Test Material:	Nitrapyrin
MRID:	49567302
Title:	Method Validation Study for the Determination of Nitrapyrin and 6-Chloropicolinic Acid (6-CPA) in Water
MRID:	49567301
Title:	Independent Laboratory Validation for the Determination of Residues of Nitrapyrin in Ground, Surface, and Drinking Water by Gas Chromatography with Mass Spectrometry and 6-Chloropicolinic Acid (6-CPA) in Ground, Surface, and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry
EPA PC Code:	069203
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** 

Date: 8/4/25

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Date: 8/4/15

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Date: 8/4/15

Analytical method for nitrapyrin and its transformation product, 6-chloropyridine-2-carboxylic acid, in water

Reports: ECM: EPA MRID No.: 49567302. Wrigley, C. 2015. Method Validation Study for the Determination of Nitrapyrin and 6-Chloropicolinic Acid (6-CPA) in Water. Dow AgroSciences LLC ID: 141215. EPL BAS Study No.: 205G907. Report prepared by EPL Bio Analytical Services (EPL BAS), Niantic, Illinois, sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 148 pages. Final report issued January 17, 2015.
ILV: EPA MRID No. 49567301. Benotti, M.J. 2015. Independent Laboratory Validation for the Determination of Residues of Nitrapyrin in Ground, Surface, and Drinking Water by Gas Chromatography with Mass Spectrometry and 6-Chloropicolinic Acid (6-CPA) in Ground, Surface, and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry. Dow AgroSciences Protocol No.: 141124. Battelle ID: 100057852. Report prepared by Battelle, Norwell, Massachusetts, sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 113 pages. Final report issued January 30, 2015.

Document No.: MRIDs 49567302 & 49567301

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards (p. 3 of MRID 49567302). 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 GLP standards (p. 3 of MRID 49567301). 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 not included; a signature page was included (p. 5).

Classification: This analytical method is classified as **UNACCEPTABLE**. For all analytes in the ECM and ILV, performance data to validate the method at 10×LOQ were not reported. Unacceptable ILV performance data were provided to validate EPL BAS Method 205G907B for analysis of 6-CPA. For the ILV, the number of trials performed was not reported. In the ILV, linearity of the calibration curves was not always satisfactory. Recoveries of 6-CPA (both ions) were corrected in the ECM. ILV representative chromatograms were of poor quality; the specificity of the method was especially difficult to assess for nitrapyrin based on these chromatograms. The sources of the ECM and ILV water matrices were not reported.

PC Code: 069203

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

Signature: HE ZHONG Digitally signed by HE ZHONG DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=HE ZHONG, dnQualifier=0000051219 Date: 2015.09.29 14:14:11 -04'00'

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, EPL BAS Method 205G907A, is designed for the quantitative determination of nitrapyrin in water at the LOQ of 0.05 µg/L using GC/MS in positive-ion electron-impact ionization mode. The analytical method, EPL BAS Method 205G907B, is designed for the quantitative determination of the nitrapyrin transformation product 6-chloropyridine-2-carboxylic acid (6-CPA) in water at the LOQ of 0.05 µg/L using LC/MS with MRM. The LOQ is less than 0.16 mg/L (Eastern Oyster NOAEC < 0.16 mg ai/L), the lowest toxicological level of concern in water for all analytes. EPL BAS Method 205G907A was validated by the ILV with only minor modifications; however, samples were not fortified at 10×LOQ, only the LOQ and 20×LOQ. Many of the chromatograms for nitrapyrin provided in the ILV were indecipherable. For EPL BAS Method 205G907B, the ILV performance data were not satisfactory to validate the method in any water sample at the LOQ and 20×LOQ. For both methods, the ILV did not specify the number of trials performed. The water matrices of the ECM and ILV were characterized, but sources were not reported.

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	49567302	49567301		Water[2]	17/01/2015	Dow AgroSciences LLC	GC/MS[3]	0.05 µg/L
6-CPA[1]							LC/MS/MS[4]	

1 6-CPA = 6-chloropyridine-2-carboxylic acid

2 Drinking, ground and surface water matrices were used in the ECM and ILV. In the ECM and ILV, water characterizations were provided, but source locations for the waters were not reported (p. 14; Appendix B, pp. 120-123 of MRID 49567302; p. 13 of MRID 49567301).

3 EPL BAS Method 205G907A with the use of positive-ion electron-impact ionization mass spectrometry detection.

4 EPL BAS Method 205G907B with the use of tandem mass spectrometry detection. This method was not validated by the ILV due to unsatisfactory performance data for all water matrices.

