Test Material:. Diflufenzopyr

MRID: 49369501

Title: Validation of Draft Analytical Method No. R0039: “The Determination of Residues of Diflufenzopyr (Reg. No. 362255) and its Metabolites, M1 (Reg. No. 363181), M2 (Reg. No. 395202), M6 (Reg. No. 395198), and M9 (Reg. No. 395207) in Water using LC-MS/MS”

MRID: 49369502

Title: Independent Laboratory Validation of BASF Analytical Method No. R0039: “The Determination of Residues of Diflufenzopyr (Reg. No. 362255) and its Metabolites, M1 (Reg. No. 363181), M2 (Reg. No. 395202), M6 (Reg. No. 395198), and M9 (Reg. No. 395207) in Water using LC-MS/MS”

EPA PC Code: 005108

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto

Signature: Lisa Muto

Date: 1/20/15

Secondary Reviewer: Lynne Binari

Signature: Lynne Binari

Date: 1/20/15

QC/QA Manager: Joan Gaidos

Signature:

Date: 1/20/15

Analytical method for diflufenzopyr and its transformation products, M1, M2, M6 and M9, in water

Reports:	ECM: EPA MRID No. 49369501. Caro, A.F., N. Tarkalanov, R. Perez. 2014. Validation of Draft Analytical Method No. R0039: “The Determination of Residues of Diflufenzopyr (Reg. No. 362255) and its Metabolites, M1 (Reg. No. 363181), M2 (Reg. No. 395202), M6 (Reg. No. 395198), and M9 (Reg. No. 395207) in Water using LC-MS/MS”. BASF Study No.: 717315. ADPEN Study No.: 2K13-903-717315. BASF Registration Document No.: 2014/7000833. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida; sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 110 pages. Final report issued April 23, 2014. ILV: EPA MRID No. 49369502. Fiorito, B., Y. Shi. 2014. Independent Laboratory Validation of BASF Analytical Method No. R0039: “The Determination of Residues of Diflufenzopyr (Reg. No. 362255) and its Metabolites, M1 (Reg. No. 363181), M2 (Reg. No. 395202), M6 (Reg. No. 395198), and M9 (Reg. No. 395207) in Water using LC-MS/MS”. BASF Study No.: 717313. Alliance Pharma Project No.: 140233. BASF Registration Document No.: 2014/7000834. Report prepared by Alliance Pharma, Malvern, Pennsylvania; sponsored and submitted by BASF Crop Protection, Research Triangle Park, North Carolina; 126 pages. Final report issued April 26, 2014.
Document No.:	MRIDs 49369501 & 49369502
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards, 40 CFR, Part 160 (p. 3 of MRID 49369501). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-5). A signatures page was also provided (p. 6). ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards, 40 CFR, Part 160 (p. 3 of MRID 49369502). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-5). A signatures page was also provided (p. 6).
Classification:	This analytical method is classified as partially acceptable. For the ECM, mean recoveries of M2 in surface water were <70% at the LOQ and 10×LOQ. The sponsor did not specify that the ground water used in the ILV was either an equivalent, or more difficult, analytical sample condition as the surface water used in the ECM. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. Linearity coefficients were <0.995 for some of the ECM calibrations and most of the ILV calibrations.
PC Code:	005108
Final EFED Reviewer:	Ronald D. Parker, Ph.D., Senior Environmental Engineer **Signature:** Ronald D. Parker 2016.02.12 17:24:07 -05'00'

