Test Material:	Chlorantraniliprole
MRID:	46889126
Title:	Validation of an Analytical Method for the Determination of DPX-E2Y45, IN-EQW78, IN-ECD73, IN-F6L99 and IN-GAZ70 in Soil, Report Amendment No. 1
MRID:	46979431
Title:	Independent Laboratory Validation of the Analytical Method, DuPont-14819, "Analytical Method for the Determination of DPX-E2Y45, IN-EQW78, IN-ECD73, IN-F6L99, and IN-GAZ70 in Soil"
EPA PC Code:	090100
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lynne Binari **Signature:** 

Date: 1/20/15

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Date: 1/20/15

Analytical method for chlorantraniliprole (DPX-E2Y45) and its transformation products IN-EQW78, IN-ECD73, IN-F6L99, and IN-GAZ70 in soil

Reports: ECM: EPA MRID No.: 46889126. Syme, G., M. Jones, and A. Doran. 2006. Validation of an Analytical Method for the Determination of DPX-E2Y45, IN-EQW78, IN-ECD73, IN-F6L99 and IN-GAZ70 in Soil, Report Amendment No. 1. Report prepared by Inveresk, Tranent, Scotland, sponsored and submitted by E.I. du Pont de Nemours and Company, Wilmington, Delaware; 283 pages. Inveresk Report No.: 23691, DuPont-14819, Revision No. 1. Final report issued November 18, 2004. Report Amendment No. 1 issued July 6, 2006.
ILV: EPA MRID No. 46979431. Neeley, M. 2005. Independent Laboratory Validation of the Analytical Method, DuPont-14819, "Analytical Method for the Determination of DPX-E2Y45, IN-EQW78, IN-ECD73, IN-F6L99, and IN-GAZ70 in Soil". Exygen Protocol and Project No.: P0001221. DuPont Study No.: DuPont-15024. Report prepared by Exygen Research, State College, Pennsylvania, sponsored and submitted by E. I. du Pont de Nemours and Company, Wilmington, Delaware; 36 pages. Final report issued May 18, 2005.

Document No.: MRIDs 46889126 & 46979431

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with OECD Good Laboratory Practice (GLP) standards and United Kingdom Department of Health Principles of GLP (p. 3 of MRID 46889126). Signed and dated Data Confidentiality, Quality Assurance, and Authenticity Certification statements were provided (pp. 2-4). The GLP statement was included as part of the Authenticity Certification (p. 3).
ILV: The study was conducted in compliance with USEPA GLP standards (p. 3 of MRID 46979431). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity Certification statements were provided (pp. 2-5).

Classification: This analytical method is classified as **Supplemental**. The LOQ maybe greater than the lowest toxicological level of concern (EC_{25} > 0.134 mg/kg) in soil. A finalized, comprehensive ECM representing the portions of the methods as validated by the independent laboratory was not submitted for review. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. The ILV did not report LODs. Soil matrices were not adequately characterized, and the registrant did not specify that the soil used in the ILV was either an equivalent, or more difficult, analytical sample condition as those used in the ECM. The ECM did not provide chromatograms for soils fortified at 10x LOQ. The ILV did not provide standard curves.

PC Code: 090100

Reviewer: Christopher M. Koper, M.S., Chemist **Date:** March 23, 2015

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Executive Summary

This analytical method, DuPont-10814, is designed for the quantitative determination of chlorantraniliprole (DPX-E2Y45) and its transformation products IN-EQW78, IN-ECD73, IN-GAZ70, and IN-F6L99 in soil using LC/MS/MS. The method is quantitative for the analytes at the stated LOQ of 0.5 µg/kg (500 mg/kg). The LOQ maybe greater than the lowest toxicological level of concern (EC_{25} > 0.134 mg/kg) in soil. The independent laboratory validated the method using the exhaustive extraction procedure for analysis of IN-EQW78 and IN-GAZ70 in soil (uncharacterized) after one trial and chlorantraniliprole and IN-ECD73 after two trials. The independent laboratory validated the method using the conventional extraction procedure for analysis of IN-F6L99 in soil after one trial. A finalized, comprehensive ECM representing the portions of the methods as validated by the independent laboratory was not submitted for review. The ILV did not report LODs. Soil matrices were not characterized, other than source location. The registrant did not specify that the soil used in the ILV was either an equivalent, or more difficult, analytical sample condition as those used in the ECM. The ECM did not provide chromatograms for soils fortified at 10x LOQ. The ILV did not provide standard curves.

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						
Chlorantraniliprole (DPX-E2Y45)	46889126	46979431		Soil	Final Report: 18/11/2004 Amendment No. 1: 06/07/2006	E. I. du Pont de Nemours and Company	LC/MS/MS	0.50 µg/kg
IN-EQW78								
IN-ECD73								
IN-GAZ70								
IN-F6L99								

I. Principle of the Method

Analytical Method DuPont-10814 utilizes two extraction procedures; a conventional extraction procedure for the determination of readily extractable residues of chlorantraniliprole (DPX-E2Y45) and its transformation products IN-EQW78, IN-ECD73, and IN-F6L99 in soil, and an exhaustive extraction procedure for the determination of difficult to extract residues of chlorantraniliprole and its products IN-EQW78, IN-ECD73, and IN-GAZ70 in soil (pp. 21, 24 of MRID 46889126).

Conventional extraction procedure: Soil (10 g) was fortified with a mixed standard of chlorantraniliprole (DPX-E2Y45), IN-EQW78, IN-ECD73, and IN-F6L99 in acetonitrile for procedural recoveries, with the fortified sample air-dried for 10 minutes to allow evaporation of the vehicle solvent (p. 27; Appendix 2, pp. 245-263 of MRID 46889126). Soil samples (10 g) are combined with 10 mL HPLC grade water, hand shaken for *ca*. 30 seconds, and allowed to sit for *ca*. 18 hours at *ca*. 4°C. The soil/water sample is extracted twice with acetonitrile:0.2% aqueous formic acid (90:10, v:v) by shaking (flatbed shaker, *ca*. 300 motions/minute) for *ca*. 60 minutes; extraction solvent volumes are 50 mL. Soil and extract are separated by centrifugation (*ca*. 3,000 rpm, 10 minutes). Extracts are combined and brought to 120 mL with the acetonitrile:0.2% formic acid extraction solvent. An aliquot (40 mL) is taken for analysis of IN-F6L99 (Aliquot B). The remaining sample (80 mL) is diluted to 220 mL with HPLC grade water and used for analysis if chlorantraniliprole, IN-EQW78, and IN-ECD73 (Aliquot A).

