Analytical method for Nitrapyrin and 6-CPA in water

Reports:	ECM: EPA MRID No.: 52085702. Senciuc, M. 2022. Method Validation for Determination of Nitrapyrin and 6-CPA in Water. Report prepared by Eurofins Agroscience Services EAG Laboratories GmbH, Ulm, Germany, sponsored and submitted by Corteva Agriscience LLC, Indianapolis, Indiana; 149 pages. Study Code: S21-09121; Sponsor Study ID.: 220060. Final report issued August 19, 2022. ILV: EPA MRID No.: 52085703. Warnick, J. 2023. Independent Laboratory Validation of Method Validation for Determination of Nitrapyrin and 6-CPA in Water. Report prepared by EPL Bio Analytical Services, Niantic, Illinois, sponsored and submitted by Corteva Agriscience LLC, Indianapolis, Indiana; 187 pages. Study No.: 205G3454, Sponsor Study ID: 221108. Final report issued January 11, 2023.
Document No.:	MRIDs 52085702 & 52085703
Guideline:	850.6100
Statements:	ECM: The study was conducted in compliance with the OECD Principles of Good Laboratory Practice (GLP; 1997), ENV/MC/CHEM(98)17, OECD, Paris, 1998, as implemented in national legislation which are compatible with the U.S. EPA FIFRA GLP Standards (40 CFR Part 160; p. 3; Appendix 5, p. 134). Signed and dated Data Confidentiality, GLP, and Quality Assurance statement were provided (pp. 2-4). An Authenticity statement was included with the Quality Assurance statement (p. 4). ILV: The study was conducted in compliance with the U.S. EPA FIFRA Principles of Good laboratory Practice (GLP; 40 CFR Part 160), which are compatible with the OECD GLP (1997), ENV/MC/CHEM(98)17, OECD, Paris, (1998; p. 3). Signed and dated Data Confidentiality, GLP, and Quality Assurance were provided (pp. 2-4). An Authenticity statement was also included with the Quality Assurance statements (p. 4).
Classification:	This analytical method is classified as **acceptable**. Since the reported LOQ in ILV was not based on scientifically acceptable procedures defined in 40 CFR Part 136; in the ILV, the LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The communications between the ECM study author and the ILV study monitor were only administrative (MRID 52424901, Amended Report). The time required to complete a set of samples was not reported in the ILV study.
PC Code:	069203
EFED Final Reviewer:	He Zhong, Ph.D., Biologist — Signature: HeZhong — Date: July 22, 2024
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PB&A/CSS JV Reviewers:	Zach C. Winfield Ph.D., Analytical Chemist — Signature: Zach C. Winfield — Date: 2/23/24

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

Executive Summary

The analytical method, Corteva Agriscience Study ID. 220060, is designed for the quantitative determination of nitrapyrin and 6-CPA (6-chloropicolinic acid) in water at the stated LOQ of 0.05 µg/L using GC-MS/MS and LC-MS/MS, respectively (see **Table 1**). The LOQ (0.05 µg ai/L) is less than daphnid NOAEC (103 µg ai/L), the lowest toxicological level of concern in water for nitrapyrin and 6-CPA. Based on the performance data submitted by the ILV and the ECM, the LLMV was equivalent to the ECM reported method LOQ for nitrapyrin and 6-CPA in the tested water matrices (0.05 µg/L).

The ECM and ILV validated the method using different water matrices; drinking, surface, and ground water matrices were included in both the ECM and ILV. The ILV validated the method for nitrapyrin and 6-CPA in water matrices in the first trial with minor modifications to the analytical parameters. The ILV modifications did not warrant an updated ECM. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for nitrapyrin and 6-CPA in test water matrices.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/year)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Nitrapyrin	52085702[1]	52085703[2]		Water	19/08/2022	Corteva Agriscience LLC	GC-MS/MS	0.05 µg/L
6-CPA							LC-MS/MS	

1 In the ECM, drinking water (pH 7.59, filterable solids 0.33 mg/L, dissolved organic carbon 0.4 mg/L, total hardness (calculated) 2.11 mmol/L, total organic carbon 0.5 mg/L, electrical conductivity at 25°C 490 µS/cm), surface water (pH 8.00, filterable solids 12.9 mg/L, dissolved organic carbon 1.4 mg/L, total hardness (calculated) 3.74 mmol/L, total organic carbon 1.6 mg/L, electrical conductivity at 25°C 773 µS/cm), and ground water (pH 6.96, filterable solids 0.10 mg/L, dissolved organic carbon 0.2 mg/L, total hardness (calculated) 3.49 mmol/L, total organic carbon 0.3 mg/L, electrical conductivity at 25°C 737 µS/cm) were used in the study (p.13; Appendix 3, pp. 124-129 of MRID 52085702). The drinking water was from Wasserhahn Labor 6/EAG Laboratories GmbH, Ulm, Germany, the surface water was from River Schussen, near Bad Schussenried, and the ground water was collected from Landeswasserversorgung Langenau. The waters were characterized by Institut Alpha, GmbH & Co. KG, Wasser- und Umweltanalytik not taken under GLP (p. 13, of MRID 52085702).