Executive Summary

This analytical method, BASF Analytical Method No. R0039, is designed for the quantitative determination of diflufenzopyr (BAS 654 H; Reg. No. 362255) and its metabolites, M1 (Reg. No. 363181), M2 (Reg. No. 395202), M6 (Reg. No. 395198), and M9 (Reg. No. 395207) in water at the LOQ of 0.05 µg/kg (ppb, µg/L) using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water. The ECM laboratory used surface (river) water and ground (well) water, while the ILV laboratory used only ground (well) water. The method was not successfully validated for M2 in surface water by the ECM. The well water of the ILV was identical in source and physical characteristics to that used in the ECM. The sponsor did not specify that the ground water was either an equivalent, or more difficult, analytical sample condition as the surface water. No major issues were discovered by the independent laboratory; the method appeared to be validated with the first trial, although the number of trials was not specifically reported.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Diflufenzopyr (BAS 654 H)	49369501	49369502		Water	4/23/2014	BASF Corporation	LC/MS/MS	0.5 µg/kg (0.5 µg/L)
M1								
M2								
M6								
M9								

I. Principle of the Method

Procedure for diflufenzopyr, M1, M6 and M9

Samples (10 mL) were fortified, then filtered through a 0.45-µm PTFE syringe filter and analyzed by LC/MS/MS (p. 18; Figure 9.2.1 p. 40 of MRID 49369501). The study authors noted that "extracts of diflufenzopyr, M1, M6 and M9 are not stable and should be injected immediately after preparation for injection" (p. 18).

Procedure for M2

Samples (100 mL) were fortified and mixed with 20 g of sodium chloride (p. 18; Figure 9.2.2 p. 40 of MRID 49369501). The solution was extracted three times with methylene chloride (3 x 20 mL). A fourth extraction can be performed, if necessary. The extract was dried with sodium sulfate and concentrated under vacuum. Acetonitrile was added to the residue, and the sample was concentrated again to remove all methylene chloride. The acetonitrile concentrate was further concentrated to *ca.* 1 mL under nitrogen at 50-60°C. The concentrated sample was reconstituted using 50 mM ammonium carbonate [$(NH_4)_2CO_3$] to a final volume of 2 mL (blank, control and LOQ samples) or 20 mL (10×LOQ samples). The sample was filtered through a 0.45-µm PTFE syringe filter and analyzed by LC/MS/MS. The study authors noted that the "extracts of M2 are stable".

Samples were analyzed for analytes using an Aglient 1290 HPLC System with large injection volume coupled to an AB Sciex 5500 Triple Quad mass spectrometer (pp. 19-20 of MRID

49369501). The HPLC/MS conditions consisted of a Phenomenex Kinetex PFP column (2.1 x 100 mm, 1.7-µm; column temperature 30.0°C), a mobile phase gradient of (A) 10 mM ammonium formate + 1% formic acid in HPLC grade water and (B) 10 mM ammonium formate + 1% formic acid in HPLC grade methanol for diflufenzopyr, M1, M6 and M9 [percent A:B (v:v) at 0.00-2.50 min. 100:0, 5.50-6.50 min. 65:35, 7.50-8.80 min. 30:70, 9.00-11.00 min. 10:90, 11.10-12.20 min. 100:0] or (A) 1% formic acid in HPLC grade water and (B) 1% formic acid in HPLC grade acetonitrile for M2 [0.00-0.50 min. 95:5, 3.00-6.50 min. 50:50, 7.00-7.40 min. 0:100, 7.50-8.60 min. 95:5] and MS/MS detection in positive ion mode. Two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored: *m/z* 335.0 → 206.1 (Q) and *m/z* 335.0 → 162.2 (C) for diflufenzopyr; *m/z* 162.2 → 102.9 (Q) and *m/z* 162.2 → 88.7 (C) for M1; *m/z* 130.1 → 83.0 (Q) and *m/z* 130.1 → 110.1 (C) for M2; *m/z* 165.9 → 148.1 (Q) and *m/z* 165.9 → 91.9 (C) for M6; and *m/z* 178.1 → 150.1 (Q) and *m/z* 178.1 → 119.1 (C) for M9. Approximate retention times were 9.7 min. for diflufenzopyr, 6.8 min. for M1, 4.4 min. for M2, 5.8 min. for M6, and 4.8 min. for M9. Injection volume was 40.0 µL.

