

**Analytical method for fluazaindolizine (DPX-Q8U80) and its metabolites IN-A5760, IN-F4106, IN-REG72, IN-RYC33, IN-QEK31, and IN-VM862 in soil**

**Reports:** ECM 1: EPA MRID No. 50932066. Swaim, L. 2015. Method Validation of DPX-Q8U80 and Its Metabolites in Soil. Report prepared by ABC Laboratories, Inc., Columbia, Missouri; and sponsored and submitted by E. I. du Pont de Nemours and Company, Newark, Delaware; 137 pages. Sponsor Study No.: DuPont-37404. ABC Laboratories, Inc.: ABC 69683. Final report issued October 28, 2015.

ECM 2: EPA MRID No. 50932065. Swaim, L., W. Fain, J.P. McClory, and E.A. Morgan. 2015. Analytical Method for the Determination of DPX-Q8U80 and Its Metabolites in Soil Using LC/ESI-MS/MS. Report prepared by ABC Laboratories, Inc., Columbia, Missouri; and sponsored and submitted by E. I. du Pont de Nemours and Company, Newark, Delaware; 116 pages. Sponsor Study No.: DuPont-34652. ABC Laboratories, Inc.: ABC 69683-M. Final report issued October 28, 2015.

ILV: EPA MRID No. 50932112. Shen, Y. 2017. Independent Laboratory Validation of DuPont-37404, "Method Validation of DPX-Q8U80 and Its Metabolites in Soil". Report prepared by Alliance Pharma, Malvern, Pennsylvania; and sponsored and submitted by E. I. du Pont de Nemours and Company, Wilmington, Delaware; 250 pages. DuPont Project ID: DuPont-43155. Alliance Pharma Project No.: 160512. Final report issued September 13, 2017.

**Document No.:** MRIDs 50932065 & 50932066 & 50932112

**Guideline:** 850.6100

**Statements:** ECM 1: The study was conducted in compliance with USEPA FIFRA (40 CFR Part 160) Good Laboratory Practices (GLP), which are compatible with OECD GLP, except that characterization of the IN-VM862 standard was not performed according to GLP standards at Sigma-Aldrich and the preparation documentation for one of the solvents was lost (p. 3 of MRID 50932066). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).

ECM 2: The study was not conducted in compliance with USEPA FIFRA (40 CFR Part 160) Good Laboratory Practices (GLP), which are compatible with OECD GLP, but it was conducted in a GLP compliant facility (p. 3 of MRID 50932065). Signed and dated Data Confidentiality, GLP, and Authenticity statements were provided (pp. 2-4). The Quality Assurance statement was not provided.

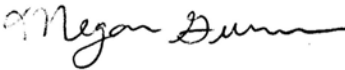
ILV: The study was conducted in compliance with USEPA FIFRA (40 CFR Part 160) GLP standards, which are compatible with OECD GLP (p. 3 of MRID 50932112). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).

**Classification:** **This analytical method is classified as supplemental.** An updated ECM should be submitted incorporating the use of lower temperature for evaporation and solvent change to methanol since these modifications were necessary for the ILV to validate the method for the analytes, especially DPX-


Q8U80, IN-VM862, and IN-RYC33. The specificity of the method for IN-VM862 and IN-RYC33 was not supported by ECM and/or ILV representative chromatograms. It could not be determined if the ILV was provided with the most difficult soil matrix with which to validate the method and if the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. The LOD was not reported in the ILV.

**PC Code:** 129777


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Signature:   
Date: 05/12/21

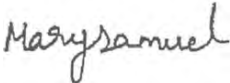
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*This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel. The CDM/CSS-Dynamac JV role does not include establishing Agency policies.*

## Executive Summary

The analytical methods, DuPont-37404 (ECM 1) and DuPont-34652 (ECM 2), are designed for the quantitative determination of fluazaindolizine and its metabolites IN-A5760, IN-F4106, IN-REG72, IN-RYC33, IN-QEK31, and IN-VM862 in soil at the stated LOQ of 1.0 µg/kg using HPLC/MS/MS. The LOQ is less the lowest toxicological level of concern in soil for all analytes. The ILV was reported performed to validate ECM 1; however, ECM 1 and ECM 2 essentially contained the same information/performance data. ECMs 1 and 2 used two characterized soil matrices obtained from fluazaindolizine terrestrial field dissipation studies; the ILV validated the method using three uncharacterized soil matrices. It could not be determined if the ILV was provided with the most difficult soil matrix with which to validate the method and if the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. ILV validated the method for all analytes in all three soils in the second trial as written, with minor modifications to the sample processing procedure and insignificant analytical instrument and equipment modifications. The submitted ECMs did not incorporate the lower temperature modification and a solvent change to methanol. An updated ECM should be submitted incorporating these modifications since they were necessary for the ILV to validate the method for the analytes, especially DPX-Q8U80, IN-VM862, and IN-RYC33. All ILV, ECM 1, and ECM 2 data regarding repeatability, accuracy, precision, and linearity were satisfactory for fluazaindolizine and its

metabolites. All ILV data regarding specificity was satisfactory for fluazaindolizine, IN-A5760, IN-F4106, IN-REG72, and IN-QEK31. The specificity of the method for IN-VM862 and IN-RYC33 was not supported by ILV representative chromatograms due to size of analyte peak and matrix interferences. All ECM 1 and ECM 2 data regarding specificity was satisfactory for all analytes, except for IN-VM862. IN-VM862 was considered the least sensitive analyte in ECM 2. The LOD was not reported in the ILV.

