

Analytical method for florasulam and its metabolite, 5-OH florasulam, in water

Reports: ECM: EPA MRID No. 50908704. Class, T. 2011. Method Validation Study for the Determination of Residues of Florasulam and its 5-OH Metabolite in Surface Water, Ground Water and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry. Dow AgroSciences Study No.: 110538. PTRL Europe ID: P 2292 G. Report prepared by PTRL Europe GmbH, Ulm, Germany, and sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 42 pages. Final report issued September 14, 2011.

ILV: EPA MRID No. 50908705. Souza, N. 2011. Independent Laboratory Validation of Dow AgroSciences LLC Method - Determination of Residues of Florasulam and its 5-OH Metabolite in Drinking Water, Ground Water and Surface Water by Liquid Chromatography with Tandem Mass Spectrometric Detection. Dow AgroSciences Study Reference No.: 110539. Report prepared by Dow AgroSciences Ind. Ltda., Mogi Mirim, SP, Brazil, and sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 79 pages. Final report issued November 3, 2011.

Document No.: MRIDs 50908704 & 50908705

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA (40 CFR Part 160), OECD, and German Good Laboratory Practice (GLP) standards (p. 3; Appendix A, p. 42 of MRID 50908704). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4; Appendix A, p. 42). A statement of the authenticity of the study report was included with the Quality Assurance statement (p. 4).

ILV: The study was conducted in accordance with USEPA and NIT-DICLA-035 (INMETRO) GLP standards (p. 3; Appendix C, p. 79 of MRID 50908705). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4; Appendix C, p. 79). A statement of the authenticity of the study report was included with the Quality Assurance statement (p. 4).

Classification: This analytical method is classified as supplemental. No ECM 10×LOQ chromatograms were provided.

PC Code: 129108

EFED Final Reviewer: Taimei Harris, Ph.D.,
Chemist

Signature:

**Harris,
Taimei** Digitally signed
by Harris, Taimei
Date: 2023.05.10
16:17:59 -04'00'

EPA Peer Reviewer: Joshua Antoline, Ph.D.,
Chemist

Signature:

**JOSHUA
ANTOLINE** Digitally signed by
JOSHUA ANTOLINE
Date: 2023.05.31
10:33:12 -04'00'

CDM/CSS-Dynamac JV Reviewers: Lisa Muto, M.S.
Environmental Scientist

Signature:

Lisa Muto

Date:

10/30/2019

Mary Samuel, M.S.,
Environmental Scientist

Signature: 

Date: 10/30/2019

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, Dow AgroSciences Study No. 110538, is designed for the quantitative determination of florasulam and its metabolite, 5-OH florasulam, in water at the Lower Limit of Method Validation (LLMV) of 0.05 µg/L using LC/MS/MS and is less than the lowest toxicological Level of Concern for aquatic plants with a NOAEC of 0.0006 mg a.i./l (0.6 µg/L) in water for florasulam and its 5-OH metabolite (USEPA, 2009; DP 356625). The reported LOQ was not based on any scientifically acceptable procedure and is instead a LLMV. The ECM and ILV were performed using characterized drinking, ground, and surface water matrices; the matrices differed between the ECM and ILV. Analytes were identified using two ion transitions. The ILV validated the method for florasulam and 5-OH florasulam in the second trial with modifications of the analytical parameters and equipment. No details on or reason for the failure of the first trial were reported. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for both analytes.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Lower Limit of Method Validation (LLMV)
	Environmental Chemistry Method	Independent Laboratory Validation					
Florasulam	50908704	50908705	Water ^{1,2}	14/09/2011	Dow AgroSciences LLC	LC/MS/MS	0.05 µg/L
5-OH Florasulam							

1 In the ECM, drinking (tap) water (pH 8.36, total hardness 2.40 mmol/L (Deutsche Härtegrade, 13.5°d), dissolved organic carbon not reported) obtained from a PTRL Europe laboratory tap, surface (river) water (pH 8.43, total hardness 2.98 mmol/L (Deutsche Härtegrade, 16.7°d), total organic carbon 0.84 mg/L, diluted organic carbon 0.64 mg/L) obtained from Brenz River in Herbrechtingen, Germany, and ground (well) water (pH 8.28, total hardness 3.37 mmol/L (Deutsche Härtegrade, 18.9°d), total organic carbon 1.1 mg/L, diluted organic carbon 1.0 mg/L) obtained from a well in Herbrechtingen, Germany, were used in the study (pp. 10-11 of MRID 50908704). The water matrices were characterized by Institute Alpha in Ulm, Germany (non-GLP).

