

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

- Reports:**
- ECM 1: EPA MRID No.: 47560226. Devine, T.J., R.M. Henze, and S.C. Nanita. 2008. Analytical Method for the Determination of DPX-KJM44, DPX-MAT28, and Metabolite in Soil using LC/MS/MS. Report prepared by E.I. du Pont de Nemours and Company, Stine-Haskell Research Center, Newark, Delaware, and sponsored and submitted by E.I. du Pont de Nemours and Company, Wilmington, Delaware; 55 pages. DuPont Study No.: DuPont-22043, Revision No. 1. Final report issued January 8, 2008; Revision No. 1 issued July 22, 2008.
- ECM 2: EPA MRID No.: 47560227. Pentz, A. and S.C. Nanita. 2008. Supplement No. 1. Analytical Method for the Determination of DPX-KJM44, DPX-MAT28, IN-LXT69 and IN-QFH57 in Soil 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; 32 pages. DuPont Study No.: DuPont-22043, Supplement No. 1. Original final report issued January 8, 2008; Supplement No. 1 issued March 26, 2008.
- ILV: EPA MRID No.: 47560233. Kinney, J. 2008. Independent Laboratory Validation of Analytical Method DuPont-22043 for the Determination of DPX-KJM44, DPX-MAT28, IN-LXT69 and IN-QFH57 in Soil using LC/MS/MS. Report prepared by Charles River, Edinburgh, United Kingdom, and sponsored and submitted by E.I. du Pont de Nemours and Company, Wilmington, Delaware; 53 pages. DuPont Study No.: DuPont-24563. Charles River Project No.: 213825. Final report issued August 14, 2008.
- Document No.:** MRIDs 47560226 & 47560227 & 47560233
- Guideline:** 850.6100
- Statements:**
- ECM 1: 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); however, the work was performed in a GLP compliant facility following Standard Operating Procedures (SOPs; p. 3 of MRID 47560226). Signed and dated Data Confidentiality, GLP, and Certification of Authenticity statements were provided (pp. 2-4). No Quality Assurance statement was included.
- ECM 2: 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); however, the work was performed in a GLP compliant facility following SOPs (p. 3 of MRID 47560227). 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 OECD Principles of GLP (1998), which are compatible with USEPA FIFRA GLP standards (40 CFR Part 160, p. 3 of MRID 47560233). 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**. It could not be determined if the ILV was conducted independently of the ECM, since the communication log did not provide the personnel who were involved. The ILV required three trials to achieve acceptable results. ECM 1 and 2 sample recoveries were corrected when residues were quantified in the controls. Two soils were reported in the ILV, but it was not reported if the soil matrices were used separately (one matrix per trial) or homogenized together. 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. The specificity of the method was not acceptable for IN-QFH57 based on ILV representative chromatograms. The LOD was not reported in the ILV.

PC Code: 288008

EFED Final Reviewer: Chuck Peck
Senior Fate Scientist

Signature:

Date:

CDM/CSS-Dynamac JV Reviewers: Lisa Muto, M.S.,
Environmental Scientist

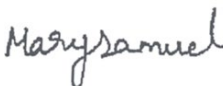
Signature:



Date: 02/05/2021

CDM/CSS-Dynamac JV Reviewers: Mary Samuel, M.S.,
Environmental Scientist

Signature:



Date: 02/05/2021

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-22043, Revision No. 1 (ECM 1), is designed for the quantitative determination of aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), and the degradate IN-LXT69 in soil at the stated LOQ of 1.0 ng/g using liquid chromatography/mass spectrometry (LC/MS/MS). Analytical method, DuPont-22043, Supplement No. 1 (ECM 2), is designed for the quantitative determination of aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), and degradates IN-LXT69 and IN-QFH57 in soil at the stated LOQ of 1.0 ng/g using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in soil 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 2, 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 soil matrices (1.0 ng/g). Based on the performance data submitted by the ILV and ECM 1, the LLMV was equivalent to the ECM reported method LOQ for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), and IN-LXT69 in the tested soil matrices (1.0 ng/g); IN-QFH57 was not included as an analyte in the original report of DuPont Study No. DuPont-22043, Revision No. 1 (ECM 1).

It could not be determined if the ILV was conducted independently of the ECM since the communication log did not provide the identities of the personnel who was involved; some technical issues were discussed regarding the outcomes of each failed trial. The ILV validated the method in the third trial for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57. The ILV performed the ECM 2 method (DuPont Study No. DuPont-22043, Supplement No. 1) as written, except for minor modifications to the sample processing (use of flat-bed shaker and elimination of filtration with 0.45 µm PTFE filters) and insignificant analytical parameters, including the re-tuned transition for IN-QFH57. The ILV modifications did not warrant an updated ECM; however, the ECM contained many critical steps/precautions. The first trial performance results were acceptable for all analytes, except for IN-QFH57; however, the study report stated that aminocyclopyrachlor (DPX-MAT28) had poor recovery, as well, since individual recoveries were <70%. The second trial performance results were unacceptable.

ECM 1 used four characterized soil matrices – two sandy loam, one clay loam and one silty clay. ECM 2 used one characterized soil matrix – sandy loam. In the ILV, two soil matrices were reported, but only one set of performance data was submitted for each analyte/trial. It was not reported if the soil matrices were used separately (one matrix per trial) or homogenized together; therefore, the characterization of the ILV soil matrix could not be determined.

All ILV, ECM 1, and ECM 2 data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for all analytes in tested soil matrices, except that specificity of the method was not acceptable for IN-QFH57 based on ILV representative chromatograms due to the size of the LOQ analyte peak. ECM 2 study report supporting data was specific for IN-QFH57. ECM 1 and 2 sample recoveries were corrected when residues were quantified in the controls. No LOD for IN-QFH57 was reported in the ECM 2. The LOD was not reported in the ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)	
	Environmental Chemistry Method	Independent Laboratory Validation							
Aminocyclopyrachlor (DPX-MAT28),	47560226 (ECM 1) ^{1,2}	None submitted	Unacceptable	Soil	08/01/2008 (ECM 1)	E.I. du Pont de Nemours and Company	LC/MS/MS	1.0 ng/g	
Aminocyclopyrachlor methyl (DPX-KJM44)					22/07/2008 (ECM 1, Revision No. 1)				
IN-LXT69									
Aminocyclopyrachlor (DPX-MAT28),	47560227 (ECM 2) ^{3,4}	47560233 ^{5,6}							08/01/2008 (ECM 2) ⁷
Aminocyclopyrachlor methyl (DPX-KJM44)					26/03/2008 (ECM 2, Supplement No. 1)				
IN-LXT69									
IN-QFH57									