2 In the ILV, surface water (Sample reference 3664-S001; pH 8.2, total organic carbon 10.6 ppm, dissolved organic carbon 10.0 ppm, hardness 377 mg/L of $CaCO_3$, conductivity 2.07 mmhos/cm, total suspended solids 20 ppm), ground water (Sample reference 3664-G001; pH 7.3, total organic carbon 8.1 ppm, dissolved organic carbon 7.7 ppm, hardness 443 mg/L of $CaCO_3$, conductivity 0.88 mmhos/cm, total suspended solids 8 ppm), and drinking water (Sample reference 3664-D001; pH 8.1, total organic carbon 5.4 ppm, dissolved organic carbon 5.3 ppm, hardness below detection limit of 1 mg/L of $CaCO_3$, conductivity 0.41 mmhos/cm, total suspended solids 10 ppm) were used in the study (p. 11; Appendix E, pp. 165-170 of MRID 52085703). The waters were collected locally by EPL Bio

Analytical Services, Niantic, Illinois. The waters were characterized by AGVISE Laboratories, Northwood, North Dakota.

The conclusions conveyed in this data evaluation record (DER)were developed in full compliance with *EPA Scientific Integrity Policy for Transparent and Objective Science*, and EPA Scientific Integrity Program's *Approaches for Expressing and Resolving Differing Scientific Opinions.* The full text of *EPA Scientific Integrity Policy for Transparent and Objective Science*, as updated and approved by the Scientific Integrity Committee and EPA Science Advisor can be found here: https://www.epa.gov/system/files/documents/2023-12/scientific_integrity_policy_2012_accessible.pdf. The full text of the EPA Scientific Integrity Program's *Approaches for Expressing and Resolving Differing Scientific Opinions* can be found here: https://www.epa.gov/scientific-integrity/approaches-expressing-and-resolving-differing-scientific-opinions.

I. Principle of the Method

Nitrapyrin

Water samples (100 mL final volume) were transferred into a 250 mL separatory funnel and were fortified with 0.50 mL of a 0.003, 0.01, and 0.1 mg/L or fortification solution, then 20 mL of each, isohexane and MTBE, and 5 g of NaCl were added to each sample, manually shaken for 1 minute and allowed phases to separate (p. 12; Appendix 1, pp. 101-107 of MRID 52085702). The aqueous phase was separated into a beaker, and the organic phase was filtered into a pre-weighed 100 mL pear-shaped flask through silanized glass wool and 10 g Na_2SO_4. The aqueous phase was returned to separatory funnel, the beaker was rinsed with 20 mL isohexane and added to the aqueous phase. The sample was shaken for 1 minute and allowed to separate. The aqueous phase was discarded, the organic phase was filtered into the same pear-shaped flask through the same silanized glass wool and 10 g Na_2SO_4. The funnel, silanized glass wool, and 10 g Na_2SO_4 were rinsed with 10 mL isohexane into the pear-shaped flask. Xylene (2 mL) was added to the combined organic phases. The organic phase was evaporated using Büchi rotary evaporator R 210 V850 at 35 °C to less than 2 mL. The final volume adjusted to 2 mL with xylene gravimetrically and analyzed by GC-MS/MS.

Nitrapyrin analysis was performed using a Thermo Scientific TSQ 8000 Evo triple quadrupole GC-MS/MS system consisting of Trace 1310 gas chromatograph equipped with electron ionization operated in the positive ion mode with multiple reaction monitoring (MRM; Appendix 1, p. 105 of MRID 52085702). The following GC conditions were used: Agilent DB-1701 column (length 30 m, i.d. 0.25 mm) with a film thickness of 0.25 µm, carrier gas: helium with a constant flow of 1.2 mL/minutes, splitless injection mode, injector temperature of 220 °C, injection volume of 1.0 µL, column oven temperature program: 50°C held for 1 minute, increased 15 °C/minute until 280 °C and held for 2.0 minutes, and transfer line temperature of 280 °C. MS ion source temperature was 250 °C. Two ion pair transitions were monitored for nitrapyrin (quantitation and confirmation, respectively): *m/z* 196→160 and *m/z* 194→158 with a reported retention time of *ca.* 10.4 minutes.

6-CPA

Water samples (20 mL final volume) were transferred into a 50 mL plastic centrifuge tube and were fortified with 0.01 mL of a 0.03, 0.1, or 1.0 mg/L fortification solution, then 2 mL 1N hydrochloric acid was added to each sample, tubes capped and mixed (p. 12; Appendix 2, pp. 112-120 of MRID 52085702). Bond Elut Mega BE C18 solid phase extraction (SPE) cartridges (1 g, 6 mL) were rinsed with 5 mL of acetonitrile and conditioned with 5 mL 0.1N HCl. The column beds were kept

wet. The entire sample extract was then transferred to the conditioned cartridges, allowed to percolate under a vacuum (*ca.* 1-2 mL/minute) and effluent was discarded. Cartridges were rinsed with 2 mL of 0.1 N HCl at *ca.* 1-2 mL/minute and dried under vacuum for 40 minutes to no more than 45 minutes (vacuum pressure ≥ -10 inches Hg) to avoid 6-CPA loss. Samples were eluted from cartridges with 5 mL acetonitrile at *ca.* 1 mL/minute flow rate into a glass culture tube. The entire eluent volume was collected using vacuum after columns stopped dripping. Extracts were evaporated to near dryness (*ca.* 100 µL) under a stream of nitrogen in a heating block at 35 °C and then manually taken to dryness using ambient conditions to prevent analyte loss. The sample was then redissolved in 0.5 mL methanol:water (25:75, v:v), vortexed for *ca.* 10 seconds, and refrigerated until analyzed by LC-MS/MS.