In the ILV, the analytes were extracted in the same manner as the ECM with the following discrepancies: samples fortified with diflufenzopyr, M1, M6 and M9 were not filtered through a 0.45-µm PTFE syringe filter prior to analysis; and the amount and timing of the addition of NaCl was altered for samples fortified with M2 (pp. 14-15 of MRID 49369502). The extracts were analyzed for analytes using a Shimadzu LC-30AD HPLC coupled to SCIEX API 5500 mass spectrometer (pp. 14-15; Table 12, pp. 35-36). The HPLC column, mobile phases, mobile gradient and injection volumes were identical to that of the ECM. Two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored: *m/z* 335.0 → 206.1 (Q) and *m/z* 335.0 → 162.2 (C) for diflufenzopyr; *m/z* 162.2 → 102.9 (Q) and *m/z* 162.2 → **93.1** (C) for M1; *m/z* 130.1 → 83.0 (Q) and *m/z* 130.1 → 110.1 (C) for M2; *m/z* 165.9 → 148.1 (Q) and *m/z* 165.9 → 91.9 (C) for M6; and *m/z* 178.1 → 150.1 (Q) and *m/z* 178.1 → 119.1 (C) for M9 (difference from ECM transitions was **bolded**). Approximate retention times were 9.7 min. for diflufenzopyr, 6.8 min. for M1, **5.6** min. for M2, **6.0** min. for M6, and **5.2** min. for M9 (differences from ECM retention times were **bolded**).

In both the ECM and ILV, the LOQ and LOD were reported as 0.5 µg/kg (0.5 µg/L) and 0.1 µg/kg (0.1 µg/L), respectively (pp. 7, 25 of MRID 49369501; p. 15 of MRID 49369502).

II. Recovery Findings

ECM (MRID 49369501): Mean recoveries and relative standard deviations (RSD) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of diflufenzopyr, M1, M6 and M9 in surface (river) and ground (well) water at the LOQ and 10×LOQ and for analysis of M2 in ground (well) water at the LOQ and 10×LOQ (Tables 8-9, pp. 23-24). In surface (river) water, mean recoveries of M2 were unsatisfactory with 65% (Q and C) at the LOQ and 67% (Q) and 68% (C) at 10×LOQ. Two parent-daughter ion transitions were monitored. Quantitative ion and confirmatory ion results were comparable. The ground water source was BD Well; the surface water source was Goose River (source location was not further specified; p. 12). Waters were fully characterized by Agvise Laboratories, Northwood, North Dakota (Appendix 9.5, pp. 88-89).

ILV (MRID 49369502): Mean recoveries and RSDs were within guidelines for analysis of diflufenzopyr, M1, M2, M6 and M9 in ground (well) water at the LOQ and 10×LOQ (no other water type was tested; pp. 7, 18-20). The number of trials was not specified, but the reviewer assumed that the method was validated with the first trial. Two parent-daughter ion transitions were monitored. Quantitative ion and confirmatory ion results were comparable. The surface water source was BD Well, the same as that of the ECM (source location was not further specified; p. 12; Appendix 2, p. 110). The water was fully characterized by Agvise Laboratories, Northwood, North Dakota. Based on the water characterization reports, the reviewer determined that the Batch/Lot of BD Well water which was used by the ILV was identical to that used by the ECM.

Table 2. Initial Validation Method Recoveries for Diflufenzopyr and its Metabolites, M1, M2, M6 and M9, in Water[1,2]

