

Analytical method for pinoxaden and its metabolites NOA407854 and NOA447204 in water

Reports: ECM GRM017.07A: EPA MRID No. 50357801. Mayer, L.C. 2017. Pinoxaden – Residue Method GRM017.07A for the Determination of Pinoxaden and Its Metabolites NOA407854 and NOA447204 in Water by LC-MS/MS Analysis – Analytical Method. Syngenta Report No. GRM017.07A and Task No. TK0285552. Report prepared, sponsored, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 62 pages. Chromatograms were generated 03/30/17, 03/31/17, 04/04/17 and 04/18/17.

ILV: EPA MRID No. 50357802. Wang, J.D. 2017. Pinoxaden - Independent Laboratory Validation of Residue Method (GRM017.07A) for the Determination of Pinoxaden and its Metabolites NOA407854 and NOA447204 in Water by LC-MS/MS Analysis– Final Report. Syngenta Report No. SR20170526A, Study No. 00136 and Task No. TK0285553. Report prepared by Symbiotic Research, LLC, Mount Olive, New Jersey, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 151 pages. Final report issued August 14, 2017.

Document No.: MRIDs 50357801 and 50357802.

Guideline: 850.6100

Statements: ECM: The study was not conducted in accordance Good Laboratory Practice (GLP) standards (pp. 3 of MRID 50357801). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3 of MRID 50357801). Quality Assurance and Authenticity statements were not included. A signed and dated Summary of Revisions to Previous Versions was included (p. 4 of MRID 50357801).

ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 50357802). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4 of MRID 50357802). A certification of authenticity was not included.

Classification: This analytical method is classified as Acceptable. The ILV representative chromatogram did not support the specificity for the metabolite NOA407854 in surface water at the LOQ. Additional clean up to reduce baseline noise may be necessary. The LOD seems to be estimated as the lowest concentration of the linearity calibration standards. As such, the actual LOD may be lower.

PC Code: 147500

EFED Final Reviewer: Ideliz Negrón-Encarnación, Chemist
Signature:
Date: 7/11/2019

CDM/CSS- Dynamac JV Reviewers: Teresa Nelis, M.S., Environmental Scientist
Signature: *Teresa Nelis*
Date: 8/13/2018

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Signature: *Lisa Muto*
Date: 8/15/2018

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, Syngenta Residue Method GRM017.07A, is designed for the quantitative determination of pinoxaden and its metabolites NOA407854 and NOA447204 in water at the LOQ of 0.05 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water, 0.0037 µg/L, which is the NOAEL for the most sensitive aquatic species, green algae *P. subcapitata*. The ECM GRM017.07A validated the method using characterized surface water and ground water. In the ECM, all analytes were identified using one ion transition; a confirmation ion transition was added in the ILV. A confirmatory method is not usually required when LC/MS and/or GC/MS are used as the primary methods to generate recovery data. All ECM data was satisfactory regarding accuracy, precision, linearity and specificity, except for linearity of the calibration curve for pinoxaden in surface water. The ILV successfully validated the method GRM017.07A for each matrix evaluated for Pinoxaden and its metabolites NOA407854 and NOA447204 at the LOQ and 10 x LOQ (0.5 µg/L) concentration levels as written, with modifications to pH adjustment and LC/MS/MS procedures, and the addition of filtration of surface water after collection. All ILV data was satisfactory regarding accuracy, precision, linearity and specificity, except for specificity of the chromatogram for NOA407854 in surface water at the LOQ, which indicates additional clean-up of the matrix or matrix-matched standards may be required. The LOD was not reported in the ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide ¹	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Pinoxaden	50357801 ²	50357802 ³		Water	08/11/2017	Syngenta Crop Protection, LLC	LC/MS/MS	0.05 µg/L
NOA407854								
NOA447204								

1 Pinoxaden = 2,2-dimethyl-propionic acid 8-(2,6-diethyl-4-niethylphenyl)-9-oxo-1,2,4,5-tetrahydro-9.H-pyrazolo[1,2,-d] oxadiaepin-7-yl ester; NOA407854 = 8-(2,6-diethyl-4-methyl-phenyl)-tetrahydropyrazolo[1,2,-d] [1,4,5] oxadiaepine-7,9-dione; NOA447204 = 8-(2,6-diethyl-4-methyl-phenyl)-8-hydroxy-tetrahydropyrazolo [1,2,-d] [1,4,5] oxadiazepine-7,9-dione.