I. Principle of the Method

EPL BAS Method 205G907A: Nitrapyrin

Samples (100 mL) of water were fortified (30.0 or 100.0 µL of 50 ng/mL solution, or 200.0 µL of 500 ng/mL solution), as necessary, then extracted twice with hexane:toluene (1:1, v:v; 10 mL x 2) via shaking in a separatory funnel for *ca.* 15 seconds (p. 15; Appendix D, pp. 131-132 of MRID 49567302). Methanol can be added drop-wise to break-up emulsions. The combined extracts were combined in a 25 mL glass test tube and reduced to less than 0.5 mL under a stream of nitrogen (N-Evap) at 35°C. The residue was purified via a deactivated silica solid-phase extraction (SPE) column (*ca*. 20 mm; pre-conditioned with 2 mL of hexane). The analyte was eluted using toluene (1 mL, 1 mL, 0.5 mL); the toluene was used to rinse the 25 mL glass tube, then applied to the SPE column. After the third toluene application, a pipet bulb should be used to push all solvent through the column. The volume of the eluate was adjusted to 3 mL with toluene then mixed via vortex; sample was stored under refrigeration when not in use. An aliquot was removed for GC/EI-MS analysis.

Samples were analyzed for nitrapyrin by gas chromatography using an Agilent Model 7890N GC with an Agilent Model 5975 MS in positive-ion electron-impact ionization mode (p. 15; Appendix D, pp. 132-133 of MRID 49567302). The following GC conditions were used: Agilent DB-5MS column (30 m x 0.25 mm, 1.00-µm film thickness), oven temperature program [130°C for 1.0 min., 10°C/min. to 210°C, 15°C/min. to 280°C, 280°C for 2.0 min.], injector temperature 180°C, detector temperature 250°C, and injection volume 2 µL. Three ions were monitored (quantitative, primary confirmation and secondary confirmation, respectively): *m/z* 194, 196 and 198 for nitrapyrin. Retention time was *ca*. 8.15-8.85 min. for nitrapyrin (Figures 13-14, pp. 87-88; Figures 17-18, pp. 91-92; Figures 21-22, pp. 96-97).

EPL BAS Method 205G907B: 6-CPA

Samples (20 mL) of water were fortified (10.0 µL of the 30 ng/mL solution, 10.0 µL of 100 ng/mL solution, or 10.0 µL or 20.0 µL of 1000 ng/mL solution), as necessary, in a plastic centrifuge tube then acidified with 2.0 mL of 1N HCl solution (pp. 15, 17-18; Appendix E, pp. 139-141 of MRID 49567302). The analyte was extracted via a C18 SPE column (rinsed with acetonitrile, pre-conditioned with 5.0 mL of 0.1N HCl). After the sample was loaded, the SPE column was washed with 2.0 mL of 0.1N HCl then dried by applying a vacuum (*ca*. 10 inches Hg) for 40-45 minutes (the study author noted that product loss can occur if the vacuum pressure is <10 inches Hg or drying time exceeded). The analyte was eluted with 5.0 mL of acetonitrile at *ca*. 1 mL/min. flow rate. The acetonitrile eluent was evaporated to dryness under a stream of nitrogen (N-Evap) at 35°C (the study author noted that product loss can occur if the temperature was exceeded or if sample overdried). For evaporation, it was recommended that the sample should be dried manually after evaporation was completed to a volume of less than 100 µL. The dry residue was dissolved in 0.5 mL of methanol:DI water (25:75, v:v) via vortex for *ca*. 10 seconds; sample was stored under refrigeration when not in use. An aliquot was removed for LC/MS/MS analysis.

Samples were analyzed for 6-CPA using an Acquity UPLC system coupled to an Acquity TQ Mass Spectrometer (MRM; pp. 17-18; Appendix E, pp. 141-142 of MRID 49567302). The instrumental conditions consisted of an HSS T3 column (2.1 x 100 mm, 1.8-µm; column temperature 40°C), a mobile phase gradient of (A) 0.01% (v:v) acetic acid in DI water and (B) methanol [percent A:B (v:v) at 0.00-0.10 min. 95:5, 4.00-4.50 min. 30:70, 4.51-5.50 min. 5:95, 5.51-6.51 min. 95:5] and MS/MS detection in electrospray ionization mode (source temperature, 120-130°C). Two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored: *m/z* 156 → 112 (Q) and *m/z* 158 → 114 (C). Retention time was *ca*. 4.65 min. for 6-CPA. Injection volume was 5.0 µL.