Samples were analyzed for 6-CPA using an Agilent 1290 series HPLC coupled with a Sciex 5500 Triple Quadrupole mass spectrometer with Turbo IonSpray ESI source operated in the negative ion mode with multiple reaction monitoring (MRM; Appendix 2, p. 118 of MRID 52085702). The following LC conditions were used: Waters Acquity UPLC HSS T3 column (2.1 mm x 100 mm, 1.8 µm; column temperature 40°C) with Phenomenex AJO 9000/AJO 8782 pre-column, mobile phase of (A) 0.01% (v:v) acetic acid in water and (B) 0.01% (v:v) acetic acid in methanol [mobile gradient phase of percent A:B (v:v) at 0.0-0.10 min. 95.0:5.0, 4.00-4.50 min. 30.0:70.0, 4.51-5.50 min. 5.0:95.0, 5.51-7.50 min. 95.0:5.0], and injection volume of 10.0 µL. The MS ion source temperature was 450°C. Two ion pair transitions were monitored for 6-CPA (quantitation and confirmation, respectively): *m/z* 156→112 and *m/z* 158→114. The reported retention time was *ca.* 3.8 minutes.

The ECM reported a sample set consisting of 11 fortified samples, 1 control blank, and 2 blank controls of either nitrapyrin or 6-CPA prepared by an experienced technicians can be prepared in 5 hours followed by an unattended overnight GC-MS/MS or LC-MS/MS analysis. Including 2 hours of date evaluation and transcription using the respective instruments, a sample set can be completed in one and a half calendar days for each analyte.

ILV

The ILV performed the ECM method as written, except for minor modifications to the instrument parameters (pp. 12-15; Appendix C, pp. 139-151; Appendix D, pp. 152-164 of MRID 52085703). A Zymark Turbovap II system replaced the rotary evaporation apparatus described in ECM. Samples were analyzed for nitrapyrin using Bruker Scion TQ - 436GC system. Two ion pair transitions were monitored for nitrapyrin (quantitation and confirmation, respectively): *m/z* 196→160 and *m/z* 194→158 with a reported retention time of *ca.* 10.4 minutes. Samples were analyzed for 6-CPA using Agilent 1290 HPLC system with an AB SCIEX 6500 triple quadrupole mass spectrometer. The LC-MS/MS parameters were similar to those of the ECM. Two ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 156→112 and *m/z* 158→114. These ion transitions were similar to those of the ECM. Reported retention time of 6-CPA was *ca.* 3.6 minutes. The ILV analytical parameters were similar to ECM method with minor modifications to mass spectrometer parameters due to use of different analytical instruments (pp. 12, 14 of MRID 52085703). These minor modifications did not warrant an updated ECM.

LOD/LOQ

The Limit of Quantification (LOQ) for nitrapyrin and 6-CPA in water was 0.050 µg/L in the ECM and ILV in all matrices (pp. 16-18 of MRID 52085702; p. 11 of MRID 52085703). In the ECM, the

Limit of Detection (LOD) for nitrapyrin and 6-CPA was 0.010 µg/L in all matrices. In the ECM, the LOD and LOQ were calculated using the standard deviation (SD) of the residue levels found in the LOQ recovery sample extracts (n = 5) using the following equations:

$$LOD_{cal} = SD \times 3$$
$$LOQ_{cal} = SD \times 10$$

In the ECM, control samples were fortified at 30% of LOQ (p. 16 of MRID 52085702). The detected chromatographic peak areas in reagent blank/unfortified control samples were less than that of the control samples fortified at 30% of LOQ for all analytes in all matrices (Figure 25, p. 75, Figure 32, p. 79, Figure 39, p. 82, Figure 46, p. 88, Figure 50, p. 92, Figure 54, p. 96 of MRID 52085702). In the ILV, the LOQ was reported to be 0.05 µg/L (p. 11 of MRID 52085703). The LOD(s) for nitrapyrin and 6-CPA were not reported. The method for defining the LOQ was not reported.

