Test Material:	Propyzamide (Pronamide, RH-23315)
MRID:	49970204
Title:	Method Validation Study for the Determination of Residues of Propyzamide and its Relevant Metabolites in Soil by Liquid Chromatography with Tandem Mass Spectrometry – AMENDED REPORT
MRID:	49970201
Title:	Propyzamide - Independent Laboratory Validation of an Analytical Method for the Determination of Propyzamide and its Relevant Metabolites in Soil by Liquid Chromatography with Tandem Mass Spectrometry
EPA PC Code:	101701
OCSPP Guideline:	850.6100

For CDM/CSS-Dynamac JV

Primary Reviewer: Lisa Muto **Signature:** Lisa Muto

Date: 10/25/16

Secondary Reviewer: Kathleen Ferguson **Signature:** Kathleen P. Ferguson

Date: 10/25/16

Quality Assurance Manager: Joan Gaidos **Signature:**

Date: 10/25/16

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

Analytical method for propyzamide and its transformation products RH-24644 and RH-24580 in soil

Reports: ECM: EPA MRID No.: 49970204. Hill, R. C. 2016. Method Validation Study for the Determination of Residues of Propyzamide and its Relevant Metabolites in Soil by Liquid Chromatography with Tandem Mass Spectrometry – AMENDED REPORT. Dow AgroSciences Study No.: 110586. Report prepared, sponsored, and submitted by Regulatory Sciences and Government Affairs – Indianapolis Lab, Dow AgroSciences LLC, Indianapolis, Indiana; 104 pages. Final report issued May 22, 2013; amended report dated March 23, 2016.

ILV: EPA MRID No. 49970201. Tessier, V. 2016. Propyzamide - Independent Laboratory Validation of an Analytical Method for the Determination of Propyzamide and its Relevant Metabolites in Soil by Liquid Chromatography with Tandem Mass Spectrometry. EAS Chem SAS Study No.: S13-03290. Dow AgroSciences Study No.: 130751. Report prepared by Eurofins AgroScience Services Chem SAS, Vergèze, France, sponsored and submitted by Regulatory Sciences and Government Affairs – Indianapolis Lab, Dow AgroSciences LLC, Indianapolis, Indiana; 100 pages. Final report issued November 15, 2013; amended report dated March 7, 2016.

Document No.: MRIDs 49970204 & 49970201

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA (40 CFR 160) and OECD Good Laboratory Practice (GLP) standards (p. 3R1 of MRID 49970204). Signed and dated GLP and Quality Assurance statements were provided (pp. 2R1-4R1). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4R1). The summary of amendment changes was provided (p. 4.1R1).

ILV: The study was conducted in accordance with OECD GLP standards and Standard Operating Procedures of EAS Chem SAS (p. 3; Appendix D, p. 100 of MRID 49970201). No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4; Appendix D, p. 100). Extremely faint signatures and dates were included with the GLP and QA statement. The Data Confidentiality statement was not signed. A statement of the authenticity of the study report was not included. The summary of amendment changes was provided (p. 5).

Classification: This analytical method is classified as supplemental. In the ECM, performance data to validate the method at 10x LOQ was not reported. Approximately half of the ECM correlation coefficients for RH-24644 were unsatisfactory ($r^2 < 0.995$).

PC Code: 101701

Reviewer: Karen Milians, Chemist **Signature:** Digitally signed by KAREN MILIANS Date: 2018.09.07 15:16:18 -04'00'
Date:

All page numbers refer to those written in the top, right corner of the pages of the study reports.

Executive Summary

This analytical method, Dow AgroSciences Study No. 110586, is designed for the quantitative determination of propyzamide and its transformation products RH-24644 and RH-24580 in soil using LC/MS/MS at the LOQ of 0.005 µg/g. The LOQ is less than the lowest toxicological level of concern in soil for all analytes. The ILV validated the method after one trial with no modifications; however, the loamy sand soil used in the ILV was not characterized. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. In the ECM, performance data to validate the method at 10x LOQ was not reported, and approximately half of the RH-24644 correlation coefficients were unsatisfactory ($r^2 < 0.995$).