2 In the ECM, the surface water (Belews Lake Surface Water, North Carolina; pH 7.5, 48 ppm total dissolved solids, 33 mg/L total hardness as CaCO₃) and ground water (Summerfield, North Carolina, Ground Water; pH 7.7, 156 ppm total dissolved solids, 54 mg/L total hardness as CaCO₃) were obtained by Syngenta (Sponsor) and characterized by Agvise Laboratories, Northwood, North Dakota (Table 1, p. 28 of MRID 50357801).

3 In the ILV, the surface water (Pequest River, New Jersey, Surface Water; pH 8.4, 8 ppm total suspended solids, 246 mg/L total hardness as CaCO₃) and ground water (Evian bottled natural spring water Ground Water; pH 8.1, 4 ppm total suspended solids, 330 mg/L total hardness as CaCO₃) were obtained by Symbiotic Research (Independent Laboratory) and characterized by Agvise Laboratories, Northwood, North Dakota (Tables 1-3, pp. 32-34 of MRID 50357802).

Page citations in this review refer to those written in the bottom-most, right-handed corner of the document pages.

ECM: GRM017.07A**I. Principle of the Method****Syngenta Residue Method GRM017.07A – MRID 50357801**

Water (20 mL) was transferred into a 50-mL polypropylene tube, acidified to pH <2 with 100 µL concentrated formic acid, and fortified, as necessary, with individual or mixed standard solutions of pinoxaden, NOA407854, and NOA447204 in acetonitrile:methanol (90:10 v/v; total volume of standard solution <1.0 mL) (10.0 µg/mL, 1.0 µg/mL or 0.1 µg/mL) for procedural recoveries (pp. 14-16; Appendix 4, p. 62 of MRID 50357801). The samples were shaken vigorously to mix well. To concentrate samples, one Waters Oasis™ HLB solid phase extraction (SPE) cartridge (60 mg, 3 mL size) per fortified sample or untreated control is first placed on the vacuum manifold, rinsed with 2 mL of methanol and percolated at a rate of 1 mL/min, the column eluate is discarded; and the column rinse is repeated with 2 mL of ultra-pure water at the same rate, again discarding column eluate.

Acidified fortified samples and untreated control are loaded onto the prepared SPE columns and percolated at a rate of 1 - 2 mL/min; pinoxaden, NOA407854 and NOA447204 are retained on the SPE cartridge (pp. 14-16; Appendix 4, p. 62 of MRID 50357801). The empty sample tubes are rinsed with ultra-pure water containing 1% formic acid (3 mL); the rinse is added to the SPE column and percolated at the same rate; the column eluate is discarded. The SPE columns are then washed with 3 mL ultra-pure water:acetonitrile (80:20 v/v), percolated at the same rate, and the eluate discarded. The cartridges are then dried under high vacuum (≤500 mbar) for 15 minutes or longer if necessary. Fifteen (15) mL collection tubes are placed under each port; 3 mL of ultra-pure water/acetonitrile (50:50 v/v) is added to each original sample tube and the tubes swirled to remove any residual analyte; and this rinsate is added to top of each cartridge and drawn through under gravity at a rate of approximately 2 mL/min and vacuumed to expel any remaining solvent. One 1 mL of the final fraction in ultra-pure water:acetonitrile (50:50 v/v) is transferred to an autosampler vial ready then analyzed directly by LC-MS/MS. The final sample concentration is 6.6 mL/mL or 0.006 mL/L.

