Test Material:	Chlorantraniliprole
MRID:	46979445
Title:	Analytical Method for the Determination of DPX-E2Y45 and Degradation Products in Water Using LC/MS/MS
MRID:	46979530
Title:	Inter laboratory validation of DuPont-16058 "Analytical method for the determination of DPX-E2Y45 and degradation products in water using LC/MS/MS"
EPA PC Code:	090100
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lynne Binari **Signature:** 

Date: 1/20/15

Secondary Reviewer: Lisa Muto **Signature:** 

Date: 1/20/15

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 1/20/15

Analytical method for chlorantraniliprole (DPX-E2Y45) and its transformation products IN-F9NO4, IN-EQW78, IN-GAZ70, IN-ECD73, and IN-F6L99 in water

Reports: ECM: EPA MRID No. 46979445. Bilas, J., and J. Stry. 2005. Analytical Method for the Determination of DPX-E2Y45 and Degradation Products in Water Using LC/MS/MS. Report prepared by E. I. du Pont de Nemours and Company, DuPont Crop Protection, Newark, Delaware, sponsored and submitted by E. I. du Pont de Nemours and Company, Wilmington, Delaware; 87 pages. DuPont Project ID: DuPont-16058. Final report issued February 18, 2005.
ILV: EPA MRID No. 46979530. Buscher, B. 2006. Inter laboratory validation of DuPont-16058 "Analytical method for the determination of DPX-E2Y45 and degradation products in water using LC/MS/MS". TNO Project No.: 010.31490, Study Code: 6449, and Report No.: V6449, Amendment 1. DuPont Study Code: DuPont-16708 Revision 1. Report prepared by TNO Quality of Life, Zeist, The Netherlands, sponsored and submitted by E. I. du Pont de Nemours and Company, Wilmington, Delaware; 74 pages. Final report issued May 22, 2006.

Document No.: MRIDs 46979445 & 46979530

Guideline: 850.6100

Statements: ECM: The study was not conducted under the restriction of compliance with USEPA Good Laboratory Practice (GLP) standards; however the study was conducted in a GLP compliant facility following Standard Operating Procedures (p. 3 of MRID 46979445). Signed and dated Data Confidentiality, GLP, and Authenticity Certification statements were provided (pp. 2-4). A Quality Assurance statement was not provided.
ILV: The study was conducted in compliance with OECD Principles of GLP (p. 3 of MRID 46979530). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included as part of the Quality Assurance statement (p. 4).

Classification: This analytical method is classified as **Supplemental**. The LOQ of 0.10 ug/L is less than the lowest toxicological level of concern (acute invertebrate aquatic life benchmark = 4.9 ug/L) in water. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. The ILV did not report LODs. For both the ECM and ILV, direct comparison could not be made between matrix control and LOQ chromatograms. The ILV ground and drinking water matrices were not characterized.

PC Code: 090100

Reviewer: Christopher M. Koper, M.S., Chemist **Signature:**
Date: March 24, 2015

Koper, Christopher
Digitally signed by Koper, Christopher
DN: cn=Koper, Christopher, email=Koper.Christopher@epa.gov
Date: 2015.03.24 15:01:08 -04'00'

Executive Summary

This analytical method, DuPont-16058, is designed for the quantitative determination of chlorantraniliprole (DPX-E2Y45) and its transformation products IN-F9NO4, IN-GAZ70, IN-EQW78, IN-ECD73, and IN-F6L99 in water using LC/MS/MS. The method is quantitative for the analytes at the stated LOQ of 0.10 µg/L. The LOQ is less than the lowest toxicological level of concern (acute invertebrate aquatic life benchmark = 4.9 ug/L) in water. The independent laboratory validated the method for analysis of IN-F6L99 in surface, ground, and drinking water matrices after one trial and for all other analytes in the three matrices after a second trial. No major modifications were made by the independent laboratory. The ILV did not report LODs. For both the ECM and ILV, direct comparison could not be made between matrix control and LOQ chromatograms. The ILV ground and drinking water matrices were not characterized.

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	46979445	46979530		Surface, ground, and drinking water	18/02/2005	E. I. du Pont de Nemours and Company	LC/MS/MS	0.10 ng/g (ppb, µg/L)
IN-F9NO4								
IN-GAZ70								
IN-EQW78								
IN-ECD73								
IN-F6L99								

I. Principle of the Method

Water samples containing sediment should be centrifuged prior to use (p. 17 of MRID 46979445). Glass labware should be used as IN-GAZ70 may adhere to plastic surfaces (p. 13).