1 ECM 1 (MRID 47560226) = DuPont Study No. DuPont-22043, Revision No. 1. In the ECM 1, Texas clay loam soil (Notebook Ref. No.: 2006-035; 34% sand, 32.8% silt, 33.2% clay; pH 7.9 in water; 1.3% organic matter), California sandy loam soil (Notebook Ref. No.: 2006-026; 58% sand, 31.2% silt, 10.8% clay; pH 7.8 in water; 1.0% organic matter), and Sassafras sandy loam soil (Notebook Ref. No.:2007-004; 70% sand, 24.0% silt, 6.0% clay; pH 5.4 in water; 2.1% organic matter) from the US and Lleida silty clay soil (Notebook Ref. No.: 2006-118; 0.8% sand, 54.4% silt, 44.8% clay; pH 7.6 in water; 3.5% organic matter) from Spain were used in the study (p. 16 of MRID 47560226). The matrices were obtained from DuPont Discovery Soil Bank at Stine-Haskell Research Center.

2 IN-QFH57 was not included as an analyte in the original report of DuPont Study No. DuPont-22043 (ECM 1).

3 ECM 2 (MRID 47560227) = DuPont Study No. DuPont-22043, Supplement No. 1. In the ECM 2, California sandy loam soil (Notebook Ref. No.: 2006-026; 58% sand, 31.2% silt, 10.8% clay; pH 7.8 in water; 1.0% organic matter) from US was used in the study (pp. 9, 12 of MRID 47560227). The soil matrix was the same as that used in ECM 1 and was obtained from DuPont Discovery Soil Bank at Stine-Haskell Research Center. The soil characterization data was not reported in this study report; data taken from ECM 1.

4 ECM 1 and ECM 2 were similar, except for a few minor modifications, as well as the addition of IN-QFH57 as an analyte.

5 In the ILV, Lleida (0-5) loam soil (685499/S1/009/III; Charles River Lab. ID: 05-159; DuPont ID No.: 2004-031A; 26% sand, 48% silt, 26% clay; pH 8 in water; pH 7.5 in 0.01M CaCl₂; 3.2% organic matter- Ashing, 4.1% organic matter- Walkley-Black) and Lleida (15-30) silty clay loam soil (685499/S1/011/III; Charles River Lab. ID: 05-165; DuPont ID No.: 2004-031C; 16% sand, 50% silt, 34% clay; pH 8.3 in water; pH 7.8 in 0.01M CaCl₂; 1.6% organic matter- Ashing, 1.9% organic matter- Walkley-Black) from Spain were used in the study (p. 13; Appendix 2, pp. 30-31 of MRID 47560233). For this study, it was not reported if the soil matrices were used separately (one matrix per trial) or homogenized together. The soil matrices were obtained from E.I. du Pont de Nemours and Company and were characterized by MDS Harris, Lincoln, Nebraska. The soil matrices were also used in another study: Charles River Laboratories Project No. 685499 (DuPont-14436).

6 The ILV performed the ECM 2 method (DuPont Study No. DuPont-22043, Supplement No. 1; see p. 15 of MRID 47560233) as written, except for minor modifications to the sample processing (use of flat-bed shaker and elimination of filtration with 0.45 µm PTFE filters) and insignificant analytical parameters (pp. 10, 13-18).

7 The original report date of ECM 1 was the same as the original report date of ECM 2. ECM 1 and ECM 2 were based on the same original report, but performance data for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), and IN-LXT69 differed between ECM 1 and ECM 2.

I. Principle of the Method

DuPont Study No. DuPont-22043, Revision No. 1

Soil samples (10 ± 0.1 g) were fortified (0.100 mL of 100 or 1000 ng/mL fortification solution), then the solvent was evaporated, and 3 metal beads were added (pp. 11-13, 16-19, 25 of MRID 47560226). The samples were extracted twice with 25 ± 1 mL of acetonitrile:0.15M ammonium acetate (aq.; 70:30, v:v) then once with 25 ± 1 mL of acetonitrile:0.2% formic acid (aq.; 80:20, v:v). Each extraction was performed by shaking at high speed on a wrist action shaker for 15 minutes, then centrifuging for 10 minutes at 3000 rpm. The volume of the combined extracts was adjusted to 75 mL with acetonitrile:0.2% formic acid (aq.; 80:20, v:v). A 5.0 mL aliquot of the combined extract was evaporated to 1 mL under nitrogen using an N-Evap at 40°C (water bath), then diluted to 6 mL total volume using 0.2% aqueous formic acid in polypropylene centrifuge tubes. The tubes were vortexed for 10 seconds and shaken by hand to dissolve any portion of the analytes on the inner wall of the tube. The samples were purified via Oasis MCX solid phase extraction (SPE) cartridges (500 mg, 6 cc) which were preconditioned with 3 mL of methanol flowing at 5 mL/min, then 2 column volumes of 0.01% aqueous formic acid (*ca.* 6 mL) at *ca.* 5 mL/min. After loading the sample at ≤ 1 mL/min. (critical step), the sample container was rinsed with 3×2 mL (maximum) 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, a 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. The cartridge was washed with 10 mL of methanol, then analytes were eluted using 15.0 mL of 50mM ammonium hydroxide in methanol (e.g., 3×5 mL) at ≤ 1 mL/min. (*ca.* 0.6-0.7 mL/min. recommended; critical step). The sample was evaporated to 1 mL under nitrogen using an N-Evap at 40°C (water bath), then diluted to 15 mL total volume using 0.01% aqueous formic acid. After vortex mixing, the samples were filtered (0.45 μ m polytetrafluoroethylene [PTFE]) then analyzed by liquid chromatography/mass spectrometry (LC/MS/MS). The method noted that final sample volume of 5.0 mL can be used depending on MS sensitivity. Transfers should be done with 0.01% aqueous formic acid rinses of the previous vessel for quantitative transfer. The method also noted that the Oasis MCX SPE cartridge could not be substituted.