Precautions for Syngenta Residue Method GRM017.07A

The method reported the following experimental precautions: a) some of the analytes have low solubility in acetonitrile alone; acetonitrile:methanol (90:10, v/v) is used to ensure complete dissolution of all analytes; b) the SPE procedure has been developed using the stated cartridges, similar cartridges from other manufacturers may be used, however, in all cases it is strongly recommended that the elution profile of the chosen batch of cartridges is checked prior to the study; c) bottled HPLC grade ultra-pure water is used to prepare the LC mobile phase, which produces a lower background noise in the MS/MS chromatograms than water taken from a laboratory water purification system; d) to prevent contamination of the instrument and to minimize possible carry-over issues, it is recommended that high level recoveries (>0.1 mg/kg) and samples with expected residues greater than 1 pg/L should be diluted so that the final analyte concentration does not exceed 0.005 pg/mL; it is recommended to include blank injections of ultra-pure water:acetonitrile (50/50, v/v) after high level samples to clear any observed carry-over greater than 10% of the LOQ; and e) additional needle and valve washes with methanol and

0.1% formic acid solution may help to reduce any significant carry-over of analytes (p. 16 of MRID 50357801).

LC/MS/MS Analysis for Syngenta Residue Method GRM017.07A

Samples were analyzed using a Waters Acquity I-Class UPLC and an Applied Biosystems (AB) QTRAP® 5500 MS with a TurboIonSpray interface (p. 17; Appendix 1, p. 59 of MRID 50357801). Note, the Agilent 1100 HPLC listed as the recommended suppliers was apparently not used (Appendix 1, p. 59 of MRID 50357801).

The following LC conditions were used in GRM017.07A: ACE C18 column (50 x 2.1 mm, 3 µm, column temperature 40°C). Mobile phase for Pinoxaden and NOA407854: (A) Ultra-Pure Water (UPW) + 0.1% formic acid and (B) methanol [percent A:B (v/v) at 0.0 min. 90:10, 4.0 min. 10:90, 5.0 min. 10:90, 5.1 min. 90:10, and 6.0 min. 90:10], and injection volume of 10 µL. Mobile phase for NOA447204: (A) 4mM Ammonium Formate in UPW and (B) methanol [percent A:B (v/v) at 0.0 min. 90:10, 4.0 min. 10:90, 5.0 min. 10:90, 5.1 min. 90:10, and 6.0 min. 90:10], and injection volume of 10 µL (pp. 17-18 of MRID 50357801).

The following MS/MS conditions were used for Pinoxaden, NOA407854, and NOA447204: positive ion mode and multiple reaction monitoring (MRM; 475°C). Analytes are identified using two ion pair transitions; one for quantitation (Q, "primary") and one for confirmation (C). Ion transitions monitored for Pinoxaden were m/z 401→317 (Q) and m/z 401→57 (C); for NOA407854 were m/z 317→115 (Q) and m/z 317→91 (C); and for NOA447204 were m/z 333→149 (Q) and m/z 333→121 (C) (pp. 19-20 of MRID 50357801).

Expected retention times were *ca.* 3.7, 2.7 and 2.8 minutes for Pinoxaden, NOA407854 and NOA447204, respectively (p. 18 of MRID 50357801).

ILV

The independent laboratory performed the ECM method GRM017.07A as written, with the exception of an additional 300 µL formic acid to acidify samples to $\text{pH} \leq 2$, and filtration of surface water after collection with Grade 1 (110 mm) Whatman Qualitative Circles filter paper (p. 21; Figures 1-72, pp. 38-73; Appendix 1, pp. 81-142; Appendix 3, pp. 148-151 of MRID 50357802).

The LC/MS/MS instrument and conditions used in independent laboratory validation were not the same as those used in ECM GRM017.07A. Samples were analyzed using an Agilent Series 1100 HPLC and an AB API 4000 triple quadrupole MS with a TurboIonSpray interface (p. 22-25; Appendix 3, pp. 148-151 of MRID 50357802). With the use of the Agilent 1100 HPLC instead of a UPLC as listed in the method, the retention times shifted by 2 minutes, and the end time was extended by 1 minute (ending at 7.0 min.). With the use of an AB API 4000, the injection volume was increased to 30 µL for Pinoxaden and NOA407854 and 50 µL for NOA447204 (pp. 22-23 of MRID 50357802). MS/MS conditions for Pinoxaden, NOA407854, and NOA447204 were positive ion mode and MRM, with an interphase temperature of 650°C (p. 24 of MRID 50357802).