Liquid-Liquid Extraction (LLE) of chlorantraniliprole (DPX-E2Y45), IN-F9NO4, IN-EQW78, IN-GAZ70, and IN-ECD73: Water (50 mL) was fortified with a mixed standard of chlorantraniliprole (DPX-E2Y45), IN-F9NO4, IN-GAZ70, IN-EQW78, and IN-ECD73 in acetonitrile for procedural recoveries (pp. 12-13, 15, 17 of MRID 46979445). Water samples (50 ± 1.0 mL) are partitioned twice with hexane:ethyl acetate (50:50, v:v); extraction solvent volumes were 100 mL for the first partition and 50 mL for the second partition (pp. 13, 17-18). Organic phases are combined and brought to volume (150 mL) with ethyl acetate. An aliquot (50 mL) is taken to dryness under nitrogen (N-Evap, 50-55°C). Resulting residues are reconstituted in 1.0 mL acetonitrile with sonication for 5 minutes, then diluted with 1.0 mL 0.01M aqueous formic acid, and filtered (Acrodisc PTFE, 0.2 µm; p. 12).

Solid-Phase Extraction (SPE) of IN-F6L99: Water (25 mL) was fortified with IN-F6L99 in acetonitrile for procedural recoveries (pp. 13, 15, 18 of MRID 46979445). Water (25 ± 1.0 mL) was acidified with 0.25 mL acetic acid, then loaded onto a Waters Oasis HLB (500 mg/6 cc) SPE) cartridge is preconditioned with methanol followed by HPLC grade water (pp. 12, 18-19). The loaded cartridge is rinsed with water, then dried under vacuum (15" Hg or 0.50 atm., 5 minutes), then IN-F6L99 residues are eluted with acetone (30 mL). The eluate is taken to dryness under nitrogen (N-Evap, 50-55°C). Resulting residues are reconstituted in 0.5 mL methanol with

sonication for 5 minutes, then diluted with 1.5 mL 0.01M aqueous formic acid, and filtered (Acrodisc PTFE, 0.2 µm).

LC/MS/MS analysis: Samples are analyzed for chlorantraniliprole (DPX-E2Y45) and its products IN-F6NO4, IN-GAZ70, IN-EQW78, IN-ECD73, and IN-F6L99 by HPLC [Agilent HP1100 LC system, Phenomenex C-18, 4.6 mm x 15 cm, 3 µm column, column temperature 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, 8.1-11.5 min. 40:60 for chlorantraniliprole, IN-F9NO4, IN-GAZ70, IN-EQW78, and IN-ECD73; 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 (Micromass Quattro II MS, atmospheric pressure chemical ionization, positive ion mode) detection and multiple reaction monitoring (MRM; pp. 11, 19-23 of MRID 46979445). Injection volumes are 0.075-0.10 mL. Analytes are identified using two or three ion transitions (p. 28; Appendix 4, pp. 73-78). Ion transitions monitored were as follows: *m/z* 484.0→453.0 ± 0.5 and *m/z* 484.0→285.8 ± 0.5 for chlorantraniliprole (DPX-E2Y45); *m/z* 470.0→452.3 ± 0.5 and *m/z* 470.0→285.1 ± 0.5 for IN-F9NO4; *m/z* 451.0→414.0 ± 0.5 and *m/z* 451.0→306.5 ± 0.5 for IN-GAZ70; *m/z* 465.9→187.8 ± 0.5 and *m/z* 465.9→75.8 ± 0.5 for IN-EQW78; *m/z* 244.0→209.0 ± 0.5, *m/z* 279.0→243.8 ± 0.5, and *m/z* 279.0→208.8 ± 0.5 for IN-ECD73; and *m/z* 203.8→172.2 ± 0.5 and *m/z* 203.8→65.9 ± 0.5 for IN-F6L99. Expected retention times were 4.7, 4.9, 6.4, 6.4, and 7.9 minutes for IN-F9NO4, chlorantraniliprole, IN-GAZ70, IN-EQW78, and IN-ECD73, respectively, and 6.2 minutes for IN-F6L99. For analyte confirmation, ion ratios of the monitored transitions from the fortified samples were compared to those of the calibration standards (p. 28; Appendix 3, pp. 68-72).