2 In the ILV, ground (well) water (Sample No. 110539-001; pH 7.22, total hardness as CaCO₃ 1.48 mmol/L, total organic carbon <2.0 mg/L, chemical oxygen demand <2.0 mg/L) obtained from a well in the Dow AgroSciences Field Station in Mogi-Mirim City, São Paulo State, Brazil, surface (river) water (Sample No. 110539-002; pH 6.46, total hardness as CaCO₃ 0.09 mmol/L, total organic carbon <1.0 mg/L, chemical oxygen demand 13.1 mg/L) obtained from River Sapezal in Mogi-Mirim City, São Paulo State, Brazil, and drinking (tap) water (Sample No. 110539-003; pH 7.29, total hardness as CaCO₃ 1.48 mmol/L, total organic carbon <2.0 mg/L, chemical oxygen demand <2.0 mg/L) obtained from a Mogi Mirim Regulatory Test Facility tap were used in the study (p. 11 of MRID 50908705). The water matrices were characterized by NL Laboratórios, Mogi-Mirim City, São Paulo State, Brazil (non-GLP facility).

I. Principle of the Method

Water samples (1.0 mL) were fortified with 100 μ L of fortification solution (florasulam in acetone or 5-OH florasulam in acetonitrile) or 100 μ L of acetonitrile in autosampler vials (pp. 12-13 of MRID 50908704). Samples were mixed via vortex prior to analysis by LC/MS/MS.

Water samples were analyzed for florasulam and its 5-OH metabolite using an Agilent Model 1200 LC system coupled to an Applied Biosystems API 5500 mass spectrometer with TurboIonSpray ionization (p. 14 of MRID 50908704). The LC/MS conditions consisted of a Supelco Ascentis Express C18-type stationary phase column (100 x 3 mm, 2.7 μ m; column temperature 40°C), Securityguard Phenomenex guard column (4 x 3 mm), a gradient mobile phase of (A) 0.1% acetic acid in water and (B) 0.1% acetic acid in acetonitrile:methanol (1:1, v:v) [percent A:B (v:v) at 0.00-1.00 min. 90:10, 5.00-9.00 min. 0:100, 9.10-13.00 min. 90:10] and MS/MS detection in negative ion mode (ionization temperature 450°C). Injection volume was 60 μ L. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 358.2 \rightarrow 167.0 and m/z 358.2 \rightarrow 152.1 for florasulam and m/z 344.1 \rightarrow 324.1 and m/z 344.1 \rightarrow 104.0 for 5-OH florasulam. Retention time was *ca.* 5.85 and 5.30 minutes for florasulam and its 5-OH florasulam, respectively (Figures 4-5, pp. 26-27).

In the ILV, the ECM was performed as written, except for modifications of the analytical parameters and equipment (pp. 14-15 of MRID 50908705). The LC/MS/MS system was an Agilent 1100 LC coupled to an MDS/Sciex API 4000 mass spectrometer. The LC/MS conditions consisted of a Phenomenex Onyx Monolithic C18 column (100 x 4.6 mm, 3.0 μ m; column temperature 40°C), a gradient mobile phase of (A) 0.1% acetic acid in water and (B) 0.1% acetic acid in acetonitrile:methanol (1:1, v:v) [percent A:B (v:v) at 0.01-1.00 min. 10:90, 5.00-8.00 min. 100:0, 8.10-10.00 min. 10:90] and MS/MS detection in ESI negative ion mode (ionization temperature 450°C). Injection volume was 100 μ L. Two ion transitions were monitored for each analyte; these were the same as those of the ECM. Retention time was *ca.* 5.7 and 5.15 minutes for florasulam and its 5-OH florasulam, respectively (Figure 3, p. 33). No other modifications to the ECM were reported.

The LLMV for florasulam and its 5-OH metabolite in water was 0.05 μ g/L in the ECM and ILV (p. 18 of MRID 50908704; pp. 9, 14 of MRID 50908705). The reported Limit of Detection (LOD) for florasulam and its 5-OH metabolite in water was 0.015 μ g/L in the ECM and ILV, calculated as 30% of the LLMV.

