

Analytical method for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), and degradates IN-LXT69 and IN-QFH57 in water

Reports: ECM: EPA MRID No.: 47560230. Pentz, A.M., and S.C. Nanita. 2008. Analytical Method for the Determination of DPX-KJM44, DPX-MAT28, IN-LXT69, and IN-QFH57 in Water using LC/MS/MS. Report prepared by E.I. du Pont de Nemours and Company, Newark, Delaware, and sponsored and submitted by E.I. du Pont de Nemours and Company, Wilmington, Delaware; 78 pages. DuPont Study No.: DuPont-22042. Final report issued May 27, 2008.

ILV: EPA MRID No.: 48333616. Rockwell, D. 2010. Independent Laboratory Validation of Analytical Method DuPont-22042, "Analytical Method for the Determination of DPX-KJM44, DPX-MAT28, IN-LXT69, and IN-QFH57 in Water using LC/MS/MS". Report prepared by Pyxant Labs Inc., Colorado Springs, Colorado, and sponsored and submitted by E.I. du Pont de Nemours and Company, Wilmington, Delaware; 65 pages. DuPont Study No.: DuPont-24562. Pyxant Labs Inc. ID: 2010. Final report issued January 19, 2010.

Document No.: MRIDs 47560230 & 48333616

Guideline: 850.6100

Statements: ECM: The study was not required to be conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160), which are compatible with OECD Principles of GLP (1998; p. 3 of MRID 47560230). Signed and dated Data Confidentiality, GLP, and Certification of Authenticity statements were provided (pp. 2-4). No Quality Assurance statement was included.

ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160), which are compatible with OECD Principles of GLP (1998; p. 3 of MRID 48333616). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Certification of Authenticity statements were provided (pp. 2-5). An Authenticity statement was also included with the Quality Assurance statement (p. 4).


Classification: This analytical method is classified as **unacceptable**. The ILV was not conducted independently of the ECM since the ILV study author communicated directly with the ECM study author regarding technical issues. The ILV water matrix was not characterized. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ. Sample recoveries were corrected when residues were quantified in the controls.

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

Executive Summary

The analytical method, DuPont Study No. DuPont-22042, is designed for the quantitative determination of aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), and degradates IN-LXT69 and IN-QFH57 in water at the stated LOQ of 0.10 ng/g (or 0.10 ng/mL, assuming a density for water of 1 g/mL) using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water for aminocyclopyrachlor, aminocyclopyrachlor methyl, IN-LXT69 and IN-QFH57.

Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57 in the tested water matrices (0.10 ng/g or 0.10 ng/mL).

The ECM validated the method using four characterized water matrices – two surface (pond and creek), one ground (well), and one drinking; the ILV validated the method using uncharacterized surface (pond) water. The ILV was not conducted independently of the ECM since the ILV study author (David Rockwell) communicated directly with the ECM study author (Sergio Nanita) regarding technical issues for the validation of IN-QFH57. The ILV performed the method with insignificant modifications to the analytical parameters. The ILV validated the method in the first trial for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), and IN-LXT69. The ILV validated the method in the second trial for IN-QFH57; performance data was acceptable in the first trial, but the RSD for the analytical standard response factors were >20%. The ILV modifications did not warrant an updated ECM; however, the ECM contained many critical steps/precautions.

All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for all analytes in tested water matrices. Sample recoveries were corrected when residues were quantified in the controls.

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						
Aminocyclopyrachlor (DPX-MAT28), Aminocyclopyrachlor methyl (DPX-KJM44) IN-LXT69 IN-QFH57	47560230 ¹	48333616 ²	Unacceptable	Water	27/05/2008	E.I. du Pont de Nemours and Company	LC/MS/MS	0.10 ng/g ³ (0.10 ng/mL)

