

Test Material: Propyzamide (Pronamide, RH-23315)

MRID: 49970202

Title: Method Validation Study for the Determination of Residues of Propyzamide and its Metabolites in Surface Water, Ground Water and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry – AMENDED REPORT

MRID: 49970203

Title: Independent Laboratory Validation of Enforcement Method for the Determination of Propyzamide and its RH-24644, RH-24655, RH-24580, RH-26059, and UK1 Metabolites in Water (surface water, ground water and drinking water) by Liquid Chromatography with Tandem Mass Spectrometry

EPA PC Code: 101701

OCSPP Guideline: 850.6100

For CDM/CSS-Dynamac JV

Primary Reviewer: Lisa Muto

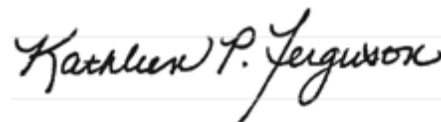
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Date: 10/25/16

Secondary Reviewer: Kathleen Ferguson

Signature:



Date: 10/25/16

Quality Assurance Manager: Joan Gaidos

Signature:



Date: 10/25/16

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Analytical method for propyzamide and its transformation products RH-24644, RH-24655, RH-24580, RH-26059 and UK1 in water

Reports: ECM: EPA MRID No.: 49970202. Li, Q. and A. Grotenhuis. 2016. Method Validation Study for the Determination of Residues of Propyzamide and its Metabolites in Surface Water, Ground Water and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry - AMENDED REPORT. Dow AgroSciences Study No.: 110587. Report prepared, sponsored, and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 105 pages. Final report issued September 6, 2011; amended report dated May 30, 2014 and March 30, 2016.

ILV: EPA MRID No. 49970203. Sears, K. 2016. Independent Laboratory Validation of Enforcement Method for the Determination of Propyzamide and its RH-24644, RH-24655, RH-24580, RH-26059, and UK1 Metabolites in Water (surface water, ground water and drinking water) by Liquid Chromatography with Tandem Mass Spectrometry. Pyxant Labs Study No.: 2340. Dow AgroSciences Study No.: 110676. Report prepared by Pyxant Labs Inc., Colorado Springs, Colorado, sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 444 pages. Final report issued September 12, 2011; amended report dated April 20, 2016.

Document No.: MRIDs 49970202 & 49970203

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards, with the exception that GLP characterizations of test substances RH-24655 and RH-26059 occurred during the course of the study rather than prior to experiment initiation (p. 3R2 of MRID 49970202). Signed and dated GLP and Quality Assurance statements were provided (pp. 3R2-4R2). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4R2). The summary of amendment changes was provided (pp. 4.1R2-4.3R2). A statement of No Data Confidentiality was not included.

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 49970203). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).

Classification: This analytical method is classified supplemental. For all analytes, performance data to validate the method at 10x LOQ was not reported. ILV quantitation ion performance data for 0.05 µg/L (LOQ) fortified UK1 (RH-23801) in ground water did not meet OCSPP Guideline 850.6100 criteria; ILV confirmatory ion performance data did not meet OCSPP Guideline 850.6100 criteria for many analyte/fortification/matrix analyses. Approximately half of the ILV correlation coefficients were unsatisfactory ($r^2 < 0.995$).

PC Code: 101701

Reviewer: Karen Milians, Chemist

Signature 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. 110587, is designed for the quantitative determination of propyzamide and its transformation products RH-24644, RH-24655, RH-24580, RH-26059, and UK1 (RH-23801) in water using LC/MS/MS at the LOQ of 0.05 µg/L. For all analytes, performance data to validate the method at 10x LOQ was not reported. The LOQ is less than the lowest toxicological level of concern in water for all analytes. Based on the quantitation ion results, the ILV validated the method after two trials, with the exception of 0.05 µg/L (LOQ) UK1 fortified ground water (RSD 22%); ILV confirmatory ion performance data was unsatisfactory for many analyte/fortification/matrix analyses. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method. Modifications to improve precision and repeatability of the method were made to the ECM by the independent laboratory; these recommended modifications were noted in the amended final ECM report. Approximately half of the ILV correlation coefficients were unsatisfactory ($r^2 < 0.995$), also the waters used in the ILV were not characterized. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method.

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	49970202 ²	49970203 ³		Water	06/09/2011 (Final Report)	Dow AgroSciences LLC	LC/MS/MS	0.05 µg/L
RH-24644								
RH-24655								
RH-24580								
RH-26059								
UK1 (RH-23801)								

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; RH-24655 = 3,5-Dichloro-N-(1,1-dimethylpropenyl)benzamide; RH-24580 = 3,5-Dichloro-N-(1,1-dimethyl-2-oxopropyl)benzamide; RH-26059 = 3-[(3,5-Dichlorobenzoyl)amino]-3-methylbutanoic acid; UK1 = RH-23801; 3-Chloro-N-(1,1-dimethylpropynyl)benzamide.

2 In the ECM, the ground (monitoring well) water (pH 8.2, hardness 330 mg equiv. CaCO₃/L, total organic carbon 3.4 ppm, dissolved organic carbon 2.2 ppm), surface (pond) water (pH 8.0, hardness 135 mg equiv. CaCO₃/L, total organic carbon 8.0 ppm, dissolved organic carbon 6.9 ppm), and drinking (tap) water (pH 8.6, hardness 4 mg equiv. CaCO₃/L, total organic carbon 3.1 ppm, dissolved organic carbon 2.6 ppm) matrices were well characterized (p. 19 of MRID 49970202).