Exhaustive extraction procedure: Soil (10 g), or the post-centrifuge soil pellet from the conventional extraction procedure, was fortified with a mixed standard of chlorantraniliprole (DPX-E2Y45), IN-EQW78, IN-ECD73, and/or IN-GAZ70 in acetonitrile for procedural recoveries (pp. 27-29; Appendix 2, pp. 249-256; Appendix 3, pp. 264-280 of MRID 46889126). Soil samples (10 g), or the post-centrifuge soil pellet from the conventional extraction procedure, are extracted once with acetonitrile:0.2% aqueous formic acid (90:10, v:v, 25 mL) by shaking (flatbed shaker, *ca*. 300 motions/minute) for 15 minutes. Soil and extract are separated by centrifugation (*ca*. 3,000 rpm, 10 minutes) and the extract decanted. The extracted soil is further extracted with 1% HCl in acetonitrile (25 mL) by shaking (as described above) for 15 minutes followed by immersion in a 60°C water bath for 30 minutes. Soil and extract are separated by centrifugation, with the second extract combined with the first extract. The extracted soil is further extracted with 5% HCl in acetonitrile (50 mL) by shaking (as described) for 5 minutes, followed by immersion in a 60°C water bath for 1 hour, then shaking (as described) for 10 minutes. Soil and extract are separated by centrifugation, with the third extract combined with the first and second extracts. The combined extract is brought to 100 mL with 1% HCl in acetonitrile, then a 35-mL aliquot is taken and brought to 115 mL with HPLC grade water.

Cleanup procedure for chlorantraniliprole (DPX-E2Y45), IN-EDQW78, IN-ECD73, and IN-GAZ70; Aliquot A from conventional extraction procedure or combined extract from exhaustive extraction procedure: A Waters Oasis HLB (1 g/20 cc) solid-phase extraction (SPE) cartridge is preconditioned with methanol followed by HPLC grade water, and a Varian SAX Mega Bond Elut (1 g/6 mL) SPE cartridge is preconditioned with HPLC grade water:acetonitrile:concentrated formic acid (70:30:0.5, v:v:v; Appendix 2, pp. 248, 253, 256-257; Appendix 3, pp. 268, 271, 273-274 of MRID 46889126). The SAX cartridge is then attached above the Oasis HLB cartridge and loaded with a sample aliquot (55 mL of Aliquot A from the conventional extraction procedure, or 40 mL of the extract from the exhaustive extraction procedure). The loaded cartridges are rinsed with the water:acetonitrile:formic acid solution, then dried under vacuum. The SAX cartridge is removed, and the analytes are eluted with acetonitrile (20 mL), followed by ethyl acetate (25 mL). The eluate is taken to dryness under nitrogen (TurboVap, *ca*. 50°C), with the residues reconstituted in acetonitrile and 0.01M aqueous formic acid (final solvent:solvent ratio of 1:1, v:v).

Cleanup procedure for IN-F6L99; Aliquot B from conventional extraction procedure: A portion (10 mL) of Aliquot B is concentrated to *ca*. 0.1 mL under nitrogen (*ca*. 35°C), reconstituted in HPLC grade water (10 mL), and loaded onto an Oasis HLB (1 g/20 cc) cartridge preconditioned with methanol and HPLC grade water (Appendix 2, pp. 257-258 of MRID 46889126). The sample tube is rinsed with HPLC grade water and the rinses passed through the SPE cartridge. The loaded cartridge is dried under vacuum, then IN-F6L99 is eluted with acetonitrile. The eluate is taken to dryness under nitrogen (TurboVap, *ca*. 50°C), with the residues reconstituted in acetonitrile and 0.01M aqueous formic acid (final acetonitrile:0.01M formic acid ratio of 0.2:1.8, v:v).

LC/MS/MS analysis: Samples are analyzed for chlorantraniliprole (DPX-E2Y45) and its products IN-EQW78, IN-ECD73, IN -GAZ70, and IN-F6L99 by HPLC [Phenomenex C18(ODS), 4.6 mm x 2.0 mm, 3 µm guard column; Phenomenex C18(2), 4.6 mm x 150 mm, 3 µm column, column temperature *ca*. 40°C] using a mobile phase of (A) 0.01M aqueous formic acid and (B) methanol [percent A:B (v:v) at 0.0-0.5 min. 40:60, 2.0 min. 20:80, 5.0-8.0 min. 2:98, 8.5-11.5 min. 40:60 for chlorantraniliprole, IN-EQW78, IN-ECD73, and IN-GAZ70; percent A:B at 0.0-0.5 min. 90:10, 5.5 min. 20:80, 5.8-8.8 min. 10:90, 9.0-11.0 min. 90:10 for IN-F6L99; flow rate 1.0 mL/minute for all analytes] with MS/MS-APCI (Applied Biosystems API 4000 MS, atmospheric pressure chemical

ionization (APCI), positive ion mode) detection and multiple reaction monitoring (MRM; Appendix 2, pp. 245-246, 253, 258-260; Appendix 3, pp. 264-265, 271, 275-277 of MRID 46889126). Injection volume is 25 µL. Analytes are identified using two ion transitions; one for quantitation (Q, "target ion") and one for confirmation (C, "confirmatory ion"). Ion transitions monitored were as follows: *m/z* 284.0→177.0 (Q) and *m/z* 484.0→452.8 (C) for chlorantraniliprole (DPX-E2Y45), *m/z* 466.0→188.0 (Q) and *m/z* 466.0→186.0 (C) for IN-EQW78, *m/z* 279.1→244.1 (Q) and *m/z* 244.0→209.0 (C) for IN-ECD73, *m/z* 451.9→416.0 (Q) and *m/z* 449.9→414.1 (C) for IN-GAZ70, and *m/z* 204.0→172.9 (Q) and *m/z* 204.0→66.0 (C) for IN-F6L99. Expected retention times were not reported in the methods.

ILV: The independent laboratory performed the methods as written using the exhaustive extraction method for chlorantraniliprole and its products IN-EQW78, IN-ECD73, and IN-GAZ70, and the conventional extraction method for product IN-F6L99 with equivalent equipment substitutions and minor modifications to optimize MS/MS conditions (pp. 15-21 of MRID 46979431). Most specifically, a Phenomenex C18(ODS) 4.0 x 3.0 mm guard column was substituted for the unavailable guard column specified in the ECMs (pp. 18, 20-21 of MRID 46979431). The independent laboratory suggested minor modifications to the methods regarding addition of concentrated formic acid to the chlorantraniliprole (DPX-E2Y45) calibration standard and mixed standard fortification solutions to provide stability for chlorantraniliprole, and increasing the acetonitrile and ethyl acetate volumes for elution of chlorantraniliprole, IN-EQW78, IN-ECD73, and IN-GAZ70 (pp. 23, 28 of MRID 46979431).