**Table 1. Analytical Method Summary**

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Fluazaindolizine (DPX-Q8U80)	50932066 <sup>1,2</sup> & 50932065 <sup>1,3</sup>	50932112 <sup>4</sup>		Soil	28/10/2015 <sup>5</sup>	E. I. du Pont de Nemours and Company	LC/MS/MS	1.0 µg/kg
IN-A5760								
IN-F4106								
IN-REG72								
IN-RYC33								
IN-QEK31								
IN-VM862								

1 In the ECM 1 and ECM 2, sand soil (Sample ID 109011; 97% sand, 3% silt, 0% clay; pH 6.4 in 1:1 soil:water ratio; 0.47% organic matter- Walkley Black) and loam soil (Sample ID 88NJ01NASCNA BA.A.001.00-02"; 28% sand, 47% silt, 25% clay; pH 6.9 in 1:1 soil:water ratio; 2.0% organic matter- Walkley Black) were obtained from fluazaindolizine terrestrial field dissipation studies from Florida (MRID 50932032; DuPont-36690) and New Jersey (MRID 50932033; DuPont-36688), characterized by Agvise Laboratories, Northwood, North Dakota, and used in the study (USDA Soil Texture Classification; pp. 21, 31; Appendix 3, pp. 94-95 of MRID 50932066; pp. 17, 27; Appendix 4, pp. 66-67 of MRID 50932065).

2 ECM 1. MRID 50932066 was considered to be the primary ECM since the ILV was reported performed to validate it (pp. 13, 40 of MRID 50932112). MRID 50932066 essentially contained the same information as ECM 2 MRID 50932065; however, both quantitation and confirmation ion transition chromatograms were included in ECM 1 MRID 50932066.

3 ECM 2. MRID 50932065 essentially contained the same information as ECM 1 MRID 50932066; however, only quantitation ion transition chromatograms were included in ECM 2 MRID 50932065.

4 In the ILV, Drummer, Tama, and Sassafras soils supplied by DuPont Crop Protection, E. I. du Pont de Nemours and Company (Newark, Delaware) were used in the study (pp. 13, 25 of MRID 50932112). The soils were not characterized or described.

5 Method date for ECMs 1 & 2, MRIDs 50932065 & 50932066.

## I. Principle of the Method

A 7.5-g ( $\pm 0.05$  g) soil sample in 50-mL centrifuge tube was fortified with 0.015 mL of 0.50 or 5.0 µg/mL fortification solutions, if necessary (pp. 16, 18, 21-23 of MRID 50932066; pp. 13-15, 18-19 of MRID 50932065). After the fortification solvent was evaporated in a fume hood for 15-20 minutes, the soil was mixed then extracted with 20 mL of acetonitrile:2% formic acid (50:50, v:v) by shaking on a Geno/grinder® (1100 shakes per minute for 3 minutes). After centrifugation (*ca.* 3000 rpm for 10 minutes), the soil pellet was extracted again in the same manner with 15 mL of acetonitrile:2% formic acid (50:50, v:v). After adjusting the volume of the combined extracts to 35 mL with HPLC water, a 7-mL aliquot of the extract is taken and diluted to *ca.* 22 mL with HPLC water. The diluted extract is then passed through an HLB SPE column [(pre-conditioned with 5 mL

of acetonitrile:water (20:80, v:v)]. The analytes are retained on the HLB SPE column. Fluazaindolizine and its metabolites are eluted from the HLB column (6-mL/500 mg) with 2 x *ca.* 6 mL of 0.1% formic acid in acetone via gravity. Full vacuum can be used for 10-15 seconds after all of the solution has passed. The extracts are evaporated to *ca.* 2 mL using N-evap® with moderate nitrogen flow and a water bath set to 35 ± 5°C. The method noted that the samples are not to be allowed to go to dryness. The solvent exchanged to acetonitrile using 2 mL of acetonitrile followed by evaporation with N-evap® with moderate nitrogen flow and a water bath set to 35 ± 5°C. The method noted that the samples are not to be allowed to go to dryness. Samples are then diluted to 10 mL with 0.1% formic acid in water, filtered (0.45 µm PTFE syringe filter), and analyzed by LC/MS/MS. Additional dilutions with acetonitrile:0.1% formic acid in water (2:8, v:v) were performed, if necessary. In ECM1, the method contained the following precautions: instruments should be well-maintained, the SPE cartridge should not be allowed to go dry after conditioning, and temperatures during nitrogen evaporation should be maintained in listed range (p. 29 of MRID 50932066). In ECM 2, the method contained the following precaution: soil pH may impact method performance and it has been found that the addition of 50 µL of 20% aqueous formic acid with vortexing during extraction and bringing the sample to 22 mL with 2% aqueous formic acid instead of water may help to improve method performance (pp. 25-26 of MRID 50932065). ECM 2 also noted that the instrument should be well-equilibrated before analysis.