3 In the ILV, ground, surface and drinking waters were used; the water matrices were not characterized, but were reported as obtained from Dow AgroSciences (p. 30 of MRID 49970203).

I. Principle of the Method

Water (4.0 mL) was fortified (40 μ L) with the appropriate fortification solution, as necessary, combined with acetonitrile (1.0 mL), vortexed for a few seconds to mix, then centrifuged (2,000 rpm, 10 minutes; p. 20 of MRID 49970202). An aliquot (1.0 mL) was transferred to an autosampler vial and analyzed by LC/MS/MS.

Samples were analyzed for propyzamide (RH-23315) and its products RH-24644, RH-24655, RH-24580, RH-26059 and UK1 by AB SCIEX API 5000 LC/MS/MS (Zorbax SB-C8, 4.6 mm x 75 mm, 3.5 μ m column; column temperature ambient) using a mobile phase of (A) acetonitrile with 0.1% formic acid and (B) water with 0.1% formic acid [percent A:B at 0 min. 10:90, 3-5 min. 100:0, 5:15-7 min. 10:90) with MS/MS-ESI (electrospray ionization) detection and multiple reaction monitoring (MRM); positive ion mode for propyzamide, RH-24644, RH-24655, RH-24580 and UK1, and negative ion mode for RH-26059 (pp. 11, 15R1-18R1 of MRID 49970202). Injection volume was 30 μ L. Analytes were identified using two ion transitions; one for quantitation (Q) and one for confirmation (C; p. 21 of MRID 49970202). Ion transitions monitored were as follows: m/z 256 \rightarrow 190 (Q) and m/z 258 \rightarrow 192 (C) for propyzamide, m/z 256 \rightarrow 172.9 (Q) and m/z 256 \rightarrow 109 (C) for RH-24644, m/z 258.1 \rightarrow 190 (Q) and m/z 258.1 \rightarrow 173 (C) for RH-24655, m/z 274 \rightarrow 173 (Q) and m/z 274 \rightarrow 109 (C) for RH-24580, m/z 288 \rightarrow 188 (Q) and m/z 290 \rightarrow 190 (C) for RH-26059, and m/z 222.1 \rightarrow 156 (Q) and m/z 222.1 \rightarrow 139 (C) for UK1.

In the ILV, the autosampler temperature was reduced from ambient to *ca.* 15°C due to possible stability issues with the analytes (p. 44; Appendix C, pp. 433-434 of MRID 49970203); no other modifications were reported. However, the ILV considered, in addition to autosampler temperature, the use of syringes and glass pipettes to fortify samples (analytes may adhere to plastic) and daily preparation of the mixed calibration standards to be critical method steps (p. 44). No other modifications of the ECM were reported.

Supplemental notes were added to the ECM in the second amended report which listed the ILV modifications to the ECM to improve precision and repeatability of the method (see above; pp. 25R2-26R2 of MRID 49970202).

The LOQ and LOD for all analytes were the same in the ECM and ILV at 0.05 μ g/L (0.05 ng/mL) and 0.015 μ g/L (0.015 ng/mL), respectively (pp. 11, 24R1 of MRID 49970202; pp. 25, 44 of MRID 49970203).

II. Recovery Findings

ECM (MRID 49970202): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of propyzamide, RH-24580, RH-24644, RH-24655, RH-26059 and UK1 in ground water, surface water and drinking water at fortification levels of 0.05 μ g/L (LOQ) and 1.0 μ g/L (20x LOQ; quantitation and confirmatory ions; Tables 50-52, pp. 48R1-50R1). No samples were prepared at 10 \times LOQ. Recoveries from samples fortified at 0.015 μ g/L (LOD) ranged (matrices combined) from 92-125% for propyzamide, 83-117% for RH-24580, 75-109% for RH-24644, 83-117% for RH-24655, 83-

109% for RH-26059, and 83-109% for UK1 (ions combined; DER Attachment 2). Performance data (recovery results) from quantitation ion analyses and confirmation ion analyses were comparable. Confirmation ratio differences [comparison of the confirmation ratio (confirmation ion peak area/quantitation ion peak area) of the recovery sample with the average confirmation ratio of the standards, p. 22R1] were used to further validate the accuracy of the method; these values were not reported in the study. The water matrices were well characterized (p. 19). Ground (monitoring well) water (pH 8.2, hardness 330 mg equiv. CaCO₃/L, total organic carbon 3.4 ppm, dissolved organic carbon 2.2 ppm), surface (pond) water (pH 8.0, hardness 135 mg equiv. CaCO₃/L, total organic carbon 8.0 ppm, dissolved organic carbon 6.9 ppm), and drinking (tap) water (pH 8.6, hardness 4 mg equiv. CaCO₃/L, total organic carbon 3.1 ppm, dissolved organic carbon 2.6 ppm) matrices were used.