ILV: The independent laboratory performed the methods as written with minor modifications to optimize LC/MS/MS conditions (pp. 15-19 of MRID 46979530). Most specifically, a ThermoElectron Surveyor LC system and TSQ Quantum MS/MS were utilized, a Phenomenex RP-C18 4 x 3 mm, 5 µm guard column was added, and injection volume was reduced to 40 µL for the LLE sample. The ground water (Netherlands) and surface water (local river/channel, Netherlands) were obtained from the Water Boards (local/regional water management, p. 12). The drinking water was Zeist tap water.

LOQ and LOD: In the ECM and ILV, the LOQ for chlorantraniliprole (DPXE2Y45), IN-F9NO4, IN-GAZ70, IN-EQW78, IN-ECD73, and IN-F6L99 was 0.10 ng/g (ppb, µg/L; pp. 9, 11, 26 of MRID 46979445; p. 22 of MRID 46979530). In the ECM, the LOD was estimated at *ca*. 0.03 ng/g for the least sensitive analyte IN-EQW78. A LOD was not specified in the ILV.

II. Recovery Findings

ECM (MRID 46979445): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of chlorantraniliprole and its transformation products IN-F9NO4, IN-GAZ70, IN-EQW78, IN-ECD73, and IN-F6L99 in surface (pond, river), ground (well) and drinking water at fortification levels of 0.10 ng/g (LOQ, n = 5) and 1.0 ng/g (10x LOQ, n = 5), with the following exceptions: 0.10 ng/g IN-EQW78 in pond water (mean 123%) and 0.10 ng/g IN-F6L99 in river water (RSD 20.8%, DER Attachment 2). All water matrices were characterized (Appendix 5, pp. 80-87). Source locations were reported as Lums Pond and Brandywine River for the surface waters, a local well for the ground water, and the drinking water was purchased at a grocery store (p. 17; Appendix 5, pp. 80-87). For chlorantraniliprole, IN-F9NO4, IN-GAZ70, IN-EQW78, and IN-ECD73, confirmation criteria (RSD of ion ratios of calibration standards ≤20% and RSD of fortified sample ion ratios ≤30% of average ratio for all calibration standards) were met (pp. 28-29; Appendix 3, pp. 68-72). Confirmation analysis results for IN-F6L99 were not provided.

ILV (MRID 46979530): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of chlorantraniliprole and its products IN-F9NO4, IN-GAZ70, IN-EQW78, IN-ECD73, and IN-F6L99 in surface (river/channel), ground, and drinking (tap) water at fortification levels of 0.10 µg/L (LOQ) and 1.0 µg/L (10x LOQ; Appendix C, pp. 58-59; Appendix D, pp. 60-74). The method was validated for IN-F6L99 (SPE method) at both fortification levels after one trial and validated for all other analytes (LLE method) at both fortification levels after a second trial (p. 21). The surface water was characterized (non-GLP), but the ground water and drinking water matrices were not (p. 22). Results from the confirmatory method were not reported.

Table 2. Initial Validation Method Recoveries for Chlorantraniliprole (DPX-E2Y45) and Its Transformation Products IN-F9NO4, IN-GAZ70, IN-EQW78, IN-ECD73, and IN-F6L99 in Water[1]

