 34-35, 39-40 of MRID 49567304). Soil and extract were separated by centrifugation (5 minutes at 2000 rpm) and decanting. The volume of the combined extracts was adjusted to 40 mL using acetone:0.1N hydrochloric acid (90:10, v:v). A 4.0-mL aliquot was mixed with 25 mL of 0.1 N hydrochloric acid and 5 g of sodium chloride then extracted with 1.0 mL of cyclohexane containing 100 ng/mL  $^{13}\text{C}_2^{15}\text{N}$ -2,3,5,6-tetrachloropyridine ( $^{13}\text{C}_2^{15}\text{N}$ -2,3,5,6-TCP) as an internal standard. After shaking via reciprocating shaker for at least 10 minutes at *ca.* 180 excursions/minute, the sample was centrifuged to separate the layers. An aliquot of the cyclohexane (top) layer was removed for GC/MS analysis.

Samples were analyzed for nitrapyrin and 2-Cl-6-DCMP by gas chromatography using an Agilent Model 6890A GC with an Agilent Model 5973N MS in positive-ion electron-impact ionization mode (p. 15; Appendix A, pp. 36-38 of MRID 49567304). The following GC conditions were used: Durabond-5MS column (30 m x 0.25 mm, 1.0- $\mu\text{m}$  film thickness), oven temperature program [70°C (initial), 20°C/min. to 320°C], injector temperature 30°C, detector temperature 230°C, and injection volume 1  $\mu\text{L}$ . Three ions were monitored for the analytes (quantitative, primary confirmation and secondary confirmation, respectively): *m/z* 194, 196 and 198 for nitrapyrin and *m/z* 160, 162 and 195 for 2-Cl-6-DCMP. Only the quantitation ion, *m/z* 220, was monitored for the internal standard,  $^{13}\text{C}_2^{15}\text{N}$ -2,3,5,6-TCP. Retention times were 9.51 min. for nitrapyrin, 8.61 min for 2-Cl-6-DCMP and 8.87 min. for the internal standard (Appendix A, Figure 5, p. 63; Appendix A, Figure 17, p. 75).

The study authors included some notes regarding the method including 1) substitutions of equivalent equipment, glassware, etc. were acceptable and 2) modifications of GC/EI-MS operating conditions for optimization were acceptable (Appendix A, p. 49 of MRID 49567304). Additionally, the study authors explained the use of the gas purifier (to purify the helium prior to entry of GC), liquid nitrogen (to cool the injection port of the GC, if necessary) and peanut oil (to decrease chromatographic peak tailing; internal standard preparation).

### ECM Method GRM 07.14

Samples (5.0 g) of soil were fortified, as necessary, then extracted twice with 0.5N potassium hydroxide/10% potassium chloride solution (25 mL then 14 mL; p. 15; Appendix B, p. 90-91, 95-97 of MRID 49567304). For each extraction, the sample was heated in a water bath (70°C) for 30 minutes, cooled to room temperature, then agitated via reciprocating shaker for 15 minutes at *ca.* 180 excursions/minute. Soil and extract were separated by centrifugation (5 minutes at 2000 rpm) and decanting. The volume of the combined extracts was adjusted to 40 mL using 0.5N potassium hydroxide/10% potassium chloride solution. A 1.0-mL aliquot was acidified with 1.5 mL of 0.5N hydrochloric acid (allowed to set for 15 minutes after mixing). The sample

was centrifuged (5 minutes at 2000 rpm), as necessary, if cloudy. A 1.0-mL aliquot of the supernatant was purified using a Phenomenex Strata-X polymeric solid phase extraction (SPE) column (pre-conditioned with methanol and 0.1 N hydrochloric acid, 1 mL each). After rinsing the column with 0.1N hydrochloric acid (3 x 1.0 mL) and drying (full vacuum at *ca.* 15 in Hg for 30 minutes), the analyte was eluted with methylene chloride (2 x 750  $\mu$ L aliquots) and combined with 100  $\mu$ L  $^{13}\text{C}_3^{15}\text{N}$ -2,3,5,6-tetrachloropyridine as an internal standard. After evaporation to dryness using an N-Evap (35°C, nitrogen flow at *ca.* 500 mL/min), the sample was derivatized with 100  $\mu$ L of N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) and 500  $\mu$ L of acetone via vortex mixing for 1-2 seconds, sonicating for 1-2 seconds, then heated at 60°C for 60 minutes. After cooling to room temperature, the volume of the sample was adjusted to 2.0 mL with cyclohexane (vortex mixing for 1-2 seconds then sonicating for 1-2 seconds). The tert-butyldimethylsilyl (TBDMS) derivatives of 6-CPA and  $^{13}\text{C}_3^{15}\text{N}$ -6-CPA were analyzed by GC/MS.