1 In the ECM, surface (White Clay Creek) water (Trial ID: Creek Water-White Clay; pH 7.3, conductivity 0.31 mmhos/cm, hardness 126 mg equiv. CaCO₃/L, total dissolved solids 322 ppm) collected from Newark, Delaware, surface (Lums Pond) water (Trial ID: Lums Pond Water; pH 6.8, conductivity 0.17 mmhos/cm, hardness 42 mg equiv. CaCO₃/L, total dissolved solids 340 ppm) collected from Bear, Delaware, drinking (Newark Drinking) water (Trial ID: Tap Water – Stine Newark; pH 7.7, conductivity 0.39 mmhos/cm, hardness 153 mg equiv. CaCO₃/L, total dissolved solids 456 ppm) collected from Stine-Haskell Research Center, Newark, Delaware, and ground (Kemblesville Well) water (Trial ID: Well Water - Kemblesville); pH 7.4, conductivity 0.17 mmhos/cm, hardness 57 mg equiv. CaCO₃/L, total dissolved solids 210 ppm) collected from Kemblesville, Pennsylvania were characterized by Agvise Laboratories, Northwood, North Dakota, and used in the study (p. 17; Appendix 4, pp. 75-78 of MRID 47560230).

2 In the ILV, uncharacterized surface water was collected from a pond in Colorado and used in the study (p. 14 of MRID 48333616).

3 Units for LOQ and fortifications was provided as “ng/g” in the ECM, which is equivalent to “ng/mL”.

I. Principle of the Method

Water samples (20 mL) were fortified (0.200 mL of 10.0 or 100 ng/mL fortification solution) and acidified by adding 0.060 mL of concentrated formic acid (*ca.* 0.3% formic acid final concentration; pp. 18-20, 26-27 of MRID 47560230). The samples were purified via Oasis MCX solid phase extraction (SPE) cartridges (500 mg, 6 cc) which were preconditioned with 5 mL of methanol then 2 column volumes of 0.01% aqueous formic acid (*ca.* 6 mL) at *ca.* 5 mL/min. Light vacuum can be used but should be removed before the column runs dry. After loading the sample at ≤1 mL/min. (critical step), the sample container was rinsed with 2 mL of 0.2% aqueous formic acid. The rinsate was added to the SPE column at ≤1 mL/min. (critical step). After the sample is completely applied to the column, vacuum is used to slowly pull the remaining solvent through the column. Polypropylene collection tubes were charged with 1.0 mL of 0.2% aqueous formic acid before collection of analytes. Analytes were eluted using 15.0 mL of 75mM ammonium hydroxide in methanol (e.g., 3 x 5 mL) at ≤1 mL/min. (critical step). After vortex mixing, the samples were reduced to *ca.* 2 mL under nitrogen gas in a water bath at 40°C (plasticware is required since glassware may lower analyte recoveries). The residue was mixed with *ca.* 2 mL of 0.01% aqueous formic acid, then the volume was adjusted to 5 mL with additional 0.01% aqueous formic acid with vigorous mixing. Samples were filtered (0.45 μm PTFE) into clean 15-mL propylene centrifuge tubes. For quantification of IN-QFH57, 1.0 mL of the filtered sample was transferred to an autosampler vial and analyzed by LC/MS/MS. For quantification of the other three analytes, autosampler vials were charged with 0.900 mL 0.01% aqueous formic acid then 0.100 mL of the filtered sample was added prior to analysis by liquid chromatography/mass spectrometry (LC/MS/MS).

Samples were analyzed for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57 using an Agilent 1100 high performance liquid chromatography (HPLC) coupled with an Applied Biosystems API-5000 triple quadrupole MS with electrospray interface (ESI) operated in the positive ion mode (DPX-MAT28, DPX-KJM44, and IN-LXT69) or negative ion mode (IN-QFH57) with multiple reaction monitoring (MRM; pp. 19-21 of MRID 47560230). The following LC conditions were used: Luna Phenyl-Hexyl column (4.6 mm x 150 mm, 3 μ m; column temperature 30°C), mobile phase of (A) 0.1% formic acid in HPLC-grade water and (B) HPLC-grade methanol [mobile gradient phase of percent A:B (v:v) at 0.00 min, 95:5, 5.00 min. 41:59, 8.00-10.00 min. 1:99, 10.10-14.50 min. 95:5] and injection volume of 60 μ L. MS source temperature was 325°C. Two ion pair transitions were monitored as follows (quantitation and confirmation, respectively): m/z 214.0 \rightarrow 68.0 and m/z 214.0 \rightarrow 101.0 for aminocyclopyrachlor (DPX-MAT28), m/z 228.0 \rightarrow 68.0 and m/z 228.0 \rightarrow 168.0 for aminocyclopyrachlor methyl (DPX-KJM44), m/z 170.0 \rightarrow 76.0 and m/z 170.0 \rightarrow 103.0 for IN-LXT69, and m/z 176.27 \rightarrow 131.9 and m/z 176.27 \rightarrow 105.0 for IN-QFH57. Reported retention times were *ca.* 5.1, 8.9, 3.8, and 8.3 minutes for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57, respectively. HPLC needle washes and thoroughly clean glassware or disposable equipment is strongly recommended (pp. 26-27).