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Chlorantraniliprole (DPX-E2Y45)	**Surface (pond) water**					
	0.10 (LOQ)	5	96-120	108	9.8	9.1
	1.0	5	93-105	99	4.4	4.5
	Surface (river) water					
	0.10 (LOQ)	5	90-121	101	12.0	11.9
	1.0	5	97-102	100	2.2	2.2
	Ground (well) water					
	0.10 (LOQ)	5	97-117	104	8.7	8.4
	1.0	5	86-116	98	11.9	12.2
	Drinking water					
	0.10 (LOQ)	5	92-103	99	4.3	4.4
	1.0	5	71-88	83	6.9	8.3
IN-F9NO4	**Surface (pond) water**					
	0.10 (LOQ)	5	95-114	102	7.6	7.5
	1.0	5	93-107	101	5.4	5.3
	Surface (river) water					
	0.10 (LOQ)	5	93-119	105	9.5	9.0
	1.0	5	99-122	105	9.6	9.1
	Ground (well) water					
	0.10 (LOQ)	5	100-109	106	3.5	3.3
	1.0	5	82-100	91	7.3	8.0
	Drinking water					
	0.10 (LOQ)	5	95-120	105	9.5	9.1
	1.0	5	70-89	82	7.4	9.0
IN-GAZ70	**Surface (pond) water**					
	0.10 (LOQ)	5	83-117	103	14.9	14.4
	1.0	5	96-117	104	8.0	7.7
	Surface (river) water					
	0.10 (LOQ)	5	86-107	95	8.3	8.8
	1.0	5	95-103	100	3.3	3.3
	Ground (well) water					
	0.10 (LOQ)	5	98-120	109	8.9	8.2
	1.0	5	89-113	99	10.3	10.4
	Drinking water					
	0.10 (LOQ)	5	91-104	96	4.8	5.0
	1.0	5	78-89	85	4.3	5.1
IN-EQW78	**Surface (pond) water**					
	0.10 (LOQ)	5	118-140[2]	**123**	9.7	7.9
	1.0	5	89-109	100	7.2	7.3
	Surface (river) water					
	0.10 (LOQ)	5	88-107	94	7.8	8.2
	1.0	5	90-108	98	7.5	7.6
	Ground (well) water					
	0.10 (LOQ)	5	84-141[2]	111	22.0	19.9
	1.0	5	93-113	101	7.8	7.8

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Drinking water					
	0.10 (LOQ)	5	99-112	105	6.1	5.8
	1.0	5	85-97	91	5.1	5.6
IN-ECD73	**Surface (pond) water**					
	0.10 (LOQ)	5	98-116	104	7.4	7.1
	1.0	5	86-103	96	6.7	6.9
	Surface (river) water					
	0.10 (LOQ)	5	93-108	100	5.6	5.6
	1.0	5	92-100	96	3.3	3.5
	Ground (well) water					
	0.10 (LOQ)	5	97-108	103	4.4	4.2
	1.0	5	91-103	97	5.4	5.5
	Drinking water					
	0.10 (LOQ)	5	86-100	95	5.7	6.0
	1.0	5	82-96	92	5.7	6.2
IN-F6L99	**Surface (pond) water**					
	0.10 (LOQ)	5	97-111	105	6.2	5.9
	1.0	5	100-112	106	5.0	4.8
	Surface (river) water					
	0.10 (LOQ)	5	62-111[2]	94	19.6	**20.8**
	1.0	5	95-108	100	5.1	5.1
	Ground (well) water					
	0.10 (LOQ)	5	107-120	114	5.4	4.8
	1.0	5	84-113	101	11.2	11.1
	Drinking water					
	0.10 (LOQ)	5	77-112	97	14.5	14.9
	1.0	5	90-101	94	4.4	4.7

Data (recovery results) were obtained from Tables 1-6, pp. 31-36 of MRID 466979445 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 (see footnote 2 below).

1 Water matrices were characterized by Agvise Laboratories, Northwood, North Dakota (Appendix 5, pp. 80-87 of MRID 46979445). The ground and surface waters were obtained locally, and the drinking water was purchased at a grocery store (p. 17).

2 The study authors considered two results "obvious sample contamination" (140% and 141% recoveries for 0.1 ng/g IN-EQW78 in pond and well water, respectively) and one result "an obvious outlier" (62% for 0.10 ng/g IN-F6L99 in river water) and excluded those recovery results from statistical analyses (pp. 10, 26; Table 4, p. 34; Table 6, p. 36). However, no supporting statistical tests, such as Grubbs' and Dixon tests, were presented to justify exclusion of the recovery results as outliers. The reviewer included all recovery results in the statistics.