ILV (MRID 49970203): For quantitative ion results, mean recoveries and RSDs were within guideline requirements for analysis of propyzamide, RH-24580, RH-24644, RH-24655, RH-26059 and UK1 in ground water, surface water and drinking water at fortification levels of 0.05 µg/L (LOQ) and 1.0 µg/L (20x LOQ), with the exception of 0.05 µg/L (LOQ) UK1 fortified ground water (RSD 22%; Tables 37-72, pp. 83-118). For confirmatory ion results, mean recoveries and RSDs were within guideline requirements for analysis of propyzamide, RH-24580, RH-24644, RH-24655, RH-26059 and UK1 in ground water, surface water and drinking water at fortification levels of 0.05 µg/L (LOQ) and 1.0 µg/L (20x LOQ), with the exception of ground water fortified at the LOQ with RH-24655 (mean 151%), RH-26059 (mean 58%) and UK1 (mean 131%); surface water fortified with RH-24580 (LOQ mean 135%), RH-26059 (LOQ mean 56%, 20xLOQ mean 67%) and UK1 (LOQ mean 134%); and drinking water fortified at the LOQ with RH-24580 (mean 124%), RH-24655 (RSD 21%) and UK1 (mean 130%). No samples were prepared at 10×LOQ. The majority of the confirmatory ion data was not comparable with the quantitation ion data. The water matrices were not characterized, but were reported as obtained from Dow AgroSciences (p. 30). Based on the quantitation ion analyses, the method for was validated, with modifications, after two trials, with the exception of 0.05 µg/L (LOQ) UK1 fortified ground water (pp. 40-42). The ECM method was performed by the ILV as written with a few critical modifications, which were included in the amended final ECM report.

Table 2. Initial Validation Method Recoveries for Propyzamide and Its Transformation Products in Water^{1,2,3,4}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground (Well) Water						
Quantitation ion						
Propyzamide (RH-23315)	0.015 (LOD)	2	92, 109	*	*	*
	0.05 (LOQ)	6	90-105	97	5.8	6.0
	1.0	6	96-106	101	3.7	3.7
RH-24580	0.015 (LOD)	2	109, 117	*	*	*
	0.05 (LOQ)	6	95-111	102	5.4	5.3
	1.0	6	100-108	103	2.8	2.7
RH-24644	0.015 (LOD)	2	92	*	*	*
	0.05 (LOQ)	6	80-115	96	14.9	15.5
	1.0	6	96-120	107	8.4	7.9
RH-24655	0.015 (LOD)	2	92, 100	*	*	*
	0.05 (LOQ)	6	98-104	101	2.6	2.6
	1.0	6	98-104	101	2.1	2.1
RH-26059	0.015 (LOD)	2	92, 100	*	*	*
	0.05 (LOQ)	6	90-95	93	2.2	2.4
	1.0	6	97-99	98	0.9	0.9
UK1 (RH-23801)	0.015 (LOD)	2	100	*	*	*
	0.05 (LOQ)	6	95-110	100	5.3	5.3
	1.0	6	99-107	103	3.1	3.0
Confirmatory ion						
Propyzamide (RH-23315)	0.015 (LOD)	2	92, 100	*	*	*
	0.05 (LOQ)	6	90-115	100	9.7	9.6
	1.0	6	100-105	102	1.8	1.8
RH-24580	0.015 (LOD)	2	100	*	*	*
	0.05 (LOQ)	6	93-103	95	4.0	4.2
	1.0	6	99-107	104	2.9	2.8
RH-24644	0.015 (LOD)	2	92	*	*	*
	0.05 (LOQ)	6	83-113	99	11.7	11.9
	1.0	6	95-119	105	9.2	8.7
RH-24655	0.015 (LOD)	2	109, 117	*	*	*
	0.05 (LOQ)	6	90-105	96	5.2	5.4
	1.0	6	101-108	104	2.7	2.6
RH-26059	0.015 (LOD)	2	100, 109	*	*	*
	0.05 (LOQ)	6	90-95	93	2.2	2.4
	1.0	6	97-100	98	0.9	0.9
UK1 (RH-23801)	0.015 (LOD)	2	83	*	*	*
	0.05 (LOQ)	6	93-105	96	4.4	4.6
	1.0	6	100-107	103	2.9	2.9
Surface (Pond) Water						
Quantitation ion						
Propyzamide (RH-23315)	0.015 (LOD)	2	100, 109	*	*	*
	0.05 (LOQ)	6	95-105	100	4.5	4.5
	1.0	6	95-101	98	2.4	2.4

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
RH-24580	0.015 (LOD)	2	100, 117	*	*	*
	0.05 (LOQ)	6	85-113	100	9.4	9.4
	1.0	6	97-104	101	2.4	2.4
RH-24644	0.015 (LOD)	2	75, 100	*	*	*
	0.05 (LOQ)	6	90-115	99	8.5	8.6
	1.0	6	101-122	108	8.9	8.2
RH-24655	0.015 (LOD)	2	92, 100	*	*	*
	0.05 (LOQ)	6	88-103	95	5.1	5.4
	1.0	6	95-102	97	2.1	2.2
RH-26059	0.015 (LOD)	2	92, 100	*	*	*
	0.05 (LOQ)	6	90-95	93	1.9	2.0
	1.0	6	93-98	96	1.6	1.7
UK1 (RH-23801)	0.015 (LOD)	2	92, 109	*	*	*
	0.05 (LOQ)	6	100-103	101	1.4	1.4
	1.0	6	96-101	98	1.7	1.7
Confirmatory ion						
Propyzamide (RH-23315)	0.015 (LOD)	2	92, 125	*	*	*
	0.05 (LOQ)	6	88-108	99	10	10.1
	1.0	6	98-103	101	1.8	1.8
RH-24580	0.015 (LOD)	2	92, 109	*	*	*
	0.05 (LOQ)	6	90-103	95	5.8	6.1
	1.0	6	97-105	100	3.3	3.3
RH-24644	0.015 (LOD)	2	83, 109	*	*	*
	0.05 (LOQ)	6	95-118	100	8.7	8.7
	1.0	6	99-122	108	8.9	8.2
RH-24655	0.015 (LOD)	2	83, 109	*	*	*
	0.05 (LOQ)	6	85-105	98	7.1	7.3
	1.0	6	99-107	101	3.0	3.0
RH-26059	0.015 (LOD)	2	92, 100	*	*	*
	0.05 (LOQ)	6	95-100	96	2.1	2.2
	1.0	6	93-98	96	1.5	1.6
UK1 (RH-23801)	0.015 (LOD)	2	92	*	*	*
	0.05 (LOQ)	6	88-100	93	4.4	4.7
	1.0	6	96-101	98	1.4	1.4
Drinking (Tap) Water						
Quantitation ion						
Propyzamide (RH-23315)	0.015 (LOD)	2	92, 109	*	*	*
	0.05 (LOQ)	6	93-105	100	4.3	4.3
	1.0	6	95-105	100	3.8	3.8
RH-24580	0.015 (LOD)	2	83, 92	*	*	*
	0.05 (LOQ)	6	95-108	103	5.3	5.2
	1.0	6	98-108	102	3.7	3.6
RH-24644	0.015 (LOD)	2	75, 100	*	*	*
	0.05 (LOQ)	6	79-100	93	8.3	8.9
	1.0	6	100-120	113	10.2	9.0
RH-24655	0.015 (LOD)	2	92, 100	*	*	*