II. Recovery Findings

ECM (MRID 52085702): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of nitrapyrin and 6-CPA at fortification levels of 0.05 µg/L (LOQ) and 0.50 µg/L (10× LOQ) in three water matrices (Tables 2-19, pp. 23-38 of MRID 52085702). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses. Drinking water (pH 7.59, filterable solids 0.33 mg/L, dissolved organic carbon 0.4 mg/L, total hardness (calculated) 2.11 mmol/L, total organic carbon 0.5 mg/L, electrical conductivity at 25°C 490 µS/cm); surface water (pH 8.00, filterable solids 12.9 mg/L, dissolved organic carbon 1.4 mg/L, total hardness (calculated) 3.74 mmol/L, total organic carbon 1.6 mg/L, electrical conductivity at 25°C 773 µS/cm); and ground water (pH 6.96, filterable solids 0.10 mg/L, dissolved organic carbon 0.2 mg/L, total hardness (calculated) 3.49 mmol/L, total organic carbon 0.3 mg/L, electrical conductivity at 25°C 737 µS/cm) were used in the study (p.13; Appendix 3, pp. 124-129 of MRID 52085702). The drinking water was from Wasserhahn Labor 6/EAG Laboratories GmbH, Ulm, Germany, the surface water was from River Schussen, near Bad Schussenried, and the ground water was collected from Landeswasserversorgung Langenau. The waters were characterized by Institut Alpha, GmbH & Co. KG, Wasser- und Umweltanalytik not taken under GLP (p. 13, of MRID 52085702).

ILV (MRID 52085703): Mean recoveries and RSDs were within guidelines for analysis of nitrapyrin and 6-CPA at fortification levels of 0.05 µg/L (LOQ) and 0.50 µg/L (10×LOQ) in one water matrix (Tables 3-18, pp. 23-38 of MRID 52085703). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses. The surface water (Sample reference 3664-S001; pH 8.2, total organic carbon 10.6 ppm, dissolved organic carbon 10.0 ppm, hardness 377 mg/L of $CaCO_3$, conductivity 2.07 mmhos/cm, total suspended solids 20 ppm), ground water (Sample reference 3664-G001; pH 7.3, total organic carbon 8.1 ppm, dissolved organic carbon 7.7 ppm, hardness 443 mg/L of $CaCO_3$, conductivity 0.88 mmhos/cm, total suspended solids 8 ppm), and drinking water (Sample reference 3664-D001; pH 8.1, total organic carbon 5.4 ppm, dissolved organic carbon 5.3 ppm, hardness below detection limit of 1 mg/L of $CaCO_3$, conductivity 0.41 mmhos/cm, total suspended solids 10 ppm) were used in the study (p. 11; Appendix E, pp. 165-170 of MRID 52085703). The waters were collected locally by EPL Bio Analytical Services, Niantic, Illinois. The waters were characterized by AGVISE Laboratories, Northwood, North Dakota. The method for nitrapyrin and 6-CPA in water was

validated in the first trial with minor modifications to the analytical parameters due to use of different analytical instruments (pp. 12, 14 of MRID 52085703). These minor modifications did not warrant an updated ECM. The ILV did not include a study of stability.

Table 2. Initial Validation Method Recoveries for Nitrapyrin and 6-CPA in Water[1,2,3]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Drinking Water					
	Quantitation ion transition					
Nitrapyrin	0.015 (LOD)[3]	1	78	--	--	--
	0.05 (LOQ)	5	71-79	74	3	4
	0.5	5	80-90	86	4	4
6-CPA	0.015 (LOD)[3]	1	81	--	--	--
	0.05 (LOQ)	5	73-90	83	7	8
	0.5	6	74-87	80	5	6
	Confirmation ion transition					
Nitrapyrin	0.015 (LOD)[3]	1	97	--	--	--
	0.05 (LOQ)	5	74-79	77	2	3
	0.5	5	83-91	87	4	4
6-CPA	0.015 (LOD)[3]	1	73	--	--	--
	0.05 (LOQ)	5	70-87	79	8	10
	0.5	6	74-88	79	6	7
	Surface Water					
	Quantitation ion transition					
Nitrapyrin	0.015 (LOD)[3]	1	74	--	--	--
	0.05 (LOQ)	5	73-86	80	6	7
	0.5	5	69-96	82	10	13
6-CPA	0.015 (LOD)[3]	1	103	--	--	--
	0.05 (LOQ)	5	86-97	90	6	6
	0.5	6	74-82	79	3	4
	Confirmation ion transition					
Nitrapyrin	0.015 (LOD)[3]	1	77	--	--	--
	0.05 (LOQ)	5	72-90	79	7	9
	0.5	5	71-97	83	10	12
6-CPA	0.015 (LOD)[3]	1	73	--	--	--
	0.05 (LOQ)	5	77-87	83	4	5
	0.5	6	71-80	77	3	4
	Ground Water					
	Quantitation ion transition					
Nitrapyrin	0.015 (LOD)[3]	1	89	--	--	--
	0.05 (LOQ)	5	68-77	74	3	4
	0.5	5	76-102	85	11	13
6-CPA	0.015 (LOD)[3]		91	--	--	--
	0.05 (LOQ)	5	73-97	90	10	11
	0.5	5	84-93	87	3	4
	Confirmation ion transition					
Nitrapyrin	0.015 (LOD)[3]	1	71	--	--	--
	0.05 (LOQ)	5	65-79	75	6	8

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.5	5	75-100	85	10	12
6-CPA	0.015 (LOD)[3]	1	89	--	--	--
	0.05 (LOQ)	5	72-95	88	9	10
	0.5	5	83-93	87	4	4

Data were obtained from p. 16; Tables 2-19, pp. 23-38 of MRID 52085702.