Table 3. Independent Validation Method Recoveries for Chlorantraniliprole (DPX-E2Y45) and Its Transformation Products IN-F9NO4, IN-GAZ70, IN-EQW78, IN-ECD73, and IN-F6L99 in Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Chlorantraniliprole (DPX-E2Y45)	**Surface (river/channel) water**					
	0.10 (LOQ)	5	83.0-92.4	87.6	3.8	4.4
	1.0	5	81.4-87.4	82.9	2.6	3.1
	Ground water					
	0.10 (LOQ)	5	84.0-97.7	89.9	5.1	5.6
	1.0	5	87.5-95.4	90.5	3.2	3.6
	Drinking (tap) water					
	0.10 (LOQ)	5	74.7-84.0	80.7	3.6	4.4
	1.0	5	74.7-79.3	76.8	1.7	2.2
IN-F9NO4	**Surface (river/channel) water**					
	0.10 (LOQ)	5	77.3-115	89.2	15.1	17.0
	1.0	5	94.0-96.5	95.1	1.0	1.1
	Ground water					
	0.10 (LOQ)	5	56.5-94.5	79.1	15.7	19.8
	1.0	5	95.5-103	98.9	3.4	3.4
	Drinking (tap) water					
	0.10 (LOQ)	5	83.9-114	97.1	11.3	11.6
	1.0	5	96.3-106	101	4.0	3.9
IN-GAZ70	**Surface (river/channel) water**					
	0.10 (LOQ)	5	92.1-115	102	8.2	8.0
	1.0	5	94.5-101	98.3	2.4	2.5
	Ground water					
	0.10 (LOQ)	5	81.3-111	100	11.4	11.4
	1.0	5	103-111	108	3.6	3.3
	Drinking (tap) water					
	0.10 (LOQ)	5	94.4-106	101	4.6	4.5
	1.0	5	97.3-107	101	3.7	3.7
IN-EQW78	**Surface (river/channel) water**					
	0.10 (LOQ)	5	85.2-105	96.4	7.0	7.3
	1.0	5	94.8-104	98.5	3.3	3.4
	Ground water					
	0.10 (LOQ)	5	71.4-113	87.8	17.0	19.4
	1.0	5	96.6-116	105	7.0	6.7
	Drinking (tap) water					
	0.10 (LOQ)	5	85.6-121	102	12.8	12.5
	1.0	5	98.1-114	104	6.3	6.0
IN-ECD73	**Surface (river/channel) water**					
	0.10 (LOQ)	5	79.9-94.2	85.2	5.4	6.4
	1.0	5	71.5-83.2	75.5	4.7	6.2
	Ground water					
	0.10 (LOQ)	5	80.5-107	94.7	10.1	10.7
	1.0	5	77.5-97.0	86.0	8.4	9.7
	Drinking (tap) water					
	0.10 (LOQ)	5	76.1-89.8	82.0	5.3	6.4
	1.0	5	72.1-83.5	77.1	4.5	5.8

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
IN-F6L99	**Surface (river/channel) water**					
	0.10 (LOQ)	5	104-114	108	4.3	4.0
	1.0	5	108-116	111	4.0	3.6
	Ground water					
	0.10 (LOQ)	5	100-108	104	3.0	2.9
	1.0	5	98.7-106	103	2.8	2.7
	Drinking (tap) water					
	0.10 (LOQ)	5	88.1-96.2	91.3	3.1	3.4
	1.0	5	84.5-106	92.7	8.2	8.9

Data (recovery results) were obtained from p. 12; Appendix C, pp. 58-59 (IN-F6L99); Appendix D, pp. 60-74 (all other analytes) of MRID 46979530.

1 The surface water was characterized (non-GLP) by Alcontrol B.V., Hoogvliet, The Netherlands (p. 22 of MRID 46979530). The ground water and drinking water matrices were not characterized. The ground water (Netherlands) and surface water (local river/channel, Netherlands) were obtained from the Water Boards (local/regional water management, p. 12). The drinking water was Zeist tap water.

III. Method Characteristics

In the ECM and ILV, the LOQ for chlorantraniliprole, IN-F9NO4, IN-GAZ70, IN-EQW78, IN-ECD73, and IN-F6L99 in water was 0.10 ng/g (ppb, µg/L; pp. 9, 11, 26-27 of MRID 46979445; p. 22 of MRID 46979530). The LOQ was defined as the lowest fortification level at which acceptable average recoveries (70-120%, RSD <20%) were achieved. The ECM estimated the LOD to be *ca*. 0.03 ng/g for IN-EQW78, the least responsive analyte. The ECM defined the LOD as the concentration of IN-EQW78 at which analyte peaks are *ca*. three times the chromatographic baseline noise observed at near the retention time, or *ca*. one-third the concentration of the LOQ. In the ILV, the LOD was not reported.