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.05 (LOQ)	6	91-104	96	4.4	4.6
	1.0	6	96-106	101	3.7	3.7
RH-26059	0.015 (LOD)	2	83, 100	*	*	*
	0.05 (LOQ)	6	93-98	95	2.2	2.4
	1.0	6	98-102	100	1.5	1.5
UK1 (RH-23801)	0.015 (LOD)	2	92	*	*	*
	0.05 (LOQ)	6	90-115	103	10	9.6
	1.0	6	99-102	100	1.2	1.2
Confirmatory ion						
Propyzamide (RH-23315)	0.015 (LOD)	2	100, 117	*	*	*
	0.05 (LOQ)	6	93-120	104	11.8	11.3
	1.0	6	99-101	100	1.1	1.1
RH-24580	0.015 (LOD)	2	83, 109	*	*	*
	0.05 (LOQ)	6	90-103	94	4.9	5.2
	1.0	6	96-105	101	3.5	3.5
RH-24644	0.015 (LOD)	2	83, 109	*	*	*
	0.05 (LOQ)	6	78-105	96	11.2	11.6
	1.0	6	97-122	114	10.3	9.1
RH-24655	0.015 (LOD)	2	92,100	*	*	*
	0.05 (LOQ)	6	90-100	95	3.5	3.7
	1.0	6	99-107	103	2.8	2.7
RH-26059	0.015 (LOD)	2	92, 100	*	*	*
	0.05 (LOQ)	6	93-103	98	3.4	3.5
	1.0	6	97-104	101	2.5	2.5
UK1 (RH-23801)	0.015 (LOD)	2	92, 100	*	*	*
	0.05 (LOQ)	6	85-105	94	7.9	8.3
	1.0	6	98-102	100	1.7	1.7

Numbers in red represent values less or higher than recommended.

Data (uncorrected recovery results, Figure 29, p. 81R1) were obtained from Tables 50-52, pp. 48R1-50R1 of MRID 49970202 and DER Attachment 2 (LOD recovery results).

* Not calculated, n = 2.

1 The ground (monitoring well) water (pH 8.2, hardness 330 mg equiv. CaCO₃/L, total organic carbon 3.4 ppm, dissolved organic carbon 2.2 ppm), surface (pond) water (pH 8.0, hardness 135 mg equiv. CaCO₃/L, total organic carbon 8.0 ppm, dissolved organic carbon 6.9 ppm), and drinking (tap) water (pH 8.6, hardness 4 mg equiv. CaCO₃/L, total organic carbon 3.1 ppm, dissolved organic carbon 2.6 ppm) matrices were well characterized (p. 19 of MRID 49970202).

2 Ion transitions monitored were as follows: *m/z* 256→190 (Q) and *m/z* 258→192 (C) for propyzamide, *m/z* 256→172.9 (Q) and *m/z* 256→109 (C) for RH-24644, *m/z* 258.1→190 (Q) and *m/z* 258.1→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* 222.1→156 (Q) and *m/z* 222.1→139 (C) for UK1.

3 Values for the quantitation ion recovery of propyzamide were recalculated in MRID 49970202 due to incorrect integration of some propyzamide chromatograms in the original ECM MRID 48599207 (pp. 4.1R2-4.2R2 of MRID 49970202).

4 For quantitation ion results, recovery range, mean, s.d. and RSD values differed slightly from those reported in Tables 20-22, pp. 48-50 of MRID 48599207; most of the differences were found for LOQ, more than 20xLOQ. For confirmatory ion results, these values were not reported in the original ECM report MRID 48599207. All LOD values were reviewer-calculated from recovery values reported in MRID 49970202; quantitation ion LOD recovery values reported in MRID 49970202 did not match those reported in the original ECM report MRID 48599207.