1 In the ECM, drinking water (pH 7.59, filterable solids 0.33 mg/L, dissolved organic carbon 0.4 mg/L, total hardness (calculated) 2.11 mmol/L, total organic carbon 0.5 mg/L, electrical conductivity at 25°C 490 µS/cm); surface water (pH 8.00, filterable solids 12.9 mg/L, dissolved organic carbon 1.4 mg/L, total hardness (calculated) 3.74 mmol/L, total organic carbon 1.6 mg/L, electrical conductivity at 25°C 773 µS/cm); and ground water (pH 6.96, filterable solids 0.10 mg/L, dissolved organic carbon 0.2 mg/L, total hardness (calculated) 3.49 mmol/L, total organic carbon 0.3 mg/L, electrical conductivity at 25°C 737 µS/cm) were used in the study (p.13; Appendix 3, pp. 124-129 of MRID 52085702). The drinking water was from Wasserhahn Labor 6/EAG Laboratories GmbH, Ulm, Germany, the surface water was from River Schussen, near Bad Schussenried, and the ground water was collected from Landeswasserversorgung Langenau. The waters were characterized by Institut Alpha, GmbH & Co. KG, Wasser- und Umweltanalytik not taken under GLP (p. 13, of MRID 52085702).

2 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 196→160 and *m/z* 194→158 for nitrapyrin and *m/z* 156→112 and *m/z* 158→114 for 6-CPA.

3 Recoveries at the LOD were reviewer-calculated based on reported quantified residues since % recoveries at the LOD fortification were not reported in the study report (MRID 52085702).

Table 3. Independent Validation Method Recoveries for Nitrapyrin and 6-CPA in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Surface Water					
	Quantitation ion transition					
Nitrapyrin	0.05 (LOQ)	5	69-108	84	15	18
	0.5	5	72-95	85	10	12
6-CPA	0.05 (LOQ)	5	78-111	95	13	14
	0.5	5	78-101	95	10	10
	Confirmation ion transition					
Nitrapyrin	0.05 (LOQ)	5	85-107	94	9	10
	0.5	5	75-99	85	11	13
6-CPA	0.05 (LOQ)	5	96-116	107	8	7
	0.5	5	75-108	96	13	13
	Ground Water					
	Quantitation ion transition					
Nitrapyrin	0.05 (LOQ)	5	70-103	84	14	16
	0.5	5	66-84	75	8	10
6-CPA	0.05 (LOQ)	5	73-88	81	6	7
	0.5	5	91-110	103	8	7
	Confirmation ion transition					
Nitrapyrin	0.05 (LOQ)	5	89-119	106	14	13
	0.5	5	72-99	84	12	14
6-CPA	0.05 (LOQ)	5	81-94	88	6	7
	0.5	5	94-110	104	6	6
	Drinking Water					
	Quantitation ion transition					

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Nitrapyrin	0.05 (LOQ)	5	75-87	80	5	6
	0.5	5	70-84	78	5	7
6-CPA	0.05 (LOQ)	5	79-92	85	5	6
	0.5	5	93-110	104	7	7
	Confirmation ion transition					
Nitrapyrin	0.05 (LOQ)	5	73-90	82	6	8
	0.5	5	70-81	76	4	6
6-CPA	0.05 (LOQ)	5	85-101	91	7	8
	0.5	5	96-115	106	7	7

Data were obtained from pp. 13-17; Tables 3-18, pp. 23-38 of MRID 52085703.

1 In the ILV, surface water (Sample reference 3664-S001; pH 8.2, total organic carbon 10.6 ppm, dissolved organic carbon 10.0 ppm, hardness 377 mg/L of $CaCO_3$, conductivity 2.07 mmhos/cm, total suspended solids 20 ppm); ground water (Sample reference 3664-G001; pH 7.3, total organic carbon 8.1 ppm, dissolved organic carbon 7.7 ppm, hardness 443 mg/L of $CaCO_3$, conductivity 0.88 mmhos/cm, total suspended solids 8 ppm); and drinking water (Sample reference 3664-D001; pH 8.1, total organic carbon 5.4 ppm, dissolved organic carbon 5.3 ppm, hardness below detection limit of 1 mg/L of $CaCO_3$, conductivity 0.41 mmhos/cm, total suspended solids 10 ppm) were used in the study (p. 11; Appendix E, pp. 165-170 of MRID 52085703). The waters were collected locally by EPL Bio Analytical Services, Niantic, Illinois. The waters were characterized by AGVISE Laboratories, Northwood, North Dakota.

2 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 196→160 and *m/z* 194→158 for nitrapyrin and *m/z* 156→112 and *m/z* 158→114 for 6-CPA.