DuPont Study No. DuPont-22043, Supplement No. 1

Soil samples (10 ± 0.1 g) were fortified (0.100 mL of 100 or 1000 ng/mL fortification solution), then the solvent was evaporated, and 3 metal beads were added (pp. 10, 13-15 of MRID 47560227). The samples were extracted twice with 25 ± 1 mL of acetonitrile:0.15M ammonium acetate (aq.; 70:30, v:v) then once with 25 ± 1 mL of acetonitrile:0.2% formic acid (aq.; 80:20, v:v). Each extraction was performed by shaking at high speed on a wrist action shaker for 15 minutes, then centrifuging for 10 minutes at 3000 rpm. The volume of the combined extracts was adjusted to 75 mL with acetonitrile:0.2% formic acid (aq.; 80:20, v:v). A 15.0 mL aliquot of the combined extract was evaporated to *ca.* 2 mL under nitrogen using an N-Evap at 40°C (water bath), then diluted to 7 mL total volume using 0.2% aqueous formic acid. The tubes were vortexed for 10 seconds and shaken by hand to dissolve any portion of the analytes on the inner wall of the tube. The samples were purified via Oasis MCX SPE cartridges (500 mg, 6 cc) which were preconditioned with 3 mL of methanol then 2 column volumes of 0.01% aqueous formic acid (*ca.* 6 mL) at *ca.* 5 mL/min. After loading the sample at ≤ 1 mL/min. (critical step), the sample container was rinsed with 2×2 mL (maximum) 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, a 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. The analyte was eluted using 15.0 mL of 75mM ammonium hydroxide in methanol (e.g., 3 x 5 mL) at ≤ 1 mL/min. (critical step). The sample was evaporated to 1 mL under nitrogen using an N-Evap at 40°C (water bath). The residue was mixed with *ca.* 3 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. After vortex mixing, the samples were filtered (0.45 μ m PTFE). For quantification of IN-QFH57, 1.0 mL of the filtered sample was transferred to an amber autosampler vial and analyzed by LC/MS/MS. For quantification of aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), and IN-LXT69, amber autosampler vials were charged with 900 μ L 0.01% aqueous formic acid then 100 μ L of the filtered sample was added prior to analysis by LC/MS/MS.

The method cited the original method DuPont Study No. DuPont-22043, Revision No. 1 for the test soil characterization, equipment list, and precautions, as well as other minor method details which were not reproduced (pp. 10, 12, 17, 20 of MRID 47560227).

DuPont Study No. DuPont-22043, Revision No. 1, and DuPont-22043, Supplement No. 1

Samples were analyzed for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57 using an Agilent 1100 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 47560226; pp. 15-17 of MRID 47560227). 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, 10.1-14.50 min, 95:5] and injection volume of 10-100 μ L or 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, 8.8 (or 8.9), 3.8 (or 4.1), 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 (p. 25 of MRID 47560226).

ILV

The ILV performed the ECM 2 method (DuPont Study No. DuPont-22043 and Supplement No. 1; see p. 15 of MRID 47560233) as written, except for minor modifications to the sample processing (use of flat-bed shaker and elimination of filtration with 0.45 μ m PTFE filters) and insignificant analytical parameters, including the re-tuned transition for IN-QFH57 (pp. 10, 13-18, 20 of MRID 47560233). Samples were analyzed for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57 using Perkin Elmer Series 200 HPLC coupled with an Applied Biosystems API 5000 Triple Quadrupole MS. The LC/MS/MS parameters were the same as those of the ECM, with the exception that a Phenomenex, C18 (ODS) guard column (4 \times 2

mm) was used and some minor MS parameters. The injection volume was 60 μ L. Two ion pair transitions were monitored as follows (quantitation and confirmation, respectively): m/z 214.2 \rightarrow 68.1 and m/z 214.2 \rightarrow 101.0 for aminocyclopyrachlor (DPX-MAT28), m/z 228.2 \rightarrow 68.1 and m/z 228.2 \rightarrow 168.1 for aminocyclopyrachlor methyl (DPX-KJM44), m/z 170.1 \rightarrow 76.0 and m/z 170.1 \rightarrow 103.1 for IN-LXT69, and m/z 176.0 \rightarrow 132.1 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.* 4.7, 8.1, 3.6, and 7.5 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 Limit of Quantification (LOQ) for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), and IN-LXT69 in soil was 1.0 ng/g in the ECM 1 and ILV (pp. 9, 24-25; Appendix 2, pp. 53-54 of MRID 47560226; pp. 10, 21 of MRID 47560233). The LOQ for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57 in soil was 1.0 ng/g in the ECM 2 and ILV (pp. 8, 20-21 of MRID 47560227; pp. 10, 21 of MRID 47560233). In the ECM 1 and ECM 2, the Limit of Detection (LOD) for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57 was estimated as 0.1, 0.01, 0.02, and 0.2 ng/g, respectively, (pp. 9, 24-25; Appendix 2, pp. 53-54 of MRID 47560226; pp. 8, 20-21 of MRID 47560227). No LOD for IN-QFH57 was reported in ECM 1. 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 1 (MRID 47560226 - DuPont Study No. DuPont-22043, Revision No. 1): 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), and IN-LXT69 at fortification levels of 1.0 ng/g (LOQ) and 10.0 ng/g (10 \times LOQ) in four soil matrices (p. 10; Tables 1-4, pp. 29-32). 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. 26-27). The calculations indicated that recoveries were to be corrected for residues quantified in the controls (pp. 21-23; Appendix 1, pp. 50-52). The study report stated that matrix interferences were not observed in the four soil matrices at the respective retention times of all of the analytes; however, the reviewer noted that one to three analytes were observed and integrated in the control samples of each of the four soil matrices in representative chromatograms (Figures 4-7, pp. 38-49). Texas clay loam soil (Notebook Ref. No.: 2006-035; 34% sand, 32.8% silt, 33.2% clay; pH 7.9 in water; 1.3% organic matter), California sandy loam soil (Notebook Ref. No.: 2006-026; 58% sand, 31.2% silt, 10.8% clay; pH 7.8 in water; 1.0% organic matter), and Sassafras sandy loam soil (Notebook Ref. No.:2007-004; 70% sand, 24.0% silt, 6.0% clay; pH 5.4 in water; 2.1% organic matter) from the US and Lleida silty clay soil (Notebook Ref. No.: 2006-118; 0.8% sand, 54.4% silt, 44.8% clay; pH 7.6 in water; 3.5% organic matter) from Spain were used in the study (p. 16). The matrices were obtained from DuPont Discovery Soil Bank at Stine-Haskell Research Center. The study report noted that Lleida silty clay soil was “analyzed as part of an internal second lab tryout performed at DuPont Stine-Haskell Research Center” (p. 27).