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						
Propyzamide	49970204[2]	49970201[3]		Soil	22/05/2013 (Final Report) 23/03/2016 (Amendment 1)	Dow AgroSciences LLC	LC/MS/MS	0.005 µg/g
RH-24644								
RH-24580								

1 Propyzamide = Pronamide; 3,5-Dichloro-N-(1,1-dimethylprop-2-ynyl)benzamide; RH-24644 = 3,5-Dichloro-N-(1,1,2-trimethylprop-2-enyl)benzamide; and RH-24580 = 3,5-Dichloro-N-(1,1-dimethyl-2-oxopropyl)benzamide.

2 In the ECM, the soil matrices were well characterized (USDA soil texture classification; p. 12; Table 2, p. 23 of MRID 49970204). Clay soil (33% sand, 26% silt, 41% clay; pH 7.7; 2.1% carbon) from Hareby County, Linconshire, United Kingdom, loam soil (47% sand, 28% silt, 25% clay; pH 7.4; 4.1% carbon) from Empingham County, Rutland, United Kingdom, silt loam soil (-003; 29% sand, 52% silt, 19% clay; pH 5.8; 3.8% carbon) from Ebbinghof County, North Rhine, Westphalia, Germany, and silt loam soil (-004; 34% sand, 60% silt, 6% clay; pH 5.8; 4.4% carbon) from Chelmorton County, Derbyshire, United Kingdom were used.

3 In the ILV, the soil was not characterized, but USDA soil texture classification was specified; the loamy sand soil was a standard soil 2.2 (batch Sp2.2 2811; EAS Chem SAS Sample Reference No. 233; p. 14; Appendix A, p. 89 of MRID 49970201).

I. Principle of the Method

Samples (5.0 ± 0.05 g) of soil were weighed into 50 mL centrifuge tubes and fortified, as necessary (p. 11; Appendix I, pp. 96-101). The samples were extracted once with 25.0 mL of acetonitrile:water (80:20, v:v) by mixing via vortex for 10 seconds then shaking for 30 minutes on a reciprocating shaker at *ca*. 180 excursions/minute. After centrifugation for 5 minutes at 2000 rpm, the supernatant was transferred to a clean 50 mL centrifuge tube. The soil pellet was extracted again with 20.0 mL of acetonitrile:water (80:20, v:v) by mixing via vortex for 10 seconds then shaking for 30 minutes on a reciprocating shaker at *ca*. 180 excursions/minute (p. 12). After centrifugation for 5 minutes at 2000 rpm, the supernatant was combined with the first extract solution. The volume of the combined extracts was adjusted to 50 mL with acetonitrile:water (80:20, v:v). After centrifugation for 5 minutes at 4000 rpm, the total extraction solution was diluted 5x with acetonitrile:water (20:80, v:v) and transferred to an autosampler vial for analysis by LC/MS/MS.

Supplemental notes were added to the ECM which indicated that modifications of the equipment, glassware. Materials, reagents and chemicals with equivalent items were allowed, as well as modifications of solution volumes and analytical conditions (Appendix I, p. 101 of MRID 49970204).

Samples were analyzed for propyzamide (RH-23315) and its products RH-24644 and RH-24580 by Agilent 1290/1260 HPLC (Zorbax SB-C8, 4.6 mm x 75 mm, 3.5 µm column; column temperature ambient, *ca*. 22°C) using a mobile phase of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid [percent A:B at 0:01 min. 75:25, 3:00-5:00 min. 10:90, 5:15-8:00 min. 75:25) with AB SCIEX QTRAP 5500 MS using MS/MS-ESI (electrospray ionization) detection and multiple reaction monitoring (MRM); positive ion mode for propyzamide, RH-24644, and RH-24580 (p. 11; Appendix I, pp. 102-103 of MRID 49970204). Injection volume was 10 µL. Analytes were identified using two ion transitions; one for quantitation (Q) and one for confirmation (C; p. 21 of MRID 49970204). Ion transitions monitored were as follows: *m/z* 256.0→190.0 (Q) and *m/z* 258.0→192.0 (C) for propyzamide, *m/z* 274.1→145.1 (Q) and *m/z* 274.1→109.0 (C) for RH-24580, and *m/z* 256.0→145.0 (Q) and *m/z* 256.0→109.0 (C) for RH-24644.

In the ILV, the ECM was performed as written using a Sciex API 5500 LC-MS/MS (pp. 16-20).

The LOQ and LOD for all analytes were the same in the ECM and ILV at 0.005 µg/g and 0.0015 µg/g, respectively (pp. 12, 18 of MRID 49970204; p. 12; Tables 1-6, pp. 28-33 of MRID 49970201).