III. Method Characteristics

The LOQ for nitrapyrin and 6-CPA in water was 0.05 µg/L in the ECM and the ILV (pp. 16-18 of MRID 52085702; p. 11 of MRID 52085703). Following the method of Keith, L. H., *et al*. (see section V. References below), the LOD and LOQ were calculated using the standard deviation (SD) of the residue levels found in the LOQ recovery sample extracts (n = 5) using the following equations in the ECM:

$$LOD_{cal} = SD \times 3$$
$$LOQ_{cal} = SD \times 10$$

Calculated (µg/L)	Nitrapyrin		6-CPA	
	m/z 196→160	m/z 194→158	m/z 156→112	m/z 158→114
Drinking water				
SD	0.00156	0.00117	0.00327	0.00399
LOD	0.00468	0.00351	0.00980	0.0120
LOQ	0.0156	0.0117	0.0327	0.0399
Surface water				
SD	0.00279	0.00388	0.00283	0.00201
LOD	0.00837	0.0116	0.00848	0.00602
LOQ	0.0279	0.0388	0.0283	0.0201
Ground water				
SD	0.00160	0.00286	0.00496	0.00459
LOD	0.00481	0.00857	0.0149	0.0138
LOQ	0.0160	0.0286	0.0496	0.0459

Data obtained from pp. 17-18 of MRID 52085702.

SD = standard deviation; LOD = Limit of Detection; LOQ = Limit of Quantification.

In the ILV, the LOQ and LOD for nitrapyrin and 6-CPA were not justified or further defined. Since the reported LOQ in ILV was not based on scientifically acceptable procedures defined in 40 CFR Part 136; in the ILV, the LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

Table 4. Method Characteristics in Water

		Nitrapyrin	6-CPA
Limit of Quantitation (LOQ)	ECM	0.05 µg/L	
	ECM (calc)	0.0156 µg/L (Q, DW) 0.0117 µg/L (C, DW) 0.0279 µg/L (Q, SW) 0.0388 µg/L (C, SW) 0.0160 µg/L (Q, GW) 0.0286 µg/L (C, GW)	0.0327 µg/L (Q, DW) 0.0399 µg/L (C, DW) 0.0283 µg/L (Q, SW) 0.0201 µg/L (C, SW) 0.0496 µg/L (Q, GW) 0.0459 µg/L (C, GW)
	ILV*	0.05 µg/L	
	ILV (calc)	--	--
Limit of Detection (LOD)	ECM	0.010 µg/L	0.010 µg/L
	ECM (calc)	0.00468 µg/L (Q, DW) 0.00351 µg/L (C, DW) 0.00837 µg/L (Q, SW) 0.0116 µg/L (C, SW) 0.00481 µg/L (Q, GW) 0.00857 µg/L (C, GW)	0.00980 µg/L (Q, DW) 0.0120 µg/L (C, DW) 0.00848 µg/L (Q, SW) 0.00602 µg/L (C, SW) 0.0149 µg/L (Q, GW) 0.0138 µg/L (C, GW)
	ILV	0.01 µg/L	0.01 µg/L
	ILV (calc)	--	--
Linearity (calibration curve r and concentration range)	ECM[1]	r = 0.9993 (Q, DW) r = 0.9980 (C, DW) r = 0.9997 (Q & C, SW) r = 0.9975 (Q, GW) r = 0.9978 (C, GW)	r = 0.9996 (Q & C, DW & SW & GW)
		0.5-50 µg/L	0.4-40 µg/L
	ILV	r = 0.9993 (Q & C, DW) r = 0.9967 (Q, SW) r = 0.9962 (C, SW) r = 0.9958 (Q, GW) r = 0.9978 (C, GW)	r = 0.99975 (Q, DW) r = 0.99970 (C, DW) r = 0.99961 (Q, SW) r = 0.99966 (C, SW) r = 0.99987 (Q, GW) r = 0.99956 (C, GW)
		0.5-50 µg/L	0.4-40 µg/L
Repeatable	ECM[2]	Yes, at LOQ (0.05 µg/L) and 10×LOQ (0.5 µg/L) (one characterized drinking water, one characterized surface water, and one characterized ground water)	Yes, at LOQ (0.05 µg/L) and 10×LOQ (0.5 µg/L) (one characterized drinking water, one characterized surface water, and one characterized ground water)
	ILV[3,4]	Yes, at LOQ (0.05 µg/L) and 10×LOQ (0.5 µg/L) (one characterized surface water, one characterized ground water, and one characterized drinking water)	Yes, at LOQ (0.05 µg/L) and 10×LOQ (0.5 µg/L) (one characterized surface water, one characterized ground water, and one characterized drinking water)
Reproducible		Yes, for 0.05 µg/L (LOQ) and 0.5 µg/L in all water matrices.	
Specific	ECM	Yes, matrix interferences were <7% of the LOQ (based on peak area) in all matrices.	Yes, matrix interferences were <3% of the LOQ (based on peak area) in all matrices.
	ILV	Yes, matrix interferences were <8% of the LOQ (based on peak height).	Yes, matrix interferences were <24% of the LOQ (based on peak area) for quantitation transition ion and peak integration was incomplete for confirmation transition ion in SW control. Also, baseline elevated for transition ion pairs in DW control. Matrix interferences

			were <4% for all other matrices.