II. Recovery Findings

ECM (MRID 50908704): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of florasulam and its 5-OH metabolite in three water matrices at the fortification level of 0.05 $\mu\text{g/L}$ (LOQ) and 0.5 $\mu\text{g/L}$ (10 \times LOQ; Tables 1-3, pp. 20-22; DER Attachment 2). Two ion transitions were quantified; performance data (recovery results) from primary and confirmatory analyses were comparable. Drinking (tap) water (pH 8.36, total hardness 2.40 mmol/L (Deutsche Härtegrade, 13.5 $^{\circ}$ d), dissolved organic carbon not reported) obtained from a PTRL Europe laboratory tap, surface (river) water (pH 8.43, total hardness 2.98 mmol/L (Deutsche Härtegrade, 16.7 $^{\circ}$ d), total organic carbon 0.84 mg/L, diluted organic carbon 0.64 mg/L) obtained from Brenz River in Herbrechtingen, Germany, and ground (well) water (pH 8.28, total hardness 3.37 mmol/L (Deutsche Härtegrade, 18.9 $^{\circ}$ d), total organic carbon 1.1 mg/L, diluted organic carbon 1.0 mg/L) obtained from a well in Herbrechtingen, Germany, were used in the study (pp. 10-11). The water matrices were characterized by Institute Alpha in Ulm, Germany (non-GLP).

ILV (MRID 50908705): Mean recoveries and RSDs were within guideline requirements for analysis of florasulam and its 5-OH metabolite in three water matrices at the fortification level of 0.05 $\mu\text{g/L}$ (LOQ) and 0.5 $\mu\text{g/L}$ (10 \times LOQ; Tables 6-9, pp. 21-24 and Tables 10-11, p. 25; DER Attachment 2). Two ion transitions were quantified; performance data (recovery results) from primary and confirmatory analyses were comparable. Ground (well) water (Sample No. 110539-001; pH 7.22, total hardness as CaCO_3 68.1 mg/L, total organic carbon <2.0 mg/L, chemical oxygen demand <2.0 mg/L) obtained from a well in the Dow AgroSciences Field Station in Mogi-Mirim City, São Paulo State, Brazil, surface (river) water (Sample No. 110539-002; pH 6.46, total hardness as CaCO_3 4.0 mg/L, total organic carbon <1.0 mg/L, chemical oxygen demand 13.1 mg/L) obtained from River Sapezal in Mogi-Mirim City, São Paulo State, Brazil, and drinking (tap) water (Sample No. 110539-003; pH 7.29, total hardness as CaCO_3 68.1 mg/L, total organic carbon <2.0 mg/L, chemical oxygen demand <2.0 mg/L) obtained from a Mogi Mirim Regulatory Test Facility tap were used in the study (p. 11). The water matrices were characterized by NL Laboratórios, Mogi-Mirim City, São Paulo State, Brazil (non-GLP facility). The method for florasulam and its 5-OH florasulam was validated in the second trial with modifications of the analytical parameters and equipment (pp. 14-15, 18). No reason for the failure of the first trial was reported.

Table 2. Initial Validation Method Recoveries for Florasulam and its 5-OH metabolite in Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Drinking (Tap) Water						
Quantitation Ion Transition						
Florasulam	0.05 (LOQ)	5	100-106	104	3	3
	0.5	5 ³	93-111	102	7	7
5-OH Florasulam	0.05 (LOQ)	5	101-105	103	2	1
	0.5	5 ³	81-103	97	9	9
Confirmation Ion Transition						
Florasulam	0.05 (LOQ)	5	101-106	104	3	2
	0.5	5 ³	91-110	103	7	7
5-OH Florasulam	0.05 (LOQ)	5	99-105	101	2	2
	0.5	5 ³	78-103	96	10	11
Surface (River) Water						
Quantitation Ion Transition						
Florasulam	0.05 (LOQ)	5	88-105	97	6	7
	0.5	5 ³	97-106	102	4	4
5-OH Florasulam	0.05 (LOQ)	5	93-106	98	5	6
	0.5	5 ³	99-109	104	4	4
Confirmation Ion Transition						
Florasulam	0.05 (LOQ)	5	87-104	95	6	6
	0.5	5 ³	95-105	100	4	4
5-OH Florasulam	0.05 (LOQ)	5	91-107	97	7	7
	0.5	5 ³	103-109	106	3	3
Ground (Well) Water						
Quantitation Ion Transition						
Florasulam	0.05 (LOQ)	5	90-95	92	3	3
	0.5	5 ³	94-97	95	1	1
5-OH Florasulam	0.05 (LOQ)	5	98-109	103	4	4
	0.5	5 ³	100-107	103	3	3
Confirmation Ion Transition						
Florasulam	0.05 (LOQ)	5	90-96	94	2	2
	0.5	5 ³	90-94	92	2	2
5-OH Florasulam	0.05 (LOQ)	5	99-107	103	4	3
	0.5	5 ³	103-108	105	2	2