Monitored ion transitions for the independent laboratory validation were the same, but approximate retention times were different, and were 5.7, 4.9 and 5.1 minutes for Pinoxaden, NOA407854 and NOA447204, respectively (pp. 24-25 of MRID 50357802).

LOQ and LOD

In the ECM and ILV, Limit of Quantification (LOQ) for Pinoxaden, NOA407854, and NOA447204 in water was 0.05 µg/L (ECM p. 23 of MRID 50357801; ILV p. 27 of MRID 50357802). The Limit of Detection (LOD) for Pinoxaden, NOA407854, and NOA447204 was 0.10 µg/L in the ECM (see Reviewer's Comment #5; p. 23 of MRID 50357801); the LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 50357801): For Syngenta Method GRM017.07A, mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of Pinoxaden and its metabolites NOA407854 and NOA447204 in two water matrices at fortification levels of 0.05 µg/L (LOQ) and 0.5 µg/L (10×LOQ) (Tables 2, 3 and 4, pp. 28-29 of MRID 50357801; DER Attachment 2). Two ion pair transitions were monitored; however, the performance data (results) of the confirmation ion analyses were not reported (p. 20 of MRID 50357801). The surface water (Belews Lake Surface Water, North Carolina; pH 7.5, 48 ppm total dissolved solids, 33 mg/L total hardness as CaCO₃) and ground water (Summerfield, North Carolina, Ground Water; pH 7.7, 156 ppm total dissolved solids, 54 mg/L total hardness as CaCO₃) were obtained by Syngenta (Sponsor) and characterized by Agvise Laboratories, Northwood, North Dakota (Table 1, p. 28 of MRID 50357801).

ILV (MRID 50357802): Mean recoveries and RSDs were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of pinoxaden and its metabolites NOA407854 and NOA447204 in two water matrices at fortification levels of 0.05 µg/L (LOQ) and 0.5 µg/L (10×LOQ) (Tables 4 and 5, pp. 35-36 of MRID 50357802). Two ion pair transitions were monitored; performance data (results) of the quantitation and confirmation ion analyses were comparable. The surface water (Pequest River, New Jersey, Surface Water; pH 8.4, 8 ppm total suspended solids, 246 mg/L total hardness as CaCO₃) and ground water (Evian bottled natural spring water Ground Water; pH 8.1, 4 ppm total suspended solids, 330 mg/L total hardness as CaCO₃) were obtained by Symbiotic Research (Independent Laboratory) and characterized by Agvise Laboratories, Northwood, North Dakota (Tables 1-3, pp. 32-34 of MRID 50357802).

Table 2. Initial Validation Method Recoveries for Pinoxaden and Its Transformation Products NOA407854 and NOA447204 in Water^{1,2}

Analyte ³	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ⁴	Relative Standard Deviation (%)
GRM017.07A						
Surface Water						
Quantitation Ion Transition						
Pinoxaden	0.05	5	83-93	90 (97) ⁵	4.1	4.6 (10.6) ⁵
	0.5	5	87-100	94	4.7	8.9
NOA407854	0.05	5	89-96	93	2.6	2.8
	0.5	5	94-98	95	1.6	2.2
NOA447204	0.05	5	94-106	100	5.0	5.1
	0.5	5	97-103	100	2.6	2.6
Ground Water						
Quantitation Ion Transition						
Pinoxaden	0.05	5	83-92	89	3.4	3.8
	0.5	5	91-98	94	2.8	3.0
NOA407854	0.05	5	95-102	98	2.5	2.6
	0.5	5	92-95	94	1.3	1.4
NOA447204	0.05	5	97-112	104	5.7	5.5
	0.5	5	104-110	109	2.7	2.5

Data were obtained from Tables 2-4, pp. 28-29 of MRID 50357801, and DER Attachment 2.