Samples are analyzed using an Applied Biosystems/Sciex API 5000 (triple quadrupole) mass spectrometer coupled with a Waters Acquity UPLC (pp. 17, 23-24 of MRID 50932066; pp. 13, 20-21 of MRID 50932065). The following LC conditions were used: Acquity HSS T3 column (2.1 mm x 50 mm, 1.8 µm; column temperature 40°C), gradient mobile phase of A) 0.1% formic acid in water and B) 0.1% formic acid in methanol [time, percent A:B; 0.00 min. 95.0:5.0, 4.00-5.00 min. 10.0:90.0, 5.01-6.00 min. 95.0:5.0], injection volume of 20 µL, MS/MS with TurboIonspray (TIS) source in positive (IN-RYC33, IN-QEK31, and IN-VM862) or negative (IN-A5760, IN-F4106, IN-REG72, and fluazaindolizine) polarity (source temperature 500°C). Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively):  $m/z$  466.1→157.1 and  $m/z$  466.1→142.0 for fluazaindolizine,  $m/z$  206.0→122.1 and  $m/z$  206.0→142.0 for IN-A5760,  $m/z$  220.0→78.0 and  $m/z$  220.0→156.1 for IN-F4106,  $m/z$  452.0→123.2 and  $m/z$  452.0→143.0 for IN-REG72,  $m/z$  264.0→157.2 and  $m/z$  264.0→219.3 for IN-RYC33,  $m/z$  265.0→157.0 and  $m/z$  265.0→219.0 for IN-QEK31, and  $m/z$  197.0→75.0 and  $m/z$  197.0→64.1 for IN-VM862 ( $m/z$  197.0→141.2 and  $m/z$  197.0→69.0 were also reported for IN-VM862; however, reported monitored ion transitions were based on Figure 1, p. 38 of MRID 50932066). Approximate retention times were 3.5, 1.2, 1.7, 3.3, 2.5, 2.4, and 2.8 minutes for fluazaindolizine, IN-A5760, IN-F4106, IN-REG72, IN-RYC33, IN-QEK31, and IN-VM862, respectively.

The ILV performed the ECM 1 & 2 method for fluazaindolizine as written, except that the methanol was used for the solvent exchange of the post-SPE, reduced extract, instead of acetonitrile, that the water bath temperature was reduced to 25 ± 5°C, instead of 35 ± 5°C, and that insignificant analytical instrument and equipment modifications were made (pp. 26-29; Appendix 2, pp. 242-244 of MRID 50932112). The LC/MS/MS instrument was an AB Sciex Triple Quad 5500 mass spectrometer coupled with a Shimadzu Nexera X2 UHPLC System. The LC conditions were the same as those of the ECMs 1 and 2, except that the injection volume was 35 µL. The ion pair transitions monitored for fluazaindolizine were the same as those of the ECMs 1 and 2, except for the monitored confirmation ion of IN-VM862:  $m/z$  197.0→69.0. Approximate retention times were 3.7, 1.4, 2.0, 3.5, 2.8, 2.7, and 3.0 minutes for fluazaindolizine, IN-A5760, IN-F4106, IN-REG72, IN-RYC33, IN-QEK31, and IN-VM862, respectively.

In the ECM 1, ECM 2 and ILV, the Limit of Quantification (LOQ) was 1.0 µg/kg for all analytes in soil matrices (pp. 11, 29 of MRID 50932066; pp. 10, 25; Appendix 2, p. 64 of MRID 50932065; p. 13 of MRID 50932112). In the ECM 1 and ECM 2, the Limit of Detection (LOD) was 0.3 µg/kg and *ca.* 0.3 µg/kg, respectively, for all analytes in soil matrices; the LOD was not reported in the ILV.

## II. Recovery Findings

ECM 1 (MRID 50932066) & ECM 2 (MRID 50932065): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of fluazaindolizine and its metabolites IN-A5760, IN-F4106, IN-REG72, IN-RYC33, IN-QEK31, and IN-VM862 at fortification levels of 1.0 µg/kg (LOQ) and 10 µg/kg (10×LOQ) in two soil matrices (Tables 1-4, pp. 32-35 of MRID 50932066; Tables 1-4, pp. 28-31 of MRID 50932065). Two ion pair transitions were monitored, one quantitation and one confirmation; quantitation and confirmation; recovery results were comparable, except for IN-REG72 and LOQ analysis of IN-VM862. The sand soil (Sample ID 109011; 97% sand, 3% silt, 0% clay; pH 6.4 in 1:1 soil:water ratio; 0.47% organic matter- Walkley Black) and loam soil (Sample ID 88NJ01NASCNA BA.A.001.00-02"; 28% sand, 47% silt, 25% clay; pH 6.9 in 1:1 soil:water ratio; 2.0% organic matter- Walkley Black) were obtained from fluazaindolizine terrestrial field dissipation studies from Florida (MRID 50932032; DuPont-36690) and New Jersey (MRID 50932033; DuPont-36688), characterized by Agvise Laboratories, Northwood, North Dakota, and used in the study (USDA Soil Texture Classification; pp. 21, 31; Appendix 3, pp. 94-95 of MRID 50932066; pp. 17, 27; Appendix 4, pp. 66-67 of MRID 50932065).