LOQ and LOD: In the ECM and ILV, the LOQ for chlorantraniliprole (DPXE2Y45), IN-EQW78, IN-ECD73, IN-GAZ70, and IN-F6L99 was 0.5 µg/kg (pp. 22, 37 of MRID 46889126; pp. 10-11 of MRID 46979431). In the ECM, the LOD was set at 0.25 ng/mL for chlorantraniliprole, IN-EQW78, IN-ECD73, and IN-GAZ70; equivalent to 0.20 µg/kg in soil matrix for the analytes using the exhaustive extraction procedure and 0.30 µg/kg for chlorantraniliprole, IN-EQW78, and IN-ECD73 using the conventional extraction procedure. The ECM set the LOD at 0.10 ng/mL for IN-F6L99; equivalent to 0.25 µg/kg in soil matrix using the conventional extraction procedure. In the ILV, LODs were not reported.

II. Recovery Findings

ECM (MRID 46889126): Using the conventional extraction procedure, mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of chlorantraniliprole and its transformation products IN-EQW78, IN-ECD73, and IN-F6L99 in Spain (Seville), France (Alsace, Burgundy), and Italy (Bologna) soils (uncharacterized, other than source location) at fortification levels of 0.5 µg/kg (LOQ, n = 5), 5.0 µg/kg (10x LOQ, n = 5) and 70 µg/kg (140x LOQ, n = 3), with the following exceptions: 5.0 µg/kg IN-ECD73 in Seville soil (RSD 21.1%) and 0.5 µg/kg IN-F6L99 in Bologna soil (RSD 38.6%; DER Attachment 2).

Using the exhaustive extraction procedure, mean recoveries and RSDs were within guidelines for analysis of chlorantraniliprole and its products IN-EQW78, IN-ECD73, and IN-GAZ70 in Spain (Seville, Lleida), France (Alsace, Burgundy), and Italy (Bologna) soils (uncharacterized) at the three fortification levels, with the following exceptions: 70 µg/kg IN-ECD73 in Seville (mean 69.1%) and Bologna (mean 65.0%) soils and 0.5 µg/kg IN-GAZ70 in Seville soil (mean 138%, RSD 52.8%; DER Attachment 2). Using the exhaustive extraction procedure and soil pellet matrix, mean recoveries and RSDs were within guidelines for analysis of chlorantraniliprole and its products IN-EQW78, and IN-ECD73 in the Seville, Alsace, Burgundy, and Bologna soils at all three fortification levels (DER Attachment 2).

For both extraction procedures, results from the confirmatory method were not reported.

ILV (MRID 46979431): Using the exhaustive extraction procedure, mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of chlorantraniliprole and its products IN-EQW78, IN-ECD73, and IN-GAZ70 in Pennsylvania soil (uncharacterized, other than source location) at fortification levels of 0.5 µg/kg (LOQ) and 5.0 µg/kg (10x LOQ; pp. 10-11). The method was validated for IN-EQW78 and IN-GAZ70 at both fortification levels after one trial and validated for chlorantraniliprole and IN-ECD73 at both fortification levels after a second trial.

Using the conventional extraction procedure, mean recoveries and RSDs were within guidelines for analysis of chlorantraniliprole product IN-F6L99 in Pennsylvania soil at both fortification levels (p. 11). The method was validated for IN-F6L99 at both fortification levels after one trial.

For both extraction procedures, results from the confirmatory method were not reported. The sponsor, E. I. du Pont de Nemours and Company, did not establish that the soil used in the ILV was either an equivalent, or more difficult, analytical sample condition as those used for the ECM.

Table 2a. Initial Validation Method Recoveries for Chlorantraniliprole (DPX-E2Y45) and Its Transformation Products IN-EQW78, IN-ECD73, and IN-F6L99 in Fresh Soil Using Conventional Extraction Procedure[1]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Chlorantraniliprole (DPX-E2Y45)	**Seville, Spain soil**					
	0.5 (LOQ)	5	73.5-111	97.1	15.5	15.9
	5.0	5	81.3-120	102	15.1	14.7
	70	**3**	95.9-108	101	6.2	6.2
	Alsace, France soil					
	0.5 (LOQ)	5	75.5-116	98.4	15.0	15.2
	5.0	5	93.8-130	105[2]	15.1	14.3[2]
	70	**3**	72.4-89.9	83.6	9.7	11.6
	Burgundy, France soil					
	0.5 (LOQ)	5	98.6-113	105	7.2	6.8
	5.0	5	79.2-99.1	91.2	7.5	8.2
	70	**3**	93.0-93.9	93.4	0.5	0.5
	Bologna, Italy soil					
	0.5 (LOQ)	5	89.4-116	104	11.7	11.3
	5.0	5	70.3-99.3	90.4	12.1	13.4
	70	**3**	84.3-104	92.2	10.4	11.3
IN-EQW78	**Seville, Spain soil**					
	0.5 (LOQ)	5	79.1-99.6	85.6	8.6	10.1
	5.0	5	79.3-117	99.8	14.5	14.5
	70	**3**	106-109	107	1.5	1.4
	Alsace, France soil					
	0.5 (LOQ)	5	98.5-120	110	7.8	7.1
	5.0	5	86.8-102	97.0	6.2	6.4
	70	**3**	84.7-93.4	88.4	4.5	5.1
	Burgundy, France soil					
	0.5 (LOQ)	5	81.4-106	91.7	9.1	9.9
	5.0	5	84.4-105	93.5	8.4	9.0
	70	**3**	95.5-103	98.2	4.1	4.2
	Bologna, Italy soil					
	0.5 (LOQ)	5	76.6-103	90.8	11.0	12.1
	5.0	5	82.1-105	94.7	8.7	9.2
	70	**3**	90.0-106	99.7	8.5	8.5
IN-ECD73	**Seville, Spain soil**					
	0.5 (LOQ)	5	73.8-85.8	81.3	4.7	5.8
	5.0	5	64.2-105	82.9	17.5	**21.1**
	70	**3**	94.3-98.6	95.9	2.4	2.5
	Alsace, France soil					
	0.5 (LOQ)	5	92.3-109	100	6.1	6.1
	5.0	5	71.4-83.4	78.2	5.1	6.6
	70	**3**	81.0-86.7	83.0	3.2	3.9
	Burgundy, France soil					
	0.5 (LOQ)	5	83.8-91.6	88.5	3.3	3.7
	5.0	5	76.0-90.1	83.0	6.6	8.0
	70	**3**	77.5-83.5	80.4	3.0	3.7
	Bologna, Italy soil					