Samples were analyzed for the tert-butyldimethylsilyl (TBDMS) derivatives of 6-CPA and  $^{13}\text{C}_3^{15}\text{N}$ -6-CPA by gas chromatography using an Agilent Model 6890A GC with an Agilent Model 5973N MS in negative-ion chemical ionization mode (p. 15; Appendix B, pp. 93-94 of MRID 49567304). The following GC conditions were used: Durabond-5MS column (30 m x 0.25 mm, 1.0- $\mu$ m film thickness), oven temperature program [40°C (initial), 20°C/min. to 320°C], injector temperature 30°C, detector temperature 150°C, and injection volume 1  $\mu$ L. Two ions were monitored (quantitative and confirmation, respectively): *m/z* 271 and 273 for 6-CPA-TBDMS. Only the quantitation ion, *m/z* 277, was monitored for the internal standard,  $^{13}\text{C}_3^{15}\text{N}$ -6-CPA-TBDMS. Retention times were 12.53 min. for 6-CPA and 12.53 min. for the internal standard (Appendix B, Figure 8, p. 119).

The study authors included some notes regarding the method including 1) substitutions of equivalent equipment, glassware, etc. were acceptable, 2) modifications of GC/NCI-MS operating conditions for optimization were acceptable, 3) that a programmable temperature vaporizer (PTV) or cool-on column injection port should be used because TBDMS derivatives of 6-CPA and  $^{13}\text{C}_3^{15}\text{N}$ -6-CPA are thermally labile, and 4) that the SPE column must be completely dry prior to eluting 6-CPA with methylene chloride (Appendix B, pp. 106-107 of MRID 49567304). Additionally, the study authors explained the use of the gas purifier (to purify the helium or methane prior to entry of GC) and liquid nitrogen (to cool the injection port of the GC, if necessary).

## ILV

In the ILV, Methods GRM 07.13 and 07.14 were performed exactly as written, except for the modification of the preparation of 0.5 N potassium hydroxide/10% potassium chloride in GRM 07.14 to allow for the correct weight of potassium hydroxide (pp. 12, 24 of MRID 49567303). Approximate retention times for the analytes were not reported and could not be determined from the provided chromatograms since they were indecipherable.

## LOQ/LOD

The LOQ for all analytes/ions was the same in the ECM Methods and ILV at 0.010 µg/g (pp. 18-20; Appendix A, pp. 45-46; Appendix B, p. 103 of MRID 49567304; pp. 12, 19, 24 of MRID 49567303). The LOD for all analytes/ions was 0.003 µg/g in the ECM Methods and ILV.

## **II. Recovery Findings**

ECM (MRID 49567304: Methods GRM 07.13 and GRM 07.14): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of nitrapyrin and 2-Cl-6-DCMP in two soils (loam and silty clay loam) and 6-CPA in two soils (sandy loam and silty clay loam) at fortification levels of 0.010 µg/g (LOQ), 0.100 µg/g (10×LOQ), 1.00 µg/g (100×LOQ) and 10.0 µg/g (1000×LOQ; Appendix A, Tables 2-7, pp. 51-56; Appendix B, Tables 2-3, pp. 109-110). Performance data (recovery results) from quantitation and confirmation ion analyses were comparable. The number of samples was insufficient for all analyses in both methods. In GRM 07.13, the number of samples was four at the LOQ and 10×LOQ and three at 100×LOQ and 1000×LOQ. In GRM 07.14, the number of samples was three at the LOQ, 10×LOQ, 100×LOQ and 1000×LOQ. Both Methods GRM 07.13 and GRM 07.14 allowed for recovery data to be corrected for residues found in the control samples; however, residues were only found in the control samples of 2-Cl-6-DCMP (quantitative ion; GRM 07.13) and 6-CPA (both ions; GRM 07.14; Appendix A, pp. 42-43; Appendix A, Tables 2-7, pp. 51-56; Appendix B, pp. 100-101; Appendix B, Tables 2-3, pp. 109-110). Recoveries from samples fortified at 0.003 µg/g (LOD) ranged (matrices/quantification and confirmation ions combined) from 103-117% for nitrapyrin, 87-110% for 2-Cl-6-DCMP and 47-93% for 6-CPA (n = 1 for each matrix/analyte/ion; DER Attachment 2). The identification of the analytes was confirmed by calculating the confirmation ratio for each confirmation ion (Appendix A, p. 47; Appendix A, Tables 2-7, pp. 51-56; Appendix B, p. 104; Appendix B, Tables 2-3, pp. 109-110). The soil matrices were partially characterized (p. 12). USDA classifications were reported, but particle percentages were not reported. For GRM 07.13 (nitrapyrin and 2-Cl-6-DCMP), loam soil (Sample No. 001-0001; 00-06"; 1.6% organic carbon) and silty clay loam (Sample No. 003-0001; 00-06"; 2.3% organic carbon) were used (p. 12; Appendix A, Tables 2-7, pp. 51-56). For GRM 07.14 (6-CPA), sandy loam soil (Sample No. 005-0002; 06-12"; 1.7% organic carbon) and silty clay loam (Sample No. 018-0002; 06-12"; 1.7% organic carbon) were used (p. 12; Appendix B, Tables 2-3, pp. 109-110).