1 In the ECM, the surface water (Belews Lake Surface Water, North Carolina; pH 7.5, 48 ppm total dissolved solids, 33 mg/L total hardness as CaCO₃) and ground water (Summerfield, North Carolina, Ground Water; pH 7.7, 156 ppm total dissolved solids, 54 mg/L total hardness as CaCO₃) were obtained by Syngenta (Sponsor) and characterized by Agvise Laboratories, Northwood, North Dakota (Table 1, p. 28 of MRID 50357801).

2 Ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 401→317 (Q) and *m/z* 401→57 (C) for Pinoxaden; *m/z* 317→115 (Q) and *m/z* 317→91 (C) for NOA407854; and *m/z* 333→149 (Q) and *m/z* 333→121 (C) for NOA447204; confirmation data were not reported in the ECM (MRID 50357801).

3 Pinoxaden = 2,2-dimethyl-propionic acid 8-(2,6-diethyl-4-niethylphenyl)-9-oxo-1,2,4,5-tetrahydro-9H-pyrazolo[1,2,-d] oxadiaepin-7-yl ester; NOA407854 = 8-(2,6-diethyl-4-methyl-phenyl)-tetrahydropyrazolo[1,2,-d] [1,4,5] oxadiaepine-7,9-dione; NOA447204 = 8-(2,6-diethyl-4-methyl-phenyl)-8-hydroxy-tetrahydropyrazolo [1,2,-d] [1,4,5] oxadiazepine-7,9-dione.

4 Standard deviations were reviewer-calculated based on data provided in the study report since the study author did not report these values (see DER Attachment 2). Rules of significant figures were followed.

5 Reported values were reviewer-calculated (see DER Attachment 2); values in parentheses were reported in the study report, but seemed erroneous.

Table 3. Independent Validation Method Recoveries for Pinoxaden and Its Transformation Products NOA407854 and NOA447204 in Water^{1,2}

Analyte ³	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
GRM017.07A						
Ground Water						
Quantitation Ion Transition						
Pinoxaden	0.05	5	77-99	91	10	11
	0.5	5	80-114	94	13	14
NOA407854	0.05	5	99-113	107	5	5
	0.5	5	99-103	101	2	2
NOA447204	0.05	5	96-112	105	7	6
	0.5	5	94-107	102	5	5
Confirmation Ion Transition						
Pinoxaden	0.05	5	79-99	91	9	10
	0.5	5	82-113	93	12	13
NOA407854	0.05	5	95-113	104	7	7
	0.5	5	97-104	101	3	3
NOA447204	0.05	5	102-118	109	6	6
	0.5	5	94-105	101	5	4
Surface Water						
Quantitation Ion Transition						
Pinoxaden	0.05	5	70-79	73	3	5
	0.5	5	83-91	86	3	4
NOA407854	0.05	5	94-105	100	4	4
	0.5	5	96-100	97	2	2
NOA447204	0.05	5	90-96	93	2	2
	0.5	5	90-96	93	3	3
Confirmation Ion Transition						
Pinoxaden	0.05	5	66-82	73	6	8
	0.5	5	83-89	86	3	3
NOA407854	0.05	5	88-101	95	5	6
	0.5	5	94-100	97	2	2
NOA447204	0.05	5	81-93	85	5	5
	0.5	5	90-97	94	2	3

Data were obtained from p. 27; Tables 4-5, pp. 35-36 of MRID 50357802.

1 The surface water (Pequest River, New Jersey, Surface Water; pH 8.4, 8 ppm total suspended solids, 246 mg/L total hardness as CaCO₃) and ground water (Evian bottled natural spring water Ground Water; pH 8.1, 4 ppm total suspended solids, 330 mg/L total hardness as CaCO₃) were obtained by Symbiotic Research (Independent Laboratory) and characterized by Agvise Laboratories, Northwood, North Dakota (Tables 1-3, pp. 32-34 of MRID 50357802).

2 Ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 401→317 (Q) and *m/z* 401→57 (C) for Pinoxaden; *m/z* 317→115 (Q) and *m/z* 317→91 (C) for NOA407854; and *m/z* 333→149 (Q) and *m/z* 333→121 (C) for NOA447204.