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.5 (LOQ)	5	69.4-105[3]	84.3	14.0	16.7
	5.0	5	75.8-80.8	78.9	1.9	2.4
	70	**3**	64.8-80.4	74.0	8.2	11.1
IN-F6L99	**Seville, Spain soil**					
	0.5 (LOQ)	5	78.3-98.0	84.3	8.4	10.0
	5.0	5	61.0-95.3	87.5	14.8	17.0
	70	**3**	106-109	108	1.7	1.6
	Alsace, France soil					
	0.5 (LOQ)	5	83.7-106	98.6	9.2	9.3
	5.0	5	83.0-106	90.8	8.9	9.8
	70	**3**	63.9-94.5	82.2	16.2	19.7
	Burgundy, France soil					
	0.5 (LOQ)	5	89.8-108	102	7.2	7.0
	5.0	5	88.8-102	94.2	5.1	5.4
	70	**3**	91.3-96.0	93.4	2.4	2.6
	Bologna, Italy soil					
	0.5 (LOQ)	5	64.3-153	91.8[2]	35.5	**38.6**[2]
	5.0	5	68.8-85.2	76.6	7.7	10.0
	70	**3**	88.3-89.4	88.7	0.6	0.7

Data (recovery results, corrected as footnoted) were obtained from Tables 11-14, pp. 52-59 of MRID 46889126 and DER Attachment 2 (all standard deviations, plus re-calculated means and relative standard deviations as necessary). Where noted, means and relative standard deviations differ from those provided by the study authors. The study authors, with no consistency or justification, would include or exclude an "atypical result" from the statistics. The reviewer included all recovery results in the statistics.

1 Soils were not characterized, other than source location (p. 25 of MRID 46889126).

2 Reviewer calculated mean and RSD differ from reported values (Table 12, p. 54; Table 14, p. 59 of MRID 46889126).

3 Recovery results corrected for residues detected in matrix control samples (Table 14, p. 59 of MRID 46889126).

Table 2b. Initial Validation Method Recoveries for Chlorantraniliprole (DPX-E2Y45) and Its Transformation Products IN-EQW78, IN-ECD73, and IN-GAZ70 in Fresh Soil Using Exhaustive Extraction Procedure[1]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Chlorantraniliprole (DPX-E2Y45)	**Seville, Spain soil**					
	0.5 (LOQ)	5	93.9-102	97.5	3.2	3.3
	5.0	5	96.0-104	99.8	3.6	3.6
	70	**3**	80.1-88.4	85.1	4.4	5.2
	Alsace, France soil					
	0.5 (LOQ)	5	91.1-120	107	14.0	13.2
	5.0	5	77.6-111	101	13.4	13.3
	70	**3**	77.1-99.5	87.9	11.2	12.8
	Burgundy, France soil					
	0.5 (LOQ)	5	78.7-114[2]	95.6	16.0	16.8
	5.0	5	85.2-108	96.4	8.3	8.6
	70	**3**	96.8-101	99.6	2.4	2.4
	Bologna, Italy soil					
	0.5 (LOQ)	5	79.4-131	107[3]	19.8	18.5[3]
	5.0	5	95.0-109	104	5.7	5.5
	70	**3**	77.9-83.6	80.2	3.0	3.7
	Lleida, Spain soil					
	0.5 (LOQ)	5	76.7-113	95.3	16.8	17.6
	5.0	5	83.2-106	97.6	9.3	9.5
	70	**3**	89.5-109	96.5	10.9	11.3
IN-EQW78	**Seville, Spain soil**					
	0.5 (LOQ)	5	99.0-110	105	5.5	5.2
	5.0	5	91.4-102	96.0	4.9	5.1
	70	**3**	87.1-97.1	91.2	5.3	5.8
	Alsace, France soil					
	0.5 (LOQ)	5	89.7-117	106	10.7	10.0
	5.0	5	71.0-104	90.7	12.4	13.6
	70	**3**	82.7-100	92.3	8.8	9.5
	Burgundy, France soil					
	0.5 (LOQ)	5	86.0-120[2]	106	13.4	12.6
	5.0	5	86.4-103	92.2	6.7	7.3
	70	**3**	102-111	107	4.5	4.2
	Bologna, Italy soil					
	0.5 (LOQ)	5	81.6-95.5	91.4	5.8	6.4
	5.0	5	91.4-106	99.5	6.4	6.4
	70	**3**	73.4-86.0	79.9	6.3	7.9
	Lleida, Spain soil					
	0.5 (LOQ)	5	70.8-91.3[2]	79.3	8.3	10.5
	5.0	5	86.4-100[2]	95.0	5.2	5.4
	70	**3**	95.1-110[2]	100	8.4	8.4
IN-ECD73	**Seville, Spain soil**					
	0.5 (LOQ)	5	76.3-86.4[2]	79.2	4.1	5.1
	5.0	5	70.1-86.1	80.6	6.3	7.8
	70	**3**	65.9-71.3	**69.1**	2.8	4.1
	Alsace, France soil					

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.5 (LOQ)	5	93.7-104	98.8	3.8	3.8
	5.0	5	59.8-82.2	75.4	8.9	11.8
	70	**3**	59.9-85.2	72.6	12.7	17.4
	Burgundy, France soil					
	0.5 (LOQ)	5	64.1-82.8	74.9	7.6	10.2
	5.0	5	68.6-83.4	75.2	5.9	7.9
	70	**3**	96.2-101	98.2	2.5	2.5
	Bologna, Italy soil					
	0.5 (LOQ)	5	75.8-99.3	89.3	8.9	10.0
	5.0	5	73.3-81.9	78.8	3.8	4.9
	70	**3**	61.7-69.7	**65.0**	4.2	6.5
	Lleida, Spain soil					
	0.5 (LOQ)	5	79.1-90.5	85.0	4.7	5.5
	5.0	5	76.6-94.6	85.3	8.2	9.6
	70	**3**	78.9-103	89.9	12.2	13.6
IN-GAZ70	**Seville, Spain soil**					
	0.5 (LOQ)	5	102-268	**138**[3]	72.8	**52.8**[3]
	5.0	5	88.1-104	97.5	6.3	6.5
	70	**3**	76.3-95.6	86.5	9.7	11.2
	Burgundy, France soil					
	0.5 (LOQ)	5	93.0-117	103	8.9	8.6
	5.0	5	96.9-109	103	5.5	5.4
	70	**3**	77.1-91.9	86.7	8.3	9.6
	Lleida, Spain soil					
	0.5 (LOQ)	5	80.5-98.3	87.5	6.9	7.9
	5.0	5	79.0-91.0	85.9	5.0	5.8
	70	**3**	82.2-92.2	86.0	5.4	6.3

Data (recovery results, corrected as footnoted) were obtained from Tables 15-18, pp. 60-67; Tables 23-25, pp. 76-78 of MRID 46889126 and DER Attachment 2 (all standard deviations, plus re-calculated means and relative standard deviations as necessary). Where noted, means and relative standard deviations differ from those provided by the study authors. The study authors, with no consistency or justification, would include or exclude an "atypical result" from the statistics. The reviewer included all recovery results in the statistics.