ILV (MRID 49567303: Methods GRM 07.13 and GRM 07.14): Mean recoveries and relative standard deviations (RSDs) were within guidelines for analysis of nitrapyrin, 2-Cl-6-DCMP and 6-CPA in loam soil at fortification levels of 0.010 µg/g (LOQ) and 10.0 µg/g (1000×LOQ), except for the secondary confirmation ion results of 2-Cl-6-DCMP (mean 46%, RSD 37.8%; Tables 21-28, pp. 37-38). Performance data (recovery results) from quantitation and confirmation ion analyses were comparable, except for the secondary confirmation ion results of 2-Cl-6-DCMP. For the individual recoveries which were lower than the acceptable range of 70-120% (see Table 3; 2-Cl-6-DCMP and 6-CPA), the study author indicated that the residues in the controls caused a negative intercept in the calibration curve which led to an incorrect calculation

of the residues in the samples (p. 18). The study author calculated “adjusted % recovery” values with correction for residues in the controls; however, the study author did not use these adjusted % recoveries for statistics. For 2-Cl-6-DCMP, adjusted % recoveries ranged 81-125% at the LOQ (unchanged at 10×LOQ; Table 18, p. 34). For 6-CPA, adjusted % recoveries ranged 83-116% at the LOQ (unchanged at 10×LOQ; Table 20, p. 36). Recoveries from samples fortified at 0.003 µg/g (LOD) ranged (matrices/quantification and confirmation ions combined) from 103-157% for nitropryrin, 90-93% for 2-Cl-6-DCMP (negative number for secondary confirmation ion) and 60-93% for 6-CPA (n = 1 for each matrix/analyte/ion; DER Attachment 2). The loam soil (33.2% sand, 40.6% silt, 26.2% clay; 2.21% organic carbon) was fully characterized (Lufa Speyer 2.4; USDA soil characterization; p. 13; Tables 21-28, pp. 37-38; Appendix 2, pp. 125-126). Although it was not specifically reported, the reviewer assumed that Methods GRM 07.13 and 07.14 were validated in the first trial (p. 24).

**Table 2. Initial Validation Method Recoveries for Nitrapyrin and Its Transformation Products in Soils<sup>1,2</sup>**

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>GRM 07.13</b>						
<b>Loam soil (00-06")</b>						
Quantitation ion						
Nitrapyrin	0.003 (LOD)	1	103	--	--	--
	0.010 (LOQ)	4	97-107	103	4	4
	0.100	4	97-104	101	3	3
	1.00	3	102-108	105	3	3
	10.0	3	107-108	107	1	1
2-Cl-6-DCMP	0.003 (LOD)	1	97	--	--	--
	0.010 (LOQ)	4	95-101	99	3	3
	0.100	4	94-97	96	1	1
	1.00	3	96-101	98	3	3
	10.0	3	103-105	104	1	1
Confirmation ion (Primary)						
Nitrapyrin	0.003 (LOD)	1	103	--	--	--
	0.010 (LOQ)	4	99-105	102	3	3
	0.100	4	97-104	101	3	3
	1.00	3	102-107	104	3	2
	10.0	3	106-108	107	1	1
2-Cl-6-DCMP	0.003 (LOD)	1	97	--	--	--
	0.010 (LOQ)	4	95-98	96	1	1
	0.100	4	95-97	96	1	1
	1.00	3	95-100	97	3	3
	10.0	3	103-104	104	1	1
Confirmation ion (Secondary)						
Nitrapyrin	0.003 (LOD)	1	103	--	--	--
	0.010 (LOQ)	4	101-106	104	2	2
	0.100	4	97-104	101	3	3
	1.00	3	102-106	104	2	2
	10.0	3	106-107	107	1	1
2-Cl-6-DCMP	0.003 (LOD)	1	87	--	--	--
	0.010 (LOQ)	4	91-103	96	5	5
	0.100	4	95-97	96	1	1
	1.00	3	94-99	96	3	3
	10.0	3	101-102	102	1	1
<b>Silty Clay Loam (00-06")</b>						
Quantitation ion						
Nitrapyrin	0.003 (LOD)	1	107	--	--	--
	0.010 (LOQ)	4	102-108	105	3	3
	0.100	4	94-102	99	3	3
	1.00	3	97-98	97	1	1
	10.0	3	99-104	102	3	3
2-Cl-6-DCMP	0.003 (LOD)	1	110	--	--	--



Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.010 (LOQ)	4	97-99	98	1	1
	0.100	4	94-99	97	2	2
	1.00	3	96-99	97	2	2
	10.0	3	100-106	104	3	3
Confirmation ion (Primary)						
Nitrapyrin	0.003 (LOD)	1	117	--	--	--
	0.010 (LOQ)	4	100-103	102	1	1
	0.100	4	94-100	98	3	3
	1.00	3	99-101	100	1	1
	10.0	3	101-107	105	3	3
2-Cl-6-DCMP	0.003 (LOD)	1	97	--	--	--
	0.010 (LOQ)	4	95-101	98	3	3
	0.100	4	94-99	97	2	2
	1.00	3	96-98	97	1	1
	10.0	3	100-105	103	3	3
Confirmation ion (Secondary)						
Nitrapyrin	0.003 (LOD)	1	113	--	--	--
	0.010 (LOQ)	4	100-104	102	2	2
	0.100	4	93-101	98	3	3
	1.00	3	98-100	99	1	1
	10.0	3	101-106	104	3	3
2-Cl-6-DCMP	0.003 (LOD)	1	103	--	--	--
	0.010 (LOQ)	4	92-102	98	4	4
	0.100	4	94-99	97	2	2
	1.00	3	95-97	96	1	1
	10.0	3	99-104	102	3	3
<b>GRM 07.14</b>						
<b>Silty Clay Loam (06-12")</b>						
Quantitation ion						
6-CPA	0.003 (LOD)	1	93	--	--	--
	0.010 (LOQ)	3	92-96	94	2	2
	0.100	3	95-99	97	2	2
	1.00	3	97-100	98	2	2
	10.0	3	99-101	100	1	1
Confirmation ion						
6-CPA	0.003 (LOD)	1	77	--	--	--
	0.010 (LOQ)	3	79-84	82	3	3
	0.100	3	89-94	91	3	3
	1.00	3	105-107	106	1	1
	10.0	3	106-108	107	1	1
<b>Sandy Loam (06-12")</b>						
Quantitation ion						
6-CPA	0.003 (LOD)	1	87	--	--	--
	0.010 (LOQ)	3	91-93	92	1	1
	0.100	3	94-96	95	1	1
	1.00	3	96-97	96	1	1

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	10.0	3	96-99	97	2	2
Confirmation ion						
6-CPA	0.003 (LOD)	1	47	--	--	--
	0.010 (LOQ)	3	76-82	78	3	4
	0.100	3	90-91	91	1	1
	1.00	3	99-100	100	1	1
	10.0	3	99-102	100	2	2

Data (results were corrected when residues were quantified in the controls) were obtained from Appendix A, Tables 2-7, pp. 51-56; Appendix B, Tables 2-3, pp. 109-110 of MRID 49567304 and DER Attachment 2 (mean, s.d. and RSD values and % recovery at LOD). 2-Cl-6-DCMP = 2-chloro-6-(dichloromethyl)pyridine; 6-CPA = 6-chloropyridine-2-carboxylic acid.

- For GRM 07.13 (nitrapyrin and 2-Cl-6-DCMP), loam soil (Sample No. 001-0001; 00-06"; 1.6% organic carbon) and silty clay loam (Sample No. 003-0001; 00-06"; 2.3% organic carbon) were used (particle percentages not reported; p. 12; Appendix A, Tables 2-7, pp. 51-56). For GRM 07.14 (6-CPA), sandy loam soil (Sample No. 005-0002; 06-12"; 1.7% organic carbon) and silty clay loam (Sample No. 018-0002; 06-12"; 1.7% organic carbon) were used (particle percentages not reported; p. 12; Appendix B, Tables 2-3, pp. 109-110).
- In GRM 07.13, three ions were monitored for the analytes (quantitative, primary confirmation and secondary confirmation, respectively):  $m/z$  194, 196 and 198 for nitrapyrin and  $m/z$  160, 162 and 195 for 2-Cl-6-DCMP. In GRM 07.14, two ions were monitored (quantitative and confirmation, respectively):  $m/z$  271 and 273 for 6-CPA-TBDMS (derivatized 6-CPA).

**Table 3. Independent Validation Method Recoveries for Nitrapyrin and Its Transformation Products in Loam Soil<sup>1,2</sup>**

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Method GRM 07.13</b>						
Quantitation ion						
Nitrapyrin	0.003 (LOD)	<b>1</b>	130	--	--	--
	0.010 (LOQ)	5	94-104	98	4.6	4.7
	10.0	5	89-115	101	12.6	12.5
2-Cl-6-DCMP	0.003 (LOD)	<b>1</b>	93	--	--	--
	0.010 (LOQ)	5	84-86	85	1.0	1.5
	10.0	5	87-97	88	4.5	5.2
Confirmation ion (Primary)						
Nitrapyrin	0.003 (LOD)	<b>1</b>	103	--	--	--
	0.010 (LOQ)	5	90-103	94	5.2	5.6
	10.0	5	90-116	101	12.8	12.6
2-Cl-6-DCMP			90			
	0.010 (LOQ)	5	81-88	83	2.7	3.2
	10.0	5	86-98	91	5.3	5.8
Confirmation ion (Secondary)						
Nitrapyrin	0.003 (LOD)	<b>1</b>	157	--	--	--
	0.010 (LOQ)	5	112-126	119	5.0	4.2
	10.0	5	91-115	101	11.7	11.5
2-Cl-6-DCMP	0.003 (LOD)	<b>1</b>	NC	--	--	--
	0.010 (LOQ)	5	28-72 <sup>3</sup>	<b>46</b>	17.4	<b>37.8</b>
	10.0	5	89-98 <sup>3</sup>	92	3.9	4.2
<b>Method GRM 07.14</b>						
Quantitation ion						
6-CPA	0.003 (LOD)	<b>1</b>	60	--	--	--
	0.010 (LOQ)	5	82-99	89	7.5	8.4
	10.0	5	94-113	101	7.6	7.5
Confirmation ion						
6-CPA	0.003 (LOD)	<b>1</b>	93	--	--	--
	0.010 (LOQ)	5	64-97 <sup>3</sup>	83	12.0	14.6
	10.0	5	88-108 <sup>3</sup>	97	9.4	9.6