The ILV performed the ECM method (DuPont Study No. DuPont-22042) as written, except for insignificant modifications to the analytical parameters (pp. 14-18 of MRID 48333616). Samples were analyzed for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57 using Shimadzu LC-20AD HPLC coupled with an MDS Sciex API 5000 LC/MS/MS. The LC/MS/MS parameters were the same as those of the ECM, with the exception that the injection volume was reduced to 35 μ L for IN-QFH57 analysis and some minor MS parameters. The reviewer noted that the dimensions of the Phenomenex Luna Phenyl-Hexyl column were incorrectly reported as 4.6 mm x 15 mm, 3 μ m, in the study report, instead of 4.6 mm x 150 mm, 3 μ m (Part No. 00F-4256-E0; <https://www.phenomenex.com/Products/Part/00F-4256-E0>). Two ion pair transitions were monitored as follows (quantitation and confirmation, respectively): m/z 214.2 \rightarrow 68.0 and m/z 214.2 \rightarrow 100.9 for aminocyclopyrachlor (DPX-MAT28), m/z 227.6 \rightarrow 68.1 and m/z 227.6 \rightarrow 168.2 for aminocyclopyrachlor methyl (DPX-KJM44), m/z 170.0 \rightarrow 76.0 and m/z 170.0 \rightarrow 103.0 for IN-LXT69, and m/z 176.0 \rightarrow 131.9 and m/z 176.0 \rightarrow 105.0 for IN-QFH57. These ion transitions were similar to those of the ECM. Reported retention times were *ca.* 4.57, 8.24, 3.69, and 7.09 minutes for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57, respectively. The ILV modifications did not warrant an updated ECM. The ECM critical steps (“Section 5.3”) were communicated to the ILV (Appendix 2, p. 64).

The Limit of Quantification (LOQ) for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57 in water was 0.10 ng/g in the ECM and ILV (pp. 9, 26; Appendix 2, pp. 65-66 of MRID 47560230; pp. 10, 21 of MRID 48333616). In the ECM, the Limit of Detection (LOD) for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57 was estimated as 0.02, 0.001, 0.005, and 0.03 ng/g for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57, respectively, in all water matrices. The LOD was not reported in the ILV. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

II. Recovery Findings

ECM (MRID 47560230): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69 and IN-QFH57 at fortification levels of 0.10 ng/g (LOQ) and 1.0 ng/g (10 \times LOQ) in four water matrices (p. 10; Tables 1-4, pp. 30-33). Two ion pair transitions were monitored; however, only recoveries from the quantitation ion transition were quantified. The quantitation ion recoveries were confirmed using the ion ratios of the two ion transitions (pp. 27-28). The calculations indicated that recoveries were to be corrected for residues quantified in the controls; residues were only quantified in the controls for aminocyclopyrachlor methyl (DPX-KJM44) in ground (well) water (pp. 22-24; Appendix 1, pp. 57-64). Units for LOQ and fortifications was provided as “ng/g” in the ECM, which is equivalent to “ng/mL” (density of water assumed to be 1 g/mL). The surface (White Clay Creek) water (Trial ID: Creek Water-White Clay; pH 7.3, conductivity 0.31 mmhos/cm, hardness 126 mg equiv. CaCO₃/L, total dissolved solids 322 ppm) collected from Newark, Delaware, surface (Lums Pond) water (Trial ID: Lums Pond Water; pH 6.8, conductivity 0.17 mmhos/cm, hardness 42 mg equiv. CaCO₃/L, total dissolved solids 340 ppm) collected from Bear, Delaware, drinking (Newark Drinking) water (Trial ID: Tap Water – Stine Newark; pH 7.7, conductivity 0.39 mmhos/cm, hardness 153 mg equiv. CaCO₃/L, total dissolved solids 456 ppm) collected from Stine-Haskell Research Center, Newark, Delaware, and ground (Kemblesville Well) water (Trial ID: Well Water - Kemblesville; pH 7.4, conductivity 0.17 mmhos/cm, hardness 57 mg equiv. CaCO₃/L, total dissolved solids 210 ppm) collected from Kemblesville, Pennsylvania were characterized by Agvise Laboratories, Northwood, North Dakota, and used in the study (p. 17; Appendix 4, pp. 75-78).