1 Soils were not characterized, other than source location (pp. 25-26 of MRID 46889126).

2 Recovery results corrected for residues detected in matrix control samples (Table 15, p. 61; Table 17, p. 64; Table 25, p. 78 of MRID 46889126).

3 Reviewer calculated mean and RSD differ from reported values (Table 18, p. 66; Table 23, p. 76 of MRID 46889126).

Table 2c. Initial Validation Method Recoveries for Chlorantraniliprole (DPX-E2Y45) and Its Transformation Products IN-EQW78 and IN-ECD73 in Soil Pellets Using Exhaustive Extraction Procedure[1]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Chlorantraniliprole (DPX-E2Y45)	**Seville, Spain soil**					
	0.5 (LOQ)	5	67.3-84.0	76.4	7.3	9.5
	5.0	5	74.2-97.2	89.7	8.9	9.9
	70	3	73.8-96.2	85.8	11.3	13.2
	Alsace, France soil					
	0.5 (LOQ)	5	92.0-104	97.3	4.5	4.6
	5.0	5	74.0-109	97.8	14.0	14.3
	70	3	86.0-99.0	94.2	7.1	7.6
	Burgundy, France soil					
	0.5 (LOQ)	5	81.7-112[2]	98.3	13.3	13.5
	5.0	5	82.3-112	94.7	12.5	13.2
	70	3	85.6-95.5	89.4	5.3	6.0
	Bologna, Italy soil					
	0.5 (LOQ)	5	76.4-110	98.4	13.0	13.2
	5.0	5	98.3-106	102	3.5	3.5
	70	3	91.6-101	97.0	4.9	5.0
IN-EQW78	**Seville, Spain soil**					
	0.5 (LOQ)	5	83.0-107	97.3	9.0	9.2
	5.0	5	91.3-106	97.2	6.2	6.4
	70	3	96.9-110	105	7.1	6.7
	Alsace, France soil					
	0.5 (LOQ)	5	94.1-113	102	8.8	8.6
	5.0	5	78.3-105	98.7	11.5	11.6
	70	3	90.7-110	101	9.8	9.7
	Burgundy, France soil					
	0.5 (LOQ)	5	77.1-97.4[2]	89.2	7.7	8.7
	5.0	5	101-119	110	7.1	6.5
	70	3	91.5-96.0	93.5	2.3	2.5
	Bologna, Italy soil					
	0.5 (LOQ)	5	74.0-122	96.8	19.1	19.7
	5.0	5	97.0-105	101	2.9	2.8
	70	3	91.0-110	104	11.0	10.6
IN-ECD73	**Seville, Spain soil**					
	0.5 (LOQ)	5	67.2-82.9[2]	74.5	6.3	8.5
	5.0	5	73.8-94.0	83.4	7.3	8.7
	70	3	73.0-88.7	81.6	7.9	9.7
	Alsace, France soil					
	0.5 (LOQ)	5	81.8-92.4	86.8	4.1	4.7
	5.0	5	68.5-105	92.4	14.2	15.4
	70	3	86.2-102	95.9	8.5	8.8
	Burgundy, France soil					
	0.5 (LOQ)	5	74.2-88.8	80.9	6.6	8.1
	5.0	5	85.1-113	96.5	10.5	10.9
	70	3	67.1-80.3	73.1	6.7	9.1
	Bologna, Italy soil					

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.5 (LOQ)	5	63.0-91.6[2]	78.4	10.7	13.7
	5.0	5	91.5-108[2]	97.0	7.0	7.2
	70	3	76.7-94.3	84.9	8.9	10.4

Data (recovery results, corrected as footnoted) were obtained from Tables 19-22, pp. 68-75 of MRID 46889126 and DER Attachment 2 (all standard deviations, plus re-calculated means and relative standard deviations as necessary). Where noted, means and relative standard deviations differ from those provided by the study authors. The study authors, with no consistency or justification, would include or exclude an "atypical result" from the statistics. The reviewer included all recovery results in the statistics.

1 Soils were not characterized, other than source location (p. 25 of MRID 46889126). Soil pellets are post-centrifuge soils from the conventional extraction procedure.

2 Recovery results corrected for residues detected in matrix control samples (Table 19, p. 69; Table 21, p. 72; Table 22, p. 75 of MRID 46889126).

Table 3. Independent Validation Method Recoveries for Chlorantraniliprole (DPX-E2Y45) and Its Transformation Products IN-EQW78, IN-ECD73, IN-GAZ70, and IN-F6L99 in Pennsylvania Soil[1]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Exhaustive Extraction Procedure						
Chlorantraniliprole (DPX-E2Y45)	0.5 (LOQ)	5	82.5-117	102	14.9	14.6
	5.0	5	86.1-113	97.6	11.4	11.7
IN-EQW78	0.5 (LOQ)	5	90.1-137	111	17.0	15.3
	5.0	5	80.5-102	92.0	8.67	9.42
IN-ECD73	0.5 (LOQ)	5	78.1-84.2	81.1	2.63	3.24
	5.0	5	78.5-87.3	82.8	3.97	4.79
IN-GAZ70	0.5 (LOQ)	5	88.2-97.6	92.4	4.65	5.03
	5.0	5	81.4-84.5	83.1	1.31	1.58
Conventional Extraction Procedure						
IN-F6L99	0.5 (LOQ)	5	97.6-102	100	2.04	2.04
	5.0	5	96.1-100	98.4	1.68	1.71

Data (uncorrected recovery results) were obtained from pp. 10-11, 22, 24-25 of MRID 46979431.

1 Soil was not characterized, other than source location; property of Paul Connolly in Centre Hall, Pennsylvania (p. 12 of MRID 46979431).

III. Method Characteristics

In the ECM and ILV, the LOQ for chlorantraniliprole, IN-EQW78, IN-ECD73, IN-GAZ70, and IN-F6L99 in soil was 0.5 µg/kg (pp. 22, 37 of MRID 46889126; pp. 10-11 of MRID 46979431). The ECM defined the LOQ as the lowest validated fortification level for each soil type and extraction procedure. The ECM set the LODs as the sample concentration equivalent to the 0.25 ng/mL calibration standard for chlorantraniliprole, IN-EQW78, IN-ECD73, and IN-GAZ70 and sample concentration equivalent to the 0.10 ng/mL calibration standard for IN-F6L99. In the ILV, no LODs were reported.