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground Water (Well)						
Quantitative ion						
Diflufenzopyr (BAS 654 H)	0.5 (LOQ)	5	90-99	96	3.8	3.9
	5	5	89-101	96	4.5	4.6
M1	0.5 (LOQ)	5	102-115	107	5.4	5.1
	5	5	99-110	104	5.4	5.1
M2	0.5 (LOQ)	5	72-84	77	5.0	6.5
	5	5	71-84	77	5.8	7.6
M6	0.5 (LOQ)	5	92-113	104	8.1	7.8
	5	5	101-114	108	4.6	4.3
M9	0.5 (LOQ)	5	91-118	102	12.3	12.1
	5	5	97-107	102	4.8	4.7
Confirmatory ion						
Diflufenzopyr (BAS 654 H)	0.5 (LOQ)	5	94-101	97	2.9	3.0
	5	5	87-100	95	4.7	4.9
M1	0.5 (LOQ)	5	96-107	103	4.4	4.2
	5	5	91-120	109	12.1	11.1
M2	0.5 (LOQ)	5	71-78	74	3.5	4.7
	5	5	64-80	72	5.9	8.2
M6	0.5 (LOQ)	5	93-112	104	7.4	7.1
	5	5	101-112	107	4.3	4.0
M9*	0.5 (LOQ)	5	86-111	100	10.3	10.3
	5	5	97-113	105	6.3	6.0
Surface Water (River)						
Quantitative ion						
Diflufenzopyr (BAS 654 H)	0.5 (LOQ)	5	80-93	88	5.0	5.7
	5	5	81-85	84	1.9	2.3
M1	0.5 (LOQ)	5	97-105	99	3.0	3.0
	5	5	90-105	96	5.8	6.0
M2	0.5 (LOQ)	5	60-73	**65**	5.3	8.1
	5	5	60-81	**67**	8.5	12.6
M6	0.5 (LOQ)	5	86-93	89	3.3	3.7
	5	5	77-90	82	4.7	5.8
M9*	0.5 (LOQ)	5	98-110	102	4.9	4.8
	5	5	102-107	105	2.4	2.3
Confirmatory ion						
Diflufenzopyr (BAS 654 H)	0.5 (LOQ)	5	80-93	89	4.9	5.6
	5	5	82-87	85	1.9	2.2

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
M1*	0.5 (LOQ)	5	87-104	97	7.2	7.4
	5	5	89-101	95	5.0	5.3
M2	0.5 (LOQ)	5	63-70	**65**	3.4	5.2
	5	5	62-72	**68**	4.5	6.7
M6	0.5 (LOQ)	5	87-93	90	2.3	2.5
	5	5	77-88	82	4.2	5.1
M9	0.5 (LOQ)	5	85-100	92	6.2	6.7
	5	5	99-104	102	2.1	2.1

Data (uncorrected recovery results, unless noted otherwise) were obtained from Tables 8-9, pp. 23-24 of MRID 49369501.

* Recovery results were corrected for residues found in the controls (Appendix 9.6, pp. 102, 106, 109).

1 The ground water source was BD Well; the surface water source was Goose River (source location was not further specified; p. 12). Waters were fully characterized by Agvise Laboratories (Appendix 9.5, pp. 88-89).

2 Ion transitions monitored were as follows (quantitative ion and confirmatory ion, respectively): *m/z* 335.0 → 206.1 (Q) and *m/z* 335.0 → 162.2 (C) for diflufenzopyr; *m/z* 162.2 → 102.9 (Q) and *m/z* 162.2 → 88.7 (C) for M1; *m/z* 130.1 → 83.0 (Q) and *m/z* 130.1 → 110.1 (C) for M2; *m/z* 165.9 → 148.1 (Q) and *m/z* 165.9 → 91.9 (C) for M6; and *m/z* 178.1 → 150.1 (Q) and *m/z* 178.1 → 119.1 (C) for M9 (pp. 19-20).

3 Only representative recovery data were provided for confirmatory ion analysis: n = 2-3, except for river samples which were not included in the representative data. It could not be determined if all samples or only selected samples were evaluated via confirmatory ion analysis.