ILV (MRID 50932112): Mean recoveries and RSDs were within guidelines for analysis of fluazaindolizine and its metabolites IN-A5760, IN-F4106, IN-REG72, IN-RYC33, IN-QEK31, and IN-VM862 at fortification levels of 1.0 µg/kg (LOQ) and 10 µg/kg (10×LOQ) in three soil matrices (pp. 14-20). Two ion pair transitions were monitored, one quantitation and one confirmation; quantitation and confirmation recovery results were comparable. Drummer, Tama, and Sassafras soils supplied by DuPont Crop Protection, E. I. du Pont de Nemours and Company (Newark, Delaware) were used in the study (pp. 13, 25). The soils were not characterized or described. The method was validated for all analytes in all three soils in the second trial as written, with the following modifications: 1) the methanol was used for the solvent exchange of the post-SPE, reduced extract, instead of acetonitrile; 2) the water bath temperature was reduced to 25 ± 5°C, instead of 35 ± 5°C; and 3) insignificant analytical instrument and equipment modifications were made (pp. 26-29, 31, 39; Appendix 2, pp. 239-250). In the first validation trial for the Sassafras Soil, the average recoveries of DPX-Q8U80, IN-VM862, and IN-RYC33 were lower than 70%. The ILV reported that, after communication with the Study Monitor, a new reconstitution solvent was used for the post-SPE, reduced extract to prepared the final extract in methanol:0.1% formic acid in water (2:8, v:v) and a change of filter (0.45-µm PTFE, 25 mm diameter) was performed. The ILV did not report the reduced temperature in the itemized method modifications. The submitted ECMs incorporated the 0.45-µm PTFE filter; however, the lower temperature and solvent change to methanol was not incorporated. An updated ECM should be submitted incorporating the use of lower temperature for evaporation and solvent change to methanol since these modifications were necessary for the ILV to validate the method for the analytes, especially DPX-Q8U80, IN-VM862, and IN-RYC33.



**Table 2. Initial Validation Method Recoveries for Fluazaindolizine and its metabolites IN-A5760, IN-F4106, IN-REG72, IN-RYC33, IN-QEK31, and IN-VM862 in Soil<sup>1,2,3</sup>**

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Florida Sand Soil</b>						
Quantitation Ion Transition						
Fluazaindolizine (DPX-Q8U80)	1.0 (LOQ)	5	81-91	85	4.2	4.9
	10	5	81-84	83	1.1	1.3
IN-REG72	1.0 (LOQ)	5	72-84	79	4.7	6.0
	10	5	79-86	82	2.5	3.0
IN-VM862	1.0 (LOQ)	5	70-96	85	9.2	10.9
	10	5	96-107	100	5.4	5.4
IN-QEK31	1.0 (LOQ)	5	108-122	116	5.4	4.6
	10	5	108-113	110	2.0	1.8
IN-F4106	1.0 (LOQ)	5	92-102	98	3.9	4.0
	10	5	96-101	98	2.3	2.4
IN-A5760	1.0 (LOQ)	5	91-107	101	6.3	6.3
	10	5	98-102	100	2.0	2.0
IN-RYC33	1.0 (LOQ)	5	92-101	97	3.4	3.5
	10	5	93-100	96	3.4	3.5
Confirmation Ion Transition						
Fluazaindolizine (DPX-Q8U80)	1.0 (LOQ)	5	74-87	83	5.2	6.2
	10	5	79-85	81	2.2	2.7
IN-REG72	1.0 (LOQ)	5	93-114	106	7.7	7.3
	10	5	71-77	74	2.4	3.3
IN-VM862	1.0 (LOQ)	5	84-104	93	8.9	9.5
	10	5	92-97	95	1.7	1.8
IN-QEK31	1.0 (LOQ)	5	87-99	95	4.8	5.0
	10	5	106-112	108	2.4	2.2
IN-F4106	1.0 (LOQ)	5	90-114	101	12.2	12.0
	10	5	99-103	101	1.6	1.5
IN-A5760	1.0 (LOQ)	5	94-108	102	5.2	5.1
	10	5	90-97	94	2.9	3.1
IN-RYC33	1.0 (LOQ)	5	92-109	101	6.5	6.5
	10	5	91-98	96	2.7	2.8
<b>New Jersey Loam Soil</b>						
Quantitation Ion Transition						
Fluazaindolizine (DPX-Q8U80)	1.0 (LOQ)	5	77-88	84	4.4	5.3
	10	5	78-85	81	2.7	3.3
IN-REG72	1.0 (LOQ)	5	80-89	84	3.6	4.3
	10	5	77-83	81	2.7	3.3
IN-VM862	1.0 (LOQ)	5	79-109	93	12.6	13.6
	10	5	91-101	96	3.6	3.8
IN-QEK31	1.0 (LOQ)	5	94-127	110	12.6	11.5
	10	5	110-117	113	2.8	2.5
IN-F4106	1.0 (LOQ)	5	89-107	100	7.2	7.2
	10	5	97-102	100	2.0	2.0
IN-A5760	1.0 (LOQ)	5	91-108	100	6.8	6.8
	10	5	97-102	99	1.9	1.9