Table 4a. Method Characteristics for Chlorantraniliprole (DPX-E2Y45) and Its Transformation Products IN-EQW78, IN-ECD73, and IN-F6L99 in Fresh Soil Using Conventional Extraction Procedure

		Chlorantraniliprole	**IN-EQW78**	**IN-ECD73**	**IN-F6L99**
Limit of Quantitation (LOQ)		0.5 µg/kg			
Limit of Detection (LOD)		0.25 ng/mL (0.30 µg/kg in soil matrix)			0.10 ng/mL (0.25 µg/kg in soil matrix)
Linearity (1/x weighting, calibration curve r^2 and concentration range)[1]	ECM:	Q ion: r^2 = 0.9996 (0.1-25 ng/mL)	Q ion: r^2 = 0.9990 (0.1-24 ng/mL)	Q ion: r^2 = 0.9986 (0.1-25 ng/mL)	Q ion: r^2 = 0.9992 (0.05-10 ng/mL)
	ILV:	Not performed.			Not reported.
Repeatable	ECM:	Yes at LOQ, 10x LOQ, and 140x LOQ, except for 5.0 µg/kg IN-ECD73 in Seville soil (RSD 21.1%) and 0.5 µg/kg IN-F6L99 in Bologna soil (RSD 38.6%).			
	ILV:	Not performed.			Yes at LOQ and 10x LOQ.
Reproducible		Not performed.			Yes; however, the study sponsor (E. I. du Pont de Nemours and Company) did not establish that the soil used in the ILV was either an equivalent, or more difficult, analytical sample condition as those used for the ECM.
Specific[2]	ECM:	Yes; no significant interferences exceeding one-third of LOQ.			
	ILV:	Not performed.			Yes

Data were obtained from pp. 22, 36-37; Tables 1-4, pp. 42-45; Figures 1-4, pp. 81-84; Figures 11-18, pp. 91-98; Figures 23-30, pp. 103-110; Figures 35-42, pp. 115-122; Figures 47-54, pp. 127-134 of MRID 46889126; pp. 10-11; Figure 7, p. 36 of MRID 46979431; and DER Attachment 2.

1 Linearity of the ECM calibration curves was verified by the reviewer (DER Attachment 2). ECM r^2 values are reviewer-generated from reported r values of 0.9993-0.9998 (Tables 1-4, pp. 42-45 of MRID 46889126; DER Attachment 2).

2 Results from confirmatory method were not provided.

Table 4b. Method Characteristics for Chlorantraniliprole (DPX-E2Y45) and Its Transformation Products IN-EQW78, IN-ECD73, and IN-GAZ70 in Fresh Soil Using Exhaustive Extraction Procedure

		Chlorantraniliprole	IN-EQW78	IN-ECD73	IN-GAZ70
Limit of Quantitation (LOQ)		0.5 µg/kg			
Limit of Detection (LOD)		0.25 ng/mL (0.20 µg/kg in soil matrix)			
Linearity (1/x weighting, calibration curve r^2 and concentration range)[1]	ECM:	Q ion: r^2 = 0.9996 (0.1-25 ng/mL)	Q ion: r^2 = 0.9990 (0.1-24 ng/mL)	Q ion: r^2 = 0.9986 (0.1-25 ng/mL)	Q ion: r^2 = 0.9992 (0.1-24 ng/mL)
	ILV:	Q ion: r^2 = 0.9974 (0.1-5 ng/mL)[2]	Not reported.		
Repeatable	ECM:	Yes at LOQ, 10x LOQ, and 140x LOQ, except for 70 µg/kg IN-ECD73 in Seville (mean 69.1%) and Bologna (mean 65.0%) soils and 0.5 µg/kg IN-GAZ70 in Seville soil (mean 138%, RSD 52.8%).			
	ILV:	Yes at LOQ and 10x LOQ.			
Reproducible		Yes; however, the study sponsor (E.I. du Pont de Nemours and Company) did not establish that the soil used in the ILV was either an equivalent, or more difficult, analytical sample condition as those used for the ECM.			
Specific[3]	ECM:	Yes; no significant interferences exceeding one-third of LOQ.			
	ILV:	Baseline noise appears >30% of peak height at LOQ.	Yes, baseline noise ≤30% of LOQ.		

Data were obtained from p. 37; Tables 1-3, pp. 42-44; Table 5, p. 46; Figures 1-3, pp. 81-83; Figure 5, p. 85; Figures 59-64, pp. 139-144; Figures 68-73, pp. 148-153; Figures 77-82, pp. 157-162; Figures 86-91, pp. 166-171; Figures 131-132, pp. 211-212; Figures 134-135, pp. 214-215; Figures 137-144, pp. 217-224 of MRID 46889126; pp. 10-11; Figures 3-5, pp. 32-34 of MRID 46979431; and DER Attachment 2.

1 Linearity of the ECM calibration curves was verified by the reviewer (DER Attachment 2). ECM r^2 values are reviewer-generated from reported r values of 0.9993-0.9998 (Tables 1-3, pp. 42-44; Table 5, p. 46 of MRID 46889126; DER Attachment 2).

2 Calibration curves were not provided in ILV MRID. Linearity determined by reviewer using representative chlorantraniliprole calibration standard data (p. 26 of MRID 46979431; DER Attachment 2).

3 Results from confirmatory method were not provided.

Table 4c. Method Characteristics for Chlorantraniliprole (DPX-E2Y45) and Its Transformation Products IN-EQW78, IN-ECD73, and IN-GAZ70 in Soil Pellets Using Exhaustive Extraction Procedure

		Chlorantraniliprole	IN-EQW78	IN-ECD73
Limit of Quantitation (LOQ)		0.5 µg/kg		
Limit of Detection (LOD)		0.25 ng/mL (0.20 µg/kg in soil matrix)		
Linearity (1/x weighting, calibration curve r^2 and concentration range)[1]	ECM:	Q ion: r^2 = 0.9996 (0.1-25 ng/mL)	Q ion: r^2 = 0.9990 (0.1-24 ng/mL)	Q ion: r^2 = 0.9986 (0.1-25 ng/mL)
	ILV:	Not performed.		
Repeatable	ECM:	Yes at LOQ, 10x LOQ, and 140x LOQ.		
	ILV:	Not performed.		
Reproducible		ILV did not use this matrix.		
Specific[2]	ECM:	Yes; no significant interferences exceeding one-third of LOQ.		
	ILV:	Not performed.		

Data were obtained from p. 37; Tables 1-3, pp. 42-44; Figures 1-3, pp. 81-83; Figures 95-100, pp. 175-180; Figures 104-109, pp. 184-189; Figures 113-118, pp. 193-198; Figures 122-127, pp. 202-207 of MRID 46889126; and DER Attachment 2.