Table 3. Independent Validation Method Recoveries for Propyzamide and Its Transformation Products in Water^{1,2,3,4}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground Water						
Quantitation ion						
Propyzamide (RH-23315)	0.05 (LOQ)	5	78-117	99	15	16
	1.0	5	73-107	89	12	14
RH-24580	0.05 (LOQ)	5	91-131	112	15	13
	1.0	5	92-102	97	4.2	4.4
RH-24644	0.05 (LOQ)	5	89-98	93	3.7	4.0
	1.0	5	96-100	98	1.8	1.8
RH-24655	0.05 (LOQ)	5	88-112	101	9.0	8.9
	1.0	5	85-105	97	7.5	7.7
RH-26059	0.05 (LOQ)	5	103-111	107	3.2	3.0
	1.0	5	103-105	104	0.84	0.80
UK1 (RH-23801)	0.05 (LOQ)	5	55-103	85	19	22
	1.0	5	82-107	95	11	12
Confirmatory ion						
Propyzamide (RH-23315)	0.05 (LOQ)	5	98-110	104	5.5	5.3
	1.0	5	95-120	107	9.5	8.9
RH-24580	0.05 (LOQ)	5	97-131	112	12	11
	1.0	5	92-108	101	6.1	6.0
RH-24644	0.05 (LOQ)	5	93-98	95	2.1	2.2
	1.0	5	97-104	100	2.9	2.9
RH-24655	0.05 (LOQ)	5	117-174	151	25	16
	1.0	5	90-97	94	3.2	3.4
RH-26059	0.05 (LOQ)	5	53-60	58	2.6	4.5
	1.0	5	71-72	72	0.67	0.93
UK1 (RH-23801)	0.05 (LOQ)	5	122-143	131	9.3	7.1
	1.0	5	104-121	113	7.7	6.8
Surface Water						
Quantitation ion						
Propyzamide (RH-23315)	0.05 (LOQ)	5	86-98	88	6.5	7.4
	1.0	5	98-108	102	4.8	4.7
RH-24580	0.05 (LOQ)	5	96-114	107	7.6	7.2
	1.0	5	95-100	98	2.3	2.3
RH-24644	0.05 (LOQ)	5	99-105	103	2.4	2.3
	1.0	5	90-98	95	3.2	3.4
RH-24655	0.05 (LOQ)	5	93-101	96	3.3	3.4
	1.0	5	94-103	97	3.5	3.6
RH-26059	0.05 (LOQ)	5	112-116	114	1.6	1.4
	1.0	5	100-107	104	2.9	2.8
UK1 (RH-23801)	0.05 (LOQ)	5	105-124	112	7.4	6.6
	1.0	5	93-105	99	4.2	4.3
Confirmatory ion						

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Propyzamide (RH-23315)	0.05 (LOQ)	5	74-97	88	9.8	11
	1.0	5	93-100	96	3.4	3.6
RH-24580	0.05 (LOQ)	5	125-165	135	17	13
	1.0	5	90-101	95	5.0	5.3
RH-24644	0.05 (LOQ)	5	97-111	102	5.3	5.1
	1.0	5	94-103	99	3.5	3.5
RH-24655	0.05 (LOQ)	5	85-113	97	12	12
	1.0	5	93-105	98	4.7	4.8
RH-26059	0.05 (LOQ)	5	51-61	56	3.5	6.3
	1.0	5	65-68	67	1.0	1.6
UK1 (RH-23801)	0.05 (LOQ)	5	95-156	134	25	19
	1.0	5	94-101	97	2.9	3.0
Drinking Water						
Quantitation ion						
Propyzamide (RH-23315)	0.05 (LOQ)	5	80-102	93	9.3	10
	1.0	5	95-103	100	3.3	3.3
RH-24580	0.05 (LOQ)	5	96-119	110	8.6	7.8
	1.0	5	102-110	107	3.6	3.3
RH-24644	0.05 (LOQ)	5	93-100	97	2.9	3.0
	1.0	5	91-101	97	4.7	4.8
RH-24655	0.05 (LOQ)	5	84-107	97	9.0	9.3
	1.0	5	99-104	102	2.0	2.0
RH-26059	0.05 (LOQ)	5	102-110	105	3.4	3.3
	1.0	5	103-108	105	2.3	2.2
UK1 (RH-23801)	0.05 (LOQ)	5	104-119	108	6.4	5.9
	1.0	5	93-100	96	3.0	3.1
Confirmatory ion						
Propyzamide (RH-23315)	0.05 (LOQ)	5	96-114	105	7.3	7.0
	1.0	5	93-106	99	4.8	4.9
RH-24580	0.05 (LOQ)	5	113-141	124	12	9.5
	1.0	5	89-104	99	7.0	7.1
RH-24644	0.05 (LOQ)	5	87-96	93	4.5	4.9
	1.0	5	95-104	100	3.9	4.0
RH-24655	0.05 (LOQ)	5	65-119	94	19	21
	1.0	5	100-108	106	3.6	3.4
RH-26059	0.05 (LOQ)	5	108-120	112	4.8	4.3
	1.0	5	104-107	105	1.3	1.3
UK1 (RH-23801)	0.05 (LOQ)	5	106-152	130	19	15
	1.0	5	99-110	104	4.1	3.9

Numbers in red represent values less or higher than recommended.

Data (uncorrected recovery results, pp. 35-36) were obtained from Tables 37-72, pp. 83-118 of MRID 49970203.

1 Water matrices were not characterized; all three control matrices were obtained from Dow AgroSciences (p. 30).

2 Ion transitions monitored were as follows: m/z 256→190 (Q) and m/z 258→192 (C) for propyzamide, m/z 256→172.9 (Q) and m/z 256→109 (C) for RH-24644, m/z 258.1→190 (Q) and m/z 258.1→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 222.1→156 (Q) and m/z 222.1→139 (C) for UK1.

³ For quantitation ion results, recovery range, mean, s.d. and RSD values were the same as those reported in Tables 19-36, pp. 55-72 of MRID 48690302. For confirmatory ion results, these values were not reported in the original ILV report MRID 48690302.