Data (uncorrected recovery results) were obtained from Tables 13-28, pp. 31-38 of MRID 49567303. 2-Cl-6-DCMP = 2-chloro-6-(dichloromethyl)pyridine; 6-CPA = 6-chloropyridine-2-carboxylic acid. NC = not calculated; a negative intercept from the calibration curve generated a negative value for residues detected at the LOD.

1 Soil matrix was fully characterized (Lufa Speyer 2.4; USDA texture classification; soil origin not reported; p. 13; Tables 21-28, pp. 37-38; Appendix 2, pp. 125-126).

2 In GRM 07.13, three ions were monitored for the analytes (quantitative, primary confirmation and secondary confirmation, respectively): *m/z* 194, 196 and 198 for nitrapyrin and *m/z* 160, 162 and 195 for 2-Cl-6-DCMP. In GRM 07.14, two ions were monitored (quantitative and confirmation, respectively): *m/z* 271 and 273 for 6-CPA-TBDMS (derivatized 6-CPA).

3 The study author indicated that the residues in the controls caused a negative intercept in the calibration curve which led to an incorrect calculation of the residues in the samples (p. 18). The study author calculated "adjusted % recovery" values with correction for residues in the controls; however, the study author did not use these adjusted % recoveries for statistics. For 2-Cl-6-DCMP, adjusted % recoveries ranged 81-125% at the LOQ

(unchanged at 10×LOQ; Table 18, p. 34). For 6-CPA, adjusted % recoveries ranged 83-116% at the LOQ (unchanged at 10×LOQ; Table 20, p. 36).

### III. Method Characteristics

In the ECM Methods and ILV, the LOQ and LOD values for nitrapyrin, 2-Cl-6-DCMP and 6-CPA in soil were established at 0.010 µg/g and 0.003 µg/g, respectively (pp. 18-20; Appendix A, pp. 45-46; Appendix B, p. 103 of MRID 49567304; pp. 12, 19, 24 of MRID 49567303).

Following the method of Keith, L. H., *et al.* (see section **V. References** below), the LOD and LOQ for determination of nitrapyrin and its transformation products in soil were calculated in the ECM Methods using the standard deviation from the 0.010 µg/g recovery results. The LOD was calculated as three times the standard deviation ( $3s$ ), and the LOQ was calculated as ten times the standard deviation ( $10s$ ) of the recovery results. The calculated values support the LOQ and LOD established for the study and are presented in **Table 4** below.

**Table 4. Method Characteristics**

Method		GRM 07.13 <sup>1</sup>		GRM 07.14 <sup>2</sup>
Analyte		Nitrapyrin	2-Cl-6-DCMP	6-CPA
Limit of Quantitation (LOQ)	Established	0.010 µg/g		
	Calculated	0.0033 µg/g (Q) 0.0019 µg/g (C1) 0.0024 µg/g (C2)	0.0022 µg/g (Q) 0.0018 µg/g (C1) 0.0046 µg/g (C2)	0.0018 µg/g (Q) 0.0028 µg/g (C)
Limit of Detection (LOD)	Established	0.003 µg/g		
	Calculated	0.0010 µg/g (Q) 0.0006 µg/g (C1) 0.0007 µg/g (C2)	0.0007 µg/g (Q) 0.0005 µg/g (C1) 0.0014 µg/g (C2)	0.0005 µg/g (Q) 0.0008 µg/g (C)
Linearity (Least squares calibration curve r and concentration range)	ECM <sup>3</sup>	r <sup>2</sup> = 0.99992 (Q) r <sup>2</sup> = 0.99993 (C1) r <sup>2</sup> = 0.99988 (C2)	r <sup>2</sup> = 0.99995 (Q) r <sup>2</sup> = 0.99997 (C1) r <sup>2</sup> = 0.99995 (C2)	r <sup>2</sup> = 0.99989 (Q) r <sup>2</sup> = 0.99944 (C)
	ILV	r <sup>2</sup> = 0.9990 (Q) r <sup>2</sup> = 0.9989 (C1) r <sup>2</sup> = 0.9991 (C2)	r <sup>2</sup> = 0.9995 (Q) r <sup>2</sup> = 0.9996 (C1) r <sup>2</sup> = 0.9996 (C2)	r <sup>2</sup> = 0.9999 (Q) r <sup>2</sup> = 0.9995 (C)
	Concentration range	1.5-500.0 ng/mL		0.075-25.0 ng/mL
Repeatable	ECM <sup>4</sup>	Yes at LOQ and 10×LOQ, but n = 4.		Yes at LOQ and 10×LOQ, but n = 3.
	ILV <sup>5</sup>	Yes at 100×LOQ and 1000×LOQ, but n = 3. Yes at LOQ and 1000×LOQ (n = 5). No samples were prepared at 10×LOQ.		
Reproducible		Yes at the LOQ. No samples were prepared at 10×LOQ. (only loam soil was tested in ILV)		
Specific	ECM/ILV	Confirmation of GC/MS analysis performed with quantification of one or two confirmation ions per analyte.		
	ECM <sup>6</sup>	Yes, no interferences were observed in the matrix control.	Yes, matrix interferences were ca.10% of the LOQ based on peak area and residue recovery.	Yes, matrix interferences were 10-25% of the LOQ based on peak area and residue recovery. <sup>7</sup>
	ILV	Yes, matrix interferences were <5% of the LOQ based on residue recovery.	Yes for Q, matrix interferences were <5% of the LOQ based on residue recovery. Could not be determined for C1 and C2; negative recovery values reported for matrix controls.	Could not be determined based on recovery data; negative recovery values reported for matrix controls.
		Could not be determined based on chromatograms. Provided		