ILV (MRID 48333616): Mean recoveries and RSDs were within guidelines for analysis of aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69 and IN-QFH57 at fortification levels of 0.10 ng/mL (LOQ) and 1.0 ng/mL (10 \times LOQ) in one water matrix (p. 11; Tables 1-3, pp. 23-25). Two ion pair transitions were monitored; however, only recoveries from the quantitation ion transition were quantified. The uncharacterized surface water was collected from a pond in Colorado and used in the study (p. 14). The method (DuPont Study No.: DuPont-22042) was validated with insignificant modifications to the analytical parameters (pp. 10, 14-21; Appendix 2, pp. 64-65). The ILV validated the method in the first trial for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), and IN-LXT69. The ILV validated the method in the second trial for IN-QFH57; performance data was acceptable in the first trial, but the RSD for the analytical standard response factors were $>$ 20%.

Table 2. Initial Validation Method Recoveries for Aminocyclopyrachlor (DPX-MAT28), Aminocyclopyrachlor methyl (DPX-KJM44), and Degradates IN-LXT69 and IN-QFH57 in Water^{1,2}

Analyte	Fortification Level (ng/g) ³	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface (Creek) Water						
Quantitation ion transition						
Aminocyclopyrachlor (DPX-MAT28)	0.10 (LOQ)	5	87-97	92	4	4
	1.0	5	80-92	87	5	6
Aminocyclopyrachlor methyl (DPX-KJM44)	0.10 (LOQ)	5	99-104	101	2	2
	1.0	5	94-100	98	2	3

Analyte	Fortification Level (ng/g) ³	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
IN-LXT69	0.10 (LOQ)	5	86-96	93	4	5
	1.0	5	95-103	99	3	3
IN-QFH57	0.10 (LOQ)	5	82-96	88	5	6
	1.0	5	94-99	97	2	2
Surface (Pond) Water						
Quantitation ion transition						
Aminocyclopyrachlor (DPX-MAT28)	0.10 (LOQ)	5	83-102	93	9	9
	1.0	5	77-98	87	10	11
Aminocyclopyrachlor methyl (DPX-KJM44)	0.10 (LOQ)	5	93-99	96	3	3
	1.0	5	88-99	92	5	5
IN-LXT69	0.10 (LOQ)	5	83-93	89	4	5
	1.0	5	92-98	94	3	3
IN-QFH57	0.10 (LOQ)	5	85-95	90	4	4
	1.0	5	88-101	96	5	5
Drinking Water						
Quantitation ion transition						
Aminocyclopyrachlor (DPX-MAT28)	0.10 (LOQ)	5	66-93	79	10	13
	1.0	5	72-80	76	3	4
Aminocyclopyrachlor methyl (DPX-KJM44)	0.10 (LOQ)	5	72-98	88	10	12
	1.0	5	77-96	84	8	10
IN-LXT69	0.10 (LOQ)	5	68-93	81	10	12
	1.0	5	82-98	87	7	8
IN-QFH57	0.10 (LOQ)	5	74-86	80	4	5
	1.0	5	78-92	83	5	7
Ground (Well) Water						
Quantitation ion transition						
Aminocyclopyrachlor (DPX-MAT28)	0.10 (LOQ)	5	72-94	78	9	12
	1.0	5	74-87	80	5	7
Aminocyclopyrachlor methyl (DPX-KJM44)	0.10 (LOQ)	5	80-106	88	10	12
	1.0	5	77-96	87	9	10
IN-LXT69	0.10 (LOQ)	5	78-101	84	10	11
	1.0	5	79-97	89	8	9
IN-QFH57	0.10 (LOQ)	5	70-93	78	9	11
	1.0	5	80-98	88	8	10

Data (calculations indicated that recoveries were to be corrected for residues quantified in the controls; residues were only quantified in the controls for aminocyclopyrachlor methyl (DPX-KJM44) in ground (well) water; pp. 22-24; Appendix 1, pp. 57-64) were obtained from p. 10; and Tables 1-4, pp. 30-33 of MRID 47560230. ***Bold italics*** text indicates value outside acceptable range.