ILV

In the ILV, the ECM extraction method for nitrapyrin and 6-CPA was performed exactly as written, with the substitution of comparable equipment and glassware and change of working solution concentrations (pp. 13-16, 22 of MRID 49567301). The analytical method was performed using an Agilent Model 7890A GC with an Agilent Model 5975C MS in positive-ion electron-impact ionization and selected ion monitoring (SIM) for nitrapyrin analysis and an Agilent 1260 SL HPLC coupled to an Applied Biosystems MDS Sciex API 5500 linear ion trap MS/MS system with TurboIonSpray (ESI) and selected reaction monitoring (SRM) for 6-CPA analysis. The same or equivalent ECM chromatographic operation parameters were used for GC/MS in the ILV. For LC/MS/MS analysis, the system was optimized in the ILV and two slightly different operating conditions were used in the course of the study. The most notable differences were that either a Waters XSelect HSS T3 column (3.0 x 100 mm, 3.5-µm) or HSS T3 column (2.1 x 100 mm, 1.8-µm) were used and the mobile phase gradient of (A) 0.01% (v:v) acetic acid in DI water and (B) methanol was optimized to [percent A:B (v:v) at 0.00-0.10 min. 95:5, 4.00-4.50 min. 30:70, 4.51-6.50 min. 5:95, 6.51-8.50 min. 95:5]. Retention time was *ca*. 4.8 min. for 6-CPA; the retention time of nitrapyrin was not reported and could not be determined from the provided chromatograms since the axes were unreadable (Figures 25-33, pp. 83-91).

LOQ/LOD

The LOQ for nitrapyrin and 6-CPA was the same in the ECM Methods and ILV at 0.05 µg/L (pp. 15, 25; Tables 3-11, pp. 30-38; Tables 15-20, pp. 42-47; Appendix D, pp. 128; Appendix E, p. 136 of MRID 49567302; p. 20; Tables 22-25, pp. 46-49 of MRID 49567301). The LOD for both analytes/ions was 0.015 µg/L in the ECM Methods and ILV.

II. Recovery Findings

ECM (MRID 49567302: EPL BAS Methods 205G907A and 205G907B): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of nitrapyrin and 6-CPA in drinking, ground and surface water matrices at fortification levels of 0.05 µg/L (LOQ) and 1.00 µg/L (20×LOQ; n = 5; Tables 3-22, pp. 30-49). Samples were not prepared at 0.50 µg/L (10×LOQ). Performance data (recovery results) from quantitation and confirmation ion analyses were comparable. Calculations allowed for recovery data to be corrected for residues found in the control samples; however, residues were only found in the control samples of 6-CPA (both ions; p. 17; Tables 3-22, pp. 30-49). Recoveries from samples fortified at 0.015 µg/L (LOD) ranged (matrices/quantification and confirmation ions combined) from 63-215% for nitrapyrin and 62-483% for 6-CPA (n = 1 for each matrix/analyte/ion; DER Attachment 2). The water matrices were well characterized by Agvise Laboratories, Northwood, ND; however, sources were not reported (p. 14; Appendix B, pp. 120-123).

ILV (MRID 49567301: EPL BAS Methods 205G907A and 205G907B): Mean recoveries and relative standard deviations (RSDs) were within guidelines for analysis of nitrapyrin in drinking, ground and surface water matrices at fortification levels of 0.05 µg/L (LOQ) and 1.00 µg/L (20×LOQ; n = 5; Tables 11-13, pp. 35-37; Tables 17-19, pp. 41-43). For 6-CPA, the mean recovery and RSD were within guidelines only for the confirmation ion analysis in the drinking water matrix at 0.05 µg/L (LOQ; n = 15; Tables 14-16, pp. 38-40; Tables 20-21, pp. 44-45). For all other analyses of 6-CPA (n = 15), mean recoveries and/or RSDs were unsatisfactory: RSDs for drinking water were 20.2% at the LOQ (quantitation ion) and 40.1-41.2% at 20×LOQ (all means were acceptable); means and RSDs for ground water (fortifications/ion combined) were 57.4-63.4% and 27.6-29.2%, respectively; and means and RSDs for surface water (fortifications/ions combined) were 53.6-58.1% and 34.8-50.7%, respectively. Samples were not prepared at 0.50 µg/L (10×LOQ). Performance data (recovery results) from quantitation and confirmation ion analyses were comparable. For the individual recoveries of 6-CPA which were outside the acceptable range of 70-120%, the study author reported that additional experimentation indicated that the low accuracy of the fortifications solutions contributed to the poor recoveries, in part (p. 20; Appendix III, pp. 108-109). The study author re-calculated the recoveries from one of the three batches of five samples to account for the degradation of the fortification solution (p. 20; Tables 14-16, pp. 38-40; Tables 20-21, pp. 44-45; DER Attachment 2). Reviewer-calculated means and RSDs using these values did not improve the results of the validation of 6-CPA in any water matrix. Mean recoveries from samples fortified at 0.015 µg/L (LOD) ranged (matrices combined) from 64.5-112.7% for 6-CPA (n = 3 for each matrix/ion; DER Attachment 2); recoveries from the LOD samples were not reported for nitrapyrin since the peak area was smaller than that of the lowest calibration standard (Tables 11-13, pp. 35-37). The water matrices were characterized, but only critical water quality parameters were reported in the study report (the full characterization and source reports were included in the study file at the laboratory; p. 13). The number of trials performed was not specified, but the reported data indicate one trial for EPL BAS Method 205G907A (nitrapyrin) and up to three trials for EPL BAS 205G907B (6-CPA; Tables 11-16, pp. 35-40).