		chromatograms were indecipherable. Matrix effects were determined to be insignificant with the internal standard (< +19.6% for nitrapyrin, < -2.7% for 2-Cl-6-DCMP and < -4.4% for 6-CPA).
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Data were obtained from pp. 18-21; Appendix A, Tables 2-7, pp. 51-56; Appendix A, Table 9, p. 57; Appendix A, Table 11, p. 58; Appendix A, Figures 2-25, pp. 60-83; Appendix B, Tables 2-3, pp. 109-110; Appendix B, Table 5, p. 111; Appendix B, Figures 2-3, pp. 113-114; Appendix B, Figures 7-9, pp. 118-120 of MRID 49567304 and pp. 12, 19, 22-24; Tables 13-28, pp. 31-38; Tables 35-42, pp. 43-45; Figures 4-11, pp. 49-56; Figures 16-18, pp. 63-71; Figures 20-22, pp. 73-75 of MRID 49567303. 2-Cl-6-DCMP = 2-chloro-6-(dichloromethyl)pyridine; 6-CPA = 6-chloropyridine-2-carboxylic acid. Q = quantitation ion; C1 = primary confirmation ion; C2 = secondary confirmation ion; C = confirmation ion.

- 1 Method GRM 07.13 with the use of gas chromatography with positive-ion electron-impact ionization mass spectrometry detection.
- 2 Method GRM 07.14 with the use of gas chromatography with negative-ion chemical ionization mass spectrometry detection.
- 3 Only representative calibration curves were presented in the study report. The study authors reported that all coefficients of determination were greater than 0.999 for analysis of nitrapyrin and 2-Cl-6-DCMP and greater than 0.998 for analysis of 6-CPA (Appendix A, p. 44; Appendix B, p. 102 of MRID 49567304).
- 4 For ECM Method GRM 07.13, loam soil (00-06"; 1.6% organic carbon) and silty clay loam (00-06"; 2.3% organic carbon) were used. For ECM Method GRM 07.14, sandy loam soil (06-12"; 1.7% organic carbon) and silty clay loam (06-12"; 1.7% organic carbon) were used (p. 12; Appendix A, Tables 2-7, pp. 51-56; Appendix B, Tables 2-3, pp. 109-110 of MRID 49567304). The soil particle distribution was not reported.
- 5 The soil matrix of the ILV was loam (26.2% clay, 2.21% organic carbon; Lufa Speyer 2.4; p. 13; Tables 21-28, pp. 37-38; Appendix 2, pp. 125-126 of MRID 49567303).
- 6 For ECM Methods GRM 07.13 and GRM 07.14, chromatograms were only provided for one of the two soil matrices tested (Appendix A, Figures 2-25, pp. 60-83; Appendix B, Figures 7-9, pp. 118-120 of MRID 49567304). The reviewer could not determine which soil matrix was provided for each method (GRM 07.13, Sample Set 071125 MV3; GRM 07.14, Sample Set 071125 MV2). The sets of chromatograms were incomplete: only one calibration chromatogram (GRM 07.13, 5.00 ng/mL; GRM 07.14, 0.25 ng/mL), the control soil sample and LOQ sample were included.
- 7 Residues in the controls were 0.0010 and 0.0011 µg/g for the quantitation ion (*ca.* 10% of the LOQ and *ca.* 33% of the LOD) and 0.0016 and 0.0019 µg/g for the confirmation ion (*ca.* 15-20% of the LOQ and *ca.* 50-60% of the LOD; Appendix B, Tables 2-3, pp. 109-110 of MRID 49567304). However, for the confirmation ion, since residue recoveries only ranged 76-84% at the LOQ and 0.0014-0.0023 µg/g at the LOD, the residues in the controls represented *ca.* 20-25% of the recovery at the LOQ and *ca.* 70-100% of the recovery at the LOD.