1 The surface (White Clay Creek) water (Trial ID: Creek Water-White Clay; pH 7.3, conductivity 0.31 mmhos/cm, hardness 126 mg equiv. CaCO₃/L, total dissolved solids 322 ppm) collected from Newark, Delaware, surface (Lums Pond) water (Trial ID: Lums Pond Water; pH 6.8, conductivity 0.17 mmhos/cm, hardness 42 mg equiv. CaCO₃/L, total dissolved solids 340 ppm) collected from Bear, Delaware, drinking (Newark Drinking) water (Trial ID: Tap Water – Stine Newark; pH 7.7, conductivity 0.39 mmhos/cm, hardness 153 mg equiv. CaCO₃/L, total dissolved solids 456 ppm) collected from Stine-Haskell Research Center, Newark, Delaware, and ground (Kemblesville Well) water (Trial ID: Well Water - Kemblesville); pH 7.4, conductivity 0.17 mmhos/cm, hardness 57 mg equiv. CaCO₃/L, total dissolved solids 210 ppm) collected from Kemblesville, Pennsylvania were characterized by Agvise Laboratories, Northwood, North Dakota, and used in the study (p. 17; Appendix 4, pp. 75-78).

2 Two ion pair transitions were monitored as follows (quantitation and confirmation, respectively): *m/z* 214.0→68.0 and *m/z* 214.0→101.0 for aminocyclopyrachlor (DPX-MAT28), *m/z* 228.0→68.0 and *m/z* 228.0→168.0 for

aminocyclopyrachlor methyl (DPX-KJM44), m/z 170.0→76.0 and m/z 170.0→103.0 for IN-LXT69, and m/z 176.27→131.9 and m/z 176.27→105.0 for IN-QFH57. Only recoveries from the quantitation ion transition were quantified.

3 Units for LOQ and fortifications was provided as “ng/g” in the ECM, which is equivalent to “ng/mL” (density of water assumed to be 1 g/mL).

Table 3. Independent Validation Method Recoveries for Aminocyclopyrachlor (DPX-MAT28), Aminocyclopyrachlor methyl (DPX-KJM44), and Degradates IN-LXT69 and IN-QFH57 in Water^{1,2}

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
Surface (Creek) Water						
Quantitation ion transition						
Aminocyclopyrachlor (DPX-MAT28)	0.10 (LOQ)	5	96-108	102	5	5
	1.0	5	96-106	99	4	4
Aminocyclopyrachlor methyl (DPX-KJM44)	0.10 (LOQ)	5	88-92	90	2	2
	1.0	5	84-89	86	2	2
IN-LXT69	0.10 (LOQ)	5	97-101	99	1	1
	1.0	5	91-100	96	4	3
IN-QFH57	0.10 (LOQ)	5	107-122	114	7	7
	1.0	5	110-117	114	3	2

Data (calculations indicated that recoveries were to be corrected for residues quantified in the controls; p. 18) were obtained from p. 11; and Tables 1-3, pp. 23-25 of MRID 48333616; and DER Excel Attachment.

1 The uncharacterized surface water was collected from a pond in Colorado and used in the study (p. 14).

2 Two ion pair transitions were monitored as follows (quantitation and confirmation, respectively): m/z 214.2→68.0 and m/z 214.2→100.9 for aminocyclopyrachlor (DPX-MAT28), m/z 227.6→68.1 and m/z 227.6→168.2 for aminocyclopyrachlor methyl (DPX-KJM44), m/z 170.0→76.0 and m/z 170.0→103.0 for IN-LXT69, and m/z 176.0→131.9 and m/z 176.0→105.0 for IN-QFH57. These ion transitions were similar to those of the ECM. Only recoveries from the quantitation ion transition were quantified.