Table 2. Initial Validation Method Recoveries for Nitrapyrin and Its Transformation Product 6-CPA in Waters[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
EPL BAS Method 205G907A						
Drinking Water						
	Quantitation ion					
Nitrapyrin	0.015 (LOD)	**1**	**148**	--	--	--
	0.05 (LOQ)	5	81.7-11	92.3	7.32	7.93
	1.00	5	85.5-90.4	88.3	2.04	2.31
	Confirmation ion (Primary)					
Nitrapyrin	0.015 (LOD)	**1**	**141**	--	--	--
	0.05 (LOQ)	5	81.2-97.7	88.8	7.38	8.31
	1.00	5	83.6-92.3	86.9	3.26	3.75
	Confirmation ion (Secondary)					
Nitrapyrin	0.015 (LOD)	**1**	**215**	--	--	--
	0.05 (LOQ)	5	93.5-102	98.1	4.18	4.26
	1.00	5	85.4-91.1	88.1	2.66	3.02
Ground Water						
	Quantitation ion					
Nitrapyrin	0.015 (LOD)	**1**	**63**	--	--	--
	0.05 (LOQ)	5	82.6-97.8	92.0	5.85	6.36
	1.00	5	83.7-90.1	86.7	2.31	2.66
	Confirmation ion (Primary)					
Nitrapyrin	0.015 (LOD)	**1**	118	--	--	--
	0.05 (LOQ)	5	78.2-101	93.0	9.31	10.0
	1.00	5	82.9-90.8	87.5	3.25	3.72
	Confirmation ion (Secondary)					
Nitrapyrin	0.015 (LOD)	**1**	114	--	--	--
	0.05 (LOQ)	5	78.9-117	104	15.8	15.2
	1.00	5	81.1-93.5	86.7	4.51	5.20
Surface Water						
	Quantitation ion					
Nitrapyrin	0.015 (LOD)	**1**	**134**	--	--	--
	0.05 (LOQ)	5	86.9-117	107	12.0	11.2
	1.00	5	74.0-83.9	79.0	3.72	4.71
	Confirmation ion (Primary)					
Nitrapyrin	0.015 (LOD)	**1**	**151**	--	--	--
	0.05 (LOQ)	5	107-116	113	3.83	3.39
	1.00	5	73.9-83.9	78.3	3.59	4.58
	Confirmation ion (Secondary)					
Nitrapyrin	0.015 (LOD)	**1**	**132**	--	--	--
	0.05 (LOQ)	5	81.8-117	97.7	17.8	18.2
	1.00	5	76.6-84.3	79.4	3.57	4.49

EPL BAS Method 205G907B						
Drinking Water						
	Quantitation ion					
6-CPA	0.015 (LOD)	**1**	**129**	--	--	--
	0.05 (LOQ)	5	71.4-90.9	79.9	7.15	8.95
	1.00	5	76.2-79.6	77.5	1.42	1.83
	Confirmation ion					
6-CPA	0.015 (LOD)	**1**	**139**	--	--	--
	0.05 (LOQ)	5	80.5-119	97.0	15.9	16.4
	1.00	5	76.5-77.9	77.4	0.639	0.826
Ground Water						
	Quantitation ion					
6-CPA	0.015 (LOD)	**1**	88	--	--	--
	0.05 (LOQ)	5	65.7-80.1	74.0	5.39	7.28
	1.00	5	73.0-80.1	76.6	3.34	4.37
	Confirmation ion					
6-CPA	0.015 (LOD)	**1**	**62**	--	--	--
	0.05 (LOQ)	5	70.9-84.0	76.7	5.56	7.25
	1.00	5	73.7-79.4	76.8	2.01	2.62
Surface Water						
	Quantitation ion					
6-CPA	0.015 (LOD)	**1**	**483**	--	--	--
	0.05 (LOQ)	5	87.9-114	102	10.6	10.4
	1.00	5	99.7-109	106	3.96	3.74
	Confirmation ion					
6-CPA	0.015 (LOD)	**1**	75	--	--	--
	0.05 (LOQ)	5	74.4-105	94.5	12.0	12.7
	1.00	5	99.6-112	105	4.59	4.39

Data (results were corrected when residues were quantified in the controls; p. 17) were obtained from Tables 3-22, pp. 30-49 of MRID 49567302 and DER Attachment 2 (% recovery at LOD). 6-CPA = 6-chloropyridine-2-carboxylic acid.