Table 3. Independent Validation Method Recoveries for Diflufenzopyr and its Metabolites, M1, M2, M6 and M9, in Water[1]

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground Water (Well)						
Quantitative ion						
Diflufenzopyr (BAS 654 H)	0.5 (LOQ)	5	102-113	109	4.5	4.1
	5	5	102-112	108	4.1	3.8
M1	0.5 (LOQ)	5	97-114	104	6.6	6.3
	5	5	109-116	113	3.3	3.0
M2	0.5 (LOQ)	5	91-113	101	8.0	7.9
	5	5	94-111	104	6.7	6.4
M6	0.5 (LOQ)	5	87-113	101	10.9	10.9
	5	5	104-110	108	2.5	2.3
M9	0.5 (LOQ)	5	80-104	93	9.0	9.7
	5	5	86-101	93	6.7	7.2
Confirmatory ion						
Diflufenzopyr (BAS 654 H)	0.5 (LOQ)	5	93-111	103	6.4	6.2
	5	5	103-111	108	2.8	2.6
M1	0.5 (LOQ)	5	89-110	101	9.4	9.3
	5	5	107-114	111	3.0	2.7
M2	0.5 (LOQ)	5	86-112	101	9.9	9.8
	5	5	96-112	106	6.5	6.2
M6	0.5 (LOQ)	5	101-117	105	6.8	6.4
	5	5	106-114	110	3.2	2.9
M9	0.5 (LOQ)	5	85-101	91	6.1	6.7
	5	5	87-101	93	5.7	6.2

Data (uncorrected recovery results) were obtained from p. 19; Tables 1-10, pp. 23-32 of MRID 49369502.

1 The surface water source was BD Well, the same as that of the ECM (source location was not further specified; p. 12; Appendix 2, p. 110). The water was fully characterized by Agvise Laboratories. Based on the water characterization report, the reviewer determined that the Batch/Lot of BD Well water which was used by the ILV was identical to that used by the ECM.

2 Ion transitions monitored were as follows (quantitative ion and confirmatory ion, respectively): *m/z* 335.0 → 206.1 (Q) and *m/z* 335.0 → 162.2 (C) for diflufenzopyr; *m/z* 162.2 → 102.9 (Q) and *m/z* 162.2 → 93.1 (C) for M1; *m/z* 130.1 → 83.0 (Q) and *m/z* 130.1 → 110.1 (C) for M2; *m/z* 165.9 → 148.1 (Q) and *m/z* 165.9 → 91.9 (C) for M6; and *m/z* 178.1 → 150.1 (Q) and *m/z* 178.1 → 119.1 (C) for M9 (pp. 14-15; Table 12, pp. 35-36).

III. Method Characteristics

In both the ECM and ILV, the LOQ and LOD were reported as 0.5 µg/kg (0.5 µg/L) and 0.1 µg/kg (0.1 µg/L), respectively (pp. 7, 25-26 of MRID 49369501; p. 15 of MRID 49369502). In the ECM, the LOQ was defined as the lowest fortification level which obtained acceptable recoveries. No other justification was provided. No calculations or comparison to background levels was provided for the LOQ or LOD. In the ILV, the LOQ and LOD were reported from the ECM, and no justification was provided.

Table 4. Method Characteristics

		Diflufenzopyr	M1	M6	M9	M2
Limit of Quantitation (LOQ)		0.5 µg/kg (0.5 µg/L)				
Limit of Detection (LOD)		0.1 µg/kg (0.1 µg/L)				
Linearity (calibration curve r^2 and concentration range)	ECM[1]	Quantification ion				
		r^2 = 0.9980	r^2 = 0.9988	r^2 = 0.9974	r^2 = 0.9952	r^2 = **0.9948**
		Confirmation ion				
		r^2 = 0.9986	r^2 = **0.9926**	r^2 = 0.9970	r^2 = 0.9958	r^2 = **0.9870**
		(0.50-10.0 ng/mL)				
	ILV[2]	Quantification ion				
		r^2 = **0.9928**	r^2 = **0.9936**	r^2 = 0.9954	r^2 = **0.9841**	r^2 = 0.9952
		r^2 = **0.9930**	r^2 = 0.9960	r^2 = **0.9928**	r^2 = **0.9888**	r^2 = **0.9948**
		(0.25-10.0 ng/mL)				
Repeatable		Yes for surface and ground water.				Yes for ground water. No[3] for surface water.
Reproducible		Yes for ground water only.[4,5]				
Specific		Yes				

Data were obtained from Tables 8-9, pp. 23-24; Figures 9.1.1-9.1.10, pp. 29-38 of MRID 49369501; and p. 19; Figures 1-5, pp. 38-42 of MRID 49369502.