#### IV. Method Deficiencies and Reviewer's Comments

1. In the ILV, no performance data were provided for 10×LOQ to validate the methods GRM 07.13 and GRM 07.14, only the LOQ and 1000×LOQ. A validation sample set should consist of, at a minimum, a reagent blank, two unspiked matrix control samples, five matrix control samples spike at the LOQ, and five matrix control samples spiked at 10×LOQ for each analyte and matrix.
2. ILV representative chromatograms were indecipherable (Figures 16-18, pp. 63-71; Figures 20-22, pp. 73-75 of MRID 49567303). In some chromatograms, a faint disjointed outline of the axis and spectra was visible, but the majority of the chromatograms were blank. Due to these representative chromatograms, the specificity of the method could not be assessed in relation to matrix and baseline interferences and contamination.

Based on data in the recovery tables, matrix interferences were <5% for nitrapyrin (all ions) and 2-Cl-6-DCMP (Q ion only). For the C1 and C2 ions of 2-Cl-6-DCMP and both ions of 6-CPA, negative residue recovery results were reported for the matrix blank (Tables 13-20, pp. 31-36). Therefore, the specificity of the methods could not be determined for 2-Cl-6-DCMP and 6-CPA based on matrix control recovery data.

3. In the ECM, the number of samples was insufficient for all analyses in both methods (Appendix A, Tables 2-7, pp. 51-56; Appendix B, Tables 2-3, pp. 109-110). In GRM 07.13, the number of samples was four at the LOQ and 10×LOQ and three at 100×LOQ and 1000×LOQ. In GRM 07.14, the number of samples was three at the LOQ, 10×LOQ, 100×LOQ and 1000×LOQ. OSCPP guidelines recommend a minimum of five samples spiked at each fortification level.
4. In the ECM, the soil matrices were only partially characterized (p. 12 of MRID 49567304). USDA classifications were reported, but particle percentages were not reported. Only pH and % organic carbon were reported; therefore, it could not be determined if the ILV was provided with the most difficult soil type with which to validate the methods. The ILV validated the methods with loam soil (26.2% clay, 2.21% organic carbon; Lufa Speyer 2.4; p. 13; Tables 21-28, pp. 37-38; Appendix 2, pp. 125-126 of MRID 49567303).
5. In the ILV, the mean recovery and RSD were 46% and 37.8%, respectively, for the secondary confirmation ion results of 2-Cl-6-DCMP (Tables 21-28, pp. 37-38). OSCPP guidelines recommend that mean recoveries are within the range 70-120% and RSDs are ≤20%. The study author calculated “adjusted % recovery” values with correction for residues in the controls; however, the study author did not use these adjusted % recoveries for statistics (p. 18). For 2-Cl-6-DCMP, adjusted % recoveries ranged 81-125% at the LOQ (unchanged at 10×LOQ; Table 18, p. 34).

6. Recoveries were corrected in the ECM. Both Methods GRM 07.13 and GRM 07.14 allowed for recovery data to be corrected for residues found in the control samples; however, residues were only found in the control samples of 2-Cl-6-DCMP (quantitative ion; GRM 07.13) and 6-CPA (both ions; GRM 07.14; Appendix A, pp. 42-43; Appendix A, Tables 2-7, pp. 51-56; Appendix B, pp. 100-101; Appendix B, Tables 2-3, pp. 109-110). Recoveries were not corrected in the ILV (Figures 12-14, pp. 57-59 of MRID 49567303).
7. In the ECM Methods GRM 07.13 and GRM 07.14, chromatograms were only provided for one of the two soil matrices tested (Appendix A, Figures 2-25, pp. 60-83; Appendix B, Figures 7-9, pp. 118-120 of MRID 49567304). The reviewer could not determine which soil matrix was provided for each method (GRM 07.13, Sample Set 071125 MV3; GRM 07.14, Sample Set 071125 MV2). The sets of chromatograms were incomplete: only one calibration chromatogram (GRM 07.13, 5.00 ng/mL; GRM 07.14, 0.25 ng/mL), the control soil sample and LOQ sample were included. Chromatograms of the 10×LOQ, 100×LOQ and 1000×LOQ samples were not included. A reagent blank was not prepared.
8. In the ECM, only representative calibration curves and data were provided for each Method [GRM 07.13, Sample Set 071125 MV3; Appendix A (Figures 2, p. 60; Figure 6, p. 64; Figure 10, p. 68; Figure 14, p. 72; Figure 18, p. 76; Figure 22, p. 80) and GRM 07.14, Sample Set 071125 MV2; Appendix B (Figures 2-3, pp. 114-115) of MRID 49567304]. The study authors reported that all coefficients of determination were greater than 0.999 for analysis of nitrapyrin and 2-Cl-6-DCMP and greater than 0.998 for analysis of 6-CPA (Appendix A, p. 44; Appendix B, p. 102 of MRID 49567304).
9. In the ECM, the study authors reported that isotopic crossover (which would interfere with ions used for quantification) was not observed in the Method GRM 07.14 due to use of a stable-isotope labeled internal standard (p. 22 of MRID 49567304).
10. In the ILV, the study author reported that communications with the sponsor were limited to the discussion of the method modification to allow for the correct weight of potassium hydroxide to be used in preparation of a solution (p. 24 of MRID 49567303).
11. In the ECM, the stability of the analyte calibration solutions was investigated in an associated terrestrial field dissipation study (ARAP-07D-002) and this validation study (pp. 22-23; Table 1, p. 28 of MRID 49567304). It was determined that the average response factor of the original calibration solutions after 117 days was within 10% of the freshly-prepared solutions for nitrapyrin and within 2% of the freshly-prepared solutions for 2-Cl-6-DCMP. For 6-CPA, it was determined that the average response factor of the original calibration solutions after 23 days was within 3% of the freshly-prepared solutions.