3 Pinoxaden = 2,2-dimethyl-propionic acid 8-(2,6-diethyl-4-niethylphenyl)-9-oxo-1,2,4,5-tetrahydro-9H-pyrazolo[1,2-d] oxadiaepin-7-yl ester; NOA407854 = 8-(2,6-diethyl-4-methyl-phenyl)-tetrahydropyrazolo[1,2,-d] [1,4,5] oxadiaepine-7,9-dione; NOA447204 = 8-(2,6-diethyl-4-methyl-phenyl)-8-hydroxy-tetrahydropyrazolo [1,2,-d] [1,4,5] oxadiazepine-7,9-dione.

III. Method Characteristics

In the ECM and ILV, the LOQ for pinoxaden and its metabolites NOA407854 and NOA447204 in water was 0.05 µg/L (p. 23 of MRID 50357801; p. 27 of MRID 50357802). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated with acceptable precision, i.e. relative standard deviation of $\leq 20\%$. Additionally, the ECM reported that the response of the LOQ peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. No justifications of the LOQ were provided in the ILV. The LOD for pinoxaden and its metabolites NOA407854 and NOA447204 was 0.10 µg/L in the ECM GRM017.07A (see Reviewer's Comment #5; p. 23 of MRID 50357801). In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. Additionally, the ECM reported that an estimate of the LOD can be taken as three times the background noise and may vary between runs and from instrument to instrument. The LOD was not reported in the ILV.

Table 4. Method Characteristics for Pinoxaden and Its Metabolites NOA407854 and NOA447204 in Water

Analyte ¹		Pinoxaden	NOA407854	NOA447204	
Limit of Quantitation (LOQ)	ECM	0.05 µg/L			
	ILV				
Limit of Detection (LOD)	ECM	0.1 µg/L			
	ILV	Not reported			
Linearity (calibration curve r ² and concentration range) ²	ECM ³	Surface	r² = 0.9934 (Q)	r ² = 0.9986 (Q)	r ² = 0.9992 (Q)
		Ground	r ² = 0.9982 (Q)	r ² = 0.9990 (Q)	r ² = 0.9990 (Q)
		Range	0.1-5.0 µg/L		
	ILV ⁴	Surface	r ² = 0.9986 (Q) r ² = 0.9986 (C)	r ² = 0.9982 (Q) r ² = 0.9994 (C)	r ² = 0.9994 (Q) r ² = 0.9997 (C)
		Ground	r ² = 0.9978 (Q) r ² = 0.9982 (C)	r ² = 0.9988 (Q) r ² = 0.9997 (C)	r ² = 0.9997 (Q) r ² = 0.9984 (C)
		Range	0.1-5.0 µg/L		
Repeatable	ECM ⁵	Only quantitation ion transition monitored. ⁷			
		Yes at LOQ and 10×LOQ using characterized surface and ground water.			
	ILV ⁶	Yes at LOQ and 10×LOQ using characterized surface and ground water.			
Reproducible		Yes at LOQ and 10×LOQ			
Specific	ECM	Surface	Yes, matrix interferences were insignificant.		
		Ground			
	ILV	Surface ⁸	Yes, matrix interferences were insignificant.	Matrix interferences for surface water, not for ground water.	Yes, matrix interferences were insignificant.
		Ground			

Data were obtained from pp. 23; Tables 9-11, pp. 32-33 (linearity); Figures 7, 18, and 29, pp. 38, 44, and 50 (calibration curves); Figures 4-36, pp. 37-54 (chromatograms) of MRID 50357801; pp. 24; Figures 73-84, pp. 74-79 (linearity and calibration curves); Figures 1-72, pp. 38-73 (chromatograms) of MRID 50357802; DER Attachment 2. Q = Quantitation ion transition; C = Confirmatory ion transition.

1 Pinoxaden = 2,2-dimethyl-propionic acid 8-(2,6-diethyl-4-niethylphenyl)-9-oxo-1,2,4,5-tetrahydro-9H-pyrazolo[1,2,-d] oxadiaepin-7-yl ester; NOA407854 = 8-(2,6-diethyl-4-methyl-phenyl)-tetrahydropyrazolo[1,2,-d] [1,4,5] oxadiaepine-7,9-dione; NOA447204 = 8-(2,6-diethyl-4-methyl-phenyl)-8-hydroxy-tetrahydropyrazolo [1,2,-d] [1,4,5] oxadiazepine-7,9-dione.