[1] ECM r^2 values are reviewer-generated from reported r values of 0.9974-0.9994 (Q) and 0.9935-0.9993 (C; Figures 9.1.1-9.1.10, pp. 29-38 of MRID 49369501; DER Attachment 2). Linearity of the ECM calibration curves was verified by the reviewer (r^2 values of 0.9933-0.9992 (Q) and 0.9876-0.9985 (C); DER Attachment 2). ECM linearity data was identified as that of surface water in the ECM. No data for ground water was provided.

[2] ILV r^2 values are reviewer-generated from reported r values of 0.9920-0.9977 (Q) and 0.9944-0.9980 (C; Figures 1-5, pp. 38-42 of MRID 49369502; DER Attachment 2). Linearity of the ECM calibration curves was verified by the reviewer (r^2 values of 0.9774-0.9997 (Q) and 0.9812-0.9996 (C); DER Attachment 2).

[3] Mean recoveries of M2 were unsatisfactory with 65% (Q and C) at the LOQ and 67% (Q) and 68% (C) at 10×LOQ.

[4] Only ground water was tested in the ILV; surface water was not tested at the sponsor's request (p. 20 of MRID 49369502).

[5] Based on the water characterization reports, the reviewer determined that the Batch/Lot of BD Well water which was used by the ILV was identical to that used by the ECM (Appendix 9.5, pp. 88-89 of MRID 49369501; Appendix 2, p. 110 of MRID 49369502).

IV. Method Deficiencies and Reviewer's Comments

1. In the ECM, mean recoveries of M2 in surface water 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) ≤20%) at the stated LOQ and at higher concentrations. Reported mean recoveries were unsatisfactory at the LOQ (means, 65%, quantitative and confirmatory) and at 10×LOQ (means, 67%, quantitative, and 68%, confirmatory; Table 9, p. 24 of MRID 49369501).

2. Surface water was not included in ILV, only ground water (p. 20 of MRID 49369502). According to the ILV study authors, this change was "made at the sponsor's request" and was "documented in Protocol Amendment 1" (p. 20). Additionally, the ILV study authors mentioned an updated version of BASF Analytical Method R0039 (Draft, March 25, 2014). Neither the updated version of the ECM or Protocol Amendment 1 were submitted for review. The sponsor did not specify that the ground water used in the ILV was either an equivalent, or more difficult, analytical sample condition as the surface water used in the ECM.

3. The estimations of the LOQ and LOD in the ECM were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. In the ECM, the LOQ was defined as the lowest fortification level which obtained average recoveries of 70-110% and a RSD <20% (pp. 7, 25 of MRID 49369501; p. 15 of MRID 49369502). No other justification was provided. No calculations or comparison to background levels was provided for the LOQ or LOD. In the ILV, the LOQ and LOD were reported from the ECM, and no justification was provided.

   Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in water were not reported. An LOQ above toxicological level of concern results in an unacceptable method classification.

4. The linearity was not always ≥0.995 in the ECM and was usually <0.995 in the ILV (see above).

5. In the ECM, a full set of chromatograms was provided (Figures 9.3.2-9.3.11, pp. 45-74 of MRID 49369501). Some minor residues were noted in several of the chromatograms. Procedural recoveries were corrected for residues in the controls for M9 in surface (quantitative) and ground water (confirmatory); and M1 in surface water (confirmatory; pp. 21-22; Appendix 9.6, pp. 102, 106, 109). Product ion spectra were also included (Figures 9.4.1-9.4.5, pp. 76-80).