II. Recovery Findings

ECM (MRID 49970204): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of propyzamide, RH-24580, and RH-24644 in four soil matrices at fortification levels of 0.005 µg/g (LOQ) and 0.5 µg/L (100x LOQ; quantitation and confirmatory ions; Tables 9-14, pp. 36-37). No samples were prepared at 10×LOQ. Recoveries from samples fortified at 0.0015 µg/g (LOD) ranged (matrices combined) from 67-93% for propyzamide, 53-93% for RH-24580 and 67-87% for RH-24644 (matrices/ions combined; DER Attachment 2). Performance data (recovery results) from quantitation ion analyses and confirmation ion analyses were comparable. The soil matrices were well characterized (USDA soil texture classification; p. 12; Table 2, p. 23 of MRID 49970204). Clay soil (33% sand, 26% silt, 41% clay; pH 7.7; 2.1% carbon) from Hareby County, Linconshire, United Kingdom, loam soil (47% sand, 28% silt, 25% clay; pH 7.4; 4.1% carbon) from Empingham County, Rutland, United Kingdom, silt loam soil (-003; 29% sand, 52% silt, 19% clay; pH 5.8; 3.8% carbon) from Ebbinghof County, North Rhine, Westphalia, Germany, and silt loam soil (-004; 34% sand, 60% silt, 6% clay; pH 5.8; 4.4% carbon) from Chelmorton County, Derbyshire, United Kingdom were used.

ILV (MRID 49970201): Mean recoveries and RSDs were within guideline requirements for analysis of propyzamide, RH-24580, and RH-24644 in one soil matrix at fortification levels of 0.005 µg/g (LOQ) and 0.05 µg/g (10x LOQ; Tables 1-6, pp. 28-33; DER Attachment 2). Performance data (recovery results) from quantitation ion analyses and confirmation ion analyses were comparable. The soil was not characterized, but USDA soil texture classification was specified; the loamy sand soil was a standard soil 2.2 (batch Sp2.2 2811; EAS Chem SAS Sample Reference No. 233; p. 14; Appendix A, p. 89). The method was validated with the first trial with insignificant modifications of the analytical method (pp. 16-20, 23).

Table 2. Initial Validation Method Recoveries for Propyzamide and Its Transformation Products in Soil[1,2]

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Clay Soil					
	Quantitation ion					
Propyzamide (RH-23315)	0.0015 (LOD)	**2**	**67**, 80	*	*	*
	0.005 (LOQ)	6	83-89	86	2.0	2.3
	0.5	6	81-92	87	4.4	5.1
RH-24580	0.0015 (LOD)	**2**	**67**, 87	*	*	*
	0.005 (LOQ)	6	84-93	87	3.7	4.3
	0.5	6	85-92	88	2.6	3.0
RH-24644	0.0015 (LOD)	**2**	**67**, 80	*	*	*
	0.005 (LOQ)	6	80-87	84	2.9	3.5
	0.5	6	84-90	87	2.9	3.4
	Confirmatory ion					
Propyzamide (RH-23315)	0.0015 (LOD)	**2**	**67**, 73	*	*	*
	0.005 (LOQ)	6	78-97	88	7.6	8.7
	0.5	6	83-90	87	3.4	3.9
RH-24580	0.0015 (LOD)	**2**	73, 93	*	*	*
	0.005 (LOQ)	6	83-94	88	4.3	4.9
	0.5	6	81-91	88	3.7	4.2
RH-24644	0.0015 (LOD)	**2**	80	*	*	*
	0.005 (LOQ)	6	84-88	86	2.0	2.4
	0.5	6	81-89	86	3.1	3.6
	Loam Soil					
	Quantitation ion					
Propyzamide (RH-23315)	0.0015 (LOD)	**2**	80, 87	*	*	*
	0.005 (LOQ)	6	81-96	86	6.2	7.1
	0.5	6	86-90	55	1.5	1.7
RH-24580	0.0015 (LOD)	**2**	**53**, 93	*	*	*
	0.005 (LOQ)	6	80-98	88	6.8	7.7
	0.5	6	86-93	90	2.3	2.5
RH-24644	0.0015 (LOD)	**2**	73, 80	*	*	*
	0.005 (LOQ)	6	81-88	85	2.5	2.9
	0.5	6	81-89	85	3.2	3.8
	Confirmatory ion					
Propyzamide (RH-23315)	0.0015 (LOD)	**2**	80	*	*	*
	0.005 (LOQ)	6	78-90	85	4.8	5.6