In the ILV, the stability of the stock, spiking and calibration solutions was also investigated after refrigeration storage (*ca.* 4°C; pp. 19-21; Tables 29-31, pp. 39-41 of MRID 49567303). After 18 days of storage, mean peak area ratios were -1.1% to 0.2% of the original for nitrapyrin (all three ions). After 18 days of storage, mean peak area ratios were -0.2% to 4.7% of the original for 2-Cl-6-DCMP (all three ions). After 26 days of storage, mean peak area ratios were -7.1% to -4.7% of the original for 6-CPA (both ions).

In the ILV, stability of the sample extracts was investigated after refrigeration storage (*ca.* 4°C) for 4 days (pp. 21-22; Tables 32-34, p. 42 of MRID 49567303). Adjusted % recovery results [mean (RSD)] were 117% (3.8%) for nitrapyrin, 88% (3.7%) for 2-Cl-6-DCMP and 92% (10.5%) for 6-CPA (quantitation ion at LOQ, n = 5 for each; confirmation(s) ion not monitored).

12. In the ECM, matrix effects (suppression) were determined to be -4% to -8% for GRM 07.13 and -3% to -12% for GRM 07.14 (p. 21 of MRID 49567304). The results showed that the matrix effects were within  $\pm 20\%$  and not significant.

In the ILV, matrix effects were determined to be insignificant, as well (<20%), with the internal standard (pp. 22-23; Tables 35-42, pp. 43-45 of MRID 49567303). Average matrix effects with the internal standard ranged 12.8 to 19.6% for nitrapyrin, -2.4 to -2.7% for 2-Cl-6-DCMP and -4.2 to -4.4% for 6-CPA. Without the internal standard, matrix effects for nitrapyrin were significant (>20%).

13. It was reported for the ILV that the analytical procedure for one set of samples (calibration samples, a reagent blank, control and fortified samples) required approximately four person hours for GRM 07.13 and approximately six person hours for GRM 07.14 (p. 16 of MRID 49567303). The entire assay time with sample processing, analysis and results work up required approximately 2.5 days for each method.

## V. References

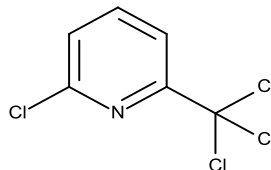
Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* 1983, 55, 2210-2218 (Appendix A, p. 49; Appendix B, p. 107 of MRID 49567304).

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.

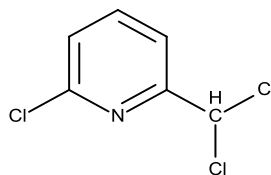
40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

**Attachment 1: Chemical Names and Structures****Nitrapyrin (Nitra)**

**IUPAC Name:** 2-Chloro-6-trichloromethylpyridine  
2-Chloro-6-(trichloromethyl)pyridine  
**CAS Name:** 2-Chloro-6-(trichloromethyl)pyridine  
**CAS Number:** 1929-82-4  
**SMILES String:** n(c(cc1)C(Cl)(Cl)Cl)c1Cl

**2-Chloro-6-(dichloromethyl)pyridine (2-Cl-6-DCMP; DCMP; DCMpyr)**

**IUPAC Name:** 2-Chloro-6-(dichloromethyl)pyridine  
2-Chloro-6-dichloromethylpyridine  
**CAS Name:** 2-Chloro-6-(dichloromethyl)pyridine  
**CAS Number:** 78152-53-1  
**SMILES String:** c1cc(nc(c1)Cl)C(Cl)Cl

**6-Chloropicolinic acid (6-CPA; 6-Chloropyridine-2-carboxylic acid)**

**IUPAC Name:** 6-Chloropyridine-2-carboxylic acid  
**CAS Name:**  
**CAS Number:** 4684-94-0  
**SMILES String:** OC(=O)c1cccc(Cl)n1