Table 4. Method Characteristics for Chlorantraniliprole (DPX-E2Y45) and Its Transformation Products IN-F9NO4, IN-GAZ70, IN-EQW78, IN-ECD73, and IN-F6L99 in Water

		DPX-E2Y45	IN-F9NO4	IN-GAZ70	IN-EQW78	IN-ECD73	IN-F6L99
Limit of Quantitation (LOQ)		0.10 ng/g (ppb, µg/L)					
Limit of Detection (LOD)		0.03 ng/g					
Linearity (calibration curve r^2 and concentration range)[1]	ECM:	r^2 = 0.9996	r^2 = 0.9997	r^2 = 0.9995	r^2 = 0.9997	r^2 = 0.9999	r^2 = 0.9971
	ILV:	r^2 = **0.9406**-0.9985	r^2 = **0.9829**-0.9989	r^2 = **0.9241**-0.997	r^2 = **0.9613**-0.9961	r^2 = **0.9649**-**0.9873**	r^2 = 0.9977
	Range:	0.5-20 ng/mL					
Repeatable	ECM:	Yes at LOQ and 10x LOQ, except for 0.10 ng/g IN-EQW78 in pond water (mean 123%) and 0.10 ng/g IN-F6L99 in river water (RSD 20.8%).					
	ILV:	Yes at LOQ and 10x LOQ.					
Reproducible		Yes					
Specific		Undetermined[2]					

Data were obtained from pp. 9, 11, 16, 25-26, 28-29; Figures 2-4, pp. 46-48; Figure 6, pp. 52-53, 55-56, 58-59; Appendix 3, pp. 68-72 of MRID 46979445; pp. 15, 22 of MRID 46979530; and DER Attachment 2.
Linearity is satisfactory when $r^2 \geq 0.995$.

1 Linearity of the ECM calibration curves was verified by the reviewer, excluding IN-F6L99 for which individual calibration data were not provided (DER Attachment 2). For the ECM, the IN-F9NO4 and IN-GAZ70 regression analysis results are inversely labeled (Figure 3, p. 47 of MRID 46979445; DER Attachment 2). ILV calibration curves were not provided; the reviewer generated r^2 values using provided calibration standard data (Appendix C, p. 58; Appendix D, pp. 60-74 of MRID 46979530; DER Attachment 2).

2 For both the ECM and ILV, chromatogram quality was poor, and direct comparison could not be made between the matrix control and LOQ chromatograms because y-axis values are 0-100% relative intensity (Figure 6, pp. 52-53, 55-56, 58-59 of MRID 46979445; Appendix B, pp. 30-47 of MRID 46979530). The ECM provided confirmation method results for all analytes, except IN-F6L99 (pp. 28-29; Appendix 3, pp. 68-72 of MRID 46979445). The ILV did not provide confirmatory method results.

IV. Method Deficiencies and Reviewer's Comments

1. The determination of the LOQ and LOD were not based on scientifically acceptable procedures. The LOQ, 0.10 ng/g (ppb, µg/L), was defined as the lowest fortification level at which acceptable average recoveries (70-120%, RSD <20%) were achieved (pp. 9, 11, 26-27 of MRID 46979445; p. 22 of MRID 46979530). The ECM estimated the LOD to be *ca*. 0.03 ng/g for IN-EQW78, the least responsive analyte. The ECM defined the LOD as the concentration of IN-EQW78 at which analyte peaks are *ca*. three times the chromatographic baseline noise observed at near the retention time, or *ca*. one-third the concentration of the LOQ. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological levels of concern in water were not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

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

3. For the ECM, the provided spectra and portions of the provided chromatograms were faint and of poor quality (Figure 1, pp. 37-45; Figures 5-6, pp. 49-60 of MRID 46979445). Direct comparison could not be made between the matrix control and LOQ chromatograms because y-axis values are 0-100% relative intensity. Standard curves were provided for all analytes, with the individual calibration standard data for all analytes except IN-F6L99 (Figures 2-4,

pp. 46-48; Appendix 2, pp. 64-68). In Figure 3 (p. 47), the IN-F9NO4 and IN-GAZ70 regression analysis results are inversely labeled (DER Attachment 2). Chromatograms of reagent blank samples were not provided.

The study authors reported interference peaks in the matrix control samples were <LOD at the retention time of each analyte (p. 25). The reviewer could not confirm this. Chromatograms of LOD equivalent calibration standards were not provided for comparison, and direct comparison could not be made between the matrix control and LOQ chromatograms.

4. For the ILV, provided chromatograms were small, faint, and of poor quality (Appendix B, pp. 30-47 of MRID 46979530). Direct comparison could not be made between the matrix control and LOQ chromatograms because y-axis values are 0-100% relative intensity. Chromatograms of calibration standards and reagent blank samples were not provided. Standard curves were not provided, but the reviewer generated curves using provided calibration standard data (Appendix C, p. 58; Appendix D, pp. 60-74). Linearity (r^2) of the calibration standards was not always ≥0.995 (see Table 4 above, DER Attachment 2).