3 Standard deviations were reviewer-calculated from the recoveries reported in the study report since the study authors did not calculate these values (see DER Excel Attachment). Rules of significant figures were followed.

III. Method Characteristics

The LOQ for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57 in water was 0.10 ng/mL (0.10 ng/g) in the ECM and ILV (pp. 9, 26; Appendix 2, pp. 65-66 of MRID 47560230; pp. 10, 21 of MRID 48333616). In the ECM, the LOQ was defined as the lowest fortification level validated at which average recoveries of 70-110% and a RSD <20% were achieved. Also, in the ECM, the LOQ was defined as the level which the analyte peak has a signal-to-noise ratio of *ca.* 5-20 to 1 for the least responsive analyte (IN-QFH57). In the ECM, the LOD was calculated as 0.02, 0.001, 0.005, and 0.03 ng/g for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57, respectively, in all water matrices from the signal-to-noise response of each analyte in matrix at the LOQ level using the following equation: $[(3/1)/LOD] = \{ \{ [sample\ response\ (cps)] / 1 \} / [sample\ recovery\ (ng/g)] \}$. The LOD was not reported in the ILV.

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

Table 4. Method Characteristics in Water

		Aminocyclopyrachlor (DPX-MAT28)	Aminocyclopyrachlor methyl (DPX-KJM44)	IN-LXT69	IN-QFH57
Limit of Quantitation (LOQ)*	ECM	0.10 ng/g (0.10 ng/mL) ¹			
	ILV				
Limit of Detection (LOD)	ECM (calc)	0.02 ng/g	0.001 ng/g	0.005 ng/g	0.03 ng/g
	ILV	Not reported			
Linearity (calibration curve r and concentration range)	ECM ²	r = 0.9999	r = 0.9987	r = 1.0000	r = 1.0000
		0.02-2.0 ng/mL			
	ILV	r = 0.997227	r = 0.999788	r = 0.999454	r = 0.996733
		0.02-2.0 ng/mL			
Repeatable	ECM ⁴	Yes at LOQ (0.10 ng/g) and 10×LOQ (1.0 ng/g) (four characterized water matrices – two surface, one ground, and one drinking)			
	ILV ^{5,6}	Yes at LOQ (0.10 ng/mL) and 10×LOQ (1.0 ng/mL) (one uncharacterized surface water matrix)			
Reproducible		Yes for 0.10 ng/g (0.10 ng/mL; LLMV)* and 1.0 ng/g (1.0 ng/mL) in water matrices			
Specific	ECM	Yes, no matrix interferences were observed.	Yes for all four water matrices. No matrix interferences were observed in three of four water matrices. In ground (well) water, matrix interferences were <5% of the LOQ (based on peak area). ⁷	Yes, no matrix interferences were observed.	
	ILV	Yes, no matrix interferences were observed.	Yes, matrix interferences were <5% of the LOQ (based on peak area).	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed. Analyte peak was small compared to baseline noise. ⁸

Data were obtained from pp. 9, 26; Appendix 2, pp. 65-66 (LOQ/LOD); p. 10; Tables 1-4, pp. 30-33 (recovery results); pp. 24-25; Figure 2, p. 36 (calibration curves); Figures 3-7, pp. 37-56 (chromatograms) of MRID 47560230; pp. 10, 21 (LOQ/LOD); p. 11; Tables 1-3, pp. 23-25 (recovery results); pp. 18-19, 21; Figures 1-2, pp. 30-33 (calibration curves); Figures 3-6, pp. 34-57 (chromatograms) of MRID 48333616; DER Excel Attachment.

* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 Units for LOQ and fortifications was provided as “ng/g” in the ECM, which is equivalent to “ng/mL”.

2 ECM correlation coefficients (r) were reviewer-calculated based on r² values reported in the study report (Figure 2, p. 36 of MRID 47560230; DER Excel Attachment). Solvent-based calibration standards were used (pp. 16-17 of MRID 47560230).

3 0.4 ng/mL calibration standard was excluded as outlier (p. 21 of MRID 48333616).