   In the ILV, a full set of chromatograms was provided (Figures 11-30, pp. 82-101 of MRID 49369502). An insignificant amount of baseline interference was noted in the LOQ chromatogram of M6 in ground water (Figure 25, p. 96). Baseline noise was significant (>LOQ peak) in the LOQ chromatogram of M9 in ground water, but this noise did not interfere with the analyte signal (Figure 29, p. 100). No correction for residues in the controls was employed (pp. 15-18; Tables 1-10, pp. 23-32).

6. The minor modifications and differences in the ILV method, which were detailed above, were not considered substantial changes to the ECM method (p. 7; pp. 14-15 of MRID 49369502). There were a few additional specific modifications of the analytical method, including the extending run time, changing calibration standard solvent and different confirmatory transition of M1 (p. 20). All of these changes were minor adjustments for optimization based on the analytical instrument or matrix effects.

7. The ECM study authors reported that matrix effects were observed for all analytes, except M1 and M9, and the recoveries of M2 were the most effected (p. 18 of MRID 49369501). The ECM study authors noted that interferences “can be alleviated by modifying the LC-MS/MS gradient before GLP analysis” (p. 26). Matrix matched standards were not required.

8. Based on the water characterization reports, the reviewer determined that the Batch/Lot of BD Well water which was used by the ILV was identical to that used by the ECM (Appendix 9.5, pp. 88-89 of MRID 49369501; Appendix 2, p. 110 of MRID 49369502).

9. It was reported for the ILV that a set consisting of 13 samples required approximately 8-person hours, not including LC/MS/MS analysis (p. 20 of MRID 49369502).

10. Communications between the ILV study authors and study sponsor were only briefly described in the ILV (p. 20 of MRID 49369502).

V. References

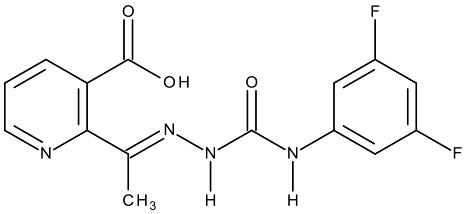
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

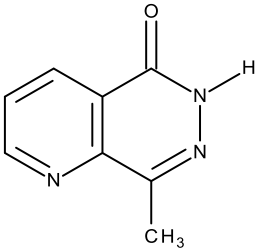
Diflufenzopyr; BAS 654 H

IUPAC Name:	2-{(E)-1-[4-(3,5-difluorophenyl)semicarbazono]ethyl}nicotinic acid
CAS Name:	2-[(1E)-1-[2-[[(3,5-difluorophenyl)amino]carbonyl]hydrazinylidene]ethyl]-3-pyridinecarboxylic acid
CAS Number:	109293-97-2
SMILES String:	OC(=O)c1cccnc1C(C)=NNC(=O)Nc2cc(F)cc(F)c2



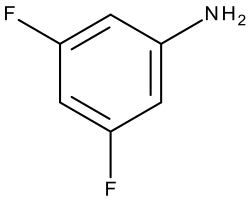
M1

IUPAC Name:	8- Methyl-pyrido[2,3-d]pyridazin-5(6H)-one
CAS Name:	Not reported
CAS Number:	90004-07-2
SMILES String:	[H]n1c(=O)c2cccnc2c(n1)C

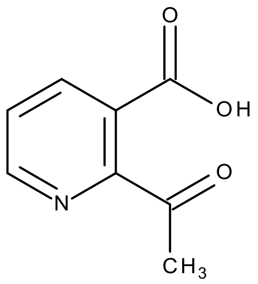


M2

IUPAC Name:	3,5-Difluoroaniline
CAS Name:	Not reported
CAS Number:	372-39-4
SMILES String:	c1c(cc(cc1F)F)N



M6
IUPAC Name: 2-Acetylnicotinic acid
CAS Name: Not reported
CAS Number: 89942-59-6
SMILES String: CC(=O)c1c(cccn1)C(=O)O



M9 (2-keto M1); Phthalazindione
IUPAC Name: 8- Methylpyrido[2,3-d]pyridazine-2,5(1H,6H)-dione
CAS Name: Not reported
CAS Number: None
SMILES String: Not found