III. Method Characteristics

In the ECM and ILV, the LOQ and LOD values for propyzamide, RH-24580, RH-24644, RH-24655, RH-26059 and UK1 in water were 0.05 µg/L and 0.015 µg/L, respectively (pp. 11, 24R1 of MRID 49970202; pp. 25, 44 of MRID 49970203). 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 water were calculated using the standard deviation from the 0.05 µg/L recovery results (p. 24R1 of MRID 49970202). The LOD was calculated as three times the standard deviation ($3s$), and the LOQ was calculated as ten times the standard deviation ($10s$) of the recovery results (Table 53, p. 51R1). The calculated values support the LOQ and LOD established for the study; only four calculation sets were above the established LOQ and LOD values (propyzamide/drinking water/confirmatory ion, RH-24644/ground water/quantitation and confirmation ions, and RH-24644/drinking water/quantitation ion).

Table 4. Method Characteristics

Analyte ¹		Propyzamide	RH-24580	RH-24644	RH-24655	RH-26059	UK1 (RH-23801)	
Limit of Quantitation (LOQ)		0.05 µg/L						
Limit of Detection (LOD)		0.015 µg/L						
Linearity (calibration curve r ² and concentration range) ²	ECM	r ² = 0.9995- 0.9996 (Q) r ² = 0.9995- 0.9997 (C)	r ² = 0.9997- 0.9999 (Q) r ² = 0.9998- 0.9999 (C)	r ² = 0.9985- 0.9994 (Q) r ² = 0.9990- 0.9991 (C)	r ² = 0.9998- 0.9999 (Q) r ² = 0.9988- 0.9993 (C)	r ² = 0.9999 (Q) r ² = 0.9998 (C)	r ² = 0.9998- 1.0000 (Q) r ² = 0.9999 (C)	
		(0.015-2.0 µg/L)						
	ILV	Surface	r ² = 0.9982 (Q) r ² = 0.9996 (C)	r ² = 0.9936 (Q) r ² = 0.9986 (C)	r ² = 0.9956 (Q) r ² = 0.9928 (C)	r ² = 0.9968 (Q) r ² = 0.9986 (C)	r ² = 0.9998 (Q) r ² = 0.9698 (C)	r ² = 0.9855 (Q) r ² = 0.9966 (C)
		Ground	r ² = 0.9926 (Q) r ² = 0.9948 (C)	r ² = 0.9872 (Q) r ² = 0.9892 (C)	r ² = 0.9972 (Q) r ² = 0.9912 (C)	r ² = 0.9910 (Q) r ² = 0.9855 (C)	r ² = 0.9998 (Q) r ² = 0.9667 (C)	r ² = 0.9990 (Q) r ² = 0.9839 (C)
		Drinking	r ² = 0.9998 (Q) r ² = 0.9990 (C)	r ² = 0.9874 (Q) r ² = 0.9974 (C)	r ² = 0.9992 (Q) r ² = 0.9970 (C)	r ² = 0.9948 (Q) r ² = 0.9988 (C)	r ² = 0.9998 (Q) r ² = 0.9984 (C)	r ² = 0.9964 (Q) r ² = 0.9962 (C)
(0.015-2.0 µg/L)								
Repeatable	ECM ³		Yes at LOQ and 20×LOQ, but no samples were prepared at 10×LOQ (Q & C)					
	ILV ⁴		No samples were prepared at 10×LOQ					
		Q	Yes at LOQ and 20×LOQ					Yes at LOQ and 20×LOQ, except at LOQ in ground water (RSD 22%)
	C	Yes at LOQ and 20×LOQ	Yes at LOQ and 20×LOQ, except at LOQ in surface water (mean 135%) and drinking water (mean 124%)	Yes at LOQ and 20×LOQ	Yes at LOQ and 20×LOQ, except at LOQ in ground water (mean 151%) and drinking water (RSD 21%)	Yes at LOQ and 20×LOQ, except in ground water (LOQ mean 58%) and surface water (LOQ mean 56% , 20xLOQ mean 67%)	Yes at LOQ and 20×LOQ, except at LOQ in ground water (mean 131%), surface water (mean 134%) and drinking water (mean 130%)	
Reproducible		Yes at LOQ and 20×LOQ, but no samples were prepared at 10×LOQ					Yes at LOQ and 20×LOQ, except ground water, but no samples were prepared at 10×LOQ	

Analyte ¹		Propyzamide	RH-24580	RH-24644	RH-24655	RH-26059	UK1 (RH-23801)
Specific	ECM	Yes, no matrix interferences were observed.					
	ILV	Yes, no matrix interferences were observed. Peak interferences were <10% of the LOQ. Some non-uniform peak integration was noted. Minor baseline noise was observed in some chromatograms.					

Numbers in red represent values less or higher than recommended.

Data were obtained from pp. 11, 20, 24R1; Tables 2-13, pp. 29.1R1-29.6R1 (calibration coefficients); Tables 50-52, pp. 48R1-50R1 (recovery data); Figures 20-28, pp. 63R1-80R1 (chromatograms) of MRID 49970202; pp. 25, 44; Tables 43-72, pp. 89-118 (recovery data); Figures 1-18, pp. 137-154 (Q calibration curves); Figures 25-120, pp. 161-256 (Q chromatograms); Figures B-1 to B-18, pp. 313-330 (C calibration curves); Figures B-25 to B-120, pp. 337-432 (C chromatograms) of MRID 49970203; DER Attachment 2. Q = quantitation ion; C = confirmatory ion.