2 Reported coefficient of determination (r²) values are reviewer-generated from reported correlation coefficient (r) values (Tables 9-11, pp. 32-22 of MRID 50357801; Figures 73-84, pp. 74-79 of MRID 50357802); see DER Attachment 2.

3 Non-matrix matched standards were used for calibration (pp. 12, 24 and 30 of MRID 50357801). The reviewer assumed that the linear regression was performed with 1/x weighting. The linearity of the calibration curve for Pinoxaden analysis on surface water

4 Non-matrix matched standards were used for calibration; the linear regression was performed with 1/x weighting (pp. 19 and 21 of MRID 50357802).

5 In the ECM, the surface water (Belews Lake Surface Water, North Carolina; pH 7.5, 48 ppm total dissolved solids, 33 mg/L total hardness as CaCO₃) and ground water (Summerfield, North Carolina, Ground Water; pH 7.7, 156 ppm total dissolved solids, 54 mg/L total hardness as CaCO₃) were obtained by Syngenta (Sponsor) and characterized by Agvise Laboratories, Northwood, North Dakota (Table 1, p. 28 of MRID 50357801).

6 In the ILV, the surface water (Pequest River, New Jersey, Surface Water; pH 8.4, 8 ppm total suspended solids, 246 mg/L total hardness as CaCO₃) and ground water (Evian bottled natural spring water Ground Water; pH 8.1, 4 ppm total suspended solids, 330 mg/L total hardness as CaCO₃) were obtained by Symbiotic Research

(Independent Laboratory) and characterized by Agvise Laboratories, Northwood, North Dakota (Tables 1-3, pp. 32-34 of MRID 50357802).

7 A confirmatory method is typically not required where GC/MS and/or LC/MS methods are used as the primary method.

8 The chromatogram for surface water primary quantitation of NOA407854 at 0.05 ug/L showed significant baseline noise (Figure 28, p. 51). Additional clean-up may be required to reduce the baseline noise or matrix-matched calibration standards should be used.

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. Communication occurred between the ILV and ECM. The ILV study report provided communication details between the ILV study author (James Wang) and the ECM study author (Louis Mayer) (Appendix 3, pp. 148-151 of MRID 50357802). These communications involved a change in solvent for LC for NOA447204, additional formic acid needed to acidify samples to $\text{pH} \leq 2$; increase in autosampler volume and extended run time as a result of differences in LC/MS/MS equipment between ECM and ILV studies; and filtration of surface water samples after collection (110 mm) for the three analytes. These adjustments in the ILV method occurred one week prior to the week that method validation was completed (pp. 148-149 of MRID 50357802).
2. In the ECM, the linearity was not satisfactory for the quantitation ion calibration curve for pinoxaden in surface water ($r^2 = 0.9934$) but was satisfactory for ground water ($r^2 = 0.9982$) (Table 9, p. 32 of MRID 50357801; DER Attachment 2). Note, in the ILV the linearity was satisfactory for the quantitation ion and confirmation ion analysis of Pinoxaden in surface water [$r^2 = 0.9986$ (Q) and $r^2 = 0.9986$ (C)] (Figures 73-84, pp. 74-79 in 50357802; DER Attachment 2).

Linearity is satisfactory when $r^2 \geq 0.995$.