1 Linearity of the ECM calibration curves was verified by the reviewer (DER Attachment 2). ECM r^2 values are reviewer-generated from reported r values of 0.9993-0.9998 (Tables 1-3, pp. 42-44 of MRID 46889126; DER Attachment 2).

2 Results from confirmatory method were not provided.

IV. Method Deficiencies and Reviewer's Comments

1. A finalized, comprehensive ECM representing the portions of the methods as validated by the independent laboratory, plus the equipment substitutions and method modifications implemented by the independent laboratory should be submitted for review.

   The sponsor, E. I. du Pont de Nemours and Company, presented the validating laboratory, Inveresk, with two ECMs. The first ECM, Analytical Method No.: 0335A Version 1, included a conventional extraction procedure for the analysis of chlorantraniliprole, IN-EQW78, IN-ECD73, and IN-F6L99 in fresh soil and an exhaustive extraction procedure for the analysis of chlorantraniliprole, IN-EQW78, and IN-ECD73 in fresh soil and soil pellets (extracted soil from the conventional extraction procedure; Appendix 2, pp. 245-263 of MRID 46889126). The second ECM, Analytical Method No.: 0335B Version 1, included the exhaustive extraction procedure for the analysis of chlorantraniliprole, IN-EQW78, IN-ECD73, and newly identified transformation product IN-GAZ70 in fresh soil, plus a procedure for analysis of the analytes in petri dishes with no soil (Appendix 3, pp. 264-280). The exhaustive extraction procedure was identical between Analytical Method No.s 0335A Version 1 and 0335B Version 1, with the exception of the addition of analyte IN-GAZ70 (p. 21). Inveresk identified the provided method as Dupont-10814, even though the appended methods were identified as Analytical Method No.: 0335A Version 1 and Analytical Method No.: 0335B Version 1 (pp. 21, 24; Appendix 2, p. 245; Appendix 3, p. 264).

   Analytical Method No.: 0335A Version 1 was reported as suitable for the determination of chlorantraniliprole (DPX-E2Y45), IN-EQW78, IN-ECD73, and IN-F6L99 in soil (Appendix 2, p. 245 of MRID 46889126). Analytical Method No.: 0335B Version 1 was reported as suitable for the determination of chlorantraniliprole, IN-EQW78, IN-ECD73, and IN-GAZ70 in soil and petri dishes with no soil (Appendix 3, p. 264). Analytical method No.: 0335B Version 1 is the "proposed method for monitoring DPX-E2Y45 and metabolites in soil"; however, that method does not analyze for product IN-F6L99 (pp. 21, 26; Appendix 3, p. 264).

   Inveresk validated the conventional extraction procedure for chlorantraniliprole, IN-EQW78, IN-ECD73, and IN-F6L99 in fresh soil, the exhaustive extraction procedure for chlorantraniliprole, IN-EQW78, IN-ECD73, and IN-GAZ70 in fresh soil, the exhaustive extraction procedure for chlorantraniliprole, IN-EQW78, and IN-ECD73 in soil pellets, and the procedure for chlorantraniliprole, IN-EQW78, IN-ECD73, and IN-GAZ70 in petri dishes with no soil (Tables 11-26, pp. 52-80 of MRID 46889126).

   The independent laboratory, Exygen Research, validated the exhaustive extraction procedure for chlorantraniliprole, IN-EQW78, IN-ECD73, and IN-GAZ70 and the conventional extraction procedure for IN-F6L99 in fresh soil (pp. 10-11 of MRID 46979431). The independent laboratory substituted an available Phenomenex C18(ODS) 4.0 x 3.0 mm guard column for the unavailable guard column specified in the ECMs (pp. 18, 20-21). The study monitor (DuPont Crop Protection) approved use of the available guard column and specified that "he would note this change in the original report" (pp. 6, 18, 20-21). The independent laboratory also suggested minor modifications to the methods regarding addition of concentrated formic acid to the chlorantraniliprole (DPX-E2Y45) calibration standard and mixed standard fortification solutions to provide stability for

chlorantraniliprole, and increasing the acetonitrile and ethyl acetate volumes for elution of chlorantraniliprole, IN-EQW78, IN-ECD73, and IN-GAZ70 (pp. 23, 28).

2. The determination of the LOQ and LOD were not based on scientifically acceptable procedures. The ECM defined the LOQ as the lowest validated fortification level for each soil type and extraction procedure (p. 37 of MRID 46889126). The ECM set the LODs as the sample concentration equivalent to the 0.25 ng/mL calibration standard for chlorantraniliprole, IN-EQW78, IN-ECD73, and IN-GAZ70 and sample concentration equivalent to the 0.10 ng/mL calibration standard for IN-F6L99. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in soil was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

3. In the ILV, LODs for the analytes were not reported.

4. For both the ECM and ILV, soil matrices were not characterized, other than source location (pp. 25-26 of MRID 46889126; p. 12 of MRID 46979431). The registrant did not specify that the Pennsylvania soil used in the ILV was either an equivalent, or more difficult, analytical sample condition as the five foreign soils (Seville and Lleida, Spain, Alsace and Burgundy, France, and Bologna, Italy) used in the ECM. Inveresk described the Lleida soil as "very clay like" (p. 39 of MRID 46889126).

5. For the ECM, chromatograms were not provided for reagent blanks or soils fortified at 10x LOQ (Figures 1-148, pp. 81-228 of MRID 46889126). Only chromatograms of the 5.0 ng/mL standard for chlorantraniliprole, IN-EQW78, IN-ECD73, and IN-GAZ70 (calibration standard range 0.1-25 ng/mL) and 2.0 ng/mL standard for IN-F6L99 (calibration standard range 0.05-10 ng/mL) were provided (p. 27). Additionally, the resolution of the submitted chromatograms was poor: the baseline was faint or invisible in all of the chromatograms.

6. For the ILV, chromatograms were not provided for reagent blanks (Figures 1-7, pp. 31-36 of MRID 46979431). Only chromatograms of the 0.1 ng/mL standard for chlorantraniliprole, IN-EQW78, IN-ECD73, and IN-GAZ70 and 0.05 ng/mL standard for IN-F6L99 were provided. Standard curves were not provided. One set of calibration standard data (range 0.10-5.0 ng/mL) was provided for chlorantraniliprole (p. 26).

7. For the ECM, an insufficient number of replicates were analyzed at the 70 µg/kg fortification level (n = 3; Tables 11-25, pp. 52-79). A minimum of five spiked replicates should be analyzed at each concentration (*i.e.*, minimally, the LOQ and 10x LOQ) for each analyte.