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
IN-RYC33	1.0 (LOQ)	5	92-105	100	5.4	5.3
	10	5	87-93	90	2.3	2.6
Confirmation Ion Transition						
Fluazaindolizine (DPX-Q8U80)	1.0 (LOQ)	5	86-97	91	4.9	5.3
	10	5	82-88	85	2.5	2.9
IN-REG72	1.0 (LOQ)	5	70-106	91	13.2	14.5
	10	5	71-78	74	2.5	3.4
IN-VM862	1.0 (LOQ)	5	96-128	114	15.1	13.2
	10	5	99-105	101	2.4	2.4
IN-QEK31	1.0 (LOQ)	5	92-135	109	16.9	15.4
	10	5	107-111	110	2.0	1.8
IN-F4106	1.0 (LOQ)	5	85-98	94	6.2	6.6
	10	5	91-97	94	2.5	2.7
IN-A5760	1.0 (LOQ)	5	78-112	98	14.4	14.7
	10	5	96-102	99	2.1	2.1
IN-RYC33	1.0 (LOQ)	5	88-101	93	5.7	6.1
	10	5	87-94	89	2.9	3.2

Data (recovery results were corrected for residues quantified in the controls; pp. 25-26 of MRID 50932066; pp. 21-23 of MRID 50932065) were obtained from Tables 1-4, pp. 32-35 of MRID 50932066; Tables 1-4, pp. 28-31 of MRID 50932065.

- The sand soil (Sample ID 109011; 97% sand, 3% silt, 0% clay; pH 6.4 in 1:1 soil:water ratio; 0.47% organic matter- Walkley Black) and loam soil (Sample ID 88NJ01NASCNA BA.A.001.00-02"; 28% sand, 47% silt, 25% clay; pH 6.9 in 1:1 soil:water ratio; 2.0% organic matter- Walkley Black) were obtained from fluazaindolizine terrestrial field dissipation studies from Florida (MRID 50932032; DuPont-36690) and New Jersey (MRID 50932033; DuPont-36688), characterized by Agvise Laboratories, Northwood, North Dakota, and used in the study (USDA Soil Texture Classification; pp. 21, 31; Appendix 3, pp. 94-95 of MRID 50932066; pp. 17, 27; Appendix 4, pp. 66-67 of MRID 50932065). The soil textures were verified by the reviewer using USDA-NRCS technical support tools.
- Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively):  $m/z$  466.1→157.1 and  $m/z$  466.1→142.0 for fluazaindolizine,  $m/z$  206.0→122.1 and  $m/z$  206.0→142.0 for IN-A5760,  $m/z$  220.0→78.0 and  $m/z$  220.0→156.1 for IN-F4106,  $m/z$  452.0→123.2 and  $m/z$  452.0→143.0 for IN-REG72,  $m/z$  264.0→157.2 and  $m/z$  264.0→219.3 for IN-RYC33,  $m/z$  265.0→157.0 and  $m/z$  265.0→219.0 for IN-QEK31, and  $m/z$  197.0→75.0 and  $m/z$  197.0→64.1 for IN-VM862.



**Table 3. Independent Validation Method Recoveries for Fluazaindolizine and its metabolites IN-A5760, IN-F4106, IN-REG72, IN-RYC33, IN-QEK31, and IN-VM862 in Soil<sup>1,2</sup>**

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Sassafras Soil</b>						
Quantitation Ion Transition						
Fluazaindolizine (DPX-Q8U80)	1.0 (LOQ)	5	88-92	90	1.6	2
	10	5	88-96	93	3.9	4
IN-REG72	1.0 (LOQ)	5	79-94	86	6.4	7
	10	5	90-98	93	2.9	3
IN-F4106	1.0 (LOQ)	5	82-105	96	11.6	12
	10	5	87-107	96	7.4	8
IN-A5760	1.0 (LOQ)	5	94-107	101	5.7	6
	10	5	98-103	100	2.0	2
IN-VM862	1.0 (LOQ)	5	77-106	90	10.4	12
	10	5	79-92	85	5.0	6
IN-QEK31	1.0 (LOQ)	5	96-105	101	3.8	4
	10	5	108-110	109	0.8	1
IN-RYC33	1.0 (LOQ)	5	77-92	83	6.2	7
	10	5	89-93	92	1.5	2
Confirmation Ion Transition						
Fluazaindolizine (DPX-Q8U80)	1.0 (LOQ)	5	86-95	90	3.7	4
	10	5	87-92	90	2.2	2
IN-REG72	1.0 (LOQ)	5	76-95	89	8.5	10
	10	5	86-94	91	2.7	3
IN-F4106	1.0 (LOQ)	5	88-102	95	6.9	7
	10	5	90-102	96	5.6	6
IN-A5760	1.0 (LOQ)	5	89-100	94	4.0	4
	10	5	98-105	102	2.5	2
IN-VM862	1.0 (LOQ)	5	84-100	93	8.0	9
	10	5	82-88	85	2.3	3
IN-QEK31	1.0 (LOQ)	5	93-100	96	2.9	3
	10	5	106-111	109	2.1	2
IN-RYC33	1.0 (LOQ)	5	86-101	93	5.6	6
	10	5	88-93	91	1.8	2
<b>Drummer Soil</b>						
Quantitation Ion Transition						
Fluazaindolizine (DPX-Q8U80)	1.0 (LOQ)	5	75-95	87	7.2	8
	10	5	82-88	84	2.4	3
IN-REG72	1.0 (LOQ)	5	88-94	92	2.1	2
	10	5	83-88	85	2.2	3
IN-F4106	1.0 (LOQ)	5	79-95	88	6.9	8
	10	5	79-90	83	4.2	5
IN-A5760	1.0 (LOQ)	5	91-98	94	3.2	3
	10	5	89-95	93	2.1	2
IN-VM862	1.0 (LOQ)	5	93-114	102	8.6	8
	10	5	71-89	79	8.3	11
IN-QEK31	1.0 (LOQ)	5	82-96	89	5.1	6
	10	5	91-95	93	1.6	2