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; RH-24655 = 3,5-Dichloro-N-(1,1-dimethylpropenyl)benzamide; RH-24580 = 3,5-Dichloro-N-(1,1-dimethyl-2-oxopropyl)benzamide; RH-26059 = 3-[(3,5-Dichlorobenzoyl)amino]-3-methylbutanoic acid; UK1 = RH-23801; 3-Chloro-N-(1,1-dimethylpropynyl)benzamide.

2 Reported correlation coefficients were reviewer-calculated from r values reported in the study report (Tables 2-13, pp. 29.1R1-29.6R1 of MRID 49970202; Figures 1-18, pp. 137-154; Figures B-1 to B-18, pp. 313-330 of MRID 49970203; DER Attachment 2). Reviewer-calculated r² values from reviewer-generated calibration curves for the provided ILV calibration curves (matrices combined) were 0.9926-0.9998 for propyzamide, 0.9873-0.9936 for RH-24580, 0.9955-0.9991 for RH-24644, 0.9909-0.9968 for RH-24655, 0.9998 (n = 3) for RH-26059, and 0.9855-0.9989 for UK1 (DER Attachment 2).

3 In the ECM, the ground (monitoring well) water (pH 8.2, hardness 330 mg equiv. CaCO₃/L, total organic carbon 3.4 ppm, dissolved organic carbon 2.2 ppm), surface (pond) water (pH 8.0, hardness 135 mg equiv. CaCO₃/L, total organic carbon 8.0 ppm, dissolved organic carbon 6.9 ppm), and drinking (tap) water (pH 8.6, hardness 4 mg equiv. CaCO₃/L, total organic carbon 3.1 ppm, dissolved organic carbon 2.6 ppm) matrices were well characterized (p. 19 of MRID 49970202).

4 In the ILV, ground, surface and drinking waters were used; the water matrices were not characterized, but were reported as obtained from Dow AgroSciences (p. 30 of MRID 49970203).

A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

Linearity is satisfactory when r² ≥ 0.995.

IV. Method Deficiencies and Reviewer's Comments

1. ECM MRID 49970202 was an amended study report of ECM MRID 48599207. ILV MRID 49970203 was an amended study report of ILV MRID 48690302. ECM MRID 48599207 and ILV MRID 48690302 had been previously submitted and reviewed by CDM Smith Primary Reviewer Lynne Binari and Secondary Reviewer Lisa Muto (08/11/2014). The review of the amended ECM MRID 4970202 and ILV MRID 49970203 was completed by CDM Smith Primary Reviewer Lisa Muto and Secondary Reviewer Kathleen Ferguson.

2. The amended ECM MRID 49970202 contained several changes to the original ECM MRID 48599207. The most significant changes were the correction of the recovery values for propyzamide, which were incorrect due to integration errors, the addition of the modifications and critical steps of the ILV, and the addition of confirmation ion recovery data, stability data and chromatograms (pp. 4.1R2-4.3R2 of MRID 49970202).

The most significant change amended ILV MRID 49970203 to the original ILV MRID 48690302 was the addition of confirmation ion recovery data and chromatograms (pp. 9-24 of MRID 49970203).

3. For both the ECM and ILV, 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.

4. For the ILV, the following fortification using the quantitation ion method did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy (mean recoveries for replicates at each spiking level between 70% and 120% and relative standard deviations (RSD) $\leq 20\%$): 0.05 $\mu\text{g/L}$ (LOQ) fortified UK1 in ground water (RSD 22%; Tables 43-72, pp. 89-118 of MRID 49970203).

For the ILV, the ILV, the following analytes/fortifications using the confirmatory ion method did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy (mean recoveries for replicates at each spiking level between 70% and 120% and relative standard deviations (RSD) $\leq 20\%$): ground water fortified at the LOQ with RH-24655 (mean 151%), RH-26059 (mean 58%) and UK1 (mean 131%); surface water fortified with RH-24580 (LOQ mean 135%), RH-26059 (LOQ mean 56%, 20xLOQ mean 67%) and UK1 (LOQ mean 134%); and drinking water fortified at the LOQ with RH-24580 (mean 124%), RH-24655 (RSD 21%) and UK1 (mean 130%; Tables 43-72, pp. 89-118 of MRID 49970203). The reviewer noted that confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

5. For the calibration curves of the ILV, the following correlation coefficients were unsatisfactory ($r^2 < 0.995$): propyzamide [ground water, $r^2 = 0.9926$ (Q), 0.9948 (C)]; RH-24580 [surface water, $r^2 = 0.9936$ (Q); ground water, $r^2 = 0.9872$ (Q), 0.9892 (C); drinking water, $r^2 = 0.9874$]; RH-24644 [surface water, $r^2 = 0.9928$ (C); ground water, r^2