ECM 2 (MRID 47560227 - DuPont Study No. DuPont-22043, Supplement No. 1): 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 1.0 ng/g (LOQ) and 10.0 ng/g (10 \times LOQ) in one soil matrix (p. 9; Table 1, p. 23). 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 for IN-QFH57 (p. 21). The calculations indicated that recoveries were to be corrected for residues quantified in the controls (pp. 17-18; Appendix 1, pp. 28-29). The study report summary was specific for IN-QFH57 and stated that matrix interferences were not observed. California sandy loam soil (Notebook Ref. No.: 2006-026; 58% sand, 31.2% silt, 10.8% clay; pH 7.8 in water; 1.0% organic matter) from US was used in the study (pp. 9, 12 of MRID 47560227). The soil matrix was the same as that used in ECM 1 and was obtained from DuPont Discovery Soil Bank at Stine-Haskell Research Center. The soil characterization data was not reported in this study report; data taken from ECM 1.

ILV (MRID 47560233): Based on the third trial performance data, mean recoveries and 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 1.0 ng/g (LOQ) and 10.0 ng/g (10 \times LOQ) in one soil 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. In the ILV, Lleida (0-5) loam soil (685499/S1/009/III; Charles River Lab. ID: 05-159; DuPont ID No.: 2004-031A; 26% sand, 48% silt, 26% clay; pH 8 in water; pH 7.5 in 0.01M CaCl₂; 3.2% organic matter- Ashing; 4.1% organic matter- Walkley-Black) and Lleida (15-30) silty clay loam soil (685499/S1/011/III; Charles River Lab. ID: 05-165; DuPont ID No.: 2004-031C; 16% sand, 50% silt, 34% clay; pH 8.3 in water; pH 7.8 in 0.01M CaCl₂; 1.6% organic matter- Ashing; 1.9% organic matter- Walkley-Black) from Spain were used in the study (p. 13; Appendix 2, pp. 30-31 of MRID 47560233). For this study, it was not reported if the soil matrices were used separately (one matrix per trial) or homogenized together. The soil matrices were obtained from E.I. du Pont de Nemours and Company and were characterized by MDS Harris, Lincoln, Nebraska. The soil matrices were also used in another study: Charles River Laboratories Project No. 685499 (DuPont-14436).

The ILV validated the method in the third trial for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57. The ILV performed the ECM 2 method (DuPont Study No. DuPont-22043, Supplement No. 1) as written, except for minor modifications to the sample processing (use of flat-bed shaker and elimination of filtration with 0.45 μ m PTFE filters) and insignificant analytical parameters, including the re-tuned transition for IN-QFH57 (pp. 10, 13-18, 20 of MRID 47560233). The first trial performance results were acceptable for all analytes, except for IN-QFH57; however, the study report stated that aminocyclopyrachlor (DPX-MAT28) had poor recovery, as well, since individual recoveries were $<$ 70% (p. 20; Tables 1-2, pp. 23-24). The second trial only included aminocyclopyrachlor (DPX-MAT28) and IN-QFH57; performance results were unacceptable for both analytes at both fortifications due to low ($<$ 70%) and variable (RSD $>$ 20%) recoveries. It should be noted that in Trials 1 and 2, matrix interference was observed for IN-QFH57 in chromatograms of unfortified control extracts, but no matrix interference was observed in Trial 3.

Table 2. Initial Validation Method Recoveries for Aminocyclopyrachlor (DPX-MAT28), Aminocyclopyrachlor methyl (DPX-KJM44), and Degradates IN-LXT69 and IN-QFH57 in Soil¹

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
MRID 47560226 - DuPont Study No. DuPont-22043, Revision No. 1^{2,3}						
Texas Clay Loam Soil						
Quantitation ion transition						
Aminocyclopyrachlor (DPX-MAT28)	1.0 (LOQ)	5	74-84	78	5	6
	10.0	5	84-93	88	4	4
Aminocyclopyrachlor methyl (DPX-KJM44)	1.0 (LOQ)	3	89-93	91	2	2
	10.0	5	87-91	90	2	2
IN-LXT69	1.0 (LOQ)	5	84-97	91	6	6
	10.0	5	83-97	90	7	8
California Sandy Loam Soil						
Quantitation ion transition						
Aminocyclopyrachlor (DPX-MAT28)	1.0 (LOQ)	5	78-121	91	18	20
	10.0	5	82-90	85	3	4
Aminocyclopyrachlor methyl (DPX-KJM44)	1.0 (LOQ)	5	84-95	90	5	5
	10.0	5	82-90	85	3	4
IN-LXT69	1.0 (LOQ)	5	76-86	84	4	5
	10.0	5	80-88	84	3	4
Sassafras Sandy Loam Soil						
Quantitation ion transition						
Aminocyclopyrachlor (DPX-MAT28)	1.0 (LOQ)	5	88-110	93	9	10
	10.0	5	84-91	87	3	3
Aminocyclopyrachlor methyl (DPX-KJM44)	1.0 (LOQ)	5	89-93	90	2	2
	10.0	5	80-86	82	2	3
IN-LXT69	1.0 (LOQ)	5	85-90	87	2	2
	10.0	5	75-84	80	4	5
Lleida Silty Clay Soil⁴						
Quantitation ion transition						
Aminocyclopyrachlor (DPX-MAT28)	1.0 (LOQ)	5	95-101	99	3	3
	10.0	5	82-97	91	6	7
Aminocyclopyrachlor methyl (DPX-KJM44)	1.0 (LOQ)	5	97-104	100	3	3
	10.0	5	91-93	92	1	1
IN-LXT69	1.0 (LOQ)	5	91-97	95	2	3
	10.0	5	82-88	86	3	3
MRID 47560227 - DuPont Study No. DuPont-22043, Supplement No. 1⁵						
California Sandy Loam Soil						
Quantitation ion transition						
Aminocyclopyrachlor (DPX-MAT28)	1.0 (LOQ)	5	99-112	103	5	5
	10.0	5	92-103	100	5	5
Aminocyclopyrachlor methyl (DPX-KJM44)	1.0 (LOQ)	5	84-97	89	5	6
	10.0	5	77-91	85	5	6
IN-LXT69	1.0 (LOQ)	5	71-87	80	6	7
	10.0	5	78-89	84	4	5

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
IN-QFH57	1.0 (LOQ)	5	95-99	96	2	2
	10.0	5	90-100	96	4	4

Data (calculations indicated that recoveries were to be corrected for residues quantified in the controls; pp. 21-23; Appendix 1, pp. 50-52 of MRID 47560226; pp. 17-18; Appendix 1, pp. 28-29 of MRID 47560227) were obtained from p. 10; and Tables 1-4, pp. 29-32 of MRID 47560226; p. 9; and Table 1, p. 23 of MRID 47560227.