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.5	6	80-90	86	4.2	4.9
RH-24580	0.0015 (LOD)	2	73, 87	*	*	*
	0.005 (LOQ)	6	78-87	83	3.5	4.2
	0.5	6	85-91	89	2.1	2.3
RH-24644	0.0015 (LOD)	2	73, 87	*	*	*
	0.005 (LOQ)	6	85-90	87	2.1	2.4
	0.5	6	84-90	87	2.0	2.3
	Silt Loam Soil (-003)					
	Quantitation ion					
Propyzamide (RH-23315)	0.0015 (LOD)	2	80, 93	*	*	*
	0.005 (LOQ)	6	85-91	89	2.2	2.4
	0.5	6	79-87	84	3.3	3.9
RH-24580	0.0015 (LOD)	2	80, 87	*	*	*
	0.005 (LOQ)	6	85-95	89	3.6	4.0
	0.5	6	85-92	88	3.0	3.4
RH-24644	0.0015 (LOD)	2	73	*	*	*
	0.005 (LOQ)	6	81-90	86	4.3	4.9
	0.5	6	80-86	83	2.2	2.6
	Confirmatory ion					
Propyzamide (RH-23315)	0.0015 (LOD)	2	87	*	*	*
	0.005 (LOQ)	6	74-90	84	5.5	6.6
	0.5	6	81-90	87	3.4	3.9
RH-24580	0.0015 (LOD)	2	67, 93	*	*	*
	0.005 (LOQ)	6	83-96	89	5.7	6.4
	0.5	6	82-91	87	3.7	4.2
RH-24644	0.0015 (LOD)	2	73, 80	*	*	*
	0.005 (LOQ)	6	83-91	87	3.4	4.0
	0.5	6	80-89	84	3.3	3.9
	Silt Loam Soil (-004)					
	Quantitation ion					
Propyzamide (RH-23315)	0.0015 (LOD)	2	80, 87	*	*	*
	0.005 (LOQ)	6	84-99	87	5.9	6.8
	0.5	6	84-93	89	3.3	3.7
RH-24580	0.0015 (LOD)	2	80, 93	*	*	*
	0.005 (LOQ)	6	87-102	95	7.2	7.6
	0.5	6	89-99	93	3.9	4.2
RH-24644	0.0015 (LOD)	2	67, 87	*	*	*

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.005 (LOQ)	6	80-97	90	6.5	7.2
	0.5	6	84-94	88	4.5	5.1
	Confirmatory ion					
Propyzamide (RH-23315)	0.0015 (LOD)	**2**	73, 80	*	*	*
	0.005 (LOQ)	6	83-99	90	5.8	6.5
	0.5	6	89-94	91	2.1	2.4
RH-24580	0.0015 (LOD)	**2**	80	*	*	*
	0.005 (LOQ)	6	84-90	87	2.4	2.7
	0.5	6	88-96	92	3.5	3.8
RH-24644	0.0015 (LOD)	**2**	80, 87	*	*	*
	0.005 (LOQ)	6	87-92	89	2.2	2.5
	0.5	6	83-90	87	3.0	3.4

Numbers in red represent values less than recommended.
Data (uncorrected recovery results, pp. 13-15) were obtained from Tables 9-14, pp. 36-37 of MRID 49970204 and DER Attachment 2 (LOD recovery results).
* Not calculated, n = 2.
1 The soil matrices were well characterized (USDA soil texture classification; p. 12; Table 2, p. 23). Clay soil (33% sand, 26% silt, 41% clay; pH 7.7; 2.1% carbon) from Hareby County, Linconshire, United Kingdom, loam soil (47% sand, 28% silt, 25% clay; pH 7.4; 4.1% carbon) from Empingham County, Rutland, United Kingdom, silt loam soil (-003; 29% sand, 52% silt, 19% clay; pH 5.8; 3.8% carbon) from Ebbinghof County, North Rhine, Westphalia, Germany, and silt loam soil (-004; 34% sand, 60% silt, 6% clay; pH 5.8; 4.4% carbon) from Chelmorton County, Derbyshire, United Kingdom were used.
2 Ion transitions monitored were as follows: *m/z* 256→190 (Q) and *m/z* 258→192 (C) for propyzamide, *m/z* 256→173 (Q) and *m/z* 258→109 (C) for RH-24644, *m/z* 258→190 (Q) and *m/z* 258→173 (C) for RH-24655, *m/z* 274→173 (Q) and *m/z* 274→109 (C) for RH-24580, *m/z* 288→188 (Q) and *m/z* 290→190 (C) for RH-26059, and *m/z* 222→156 (Q) and *m/z* 222→139 (C) for UK1.