Data (uncorrected recovery results, pp. 15-16) were obtained from Tables 1-3, pp. 20-22 of MRID 50908704 and DER Attachment 2.

1 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 358.2→167.0 and m/z 358.2→152.1 for florasulam and m/z 344.1→324.1 and m/z 344.1→104.0 for 5-OH florasulam.

2 The drinking (tap) water (pH 8.36, total hardness 2.40 mmol/L (Deutsche Härtegrade, 13.5°d), dissolved organic carbon not reported) obtained from a PTRL Europe laboratory tap, surface (river) water (pH 8.43, total hardness 2.98 mmol/L (Deutsche Härtegrade, 16.7°d), total organic carbon 0.84 mg/L, diluted organic carbon 0.64 mg/L) obtained from Brenz River in Herbrechtingen, Germany, and ground (well) water (pH 8.28, total hardness 3.37 mmol/L (Deutsche Härtegrade, 18.9°d), total organic carbon 1.1 mg/L, diluted organic carbon 1.0 mg/L) obtained from a well in Herbrechtingen, Germany, were used in the study (pp. 10-11). The water matrices were characterized by Institute Alpha in Ulm, Germany (non-GLP).

3 The recovery result for the fifth sample was an average of two injections.

Table 3. Independent Validation Method Recoveries for Florasulam and its 5-OH metabolite in Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground (Well) Water						
Quantitation Ion Transition						
Florasulam	0.05 (LOQ)	6	93-109	101	6	6
	0.5	6	101-104	103	1	1
5-OH Florasulam	0.05 (LOQ)	6	98-114	109	8	7
	0.5	6	96-105	102	3	3
Confirmation Ion Transition						
Florasulam	0.05 (LOQ)	6	84-116	99	14	15
	0.5	6	96-111	104	5	5
5-OH Florasulam	0.05 (LOQ)	6	83-115	102	13	12
	0.5	6	98-110	104	4	4
Surface (River) Water						
Quantitation Ion Transition						
Florasulam	0.05 (LOQ)	6	96-116	104	7	7
	0.5	6	99-103	101	2	2
5-OH Florasulam	0.05 (LOQ)	6	96-109	102	6	5
	0.5	6	97-104	100	3	3
Confirmation Ion Transition						
Florasulam	0.05 (LOQ)	6	83-113	102	12	13
	0.5	6	91-103	99	4	4
5-OH Florasulam	0.05 (LOQ)	6	76-114	100	13	13
	0.5	6	98-105	103	3	3
Drinking (Tap) Water						
Quantitation Ion Transition						
Florasulam	0.05 (LOQ)	6	86-114	101	9	9
	0.5	6	96-102	100	2	2
5-OH Florasulam	0.05 (LOQ)	6	96-114	103	6	6
	0.5	6	95-101	99	2	2
Confirmation Ion Transition						
Florasulam	0.05 (LOQ)	6	87-118	100	11	11
	0.5	6	96-104	101	3	3
5-OH Florasulam	0.05 (LOQ)	6	75-94	88	7	8
	0.5	6	96-105	101	3	3

Data (uncorrected recovery results, pp. 16-17) were obtained from Tables 1-3, pp. 23-25 of MRID 50908705.

1 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 358.2→167.0 and m/z 358.2→152.1 for florasulam and m/z 344.1→324.1 and m/z 344.1→104.0 for 5-OH florasulam. These were the same as those of the ECM.