1 Water matrices were fully characterized by Agvise Laboratories, Northwood, ND; however, sources were not reported (p. 14; Appendix B, pp. 120-123).

2 EPL BAS Method 205G907A with the use of positive-ion electron-impact ionization mass spectrometry detection. EPL BAS Method 205G907B with the use of tandem mass spectrometry detection.

Table 3. Independent Validation Method Recoveries for Nitrapyrin and Its Transformation Product 6-CPA in Waters[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
EPL BAS Method 205G907A						
Drinking Water						
	Quantitation ion					
Nitrapyrin	0.015 (LOD)	1	NR	--	--	--
	0.05 (LOQ)	5	76-88	82	4.7	5.8
	1.00	5	75-93	83	6.7	8.0
	Confirmation ion (Primary)					
Nitrapyrin	0.015 (LOD)	1	NR	--	--	--
	0.05 (LOQ)	5	81-92	87	3.9	4.4
	1.00	5	75-93	83	7.0	8.4
	Confirmation ion (Secondary)					
Nitrapyrin	0.015 (LOD)	1	NR	--	--	--
	0.05 (LOQ)	5	91-97	94	2.6	2.7
	1.00	5	70-95	84	7.5	9.0
Ground Water						
	Quantitation ion					
Nitrapyrin	0.015 (LOD)	1	NR	--	--	--
	0.05 (LOQ)	5	90-103	98	5.1	5.2
	1.00	5	68-75	71	3.2	4.6
	Confirmation ion (Primary)					
Nitrapyrin	0.015 (LOD)	1	NR	--	--	--
	0.05 (LOQ)	5	84-96	93	5.0	5.4
	1.00	5	67-74	71	2.8	3.9
	Confirmation ion (Secondary)					
Nitrapyrin	0.015 (LOD)	1	NR	--	--	--
	0.05 (LOQ)	5	91-97	94	2.6	2.7
	1.00	5	76-95	84	7.5	9.0
Surface Water						
	Quantitation ion					
Nitrapyrin	0.015 (LOD)	1	NR	--	--	--
	0.05 (LOQ)	5	77-89	82	4.7	5.8
	1.00	5	77-94	85	7.4	8.7
	Confirmation ion (Primary)					
Nitrapyrin	0.015 (LOD)	1	NR	--	--	--
	0.05 (LOQ)	5	78-101	93	8.8	9.5
	1.00	5	78-95	87	7.1	8.2
	Confirmation ion (Secondary)					
Nitrapyrin	0.015 (LOD)	1	NR	--	--	--
	0.05 (LOQ)	5	79-102	92	9.5	10.3
	1.00	5	66-77	72	4.6	6.4
EPL BAS Method 205G907B						
Drinking Water						

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Quantitation ion					
6-CPA	0.015 (LOD)	**3**	51.5-130.8	90.9	39.7	**43.6**
	0.05 (LOQ)	15	67.9-137.7	89.4	18.1	**20.2**
	1.00	15	51.0-173.1	77.6	31.1	**40.1**
	Confirmation ion					
6-CPA	0.015 (LOD)	**3**	48.5-194.3	112.7	74.4	**66.1**
	0.05 (LOQ)	15	66.7-136.3	96.4	16.7	17.3
	1.00	15	50.6-173.0	76.0	31.3	**41.2**
Ground Water						
	Quantitation ion					
6-CPA	0.015 (LOD)	**3**	47.7-138.2	84.6	47.5	**56.2**
	0.05 (LOQ)	15	32.0-94.4	**63.4**	18.5	**29.2**
	1.00	15	32.6-83.4	**58.2**	16.1	**27.7**
	Confirmation ion					
6-CPA	0.015 (LOD)	**3**	55.5-112.3	77.9	30.2	**38.8**
	0.05 (LOQ)	15	29.9-89.6	**61.8**	17.1	**27.7**
	1.00	15	32.7-82.4	**57.4**	15.8	**27.6**
Surface Water						
	Quantitation ion					
6-CPA	0.015 (LOD)	**3**	55.8-109.8	75.3	30.0	**39.8**
	0.05 (LOQ)	15	24.5-102.4	**58.1**	20.5	**35.3**
	1.00	15	16.2-106.5	**54.7**	27.2	**49.6**
	Confirmation ion					
6-CPA	0.015 (LOD)	**3**	38.8-84.2	**64.5**	23.3	**36.1**
	0.05 (LOQ)	15	26.3-80.6	**54.2**	18.8	**34.8**
	1.00	15	15.5-107.0	**53.6**	27.1	**50.7**

Data (uncorrected recovery results; Figures 18-19, pp. 74-77) were obtained from Tables 11-21, pp. 35-45 of MRID 49567301 and DER Attachment 2 (reviewer-calculated mean, s.d. and RSDs for 6-CPA with n = 15 since study author calculated statistics based on n = 5 for 3 batches). 6-CPA = 6-chloropyridine-2-carboxylic acid. NR = not reported; peak area was smaller than that of the lowest calibration standard.