4 In the ECM, surface (White Clay Creek) water (Trial ID: Creek Water-White Clay; pH 7.3, conductivity 0.31 mmhos/cm, hardness 126 mg equiv. CaCO₃/L, total dissolved solids 322 ppm) collected from Newark, Delaware, surface (Lums Pond) water (Trial ID: Lums Pond Water; pH 6.8, conductivity 0.17 mmhos/cm, hardness 42 mg equiv. CaCO₃/L, total dissolved solids 340 ppm) collected from Bear, Delaware, drinking (Newark Drinking) water (Trial ID: Tap Water – Stine Newark; pH 7.7, conductivity 0.39 mmhos/cm, hardness 153 mg equiv. CaCO₃/L, total

dissolved solids 456 ppm) collected from Stine-Haskell Research Center, Newark, Delaware, and ground (Kemblesville Well) water (Trial ID: Well Water - Kemblesville); pH 7.4, conductivity 0.17 mmhos/cm, hardness 57 mg equiv. CaCO₃/L, total dissolved solids 210 ppm) collected from Kemblesville, Pennsylvania were characterized by Agvise Laboratories, Northwood, North Dakota, and used in the study (p. 17; Appendix 4, pp. 75-78 of MRID 47560230).

- 5 In the ILV, uncharacterized surface water was collected from a pond in Colorado and used in the study (p. 14 of MRID 48333616).
- 6 The method (DuPont Study No.: DuPont-22042) was validated with insignificant modifications to the analytical parameters (pp. 10, 14-21; Appendix 2, pp. 64-65 of MRID 48333616). The ILV validated the method in the first trial for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), and IN-LXT69. The ILV validated the method in the second trial for IN-QFH57; performance data was acceptable in the first trial, but the RSD for the analytical standard response factors were >20%. The ILV modifications did not warrant an updated ECM.
- 7 See Appendix 1, p. 61 of MRID 47560230. Well-Control-012108 DPX-KJM44 peak area = 13580 counts. The calculations indicated that recoveries were to be corrected for residues quantified in the controls; residues were only quantified in the controls for aminocyclopyrachlor methyl (DPX-KJM44) in ground (well) water (pp. 22-24; Appendix 1, pp. 57-64).
- 8 See Figure 6, p. 56 of MRID 48333616.

IV. Method Deficiencies and Reviewer's Comments

1. The ILV was not conducted independently of the ECM since the ILV study author (David Rockwell) communicated directly with the ECM study author (Sergio Nanita) via email and phone (pp. 1, 9 of MRID 47560230; pp. 1, 6; Appendix 2, pp. 64-65 of MRID 48333616). The communications between the ILV study authors ILV study author (David Rockwell) and the ECM study author (Sergio Nanita) occurred after trial 1 and prior to trial 2 and appeared to involve technical troubleshooting (p. 1; Appendix 2, pp. 64-65 of MRID 48333616). Communications were summarized; details were not provided.

OCSPP guidelines state that the two laboratories must have been distinct and operated separately and without collusion. Also, the analysts and study director of the ILV must have been unfamiliar with the method both in its development and subsequent use in field studies.

Communication also involved Brian Graham and Audrey Knobloch who are Pyxant Lab personnel (pp. 1, 6; Appendix 2, pp. 64-65 of MRID 48333616). Other personnel involved in the communications between the ECM and ILV were Del Koch, John May, and Kristin Milby, whose roles and company affiliation could not be found.

2. The ILV used one uncharacterized surface water collected from a pond in Colorado in the study (p. 14 of MRID 48333616). The ILV study report noted that pond water was chosen since it is expected to be one of the more difficult water sources to analyze.
3. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ (pp. 9, 26; Appendix 2, pp. 65-66 of MRID 47560230; pp. 10, 21 of MRID 48333616). 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 aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57 in the tested water matrices (0.10 ng/g or 0.10 ng/mL). Units for LOQ and fortifications was provided as "ng/g" in the ECM, which is equivalent to "ng/mL".