1 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. Ion transitions were the same for ECM 1 and ECM 2.

2 ECM 1 (MRID 47560226) = DuPont Study No. DuPont-22043, Revision No. 1. In the ECM 1, Texas clay loam soil (Notebook Ref. No.: 2006-035; 34% sand, 32.8% silt, 33.2% clay; pH 7.9 in water; 1.3% organic matter), California sandy loam soil (Notebook Ref. No.: 2006-026; 58% sand, 31.2% silt, 10.8% clay; pH 7.8 in water; 1.0% organic matter), and Sassafras sandy loam soil (Notebook Ref. No.:2007-004; 70% sand, 24.0% silt, 6.0% clay; pH 5.4 in water; 2.1% organic matter) from the US and Lleida silty clay soil (Notebook Ref. No.: 2006-118; 0.8% sand, 54.4% silt, 44.8% clay; pH 7.6 in water; 3.5% organic matter) from Spain were used in the study (p. 16 of MRID 47560226). The matrices were obtained from DuPont Discovery Soil Bank at Stine-Haskell Research Center. The source of the characterization data was not reported. The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

3 IN-QFH57 was not included as an analyte in the original report of DuPont Study No. DuPont-22043, Revision No. 1 (ECM 1).

4 Lleida silty clay soil was “analyzed as part of an internal second lab tryout performed at DuPont Stine-Haskell Research Center” (p. 27 of MRID 47560226). Lab-specific information was not reported for this second lab tryout.

5 ECM 2 (MRID 47560227) = DuPont Study No. DuPont-22043, Supplement No. 1. In the ECM 2, California sandy loam soil (Notebook Ref. No.: 2006-026; 58% sand, 31.2% silt, 10.8% clay; pH 7.8 in water; 1.0% organic matter) from US was used in the study (pp. 9, 12 of MRID 47560227). The soil matrix was the same as that used in ECM 1 and was obtained from DuPont Discovery Soil Bank at Stine-Haskell Research Center. The soil characterization data was not reported in this study report; data taken from ECM 1. The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

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

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Lleida Soil (Loam and Silty Clay Loam) – Trial 1						
Quantitation ion transition						
Aminocyclopyrachlor (DPX-MAT28)	1.0 (LOQ)	5	67.6-104	82.6	13.4	16.2
	10.0	5	64.2-85.1	75.7	8.9	11.8
Aminocyclopyrachlor methyl (DPX-KJM44)	1.0 (LOQ)	5	95.1-111	103	7.3	7.1
	10.0	5	95.4-101	97.1	2.3	2.4
IN-LXT69	1.0 (LOQ)	5	91.6-115	104	10.3	9.9
	10.0	5	93.8-103	98.7	3.3	3.3
IN-QFH57	1.0 (LOQ)	5	70.7- 122	92.1	20.7	22.5
	10.0	5	59.6 -81.4	74.9	9.5	12.7
Lleida Soil (Loam and Silty Clay Loam) – Trial 2³						
Quantitation ion transition						
Aminocyclopyrachlor (DPX-MAT28)	1.0 (LOQ)	5	52.1 -91.3	75.1	19.2	25.6
	10.0	5	52.3 -76.2	61.1	9.7	15.9
IN-QFH57	1.0 (LOQ)	5	41.1 -94.5	73.6	19.9	27.0
	10.0	5	63.1 -73.0	68.5	4.3	6.3
Lleida Soil (Loam and Silty Clay Loam) – Trial 3						
Quantitation ion transition						
Aminocyclopyrachlor (DPX-MAT28)	1.0 (LOQ)	5	89.3-94.2	92.8	2.0	2.2
	10.0	5	86.4-96.5	93.4	4.0	4.3
Aminocyclopyrachlor methyl (DPX-KJM44)	1.0 (LOQ)	5	92.7-96.1	94.7	1.3	1.4
	10.0	5	86.4-91.1	89.4	2.1	2.3
IN-LXT69	1.0 (LOQ)	5	82.5-102	91.0	8.2	9.0
	10.0	5	81.7-87.9	83.3	2.6	3.1
IN-QFH57	1.0 (LOQ)	5	100-118	108	6.7	6.2
	10.0	5	96.5-103	98.7	2.6	2.6

Data (uncorrected recovery results; pp. 18-19) were obtained from p. 11; and Tables 1-3, pp. 23-25 of MRID 47560233; and DER Excel Attachment.

Bold, italic font indicates deviation from the guideline.

1 Lleida (0-5) loam soil (685499/S1/009/III; Charles River Lab. ID: 05-159; DuPont ID No.: 2004-031A; 26% sand, 48% silt, 26% clay; pH 8 in water; pH 7.5 in 0.01M CaCl₂; 3.2% organic matter- Ashing, 4.1% organic matter- Walkley-Black) and Lleida (15-30) silty clay loam soil (685499/S1/011/III; Charles River Lab. ID: 05-165; DuPont ID No.: 2004-031C; 16% sand, 50% silt, 34% clay; pH 8.3 in water; pH 7.8 in 0.01M CaCl₂; 1.6% organic matter- Ashing, 1.9% organic matter- Walkley-Black) from Spain were used in the study (p. 13; Appendix 2, pp. 30-31). For this study, it was not reported if the soil matrices were used separately (one matrix per trial) or homogenized together. The soil matrices were obtained from E.I. du Pont de Nemours and Company and were characterized by MDS Harris, Lincoln, Nebraska. The soil textures were verified by the reviewer using USDA-NRCS technical support tools. The soil matrices were also used in another study: Charles River Laboratories Project No. 685499 (DuPont-14436). The soil matrices

2 Two ion pair transitions were monitored as follows (quantitation and confirmation, respectively): *m/z* 214.2→68.1 and *m/z* 214.2→101.0 for aminocyclopyrachlor (DPX-MAT28), *m/z* 228.2→68.1 and *m/z* 228.2→168.1 for aminocyclopyrachlor methyl (DPX-KJM44), *m/z* 170.1→76.0 and *m/z* 170.1→103.1 for IN-LXT69, and *m/z* 176.0→132.1 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 Trial 2 results included for completeness. Performance data was unacceptable for both analytes in the test soil.