   The study author reported no peaks were observed at the retention time of each analyte (pp. 9, 21, 23). It could not be determined if interferences with peak areas were <50% at the LOD because LODs for the analytes were not established in the ILV, and direct comparison could not be made between matrix control and LOQ chromatograms. NL values of matrix control samples were <12% of LOQ samples (Appendix B, pp. 30-47).

   Matrix blank samples fortified after sample extraction were included in the validation sample set to assess matrix effects, but results from those analyses were not reported (p. 13).

5. For the ILV, the surface water matrix was characterized (non-GLP), but the ground water and drinking water matrices were not (p. 22 of MRID 46979530).

6. For the ECM, ion ratios of the monitored parent-daughter transitions from the fortified samples were compared to those of the calibrations standards for analyte confirmation (p. 28 of MRID 46979445). Confirmation analysis results were provided for all analytes, except IN-F6L99 (pp. 28-29; Appendix 3, pp. 68-72).

7. Communication between the independent laboratory and study sponsor was not documented in the ILV study report. The only noted communication in the ILV study report was the fact that the decrease of HPLC/MS/MS injection volume (from 100 µL to 40 µL) for the second validation run of the LLE was at the request of the sponsor (p. 20).

8. The ILV did not report the time required to complete a sample set (typically thirteen samples consisting of one reagent blank, two matrix control samples, and ten fortified samples). It was reported in the ECM study report that a sample set consisting of twelve to twenty samples typically can be prepared during an 8-hour day, with LC/MS/MS analyses run unattended overnight (p. 27 of MRID 46979445).

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.

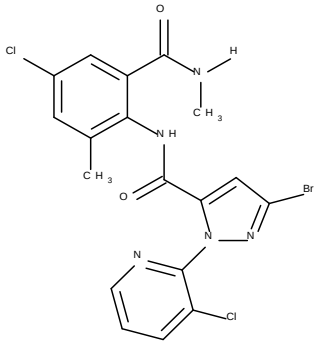
40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.


calcs

Attachment 1: Chemical Names and Structures

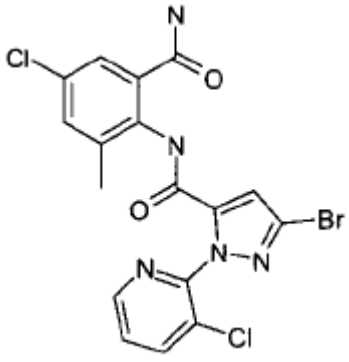
Chlorantraniliprole (DPX-E2Y45)

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



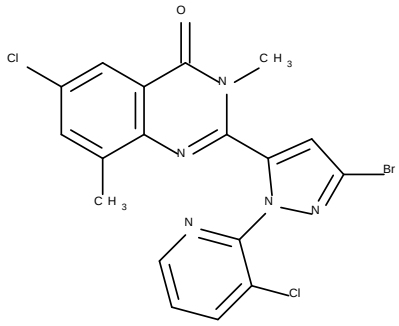
IN-F9NO4

IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.
SMILES String: Not found.



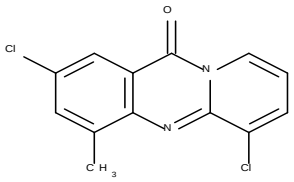
IN-EQW78

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: Not reported.
SMILES String: Cc1cc(cc2c1nc(n(c2=O)C)c3cc(nn3c4c(cccn4)Cl)Br)Cl



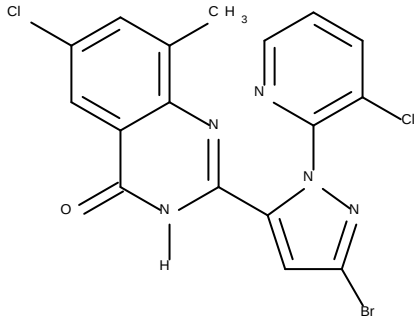
IN-ECD73

IUPAC Name: 2,6-Dichloro-4-methyl-pyrido[2,1-b]quinazolin-11-one
CAS Name: Not reported.
CAS Number: Not reported.
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: Not reported.
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: Not reported.
SMILES String: Not found.