Table 3. Independent Validation Method Recoveries for Propyzamide and Its Transformation Products in Soil[1,2]

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	Loamy Sand Soil					
	Quantitation ion					
Propyzamide (RH-23315)	0.005 (LOQ)	5	89-104	97	7	7
	0.05	5	88-102	98	6	6
RH-24580	0.005 (LOQ)	5	81-99	87	8	9
	0.05	5	88-97	95	4	4
RH-24644	0.005 (LOQ)	5	102-121	109	8	7
	0.05	5	100-113	106	5	5
	Confirmatory ion					
Propyzamide (RH-23315)	0.005 (LOQ)	5	91-104	97	5	5
	0.05	5	90-104	98	5	5
RH-24580	0.005 (LOQ)	5	88-106	97	7	7
	0.05	5	92-104	99[4]	5	5
RH-24644	0.005 (LOQ)	5	110-115	113	3	2
	0.05	5	101-114	109	5	5

Numbers in red represent values less than recommended.

Data (uncorrected recovery results, pp. 21-22) were obtained from Tables 1-6, pp. 28-33 of MRID 49970201 and DER Attachment 2 (s.d. values).

1 The soil was not characterized, but USDA soil texture classification was specified; the loamy sand soil was a standard soil 2.2 (batch Sp2.2 2811; EAS Chem SAS Sample Reference No. 233; p. 14; Appendix A, p. 89).

2 Ion transitions monitored were as follows: *m/z* 256→190 (Q) and *m/z* 258→192 (C) for propyzamide, *m/z* 256→173 (Q) and *m/z* 258→109 (C) for RH-24644, *m/z* 258→190 (Q) and *m/z* 258→173 (C) for RH-24655, *m/z* 274→173 (Q) and *m/z* 274→109 (C) for RH-24580, *m/z* 288→188 (Q) and *m/z* 290→190 (C) for RH-26059, and *m/z* 222→156 (Q) and *m/z* 222→139 (C) for UK1.

3 The reported standard deviations were reviewer-calculated since they were not reported in the study report (see DER Attachment 2).

4 The mean value reported in the study report was 88%; however, the reviewer considered this to be a typographical error since the recovery range was 92-104% (Table 4, p. 31; Table 7, p. 34). The reviewer reported the reviewer-calculated mean value (see DER Attachment 2). Also, the overall mean for the confirmatory ion RH-24580 appeared to be a typographical error, as well (87% mean, 88-106% range).

III. Method Characteristics

In the ECM and ILV, the LOQ and LOD values for propyzamide, RH-24580, and RH-24644 in soil were 0.005 µg/g and 0.0015 µg/g, respectively (pp. 12, 18 of MRID 49970204; p. 12; Tables 1-6, pp. 28-33 of MRID 49970201). Following the method of Keith, L. H., *et al*. (see section **V. References** below), the LOD and LOQ for determination of propyzamide and its transformation products in soil were calculated using the standard deviation from the 0.005 µg/g recovery results (p. 18 of MRID 49970204). The LOD was calculated as three times the standard deviation (3*s*), and the LOQ was calculated as ten times the standard deviation (10*s*) of the recovery results. The calculated values support the LOQ and LOD established for the study.