III. Method Characteristics

The LOQ for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), and IN-LXT69 in soil was 1.0 ng/g in the ECM 1 and ILV (pp. 9, 24-25; Appendix 2, pp. 53-54 of MRID 47560226; pp. 10, 21 of MRID 47560233). The LOQ for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57 in soil was 1.0 ng/g in the ECM 2 and ILV (pp. 8, 20-21 of MRID 47560227; pp. 10, 21 of MRID 47560233). In the ECM 1 and ECM 2, the LOQ was defined as the lowest fortification level validated at which average recoveries of 70-110% and an RSD <20% were achieved (pp. 9, 24-25; Appendix 2, pp. 53-54 of MRID 47560226; pp. 8, 20-21 of MRID 47560227). 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 ECM 1 and ECM 2, the LOD was calculated as 0.1, 0.01, 0.02, and 0.2 ng/g for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57, respectively, from the signal-to-noise response of each analyte at the LOQ level using the following equation: $[(3/1)/LOD] = \{ \{ [\text{sample response (cps)}] / 1 \} / [\text{sample recovery (ng/g)}] \}$. 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 Soil

		Aminocyclopyrachlor (DPX-MAT28)	Aminocyclopyrachlor methyl (DPX-KJM44)	IN-LXT69	IN-QFH57
Limit of Quantitation (LOQ)*	ECM 1 ¹	1.0 ng/g			Not included ²
	ECM 2 ³	1.0 ng/g			
	ILV	1.0 ng/g			
Limit of Detection (LOD)	ECM 1 (calc)	0.1 ng/g	0.01 ng/g	0.02 ng/g	Not included
	ECM 2 (calc)				0.2 ng/g
	ILV	Not reported			
Linearity (calibration curve r and concentration range)	ECM 1 ⁴	r = 0.9994	r = 1.0000	r = 0.9999	Not included
		0.02-2.0 ng/mL			
	ECM 2 ⁴	Not reported ⁵			r = 0.9999
					0.2-20 ng/mL
	ILV	r = 1.0000	r = 1.0000	r = 0.9999	r = 0.9999
		ca. 0.02-2.0 ng/mL			ca. 0.2-20.0 ng/mL
Repeatable	ECM 1 ⁶	Yes at LOQ (1.0 ng/g) and 10×LOQ (1.0 ng/g) (four characterized soil matrices – two sandy loam, one clay loam and one silty clay)			Not included
	ECM 2 ^{7,8}	Yes at LOQ (1.0 ng/g) and 10×LOQ (1.0 ng/g) (one characterized soil matrix – sandy loam)			
	ILV ^{9,10}	Yes at LOQ (1.0 ng/mL) and 10×LOQ (1.0 ng/mL) (one poorly characterized soil matrix - loam and silty clay loam mixture) ¹¹			
Reproducible		Yes for 1.0 ng/g (1.0 ng/mL; LLMV)* and 1.0 ng/g (1.0 ng/mL) in soil matrices			
Specific	ECM 1	Yes, matrix interferences were only observed in the clay loam soil where residues in the controls measured ca. 15% of the LOQ (based on quantified residues). ¹²	Yes, matrix interferences were <10% of the LOQ (based on peak height and quantified residues). ¹² Matrix interferences were observed in three of the four soil matrices.	Not included	
	ECM 2	None provided ⁵			Yes, no matrix interferences were observed.
	ILV	Yes, no matrix interferences were observed.			No , no matrix interferences were observed; however, LOQ analyte peak was extremely small compared to baseline noise. ¹³

Data were obtained from pp. 9, 24-25; Appendix 2, pp. 53-54 (ECM 1 LOQ/LOD); p. 10; Tables 1-4, pp. 29-32 (ECM 1 recovery results); pp. 15-16, 23; Figure 2, p. 34

(ECM 1 calibration curves); Figures 3-7, pp. 37-56 (ECM 1 chromatograms) of MRID 47560226; pp. 8, 20-21 (ECM 2 LOQ/LOD); p. 10; Tables 1-4, pp. 29-32 (ECM 2 recovery results); pp. 18-19; Figure 2, p. 25 (ECM 2 calibration curves); Figures 3-4, pp. 26-27 (ECM 2 chromatograms) of MRID 47560227; pp. 10, 21 (ILV LOQ/LOD); p. 11; Tables 1-3, pp. 23-25 (ILV recovery results); pp. 20-21; Appendix 4, pp. 34-37 (ILV calibration curves); Appendix 5, pp. 42-53 (ILV chromatograms) of MRID 47560233; DER Excel Attachment.

Bold font indicates deviation from the guidelines.

* 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 ECM 1 (MRID 47560226) = DuPont Study No. DuPont-22043, Revision No. 1.

2 IN-QFH57 was not included as an analyte in the original report of DuPont Study No. DuPont-22043, Revision No. 1 (ECM 1).

3 ECM 2 (MRID 47560227) = DuPont Study No. DuPont-22043, Supplement No. 1.

4 ECM 1 and ECM 2 correlation coefficients (r) were reviewer-calculated based on r^2 values reported in the study report (Figure 2, p. 34 of MRID 47560226; Figure 2, p. 25 of MRID 47560227; DER Excel Attachment). Solvent-based calibration standards were used (pp. 15-16 of MRID 47560226; p. 17 of MRID 47560227).

5 The ECM 2 study report supporting data was specific for IN-QFH57.

6 In the ECM 1, Texas clay loam soil (Notebook Ref. No.: 2006-035; 34% sand, 32.8% silt, 33.2% clay; pH 7.9 in water; 1.3% organic matter), California sandy loam soil (Notebook Ref. No.: 2006-026; 58% sand, 31.2% silt, 10.8% clay; pH 7.8 in water; 1.0% organic matter), and Sassafras sandy loam soil (Notebook Ref. No.: 2007-004; 70% sand, 24.0% silt, 6.0% clay; pH 5.4 in water; 2.1% organic matter) from the US and Lleida silty clay soil (Notebook Ref. No.: 2006-118; 0.8% sand, 54.4% silt, 44.8% clay; pH 7.6 in water; 3.5% organic matter) from Spain were used in the study (p. 16 of MRID 47560226). The matrices were obtained from DuPont Discovery Soil Bank at Stine-Haskell Research Center.