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
IN-RYC33	1.0 (LOQ)	5	60-78	72	6.7	9
	10	5	74-81	77	2.9	4
<b>Confirmation Ion Transition</b>						
Fluazaindolizine (DPX-Q8U80)	1.0 (LOQ)	5	72-89	82	6.3	8
	10	5	81-86	83	2.5	3
IN-REG72	1.0 (LOQ)	5	83-100	91	6.2	7
	10	5	85-90	88	2.2	2
IN-F4106	1.0 (LOQ)	5	72-91	84	7.9	9
	10	5	80-91	86	4.9	6
IN-A5760	1.0 (LOQ)	5	83-88	85	2.1	2
	10	5	89-96	93	2.7	3
IN-VM862	1.0 (LOQ)	5	78-96	88	8.0	9
	10	5	66-85	74	8.7	12
IN-QEK31	1.0 (LOQ)	5	91-98	95	2.4	3
	10	5	90-97	94	3.2	3
IN-RYC33	1.0 (LOQ)	5	79-89	84	4.0	5
	10	5	76-82	78	2.4	3
<b>Tama Soil</b>						
<b>Quantitation Ion Transition</b>						
Fluazaindolizine (DPX-Q8U80)	1.0 (LOQ)	5	80-96	89	7.3	8
	10	5	96-109	101	4.8	5
IN-REG72	1.0 (LOQ)	5	90-106	97	7.1	7
	10	5	94-102	99	3.2	3
IN-F4106	1.0 (LOQ)	5	87-94	90	3.1	3
	10	5	89-97	93	3.3	4
IN-A5760	1.0 (LOQ)	5	90-98	94	3.3	4
	10	5	97-104	100	2.7	3
IN-VM862	1.0 (LOQ)	5	77-99	93	9.3	10
	10	5	89-100	95	4.2	4
IN-QEK31	1.0 (LOQ)	5	96-104	101	3.3	3
	10	5	95-106	100	4.0	4
IN-RYC33	1.0 (LOQ)	5	71-109	91	16.1	18
	10	5	95-99	97	1.5	2
<b>Confirmation Ion Transition</b>						
Fluazaindolizine (DPX-Q8U80)	1.0 (LOQ)	5	79-102	90	8.5	9
	10	5	94-101	97	2.6	3
IN-REG72	1.0 (LOQ)	5	95-116	106	8.7	8
	10	5	98-103	100	2.0	2
IN-F4106	1.0 (LOQ)	5	74-93	83	9.3	11
	10	5	95-104	98	3.8	4
IN-A5760	1.0 (LOQ)	5	90-111	102	8.9	9
	10	5	95-102	99	2.7	3
IN-VM862	1.0 (LOQ)	5	88-108	96	8.1	8
	10	5	98-107	101	3.9	4
IN-QEK31	1.0 (LOQ)	5	96-111	104	6.1	6
	10	5	98-104	101	2.4	2

Analyte	Fortification Level ( $\mu\text{g}/\text{kg}$ )	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
IN-RYC33	1.0 (LOQ)	5	85-106	94	8.9	9
	10	5	98-105	100	2.9	3

Data (recovery results were corrected for residues quantified in the controls; pp. 30-31) were obtained from pp. 14-20 of MRID 50932112.

- 1 The Drummer, Tama, and Sassafras soils supplied by DuPont Crop Protection, E. I. du Pont de Nemours and Company (Newark, Delaware) were used in the study (pp. 13, 25 of MRID 50932112). The soils were not characterized or described. The soil textures could not be verified by the reviewer using USDA-NRCS technical support tools.
- 2 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively):  $m/z$  466.1 $\rightarrow$ 157.1 and  $m/z$  466.1 $\rightarrow$ 142.0 for fluazaindolizine,  $m/z$  206.0 $\rightarrow$ 122.1 and  $m/z$  206.0 $\rightarrow$ 142.0 for IN-A5760,  $m/z$  220.0 $\rightarrow$ 78.0 and  $m/z$  220.0 $\rightarrow$ 156.1 for IN-F4106,  $m/z$  452.0 $\rightarrow$ 123.2 and  $m/z$  452.0 $\rightarrow$ 143.0 for IN-REG72,  $m/z$  264.0 $\rightarrow$ 157.2 and  $m/z$  264.0 $\rightarrow$ 219.3 for IN-RYC33,  $m/z$  265.0 $\rightarrow$ 157.0 and  $m/z$  265.0 $\rightarrow$ 219.0 for IN-QEK31, and  $m/z$  197.0 $\rightarrow$ 75.0 and  $m/z$  197.0 $\rightarrow$ 69.0 for IN-VM862.