3. In the ILV, the chromatogram for surface water for the primary quantitation of NOA407854 0.05 $\mu\text{g/L}$ showed appreciable baseline noise (Figure 28, p. 51 of MRID 50357802). Additional clean-up may be required to reduce the baseline noise or matrix-matched calibration standards should be used. Similar issues were observed with the confirmatory analyses, in surface water of NOA407584, 0.05 $\mu\text{g/L}$ (Figure 34, p. 54) and NOA447204, 0.05 $\mu\text{g/L}$ (Figure 58, p. 66).
4. Syngenta indicated in an email to the ILV that pinoxaden may adhere to plastic, so the elution solvent rinse of the original sample vessel prior to elution on SPE is important (p. 149 of MRID 50357802).
5. In the ECM, the reviewer assumed that the ECM means that the LOD is equivalent to the lowest calibration standard, i.e. 0.1 $\mu\text{g/L}$, which is equivalent to **1 pg** injected on the column (p. 23; Table 9, p. 32 of MRID 50357801). This value is *ca.* 30% of the LOQ response on the column (33679 ct vs. 82119 ct; Table 9, p. 32 and Figure 10, p. 40). Even though the injection volume of the calibration standard and LOQ sample are the same (10 μL) in the ECM, the sample processing of the LOQ sample has a final concentration step (see steps g and h on p. 15). So, the 0.05 $\mu\text{g/L}$ sample concentration was increased which would explain why the 0.05 $\mu\text{g/L}$ LOQ sample response is larger than the 0.1 $\mu\text{g/L}$ calibration standard response.
6. The reviewer noted that the following ecotoxicological data was reported in the ECM: Aquatic plants and algae NOAEL = 3.7 ppb; Terrestrial plants non-target NOAEL = 8.0 ppb; Flathead minnow NOEC = >1.0 ppm; and Mysid shrimp NOEC = 0.87 ppm.

7. In the ECM, all analytes were identified using one ion transition; a confirmation ion transition was added in the ILV. A confirmatory method is not usually required when LC/MS and/or GC/MS are used as the primary methods to generate recovery data.
8. The LOD was not reported in the ILV (MRID 50357802).
9. The stability of the analytes in the water samples was studied in the ECM. The ECM determined surface water and ground water extracts were stable for storage periods up to 14 days (p. 24, Tables 6-8, pp. 31-32 of MRID 50357801).
10. It was reported for the ECM that a single analyst can complete a set of twenty samples in eight working-hours (p. 25 of MRID 50357801). It was reported for the ILV that a single analyst can complete a set of thirteen samples in eight working-hours (p. 28 of MRID 50357802).

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.

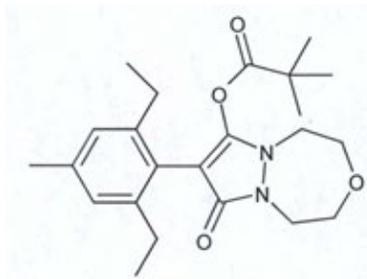
Attachment 1: Chemical Names and Structures [pp. 35-36 of ECM; pp. 16-17, 143-147 of ILV]**Pinoxaden (NOA407855)**

IUPAC Name: [8-(2,6-diethyl-4-methylphenyl)-7-oxo-1,2,4,5-tetrahydropyrazolo[1,2-d][1,4,5]oxadiazepin-9-yl] 2,2-dimethylpropanoate

CAS Name: Propanoic acid, 2,2-dimethyl-,8-(2,6-diethyl-4-methylphenyl)-1,2,4,5-tetrahydro-7-oxo-7H-pyrazolo[1,2-d][1,4,5] oxadiazepin-9-yl ester

CAS Number: 243973-20-8

SMILES String: Not found

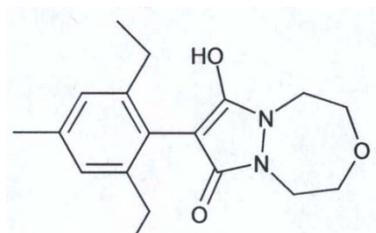
**NOA407854**

IUPAC Name: 8-(2,6-diethyl-4-methyl-phenyl)-tetrahydropyrazolo[1,2,-d] [1,4,5] oxadiazepine-7,9-dione

CAS Name: Not reported

CAS Number: 314020-44-5

SMILES String: Not found

**NOA447204**

IUPAC Name: 8-(2,6-diethyl-4-methyl-phenyl)-8-hydroxy-tetrahydropyrazolo[1,2,-d] [1,4,5] oxadiazepine-7,9-dione

CAS Name: Not reported

CAS Number: Not assigned

SMILES String: Not found