7 In the ECM 2, California sandy loam soil (Notebook Ref. No.: 2006-026; 58% sand, 31.2% silt, 10.8% clay; pH 7.8 in water; 1.0% organic matter) from US was used in the study (pp. 9, 12 of MRID 47560227). The soil matrix was the same as that used in ECM 1 and was obtained from DuPont Discovery Soil Bank at Stine-Haskell Research Center. The soil characterization data was not reported in this study report; data taken from ECM 1.

8 ECM 1 and ECM 2 were similar, except for a few minor modifications, as well as the addition of IN-QFH57 as an analyte.

9 In the ILV, Lleida (0-5) loam soil (685499/S1/009/III; Charles River Lab. ID: 05-159; DuPont ID No.: 2004-031A; 26% sand, 48% silt, 26% clay; pH 8 in water; pH 7.5 in 0.01M CaCl₂; 3.2% organic matter- Ashing, 4.1% organic matter- Walkley-Black) and Lleida (15-30) silty clay loam soil (685499/S1/011/III; Charles River Lab. ID: 05-165; DuPont ID No.: 2004-031C; 16% sand, 50% silt, 34% clay; pH 8.3 in water; pH 7.8 in 0.01M CaCl₂; 1.6% organic matter- Ashing, 1.9% organic matter- Walkley-Black) from Spain were used in the study (p. 13; Appendix 2, pp. 30-31 of MRID 47560233). For this study, it was not reported if the soil matrices were used separately (one matrix per trial) or homogenized together. The soil matrices were obtained from E.I. du Pont de Nemours and Company and were characterized by MDS Harris, Lincoln, Nebraska. The soil matrices were also used in another study: Charles River Laboratories Project No. 685499 (DuPont-14436).

10 The ILV validated the method in the third trial for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57. The ILV performed the ECM 2 method (DuPont Study No. DuPont-22043, Supplement No. 1) as written, except for minor modifications to the sample processing (use of flat-bed shaker and elimination of filtration with 0.45 μ m PTFE filters) and insignificant analytical parameters, including the re-tuned transition for IN-QFH57 (pp. 10, 13-18, 20 of MRID 47560233). The first trial performance results were acceptable for all analytes, except for IN-QFH57; however, the study report stated that aminocyclopyrachlor (DPX-MAT28) had poor recovery, as well, since individual recoveries were <70% (p. 20; Tables 1-2, pp. 23-24). The second trial only included aminocyclopyrachlor (DPX-MAT28) and IN-QFH57; performance results were unacceptable for both analytes at both fortifications due to low (<70%) and variable (RSD >20%) recoveries. The ILV modifications did not warrant an updated ECM.

11 In the ILV, it was not reported if the two soil matrices were used separately (one matrix per trial) or homogenized together.

12 Raw data was only provided for the Texas clay loam soil (Appendix 1, pp. 50-52 of MRID 47560226).

13 See Appendix 5, p. 49 of MRID 47560233.

IV. Method Deficiencies and Reviewer's Comments

1. It could not be determined if the ILV was conducted independently of the ECM since the communication log did not provide the identities of the personnel who were involved (Appendix 3, pp. 32-33 of MRID 47560233). The communications between the Charles River Laboratories and E.I. du Pont de Nemours and Company were listed by date with media used and content discussed. Some technical issues were discussed regarding the outcomes of each failed trial; however, the "Sponsor Monitor" from the communications was not identified in the ILV study report (pp. 1, 6; Appendix 3, pp. 32-33 of MRID 47560233).

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.

2. The ILV validated the method in the third trial for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), IN-LXT69, and IN-QFH57. The first trial performance results were acceptable for all analytes, except for IN-QFH57; however, the study report stated that aminocyclopyrachlor (DPX-MAT28) had poor recovery, as well, since individual recoveries were <70% (p. 20; Tables 1-2, pp. 23-24). The second trial only included aminocyclopyrachlor (DPX-MAT28) and IN-QFH57 and performance results were unacceptable for both analytes at both fortifications due to low (<70%) and variable (RSD >20%) recoveries. In Trials 1 and 2 matrix interference was observed for IN-QFH57 in chromatograms of unfortified control extracts, but no matrix interference was observed in Trial 3.
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, 24-25; Appendix 2, pp. 53-54 of MRID 47560226; pp. 8, 20-21 of MRID 47560227; pp. 10, 21 of MRID 47560233). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM 2, 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 soil matrices (1.0 ng/g). Based on the performance data submitted by the ILV and ECM 1, the LLMV was equivalent to the ECM reported method LOQ for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), and IN-LXT69 in the tested soil matrices (1.0 ng/g); IN-QFH57 was not included as an analyte in the original report of DuPont Study No. DuPont-22043 (ECM 1).
4. The ILV test soil was poorly characterized, and it could not be determined if the ILV was provided with the most difficult matrices with which to validate the method. In the ILV, two Lleida soil matrices were reported: Lleida (0-5) loam soil (26% clay, 3.2% organic matter- Ashing, 4.1% organic matter- Walkley-Black, 1.9-2.4% organic carbon, assuming organic carbon equals organic matter divided by 1.72) and Lleida (15-30) silty clay loam soil (34% clay, 1.6% organic matter- Ashing, 1.9% organic matter- Walkley-Black, 0.9-1.1% organic carbon; p. 13; Appendix 2, pp. 30-31 of MRID 47560233). However, it was not reported if the soil matrices were used separately (one matrix per trial) or homogenized together. Overall, the ILV soil matrix could not be determined.

OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (e.g., high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs. Submitted terrestrial field dissipation (TFD) and aquatic field deposition (AFD) studies are summarized in the tables below. Organic carbon content ranged from 0.04 to 3.8%, indicating that the ILV, while not conducted with soil of the highest organic carbon content, was in the upper range of the field studies conducted. OCSPP guidelines do not specify the number of soil matrices required for ILV validation, but the soil matrices are meant to be representative of those used in test chemical field studies.