### III. Method Characteristics

In the ECM 1, ECM 2, and ILV, the LOQ was 1.0 µg/kg for all analytes in soil matrices (pp. 11, 29 of MRID 50932066; pp. 10, 25; Appendix 2, p. 64 of MRID 50932065; p. 13 of MRID 50932112). In the ECMs 1 and 2, the LOQ was defined as the lowest fortification level evaluated at which acceptable average recoveries (70-120%, RSD <20%) were obtained, as well as the fortification level at which analyte peaks are consistently generated at a level of *ca.* 3-20x the signal at the analytes in the untreated controls. The ECM 1, the method LOD was 0.3 µg/kg, or 1/3 of the LOQ. In the ECM 2, the LOD was defined as the concentration of the least responsive analyte (IN-VM862) at which analyte peaks are *ca.* 3x the chromatographic baseline noise observed near the retention time or *ca.* one-third the concentration of the LOQ. In ECM 2, the LOD was set as *ca.* 0.3 µg/kg for all analytes in soil matrices, based on the signal-to-noise ratio of 3.5 in the IN-VM862 chromatogram at the LOQ. The LOD was not reported in the ILV. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM 1, ECM 2 or ILV.

**Table 4. Method Characteristics Fluazaindolizine and its metabolites IN-A5760, IN-F4106, IN-REG72, IN-RYC33, IN-QEK31, and IN-VM862 in Soil**

Test Material		Fluazaindolizine	IN-REG72	IN-VM862	IN-QEK31	IN-F4106	IN-A5760	IN-RYC33	
Limit of Quantitation (LOQ)	ECM 1 <sup>1</sup>	1.0 µg/kg							
	ECM 2 <sup>2</sup>								
	ILV								
Limit of Detection (LOD)	ECM 1	0.3 µg/kg							
	ECM 2	ca. 0.3 µg/kg (calculated)							
	ILV	<b>Not reported</b>							
Linearity (calibration curve r and concentration range)	ECM 1 & ECM 2	r = 0.9995 (Q)	r = 0.9998 (Q)	r = 0.9989 (Q)	r = 0.9995 (Q & C)	r = 0.9997 (Q)	r = 0.9997 (Q)	r = 0.9983 (Q)	
		r = 0.9994 (C)	r = 0.9996 (C)	r = 0.9994 (C)		r = 0.9989 (C)	r = 0.9991 (C)	r = 0.9991 (C)	
	0.10-5.0 ng/mL								
	ILV	r = 0.9996 (Q)	r = 0.9996 (Q)	r = 0.9977 (Q)	r = 0.9997 (Q)	r = 0.9979 (Q)	r = 0.9996 (Q)	r = 0.9982 (Q)	
r = 0.9993 (C)		r = 0.9988 (C)	r = 0.9985 (C)	r = 0.9998 (C)	r = 0.9962 (C)	r = 0.9997 (C)	r = 0.9998 (C)		
		0.10-5.0 ng/mL							
Repeatable	ECM 1 & ECM 2 <sup>3</sup>	Yes at LOQ and 10×LOQ in two characterized soil matrices.							
	ILV <sup>4,5</sup>	Yes at LOQ and 10×LOQ in three uncharacterized soil matrices.							
Reproducible		Yes at LOQ and 10×LOQ.							
Specific	ECM 1 & ECM 2	Yes, no matrix interferences were observed, but notable baseline noise was observed.	Yes, no matrix interferences were observed. Nearby contaminant (peak height ca. LOQ peak height) noted in C ion which co-eluted with analyte peak. <sup>6,7</sup>	<b>No</b> , no matrix interferences were observed; however, LOQ peak was very small compared to baseline noise and baseline noise significantly interfered with peak attenuation and integration. <sup>8</sup>	Yes, no matrix interferences were observed, but LOQ peak was relatively small compared to baseline noise. <sup>9</sup>	Yes, no matrix interferences were observed. Contaminant (peak height ca. LOQ peak height) noted.	Yes, no matrix interferences were observed		
		All chromatograms showed at least some minor baseline noise interference at the LOQ which was more prominent in the representative chromatograms from the confirmation ion analysis.							