Data were obtained from pp. 16-18 (LOQ/LOD); Tables 2-19, pp. 23-38 (recovery results); pp. 13-16; Figures 3, 5, 7, 9, 11, 13, 15, pp. 56, 58, 60, 62, 64, 66, 68, (calibration curves); Figures 20-56, pp. 73-98 (chromatograms) of MRID 52085702; and p. 11 (LOQ/LOD); pp. 13-17; Tables 3-18, pp. 23-38 (recovery results); pp. 12, 14, 16; Figures 1-12, pp. 44-55 (calibration curves); Figures 13-84, pp. 55-127 (chromatograms) of MRID 52085703. Q = quantitation ion transition; C = confirmation ion transition; DW = drinking water; GW = ground water; SW = surface water; -- = not reported.

* Since the reported LOQ in ILV was not based on scientifically acceptable procedures defined in 40 CFR Part 136; in the ILV, the LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

1 ECM correlation coefficients (r) for nitrapyrin were reviewer-calculated based on r^2 values reported in the study report (Figures 3, 5, 7, 9, 11, 13, pp. 56, 58, 60, 62, 64, 66 of MRID 52085702; DER Attachment 2). Matrix-matched calibration standards were used for nitrapyrin, and non-matrix-matched calibration standards were used for 6-CPA in the ECM and ILV (p. 19; Tables 20-21, pp. 39-40 of MRID 52085702; p. 17; Tables 21-22, pp. 41-42 of MRID 52085703).

2 In the ECM, drinking water (pH 7.59, filterable solids 0.33 mg/L, dissolved organic carbon 0.4 mg/L, total hardness (calculated) 2.11 mmol/L, total organic carbon 0.5 mg/L, electrical conductivity at 25°C 490 µS/cm); surface water (pH 8.00, filterable solids 12.9 mg/L, dissolved organic carbon 1.4 mg/L, total hardness (calculated) 3.74 mmol/L, total organic carbon 1.6 mg/L, electrical conductivity at 25°C 773 µS/cm); and ground water (pH 6.96, filterable solids 0.10 mg/L, dissolved organic carbon 0.2 mg/L, total hardness (calculated) 3.49 mmol/L, total organic carbon 0.3 mg/L, electrical conductivity at 25°C 737 µS/cm) were used in the study (p.13; Appendix 3, pp. 124-129 of MRID 52085702). The drinking water was from Wasserhahn Labor 6/EAG Laboratories GmbH, Ulm, Germany, the surface water was from River Schussen, near Bad Schussenried, and the ground water was collected from Landeswasserversorgung Langenau. The waters were characterized by Institut Alpha, GmbH & Co. KG, Wasser- und Umweltanalytik not taken under GLP (p. 13, of MRID 52085702).

3 In the ILV, surface water (Sample reference 3664-S001; pH 8.2, total organic carbon 10.6 ppm, dissolved organic carbon 10.0 ppm, hardness 377 mg/L of $CaCO_3$, conductivity 2.07 mmhos/cm, total suspended solids 20 ppm); ground water (Sample reference 3664-G001; pH 7.3, total organic carbon 8.1 ppm, dissolved organic carbon 7.7 ppm, hardness 443 mg/L of $CaCO_3$, conductivity 0.88 mmhos/cm, total suspended solids 8 ppm); and drinking water (Sample reference 3664-D001; pH 8.1, total organic carbon 5.4 ppm, dissolved organic carbon 5.3 ppm, hardness below detection limit of 1 mg/L of $CaCO_3$, conductivity 0.41 mmhos/cm, total suspended solids 10 ppm) were used in the study (p. 11; Appendix E, pp. 165-170 of MRID 52085703). The waters were collected locally by EPL Bio Analytical Services, Niantic, Illinois. The waters were characterized by AGVISE Laboratories, Northwood, North Dakota.

4 The ILV validated the method for nitrapyrin and 6-CPA in water in the first trial with minor modifications to the analytical parameters (pp. 10, 16-17 of MRID 52085703). The ILV modifications did not warrant an updated ECM.

IV. Method Deficiencies and Reviewer's Comments

1. In the ECM, LOQ was based on scientifically acceptable procedures defined in 40 CFR Part 136. Since the reported LOQ in ILV was not based on scientifically acceptable procedures defined in 40 CFR Part 136; in the ILV, the LOQ is the lowest level of method validation (LLMV) rather than an LOQ (pp. 16-18 of MRID 52085702; p. 11 of MRID 52085703). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for nitrapyrin and 6-CPA in the tested water matrices (0.05 µg/L).

2. The ILV performed the ECM method as written, except for minor modifications to the analytical parameters due to use of different analytical instruments (pp. 12-15; Appendix C, pp. 139-151; Appendix D, pp. 152-164 of MRID 52085703). The ILV modifications did not warrant an updated ECM; analytical instrument optimization is expected (pp. 12, 14 of MRID 52085703).

3. The communication between the ILV study personnel and ECM study author were only administrative (MRID 52424901, Amended Report). The OCSPP 850.6100 guideline requires all communications between ILV personnels and ECM developers or previous users be logged and reported to the agency documenting that such communication did not compromise the independent evaluation.