Aminocyclopyrachlor (DPX-MAT28) Formulation

Study type	MRIDs	Soil source	Soil description	References
TFD	49359301/49614601	California	Sandy loam (6-8% clay, 0.18-1.2% organic matter)	Table 2, p. 39 of MRID 49359301 & Table 2, p. 41 of MRID 49614601
	49359302/49614602	North Carolina	Loamy sand & sandy loam (5-15% clay, 0.04-0.54% organic matter)	Table 2, p. 39 of MRID 49359302 & Table 2, p. 41 of MRID 49614602

Aminocyclopyrachlor methyl (DPX-KJM44) Formulation

Study type	MRID	Soil source	Soil description	References
TFD	48333611	Manitoba, Canada	Clay loam & loam (22-30% clay, 0.3-3.8% organic carbon)	Table 1, p. 40 of MRID 48333611
	48333612	Washington	Sand & loamy sand (2-4% clay, 0.06-0.3% organic carbon)	Table 1, p. 39 of MRID 48333612
	48333613	Ontario, Canada	Silt loam, clay loam, & silty clay loam (14-38% clay, 0.23-1.04% organic carbon)	Table 1, p. 39 of MRID 48333613
	48333614	California	Silt loam (9-11% clay, 0.1-0.4% organic carbon)	Table 1, p. 39 of MRID 48333614
	47560222	Georgia	Sand, loamy sand, sandy clay loam, & sandy clay (3-39% clay, 0.5-1.5% organic matter)	DER Table 3 (dated 04/09.2009; MRID not found)
	47560223	Ontario, Canada	Loam, silt loam, & silty clay loam (10-36% clay, 0.4-4.4% organic matter)	DER Table 3 (dated 04/09.2009; MRID not found)
	47560224*	Ontario, Canada	Silt loam, clay loam, & silty clay loam (14-38% clay, 0.23-1.04% organic carbon)	DER Table 3 (dated 04/23.2009; MRID not found)
AFD	48333615	California (pond)	Clay loam, clay, & sandy clay loam (31-43% clay, 0.1-0.87% organic carbon)	Table 1, p. 42 of MRID 48333615
Runoff	49656901/49409801	Texas	Clay	Table 2, p. 62 of

			(62-66% clay, 0.7-1.7% organic carbon)	MRID 49409801
	49656902/49409802	North Carolina	Sandy loam & sandy clay loam (5-39% clay, 0.3-3.5% organic carbon)	Table 2, p. 65 of MRID 49409802

* Comparison of Table 6 of MRID 48333613 with Table 6 of the DER for MRID 47560224 indicated that MRID 48333613 and MRID 47560224 were based on the same original report. MRID 47560224 appeared to be the interim report for MRID 48333613.

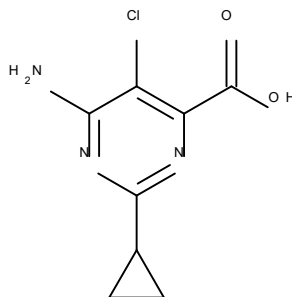
5. The specificity of the method was not acceptable for IN-QFH57 based on ILV representative chromatograms due to the fact that the LOQ analyte peak was extremely small compared to baseline noise (Appendix 5, p. 49 of MRID 47560233).
6. The method calculations for DuPont Study No. DuPont-22043, Revision No. 1 and DuPont Study No. DuPont-22043, Supplement No. 1 indicated that recoveries were to be corrected for residues quantified in the controls (pp. 21-23; Appendix 1, pp. 50-52 of MRID 47560226; pp. 17-18; Appendix 1, pp. 28-29 of MRID 47560227). In ECM 1, the study report stated that matrix interferences were not observed in the four soil matrices at the respective retention times of all of the analytes; however, the reviewer noted that one to three analytes were observed and integrated in the control samples of each of the four soil matrices in representative chromatograms (Figures 4-7, pp. 38-49 of MRID 47560226). Raw data for ECM 1 only included the Texas clay loam soil, but residues were quantified at *ca.* 6-15% of the LOQ in that soil matrix (Appendix 1, pp. 50-52). In ECM 2, the study report summary was specific for IN-QFH57 and stated that matrix interferences were not observed (p. 19; Appendix 1, pp. 28-29 of MRID 47560227).
7. The reviewer noted that the ECM 2 study report supporting data was specific for IN-QFH57; therefore, even though the recovery data for aminocyclopyrachlor (DPX-MAT28), aminocyclopyrachlor methyl (DPX-KJM44), and IN-LXT69 differed from ECM 1 recovery data, no linearity data, representative chromatograms, or raw data was provided for these three analytes.
8. The revisions to ECM 1 MRID 47560226 were reported in the study report as corrections of the ion transitions of aminocyclopyrachlor (DPX-MAT28) and aminocyclopyrachlor methyl (DPX-KJM44), common name inclusions for aminocyclopyrachlor (DPX-MAT28) and aminocyclopyrachlor methyl (DPX-KJM44), updated stability of stock standard solutions, clarification of critical steps, and correction of other minor typographical errors (p. 8 of MRID 47560226).
9. The reviewer noted that the ILV communications indicated that discussion to attempt DuPont-22043 was offered by the Sponsor Monitor if trial 3 was unsuccessful (Appendix 3, p. 33 of MRID 47560233).
10. The total time required to complete one batch of samples (12 samples per batch) was reported in the ILV as one 8-hour day (pp. 10, 21 of MRID 47560233). LC/MS/MS analyses were run unattended overnight and during the next day. In the ECM 1, it was reported that 6-12 samples could be prepared over one 8-hour day with LC/MS/MS analyses run overnight (pp. 9, 25 of MRID 47560226).

V. References

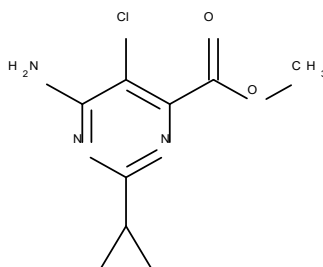
- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- USEPA. 2012. *Environmental Chemistry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

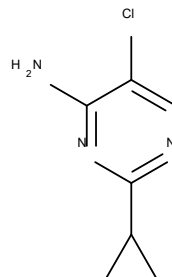
**Aminocyclopyrachlor methyl (DPX-KJM44; Aminocyclopyrachlor methyl ester; Aminocyclopyrachlor ME)**

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