4. The method calculations for DuPont Study No. DuPont-22042 indicated that recoveries were to be corrected for residues quantified in the controls (pp. 22-24 of MRID 47560230). In the ILV, the study report indicated that the ECM calculations were followed (p. 18 of MRID 48333616). In the ECM, residues were only quantified in the controls for aminocyclopyrachlor methyl (DPX-KJM44) in ground (well) water (Appendix 1, p. 61 of MRID 47560230). Well-Control-012108 DPX-KJM44 peak area = 13580 counts. In the ILV, no residues appeared to have been quantified in the controls; however, the raw data sheets were difficult to read (Appendix 1, pp. 59-63 of MRID 48333616).
5. The reviewer noted that the successful validation of IN-QFH57 was performed with Batch 3 in trial 2 after excluding the 0.4 ng/mL calibration standard as an outlier (p. 21 of MRID 48333616). Performance data from the first trial of the validation of IN-QFH57 was reported (LOQ, range 100-108%, mean \pm RSD, 103 \pm 3.6%; 10 \times LOQ, range 77-88%, mean \pm RSD 84 \pm 4.4%; p. 20). Performance data was acceptable in the first trial, but the RSD for the analytical standard response factors were >20% (pp. 19-20).
6. Reagent blank not included in the ECM.
7. The reviewer noted that the dimensions of the Phenomenex Luna Phenyl-Hexyl column were incorrectly reported as 4.6 mm x 15 mm, 3 μ m, in the study report, instead of 4.6 mm x 150 mm, 3 μ m (Part No. 00F-4256-E0; <https://www.phenomenex.com/Products/Part/00F-4256-E0>; pp. 15, 17 of MRID 48333616).
8. The reviewer noted that the acidification step did not report a specific target final pH, but rather a target final concentration of *ca.* 0.3% formic acid (pp. 18-20, 26-27 of MRID 47560230).
9. The total time required to complete one batch of samples (13 to 15 samples per batch) was reported in the ILV as one 8-hour day (pp. 12, 21 of MRID 48333616). LC/MS/MS analyses were run unattended overnight and during the day; LC/MS/MS run time was considered to be the rate-determining step. Additional processing and verification required *ca.* 4 person-hours on the following day. Each batch required *ca.* 8 to 10 person-hours over the course of two calendar days. In the ECM, it was reported that *ca.* 24 samples could be prepared over one 8-hour day with LC/MS/MS analyses run overnight (p. 26 of MRID 47560230).

V. References

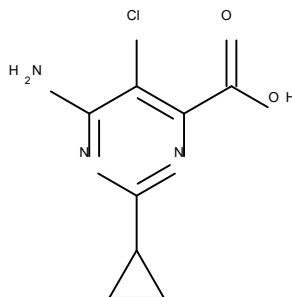
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- USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at:

<https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides>.

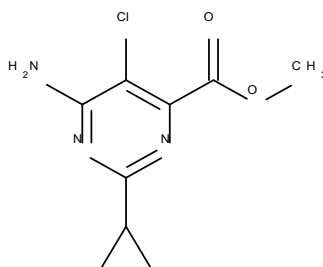
40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 344-347, and Revision 2; 2015 and 2016.

Attachment 1: Chemical Names and Structures**Aminocyclopyrachlor (DPX-MAT28; Aminocyclopyrachlor acid)**

IUPAC Name: 6-Amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylic acid
CAS Name: 6-Amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid
CAS Number: 858956-08-8
SMILES String: C1CC1c2nc(c(c2)N)Cl)C(=O)O

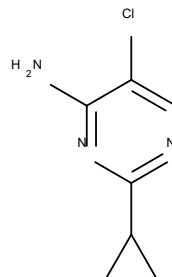
**Aminocyclopyrachlor methyl (DPX-KJM44; Aminocyclopyrachlor methyl ester; Aminocyclopyrachlor ME)**

IUPAC Name: Methyl 6-amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylate
CAS Name: Methyl 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylate
CAS Number: 858954-83-3
SMILES String: COC(=O)c1c(c(nc(n1)C2CC2)N)Cl



IN-LXT69

IUPAC Name: 5-Chloro-2-cyclopropyl-pyrimidin-4-amine
CAS Name: Not reported
CAS Number: Not reported
SMILES String: c1c(nc(n1)C2CC2)N)Cl

**IN-QFH57**

IUPAC Name: 4-Cyano-2-cyclopropyl-1H-imidazole-5-carboxylic acid
CAS Name: Not reported
CAS Number: Not reported
SMILES String: C1CC1c2[nH]c(c(n2)C#N)C(=O)O