4. The determinations of the LOD and LOQ in the ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 11 of MRID 52085703). In the ECM, the LOD and LOQ were calculated using the standard deviation (SD) of the residue levels found in the LOQ recovery sample extracts (n = 5) using the following equations:

$$LOD_{cal} = SD \times 3$$
$$LOQ_{cal} = SD \times 10$$

   In the ILV, the LOD for nitrapyrin and 6-CPA was not justified or further defined. Since the reported LOQ in ILV was not based on scientifically acceptable procedures defined in 40 CFR Part 136; in the ILV, the LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

5. Matrix effects were calculated in the ECM study at 5.0 and 50 ng/mL for both nitrapyrin and 6-CPA. In the ILV, matrix effects at 10×LOQ were evaluated at 25 ng/mL. Matrix effects for the quantitative and confirmation transitions were calculated for nitrapyrin and 6-CPA in all three water matrices to standards. Significant matrix effects (up to 1000%) were observed in all matrices for nitrapyrin at 50 ng/mL, suggesting signal enhancement. Significant matrix effects were not observed for 6-CPA at 20 ng/mL (p. 19 and pp. 39-40 of MRID 52085702). The ECM did not discuss the impact of this matrix enhancement on the performance of the method. The ILV reported that matrix effects were <15% at 25 ng/mL (p. 17 and pp. 39-42 of MRID 52085703.

   The reviewer noted that the ECM included the matrix effects equation in the footnotes of Tables 20 and 21 (pp. 38-39 of MRID 52085702). The equation did not include the calculated

value multiplied by 100, however, the calculation was performed correctly within the tabulated results.

6. The matrix interferences observed in the representative chromatograms were determined to be insignificant (<24%) in the ECM and ILV for nitrapyrin and 6-CPA. Non-matrix-matched calibration standards were used in the ECM and ILV for 6-CPA but not for nitrapyrin (p. 19; Tables 20-21, pp. 39-40 of MRID 52085702; and p. 17; Tables 21-22, pp. 41-42 of MRID 52085703).

7. The total time required to complete one set of samples (n = 14) was reported as one and a half working days for nitrapyrin and 6-CPA in the ECM (p. 15 of MRID 52085702) but was not reported in the ILV.

8. The confirmation ion had a higher recovery in multiple matrices reported in the ECM. Using the confirmation ion pair transition may lower LOD and LOQ.

9. The stability of working standard solutions and final sample volumes was evaluated by the ECM (pp. 18-19, 41-42 of MRID 52085702). The stability of nitrapyrin and 6-CPA, both at 0.50 µg/L, in final sample volumes were found to be stable at 10×LOQ (0.50 µg/L) at least 11 days when stored in a dark refrigerator. The mean recoveries in the stored samples were within the range of 70-120% and had a relative standard deviation ≤ 20%. Nitrapyrin and 6-CPA standard solutions in solvents were stable (<10%) when stored in dark refrigerator for up to 17 days and 48 days, respectively.

10. The ECM conducted a stability study of standard in varied solvents and of final volume samples. The ILV did not contain a stability study.

V. References

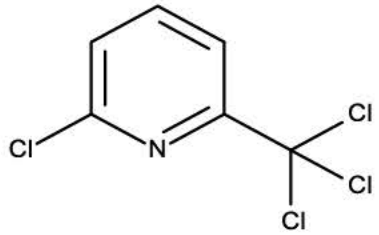
Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* 1983, *55*, 2210-2218 (Appendix D, p. 66 of MRID 51120701).

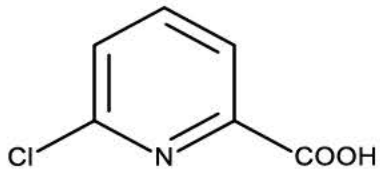
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

USEPA. 2012. Environmental Chemistry Method Guidance. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 344-347, and Revision 2; 2015 and 2016.

DER ATTACHMENT 1. Nitrapyrin and Its Environmental Transformation Products. [A]

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)	Final %AR (study length)
PARENT						
Nitrapyrin (Nitra)	**IUPAC:** 2-Chloro-6-trichloromethylpyridine **CAS:** 2-Chloro-6-(trichloromethyl)pyridine **CAS No.:** 1929-82-4 **Formula:** $C_6H_3Cl_4N$ **MW:** 230.9 g/mol **SMILES:** n(c(ccc1)C(Cl)(Cl)Cl)c1Cl		850.6100 ECM/ILV Water	52085702 52085703	PRT	PRT

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)	Final %AR (study length)
6-Chloropicolinic acid (6-CPA)	**IUPAC:** 6-Chloropyridine-2-carboxylic acid **CAS No.:** 4684-94-0 **Formula:** $C_6H_4ClNO_2$ **MW:** 157.56 g/mol **SMILES:** OC(=O)c1cccc(Cl)n1		850.6100 ECM/ILV Water	52085702 52085703	PRT	PRT
MAJOR (>10%) TRANSFORMATION PRODUCTS						
No major transformation products were identified.						
MINOR (<10%) TRANSFORMATION PRODUCTS						
No minor transformation products were identified.						
REFERENCE COMPOUNDS NOT IDENTIFIED						
All compounds used as reference compounds were identified.						

[A] AR means “applied radioactivity”. MW means “molecular weight”. PRT means “parent”. ECM means "environmental chemical methods". ILV means "independent laboratory validation".