1 Water matrices were characterized; however, sources were not reported (p. 13).

2 EPL BAS Method 205G907A with the use of positive-ion electron-impact ionization mass spectrometry detection. EPL BAS Method 205G907B with the use of tandem mass spectrometry detection.

III. Method Characteristics

In the ECM Methods and ILV, the LOQ and LOD values for nitrapyrin and 6-CPA in water were 0.05 µg/L and 0.015 µg/L, respectively (pp. 15, 25; Tables 3-11, pp. 30-38; Tables 15-20, pp. 42-47; Appendix D, pp. 128; Appendix E, p. 136 of MRID 49567302; p. 20; Tables 22-25, pp. 46-49 of MRID 49567301). The LOD and LOQ for determination of nitrapyrin and its transformation products in water were calculated in the ILV study report using the standard deviation from the 0.05 µg/L recovery results. The LOD was calculated as three times the standard deviation (3*s*), and the LOQ was calculated as ten times the standard deviation (10*s*) of the recovery results. The method for calculating the LOQ and LOD were based on "established practices"; however, the reference was not provided (pp. 20, 24 of MRID 49567301). The reviewer assumed that the reference was Keith, L. H., *et. al*. (1983). The calculated values support the LOQ and LOD established for the study and are presented in **Table 4** below.

Table 4. Method Characteristics

Method		**EPL BAS Method 205G907A[1]**	**EPL BAS Method 205G907B[2]**
Analyte		**Nitrapyrin**	**6-CPA**
Limit of Quantitation (LOQ)	Established	0.05 µg/L	
	Calculated	0.0236-0.0254 µg/L (Q) 0.0194-0.0442 µg/L (C1) 0.0129-0.0701 µg/L (C2)	0.0274-0.1048 µg/L (Q) 0.0492-0.1090 µg/L (C)
Limit of Detection (LOD)	Established	0.015 µg/L	
	Calculated	0.0071-0.0076 µg/L (Q) 0.0058-0.0133 µg/L (C1) 0.0039-0.0210 µg/L (C2)	0.0082-0.0315 µg/L (Q) 0.0138-0.0327 µg/L (C)
Linearity (Least squares calibration curve r and concentration range)	ECM[3]	r^2 = 0.998 (Q) r^2 = 0.999 (C1) r^2 = 0.999 (C2)	r^2 = 1.000 (Q) r^2 = 0.999 (C)
	ILV[4]	r^2 = **0.9910**-0.9972 (Q) r^2 = **0.9922**-0.9962 (C1) r^2 = **0.9922**-0.9968 (C2)	r^2 = **0.8680**-0.9993 (Q) r^2 = **0.8706**-0.9984 (C)
	Concentration range	0.6-50 ng/mL	0.5-100 ng/mL
Repeatable	ECM	Yes at LOQ and 20×LOQ (n = 5). No samples were prepared at 10×LOQ. [drinking, ground and surface water matrices (source not specified)][5]	
	ILV	Yes at LOQ and 20×LOQ (n = 5).	**No** at LOQ and 20×LOQ (n = 15).
		No samples were prepared at 10×LOQ. [drinking, ground and surface water matrices (source not specified)][5]	
Reproducible		Yes	**No**
Specific	ECM/ILV	Confirmation of GC/MS analysis performed with quantification of one or two confirmation ions per analyte.	Two MRM transitions were monitored in LC/MS/MS analysis.
	ECM	No interferences were observed in the matrix control at the analyte retention time; however, LOQ peak was very small.	Matrix interferences were *ca*. 0-75% of the LOQ based on peak area and residue recovery.[6]
		Chromatograms of 10×LOQ were not provided. Matrix effects were insignificant (< 15%).	
	ILV	No interferences were observed; however, most of the provided chromatograms were of poor quality. Residues were quantified as <LOD in the controls.	
		Matrix effects were significant for drinking and ground water (26-64%), but insignificant for surface water (< -4%).	Matrix effects were significant for drinking, ground and surface water (-93.2 to 133.9%).
		Matrix-matched standards were used for all calibration curves.	