2 The ground (well) water (Sample No. 110539-001; pH 7.22, total hardness as CaCO₃ 68.1 mg/L, total organic carbon <2.0 mg/L, chemical oxygen demand <2.0 mg/L) obtained from a well in the Dow AgroSciences Field Station in Mogi-Mirim City, São Paulo State, Brazil, surface (river) water (Sample No. 110539-002; pH 6.46, total hardness as CaCO₃ 4.0 mg/L, total organic carbon <1.0 mg/L, chemical oxygen demand 13.1 mg/L) obtained from River Sapezal in Mogi-Mirim City, São Paulo State, Brazil, and drinking (tap) water (Sample No. 110539-003; pH 7.29, total hardness as CaCO₃ 68.1 mg/L, total organic carbon <2.0 mg/L, chemical oxygen demand <2.0 mg/L) obtained from a Mogi Mirim Regulatory Test Facility tap were used in the study (p. 11). The water matrices were characterized by NL Laboratórios, Mogi-Mirim City, São Paulo State, Brazil (non-GLP facility).

III. Method Characteristics

The LOQ for florasulam and its 5-OH metabolite in water was 0.05 µg/L in the ECM and ILV (p. 18 of MRID 50908704; pp. 9, 14 of MRID 50908705). No justification of the LOQ was reported in the ECM. The LOD for florasulam and its 5-OH metabolite in water was 0.015 µg/L in the ECM and ILV. In the ECM, the LOD was estimated based on the lowest calibration concentration, as well as 30% of the LOQ. No justifications or calculations were provided to support the LOD and LOQ in the ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136, therefore, the reported LOD is actually the LLMV of the method.

Table 4. Method Characteristics

Analyte		Florasulam	5-OH Florasulam
Lower Limit of Method Validation (LLMV)	ECM	0.05 µg/L	
	ILV		
Limit of Detection (LOD)	ECM	0.015 µg/L	
	ILV		
Linearity (calibration curve r^2 and concentration range) ¹	ECM	$r^2 = 0.9996$ (Q) $r^2 = 0.9998$ (C)	$r^2 = 0.9998$ (Q) $r^2 = 0.9994$ (C)
	ILV	$r^2 = 0.9997$ (Q) $r^2 = 0.9992$ (C)	$r^2 = 0.9997$ (Q) $r^2 = 0.9988$ (C)
	Concentration Range	0.01-1 ng/mL	
Repeatable	ECM ²	Yes at LOQ and 10×LOQ (drinking, surface, and ground water matrices)	
	ILV ^{3,4}		
Reproducible	Yes at LOQ and 10×LOQ.		
Specific	ECM	Yes, no matrix interferences were observed. Some minor baseline noise was observed at LOQ in surface and ground water matrices. No 10×LOQ representative chromatograms provided.	
	ILV	Yes, no matrix interferences were observed. Some minor baseline noise was observed at LOQ in all water matrices.	Yes, no matrix interferences were observed.

Data were obtained from p. 18 (LOQ/LOD); Tables 1-3, pp. 20-22 (recovery data); p. 12; Figures 1-2, pp. 23-24 (calibration curves); Figures 4-17, pp. 26-39 (chromatograms) of MRID 50908704; pp. 9, 14 (LOQ/LOD); Tables 1-3, pp. 23-25 (recovery data); Figures 1-2, pp. 29-32 (calibration curves); Figures 3-13, pp. 33-43 (chromatograms) of MRID 50908705. Q = Quantitation ion transition; C = Confirmatory ion transition.

¹ ECM reported correlation coefficients (r^2) were reviewer-calculated based on the reported r values in the study report (Figures 1-2, pp. 23-24 of MRID 50908704). Solvent calibration standards were used in the ECM and ILV (p. 12 of MRID 50908704; p. 13 of MRID 50908705).

² In the ECM, drinking (tap) water (pH 8.36, total hardness 2.40 mmol/L (Deutsche Härtegrade, 13.5°d), dissolved organic carbon not reported) obtained from a PTRL Europe laboratory tap, surface (river) water (pH 8.43, total hardness 2.98 mmol/L (Deutsche Härtegrade, 16.7°d), total organic carbon 0.84 mg/L, diluted organic carbon 0.64 mg/L) obtained from Brenz River in Herbrechtingen, Germany, and ground (well) water (pH 8.28, total hardness 3.37 mmol/L (Deutsche Härtegrade, 18.9°d), total organic carbon 1.1 mg/L, diluted organic carbon 1.0 mg/L) obtained from a well in Herbrechtingen, Germany, were used in the study (pp. 10-11 of MRID 50908704). The water matrices were characterized by Institute Alpha in Ulm, Germany (non-GLP).