Table 4. Method Characteristics

Analyte[1]		**Propyzamide**	**RH-24580**	**RH-24644**
Limit of Quantitation (LOQ)		0.005 µg/g		
Limit of Detection (LOD)		0.0015 µg/g		
Linearity (calibration curve r^2 and concentration range)	ECM[2]	r^2 = 0.9960-0.9980 (Q) r^2 = 0.9980-0.9996 (C)	r^2 = 0.9960-0.9980 (Q) r^2 = 0.9976-0.9980 (C)	r^2 = **0.9883**-0.9960 (Q) r^2 = **0.9935**-0.9980 (C)
		(0.015-2.0 µg/L)		
	ILV	r^2 = 0.9991 (Q & C)	r^2 = 0.9997 (Q) r^2 = 0.9968 (C)	r^2 = 0.9982 (Q) r^2 = 0.9991 (C)
		(0.03-20.00 ng/mL)		
Repeatable	ECM[3]	Yes at LOQ and 20×LOQ, but **no** samples were prepared at 10×LOQ		
	ILV[4]	Yes at LOQ and 10×LOQ		
Reproducible		Yes at LOQ and 10×LOQ		
Specific	ECM	Yes, matrix interferences were *ca.* <10% of the LOQ (based on peak area) for all soil matrices, except for the loam soil in which matrix interferences were *ca.* <15% of the LOQ (based on peak area).		
	ILV[5]	Yes, no matrix interferences were observed. Minor baseline noise was observed in LOQ chromatograms which interfered with peak integration.	Yes, matrix interferences were <10% of the LOQ (quantitation ion only; based on peak area and height). Minor baseline noise was observed in LOQ chromatograms which interfered with peak integration.	Yes, matrix interferences were <10% of the LOQ (based on peak area and height). Some non-uniform peak integration was noted in the LOQ chromatograms.

Numbers in red represent values less than recommended.
Data were obtained from pp. 12, 18; Tables 9-14, pp. 36-37 (recovery data); Tables 28-32, pp. 51-52 (calibration coefficients); Figures 1-6, pp. 53-58 (calibration curves); Figures 11-14, pp. 74R1-91R1 (chromatograms) of MRID 49970204; p. 12; Tables 1-6, pp. 28-33 (recovery data); Figures 1-6, pp. 36-41 (calibration curves); Figures 11-48, pp. 46-83 (chromatograms) of MRID 49970201; DER Attachment 2. Q = quantitation ion; C = confirmatory ion.

1 Propyzamide = Pronamide; 3,5-Dichloro-N-(1,1-dimethylprop-2-ynyl)benzamide; RH-24580 = 3,5-Dichloro-N-(1,1-dimethyl-2-oxopropyl)benzamide; and RH-24644 = 3,5-Dichloro-N-(1,1,2-trimethylprop-2-enyl)benzamide.

2 Reported correlation coefficients were reviewer-calculated from r values reported in the study report (Tables 28-32, pp. 51-52; Figures 1-6, pp. 53-58 of MRID 49970204; DER Attachment 2). Reported values included correlation coefficients for S01 (clay), S02 (loam) and S03 (silt loam); correlation coefficients for S04 (silt loam) were not reported.

3 In the ECM, the soil matrices were well characterized (USDA soil texture classification; p. 12; Table 2, p. 23 of MRID 49970204). Clay soil (33% sand, 26% silt, 41% clay; pH 7.7; 2.1% carbon) from Hareby County, Linconshire, United Kingdom, loam soil (47% sand, 28% silt, 25% clay; pH 7.4; 4.1% carbon) from Empingham County, Rutland, United Kingdom, silt loam soil (-003; 29% sand, 52% silt, 19% clay; pH 5.8; 3.8% carbon) from Ebbinghof County, North Rhine, Westphalia, Germany, and silt loam soil (-004; 34% sand, 60% silt, 6% clay; pH 5.8; 4.4% carbon) from Chelmorton County, Derbyshire, United Kingdom were used.

4 In the ILV, the soil was not characterized, but USDA soil texture classification was specified; the loamy sand soil was a standard soil 2.2 (batch Sp2.2 2811; EAS Chem SAS Sample Reference No. 233; p. 14; Appendix A, p. 89 of MRID 49970201).

5 Representative ILV chromatograms were faint, and the data results and baseline were difficult to interpret. The reviewer used a combination of the peak area data result and the peak height to evaluate the matrix interferences.

A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.
Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. The study reports ECM MRID 49970204 and ILV MRID 49970201 were amended to include the reagent blank chromatograms (p. 4.1R1 of MRID 49970204; p. 5 of MRID 49970201). Changes to the study report were made as a consequence of that addition.

2. For the ECM, performance data at 10x LOQ was not reported to validate the method. A validation sample set should consist of, at a minimum, a reagent blank, two unspiked matrix control samples, five matrix control samples spike at the LOQ, and five matrix control samples spiked at 10x LOQ for each analyte and matrix.