Data were obtained from pp. 15, 22-25; Tables 3-22, pp. 30-49; Tables 43-47, pp. 69-73; Figures 3-7, pp. 77-81; Figures 11-22, pp. 85-97; Figures 25-37, pp. 100-112; Appendix D, pp. 128-129; Appendix E, pp. 136-138 of MRID 49567302; pp. 16-21; Tables 2-25, pp. 26-49; Tables 26-32, pp. 50-56; Figures 25-33, pp. 83-91; Appendix I, p. 96;

Appendix II, pp. 102-103 of MRID 49567301 and DER Attachment 2. 6-CPA = 6-chloropyridine-2-carboxylic acid. Q = quantitation ion; C1 = primary confirmation ion; C2 = secondary confirmation ion; C = confirmation ion. Linearity is satisfactory when $r^2 \geq 0.995$.

1 EPL BAS Method 205G907A with the use of positive-ion electron-impact ionization mass spectrometry detection.

2 EPL BAS Method 205G907B with the use of tandem mass spectrometry detection.

3 Only representative calibration curves were presented in the study report; the calibration curve for each ion of each analyte was shown for a different water matrix. The study authors reported that all coefficients of determination were greater than 0.995 for analysis of nitrapyrin and 6-CPA (p. 22; Figures 3-7, pp. 77-81 of MRID 49567302).

4 Solvent-based standards were used. ILV r^2 values are reviewer-generated from reported r values of 0.9955-0.9986 for the quantitative and confirmatory ions of nitrapyrin and 0.93164-0.99967 for the quantitative and confirmatory ions of 6-CPA (Figures 7-9, pp. 48-50 of MRID 49567301; DER Attachment 2).

5 Water characterizations were provided, but source locations for the waters were not reported (p. 14; Appendix B, pp. 120-123 of MRID 49567302; p. 13 of MRID 49567301).

6 Residues in the controls were 0.00225-0.0393 µg/L for the quantitation ion (*ca*. 5-79% of the LOQ and *ca*. 15-250% of the LOD) and 0.0000-0.0208 µg/g for the confirmation ion (*ca*. 0-42% of the LOQ and *ca*. 0-139% of the LOD; Tables 15-20, pp. 42-47 of MRID 49567302).

IV. Method Deficiencies and Reviewer's Comments

1. In the ECM and ILV, no performance data were provided for 10×LOQ to validate the methods EPL BAS Method 205G907A and EPL BAS Method 205G907B, only the LOQ and 20×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. EPL BAS Method 205G907B was not validated by the ILV for the analysis of 6-CPA. Mean recoveries and/or RSDs were unsatisfactory for all analyses, except for the LOQ confirmation ion analysis in drinking water (n = 15; Tables 14-16, pp. 38-40; Tables 20-21, pp. 44-45 of MRID 49567301). RSDs for drinking water were 20.2% at the LOQ (quantitation ion) and 40.1-41.2% at 20×LOQ (both ions); the means were acceptable. Means and RSDs for ground water (both ions) were 57.4-63.4% and 27.6-29.2%, respectively, at the LOQ and 20×LOQ. Means and RSDs for surface water (both ions) were 53.6-58.1% and 34.8-50.7%, respectively, at the LOQ and 20×LOQ. For the individual recoveries of 6-CPA which were outside the acceptable range of 70-120%, the study author reported that additional experimentation indicated that the low accuracy of the fortifications solutions contributed to the poor recoveries, in part (p. 20; Appendix III, pp. 108-109). The study author re-calculated the recoveries from one of the three batches of five samples to account for the degradation of the fortification solution (p. 20; Tables 14-16, pp. 38-40; Tables 20-21, pp. 44-45; DER Attachment 2). Reviewer-calculated means and RSDs using these values did not improve the results of the validation of 6-CPA in any water matrix: means and RSDs at the LOQ and 20×LOQ ranged 60.8-103.5% and 22.9-61.0%, respectively, for all water matrices (both ions).

   The ILV study author calculated statistics for 6-CPA based on n = 5 for each of three batches (Tables 20-21, pp. 44-45 of MRID 49567301). Means and RSDs (batches/fortifications/ions combined) were 55-108% and 4.9-38.5%, respectively, for drinking water, 52-83% and 10.1-33.3%, respectively, for ground water, and 26-84% and 9.1-19.9%, respectively, for surface water. The recalculation of the third batch yielded means and RSDs ranging 71-129% and 12.6-45.5%, respectively (matrices/ions combined). Overall, the only batches which yielded acceptable results for the LOQ (n = 5) were drinking water Batches 14-0606 and 15-0003 (both ions), ground water Batch 14-0618 (both ions) and surface water Batch 14-0625 (quantification ion only). After re-calculation, acceptable results at the LOQ (n = 5) were obtained with ground water Batch 15-0027 (both ions) and surface water Batch 15-0028 (both ions). The study author concluded that "the method showed reasonable accuracy [for 6-CPA] over the concentration range of 0.05-1.0 µg/L…when correcting for accuracy of fortification solutions" (p. 23).