³ In the ILV, ground (well) water (Sample No. 110539-001; pH 7.22, total hardness as CaCO₃ 68.1 mg/L, total organic carbon <2.0 mg/L, chemical oxygen demand <2.0 mg/L) obtained from a well in the Dow AgroSciences Field Station in Mogi-Mirim City, São Paulo State, Brazil, surface (river) water (Sample No. 110539-002; pH

6.46, total hardness as CaCO₃ 4.0 mg/L, total organic carbon <1.0 mg/L, chemical oxygen demand 13.1 mg/L) obtained from River Sapezal in Mogi-Mirim City, São Paulo State, Brazil, and drinking (tap) water (Sample No. 110539-003; pH 7.29, total hardness as CaCO₃ 68.1 mg/L, total organic carbon <2.0 mg/L, chemical oxygen demand <2.0 mg/L) obtained from a Mogi Mirim Regulatory Test Facility tap were used in the study (p. 11 of MRID 50908705). The water matrices were characterized by NL Laboratórios, Mogi-Mirim City, São Paulo State, Brazil (non-GLP facility).

⁴ In the ILV, the method for florasulam and its 5-OH florasulam was validated in the second trial with modifications of the analytical parameters and equipment (pp. 14-15, 18 of MRID 50908705). No reason for the failure of the first trial was reported.

IV. Method Deficiencies and Reviewer's Comments

1. In the ECM, no 10×LOQ chromatograms were provided. Representative chromatograms from all matrices/ions/fortifications should be provided for review in order to fully assess the specificity of the method.
2. The ILV validated the method for florasulam and its 5-OH florasulam was validated in the second trial with modifications of the analytical parameters and equipment (pp. 14-15, 18 of MRID 50908705). No reason for the failure of the first trial was reported.
3. The matrix effects were found to be insignificant (<20%) for florasulam and its 5-OH metabolite in the ECM and ILV (p. 17 of MRID 50908704; p. 19 of MRID 50908705). Solvent calibration standards were used in the ECM and ILV.

In the ECM and ILV, the final sample extracts were found to be stable after up to 5 or 9 days of refrigeration (p. 18; Tables 1-3, pp. 20-22 of MRID 50908704; p. 20; Table 5, p. 28 of MRID 50908705).

4. The ILV study author reported that communications between the ILV and ECM did not occur (p. 20 of MRID 50908705).
5. The determinations of LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 18 of MRID 50908704; pp. 9, 14 of MRID 50908705). No justification of the LOQ was reported in the ECM. In the ECM, the LOD was estimated based on the lowest calibration concentration, as well as 30% of the LOQ. No justifications or calculations were provided to support the LOD and LOQ in the ILV. Therefore, the reported LOD is actually the LLMV.
6. It was reported for the ILV that one sample set of 15 specimens required *ca.* 1 hour for preparation, *ca.* 6 hours for LC/MS/MS analysis, and *ca.* 2 for LC/MS/MS evaluation (p. 20 of MRID 50908705). Therefore, performing the method for one sample set of 15 specimens required *ca.* 8 person-hours or one calendar day.

V. References

USEPA. 2009. *Environmental Fate and Ecological Risk Assessment for Proposed Section 3 New Use Reregistration for Florasulam Use on Turfgrass*. DP Barcode 356625. Washington,

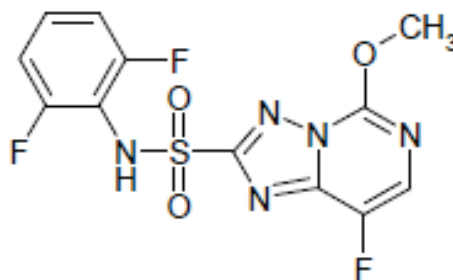
DC: US EPA Office of Pesticide Programs.

USEPA. 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**Florasulam**

IUPAC Name: Not reported
CAS Name: *N*-(2,6-difluorophenyl)-8-fluoro-5-methoxy[1,2,4]triazolo[1,5-*c*]pyrimidine-2-sulfonamide
CAS Number: 145701-23-1
SMILES String: Not found

**5-OH Florasulam**

IUPAC Name: Not reported
CAS Name: *N*-(2,6-difluorophenyl)-8-fluoro-5-hydroxy[1,2,4]triazolo[1,5-*c*]pyrimidine-2-sulfonamide
CAS Number: Not reported
SMILES String: Not found