- = 0.9912 (C)]; RH-24655 [ground water, $r^2 = 0.9910$ (Q), 0.9855 (C); drinking water, $r^2 = 0.9948$ (Q)]; RH-26059 [surface water, $r^2 = 0.9698$ (C); ground water, $r^2 = 0.9667$ (C)]; and UK1 [surface water, $r^2 = 0.9855$ (Q); ground water, $r^2 = 0.9839$ (C); Figures 1-18, pp. 137-154; Figures B-1 to B-18, pp. 313-330 of MRID 49970203; DER Attachment 2].
6. The waters used in the ILV were not characterized. The water matrices were reported as obtained from Dow AgroSciences (p. 30 of MRID 49970203). It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method.
 7. The ILV implemented several modifications to the ECM to improve precision and repeatability of the method (p. 44; Appendix C, pp. 433-434 of MRID 49970203). The autosampler temperature was reduced from ambient to *ca.* 15°C due to possible stability issues with the analytes. Additionally, the ILV considered the use of syringes and glass pipettes to fortify samples (analytes may adhere to plastic) and daily preparation of the mixed calibration standards to be critical method steps. The modifications recommended by the independent laboratory were implemented in the amended final ECM report as supplemental notes (pp. 25R2-26R2 of MRID 49970202).
 8. In the ECM, confirmation ratio differences [comparison of the confirmation ratio (confirmation ion peak area/quantitation ion peak area) of the recovery sample with the average confirmation ratio of the standards, p. 22R1 of MRID 49970202] were used to further validate the accuracy of the method; these values were not reported in the amended ECM study report. However, in the original ECM MRID 48599207, these values were reported and were $\leq 20\%$ for all analytes at both fortification levels in the three water matrices, with the exception of one 0.05 µg/L RH-24655 fortified surface water sample (Tables 2-19, pp. 30-47 of MRID 48599207).
 9. Communications between the ILV and study sponsor were provided (see above; p. 44; Appendix C, pp. 433-434 of MRID 49970203).
 10. In the ECM, no significant matrix effects were observed; solvent calibration standards were used (pp. 20, 25R2; Tables 78-83, pp. 51.9R1-51.11R1 of MRID 49970202).
 11. In the ECM, the stability of the analytes prepared in acetonitrile and stored at -20°C was investigated (pp. 24R1-25R2 of MRID 49970202). Quantitation ion analyses found propyzamide (RH-23315, 97.1% recovered) and RH-24580 (100.2%) were stable up to 57 days, RH-24644 (96.2%), RH-24655 (96.4%) and RH-26059 (110.2%) up to 56 days and UK1 (97.8%) up to 41 days. Recoveries from confirmation ion analyses were also determined: propyzamide (RH-23315, 97.2% recovered), RH-24580 (103.3%), RH-24644 (96.1%), RH-24655 (98.8%), RH-26059 (108.7%), and UK1 (98.9%; Tables 54-59, pp. 51.1R1-51.2R1).
- In addition, stability of the sample extracts was investigated (p. 25R2; Tables 60-77, pp. 51.3R1-51.8R1 of MRID 49970202). Following initial analyses, the samples were stored at 4°C for 3-6 days, then re-analyzed. Quantitation ion analyses determined mean recoveries for all analytes ranged from 97-107% with RSDs of 1.3-6.5%. Propyzamide (RH-23315), RH-24644, RH-24655, RH-24580 and UK1 were found to be stable up to 3

days and RH-26059 up to 6 days after sample preparation. Confirmatory ion analyses determined mean recoveries for all analytes ranged from 93-105% with RSDs of 3.5-10.5%.

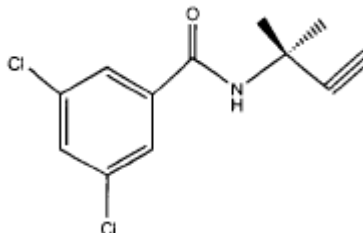
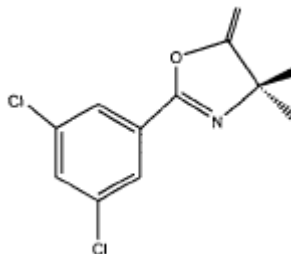
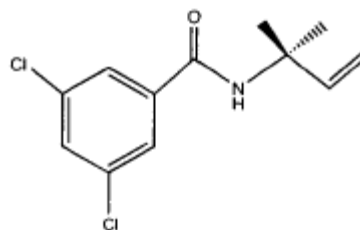
12. It was reported for the ILV that one batch of thirty-eight samples required approximately four person hours over one calendar day to complete the preparation (p. 44 of MRID 49970203). Instrument analysis of water samples was three hours. Data manipulation required an additional one to two person hours. Initial solution preparation required approximately three person hours.

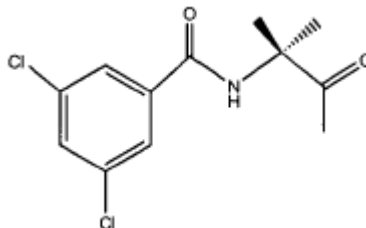
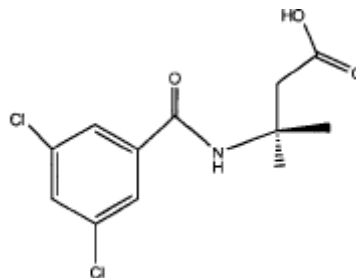
V. References

Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* 1983, 55, 2210-2218 (p. 27R2 of MRID 49970202).

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 oxazoline**CAS Number:** 29918-40-9**SMILES String:** Not reported.**RH-24655****IUPAC Name:** 3,5-Dichloro-N-(1,1-dimethylpropenyl)benzamide**CAS Name:** Not reported.**CAS Number:** Not reported.**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 acetyl)-3,5-dichloro-benzamide**CAS Number:** 29918-41-0**SMILES String:** Not reported.**RH-26059****IUPAC Name:** 3-[(3,5-Dichlorobenzoyl)amino]-3-methylbutanoic acid**CAS Name:** Not reported.**CAS Number:** Not reported.**SMILES String:** Not reported.**RH-23801 (UK1, 3-chloro kerb)****IUPAC Name:** 3-Chloro-N-(1,1-dimethylpropynyl)benzamide**CAS Name:** Not reported.**CAS Number:** Not reported.**SMILES String:** Not reported.