3. For the calibration curves of the ECM, the following RH-24644 correlation coefficients were unsatisfactory ($r^2 < 0.995$): clay soil, $r^2 = 0.9883$ (Q), 0.9935 (C); and loam soil, $r^2 = 0.9920$ (Q) (Tables 28-32, pp. 51-52; Figures 1-6, pp. 53-58 of MRID 49970201; DER Attachment 2). Reported values included correlation coefficients for S01 (clay), S02 (loam) and S03 (silt loam); correlation coefficients for S04 (silt loam) were not reported.

4. The soil used in the ILV were not characterized, but USDA soil texture classification was specified; the loamy sand soil was a standard soil 2.2 (batch Sp2.2 2811; EAS Chem SAS Sample Reference No. 233; p. 14; Appendix A, p. 89 of MRID 49970201). It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method.

5. Representative ILV chromatograms were faint, and the data results and baseline were difficult to interpret (Figures 11-48, pp. 46-83 of MRID 49970201).

6. Communications between the ILV and study sponsor were provided (p. 24 of MRID 49970201). Communication only occurred prior to the study to confirm the extraction solvent.

7. In the ECM, no significant matrix effects were observed; solvent calibration standards were used (p. 20; Table 21, p. 44; Appendix I, p. 99 of MRID 49970204). In the ILV, no significant matrix effects were observed, as well (p. 23; Table 8, p. 34 of MRID 49970201).

8. In the ECM, the stability of the analytes prepared in acetonitrile and stored at -20°C was investigated (p. 19 of MRID 49970204). It was determined that propyzamide (RH-23315) and RH-24580 were stable up to 57 days and RH-24644 up to 56 days.

   In addition, stability of the sample extracts was investigated (p. 19; Tables 15-20, pp. 38-43 of MRID 49970204). Following initial analyses, the samples were stored at 4°C for 4 days, then re-analyzed. Quantitation and confirmatory ion analyses determined mean recoveries for all analytes ranged from 72-97% with RSDs of 2.5-12.0%. Propyzamide, RH-24644 and RH-24580 were found to be stable up to 4 days.

In the ILV, extract stability was also investigated (pp. 23-24; Tables 9-10, p. 35 of MRID 49970201). Propyzamide, RH-24644 and RH-24580 were found to be stable up to 7 days when stored at 4°C.

9. It was reported for the ILV that one batch of fourteen samples required approximately five person hours (p. 24 of MRID 49970201). Instrument analysis of soil samples was two hours. Data manipulation required an additional two person hours. The entire set was completed in approximately one and a half calendar days.

V. References

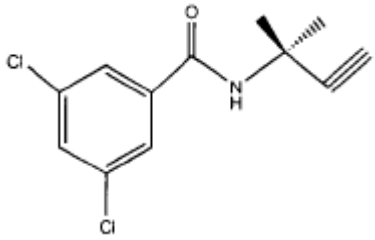
Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* 1983, *55*, 2210-2218 (p. 21 of MRID 49970204).

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.

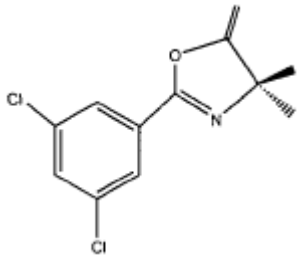
40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

Propyzamide (Kerb, pronamide, RH-23315, RH-3315, RH-315)
IUPAC Name: 3,5-Dichloro-N-(1,1-dimethylprop-2-ynyl)benzamide
CAS Name: 3,5-Dichloro-N-(1,1-dimethyl-2-propynyl)-benzamide
CAS Number: 23950-58-5
SMILES String: CC(C)(NC(=O)c1cc(Cl)cc(Cl)c1)C#C (EPI Suite, v3.12 SMILES).



RH-24644 (RH-4644)
IUPAC Name: 3,5-Dichloro-N-(1,1,2-trimethylprop-2-enyl)benzamide
CAS Name: 2-(3,5-Dichlorophenyl)-4,4-dimethyl-5-methylene oxozoline
CAS Number: 29918-40-9
SMILES String: Not reported.



RH-24580 (RH-4580)
IUPAC Name: 3,5-Dichloro-N-(1,1-dimethyl-2-oxopropyl)benzamide
CAS Name: N-(1,1-Dimethyl acetonyl)-3,5-dichloro-benzamide
CAS Number: 29918-41-0
SMILES String: Not reported.