   In the ECM, the EPL BAS Method 205G907B stated that stock, calibration and fortification solutions of 6-CPA should be "maintained refrigerated up to 365 days when not needed in the laboratory" (Appendix E, pp. 137-138 of MRID 49567302).

3. For the ILV, the number of trials performed to validate the methods was not reported. The reported data indicate one trial for EPL BAS Method 205G907A (nitrapyrin) and up to three trials for EPL BAS 205G907B (6-CPA; Tables 11-16, pp. 35-40).

4. In the ILV, linearity was not always satisfactory ($r^2 \geq 0.995$).

5. ILV representative chromatograms were of poor quality (Figures 25-33, pp. 83-91 of MRID 49567301). Chromatograms of nitrapyrin were faint to indecipherable, and the axes were unreadable. Chromatograms of 6-CPA were of poor quality to faint, but the axes were somewhat readable or mostly decipherable. Due to these representative chromatograms, the specificity of the method was difficult to assess, in some cases, in relation to matrix and baseline interferences and contamination.

6. Recoveries of 6-CPA (both ions) were corrected in the ECM (residues were not found in the control samples of nitrapyrin; p. 17; Tables 3-22, pp. 30-49 of MRID 49567302). Residues in the controls were 0.00225-0.0393 µg/L for the quantitation ion (*ca*. 5-79% of the LOQ and *ca*. 15-250% of the LOD) and 0.0000-0.0208 µg/g for the confirmation ion (*ca*. 0-42% of the LOQ and *ca*. 0-139% of the LOD; Tables 15-20, pp. 42-47 of MRID 49567302). Recoveries were not corrected in the ILV (Figures 18-19, pp. 74-77 of MRID 49567301).

7. In the ECM and ILV, the water matrices were characterized, but the sources were not reported (p. 14; Appendix B, pp. 120-123 of MRID 49567302; p. 13 of MRID 49567301).

8. Most of the ILV calculated values for nitrapyrin support the LOQ and LOD established in the study; however, many of the calculated values for 6-CPA were greater than the established LOQ and LOD values (see Table V above; p. 20; Tables 22-25, pp. 46-49 of MRID 49567301).

9. Representative chromatograms of the reagent blank were not included in the ILV (Figures 25-33, pp. 83-91 of MRID 49567301).

10. In the ILV, the study author reported that communications with the sponsor were limited to the discussion of EPL BAS Method 205G907B for the validation of 6-CPA (pp. 21-22; Appendix IV, pp. 110-113 of MRID 49567301). This communication lead the ILV to change HPLC columns and perform the accuracy checks of the fortification solutions.

11. In the ECM, the stability of the final extracts and working standard solutions were investigated; temperatures and lighting conditions of storage were not reported (pp. 22-23; Tables 23-42, pp. 50-68 of MRID 49567302). The extracts of nitrapyrin were shown to be stable for two days (drinking water) and nine days (ground and surface water) under refrigerated storage. The extracts of 6-CPA were shown to be stable for three days (drinking, ground and surface water) under refrigerated storage. The aged working standards of nitrapyrin were shown to be stable for 126 days under frozen storage. The aged working standards of 6-CPA were shown to be stable for nine days under refrigerated storage.

    In the ILV, the stability of the 6-CPA fortification solutions was investigated due to the poor recoveries yielded in the validation experiment (p. 20; Appendix III, pp. 108-109 of MRID 49567301). The ILV study author concluded that degradation was occurring during experimentation (Appendix III, pp. 108-109; Appendix IV, p. 113).

12. The reviewer noted the following errors in the ILV study report: 1) the listing of mean "corrected" recoveries for the quantitative and confirmatory batches should have been reported as "[]% for drinking water, []% for groundwater, and []% for surface water", instead of "[]% for drinking water, []% for groundwater, and []% for drinking water"; and 2) Reference 6 was not included in the References Section in order to identify the method for LOQ/LOD calculation; the reviewer assumed that the reference was Keith, L. H., *et. al*. (1983; pp. 20, 24 of MRID 49567301).

13. It was reported for the ILV that the analytical procedure for one set of samples [calibration samples, a reagent blank, two controls and fortified samples (LOD, LOQ and 20×LOQ)] required approximately eight person hours for EPL BAS Method 205G907A or EPL BAS Method 205G907B (p. 18 of MRID 49567301). The analysis and results work up was performed the following day for each method.

V. References

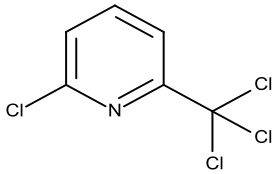
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

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(ccc1)C(Cl)(Cl)Cl)c1Cl



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