8. For the ECM validation, analysis of fortified soil extracts found no significant matrix enhancement or suppression; mean percent deviation was reported as 85-115% for all soil types (individual data not reported; pp. 29-30, 36 of MRID 46889126). Prior to the trial, the independent lab verified that the soil matrix was "free of interferences and that no matrix effects were observed at the retention time of the analytes"; "no response greater than 30% of the proposed LOQ was observed" (p. 21 of MRID 46979431). However, for chlorantraniliprole, baseline noise appears >30% of peak height at LOQ (Figure 3, p. 32 of MRID 46979431). For IN-EQW78, IN-ECD73, IN-GAZ70, and IN-F6L99, baseline noise appears ≤30% of peak height at LOQ (Figures 4-7, pp. 33-36 of MRID 46979431).

9. As part of the ECM validation, LC/MS/MS system precision (coefficient of variation) was determined to be 2.2-8.5% for the analytes using the 5.0 ng/mL calibration standard for chlorantraniliprole, IN-EQW78, IN-ECD73, and IN-GAZ70 and the 2.0 ng/mL calibration standard for IN-F6L99 (p. 33; Tables 6-10, pp. 47-51 of MRID 46889126).

10. As part of the ECM validation, extraction of chlorantraniliprole and its products IN-EQW78, IN-ECD73, and IN-GAZ70 from petri dishes with no soil was investigated (Appendix 3, pp. 264-280 of MRID 46889126). Fortified petri dishes were extracted with acetonitrile:aqueous formic acid (90:10, v:v) using sonication. An aliquot of the extract was diluted with water to 50:50 (v:v) acetonitrile:water, then analyzed by LC/MS/MS as described above. Recovery results are presented below.

Table 5. Initial Validation Method Recoveries for Chlorantraniliprole (DPX-E2Y45) and Its Transformation Products IN-EQW78, IN-ECD73, and IN-GAZ70 in Petri Dishes Without Soil

Analyte	Fortification Level (µg/dish)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Chlorantraniliprole (DPX-E2Y45)	50	4	88.0-106	97.8	7.4	7.6
	600	4	85.7-100	92.8	7.8	8.4
IN-EQW78	50	4	92.4-107	98.4	6.6	6.7
IN-ECD73	50	4	88.6-93.9	92.0	2.4	2.6
IN-GAZ70	50	4	87.1-99.4	93.4	6.6	7.1

Data were obtained from Table 26, p. 80 of MRID 46889126 and DER Attachment 2 (standard deviation).

11. As part of the ECM validation, soil homogenization procedures were investigated. Soils from various depths (0-5, 5-15, 15-30, 30-50, 50-70, 70-90 cm) were homogenized, using various methods, with Evans Blue dye (pp. 31-32 of MRID 46889126). The dye was then extracted with water and quantified via spectrophotometer. Seville and Burgundy soils (0-5, 5-15 cm) could be homogenized by hand (p. 23). Seville (15-70 cm), Burgundy (15-90 cm), Alsace (0-90 cm), Bologna (0-90 cm), and Lleida (0-30 cm) soils should be homogenized by air-drying and sieving. Large (1 kg) soil samples (Lleida, 0-20 cm) could also be homogenized by air-drying and sieving. Mean precision (coefficient of variation) for the homogenization methods was reported as ≤20%.

12. It was reported for the ILV that a single set of thirteen samples required *ca*. 10 person hours for the exhaustive extraction procedure and *ca*. 7 person hours for the conventional extraction procedure, followed by *ca*. 4 hours of unattended overnight LC/MS/MS analysis (p. 11 of MRID 46979431).

V. References

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

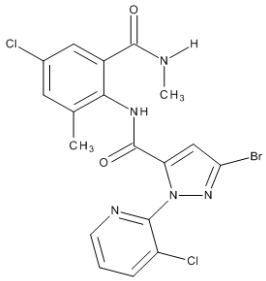
40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.


calcs

Attachment 1: Chemical Names and Structures

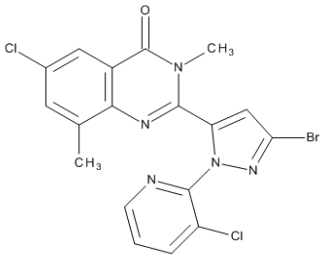
Chlorantraniliprole (DPX-E2Y45)

IUPAC Name:	3-Bromo-4′-chloro-1-(3-chloro-2-pyridyl)-2′-methyl-6′-(methylcarbamoyl)pyrazole-5-carboxanilide
CAS Name:	3-Bromo-N-[4-chloro-2-methyl-6-[(methylamino)carbonyl]phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide
CAS Number:	500008-45-7
SMILES String:	Cc1cc(cc(c1NC(=O)c2cc(nn2c3c(cccn3)Cl)Br)C(=O)NC)Cl



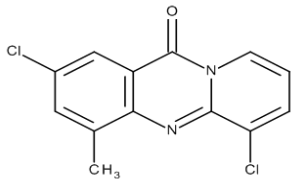
IN-EQW78

IUPAC Name:	2-[5-Bromo-2-(3-chloro-2-pyridyl)pyrazol-3-yl]-6-chloro-3,8-dimethyl-quinazolin-4-one
CAS Name:	Not reported.
CAS Number:	N/A
SMILES String:	Cc1cc(cc2c1nc(n(c2=O)C)c3cc(nn3c4c(cccn4)Cl)Br)Cl



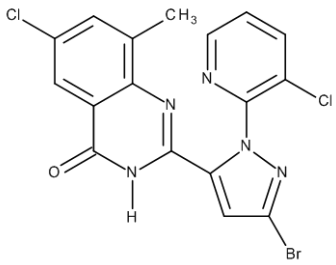
IN-ECD73

IUPAC Name:	2,6-Dichloro-4-methyl-pyrido[2,1-b]quinazolin-11-one
CAS Name:	Not reported.
CAS Number:	N/A
SMILES String:	Cc1cc(cc2c1nc3c(cccn3c2=O)Cl)Cl



IN-GAZ70

IUPAC Name:	2-[5-Bromo-2-(3-chloro-2-pyridyl)pyrazol-3-yl]-6-chloro-8-methyl-3H-quinazolin-4-one
CAS Name:	Not reported.
CAS Number:	N/A
SMILES String:	[H]n1c(=O)c2cc(cc(c2nc1c3cc(nn3c4c(cccn4)Cl)Br)C)Cl



IN-F6L99

IUPAC Name:	(2-[3-Bromo-1-(3-chloropyridin-2-yl)-1H-pyrazol-5-yl]-6-chloro-8-methylquinazolin-4(1H)-one
CAS Name:	Not reported.
CAS Number:	N/A
SMILES String:	Not found.