Test Material		Fluazaindolizine	IN-REG72	IN-VM862	IN-QEK31	IN-F4106	IN-A5760	IN-RYC33
	ILV	Yes, no matrix interferences were observed. Nearby minor contaminant (peak height <i>ca.</i> 25% of LOQ peak height) noted.	Yes, no matrix interferences were observed.	<b>No</b> , matrix interferences were <15% of the LOQ (based on peak area); however, LOQ peak was very small compared to baseline noise and baseline noise significantly interfered with peak attenuation and integration. <sup>10</sup> Nearby contaminant (peak height <i>ca.</i> LOQ peak height) noted.	Yes, no matrix interferences were observed. Nearby minor contaminant (peak height <i>ca.</i> 5% of LOQ peak height) noted which interfered with peak attenuation and integration	Yes, no matrix interferences were observed, but baseline interfered with peak attenuation and integration.	Yes, no matrix interferences were observed.	<b>No</b> , matrix interferences were <16% (Q) and <18% (C) of the LOQ (based on peak area); however, LOQ peak was small compared to baseline noise and baseline noise significantly interfered with peak attenuation and integration. <sup>11</sup> Nearby contaminants (peak height <i>ca.</i> 30-70% of LOQ peak height) noted.

Data were obtained from pp. 11, 29 (LOQ/LOD); Tables 1-4, pp. 32-35 (recovery results); pp. 20-21; Figure 1, pp. 36-42 (calibration curves); Figures 2-4, pp. 43-84 (chromatograms) of MRID 50932066; pp. 10, 25; Appendix 2, p. 64 (LOQ/LOD); Tables 1-4, pp. 28-31 (recovery results); Figure 1, pp. 32-35 (calibration curves); Figures 2-4, pp. 36-56 (chromatograms) of MRID 50932065; p. 13 (LOQ/LOD); pp. 14-20 (recovery results); Figure 8, pp. 139-145 (calibration curves); Figures 9-30, pp. 146-229 (chromatograms) of MRID 50932112; DER Attachment 2. Q = quantitation ion transition; C = confirmation ion transition.

1 MRID 50932066 was considered to be the primary ECM since the ILV was reported performed to validate it (pp. 13, 40 of MRID 50932112). MRID 50932066 essentially contained the same information as ECM 2 MRID 50932065; however, both quantitation and confirmation ion transition chromatograms were included in ECM 1 MRID 50932066.

2 MRID 50932065 essentially contained the same information as ECM 1 MRID 50932066; however, only quantitation ion transition chromatograms were included in ECM 2 MRID 50932065.

3 In the ECM 1 and ECM 2, sand soil (Sample ID 109011; 97% sand, 3% silt, 0% clay; pH 6.4 in 1:1 soil:water ratio; 0.47% organic matter- Walkley Black) and loam soil (Sample ID 88NJ01NASCNA BA.A.001.00-02"; 28% sand, 47% silt, 25% clay; pH 6.9 in 1:1 soil:water ratio; 2.0% organic matter- Walkley Black) were obtained from fluazaindolizine terrestrial field dissipation studies from Florida (MRID 50932032; DuPont-36690) and New Jersey (MRID 50932033; DuPont-36688), characterized by Agvise Laboratories, Northwood, North Dakota, and used in the study (USDA Soil Texture Classification; pp. 21, 31; Appendix 3, pp. 94-95 of MRID 50932066; pp. 17, 27; Appendix 4, pp. 66-67 of MRID 50932065).

4 In the ILV, Drummer, Tama, and Sassafras soils supplied by DuPont Crop Protection, E. I. du Pont de Nemours and Company (Newark, Delaware) were used in the study (pp. 13, 25 of MRID 50932112). The soils were not characterized or described.

5 The ILV validated the method for all analytes in all three soils in the second trial as written, with the following modifications: 1) the methanol was used for the solvent exchange of the post-SPE, reduced extract, instead of acetonitrile; 2) the water bath temperature was reduced to  $25 \pm 5^\circ\text{C}$ , instead of  $35 \pm 5^\circ\text{C}$ ; and 3) insignificant analytical instrument and equipment modifications were made (pp. 26-29, 31, 39; Appendix 2, pp. 239-250 of MRID 50932112). In the first validation trial for the Sassafras Soil, the average recoveries of DPX-Q8U80, IN-VM862, and IN-RYC33 were lower than 70%. The ILV reported that, after communication with the Study Monitor, a new reconstitution solvent was used for the post-SPE, reduced extract to prepared the final extract in methanol:0.1% formic acid in water (2:8, v:v) and a change of filter (0.45- $\mu\text{m}$  PTFE, 25 mm diameter) was performed. The ILV did not report the reduced temperature in the itemized method modifications. The submitted ECMs incorporated the 0.45- $\mu\text{m}$  PTFE filter; however, the lower temperature and solvent change to methanol was not incorporated. An updated ECM should be submitted incorporating the use of lower temperature for evaporation and solvent change to methanol since these modifications were necessary for the ILV to validate the method for the analytes, especially DPX-Q8U80, IN-VM862, and IN-RYC33.

6 Based on Figure 3, p. 60; Figure 4, p. 74, of MRID 50932066.

7 Deviations of acceptability in the confirmation ion analysis did not affect the validity of the method since a confirmation method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.

8 Based on Figure 3, pp. 61-62; Figure 4, pp. 75-76, of MRID 50932066.

9 Based on Figure 3, pp. 63-64; Figure 4, pp. 77-78, of MRID 50932066.

10 Based on Figure 13, pp. 158-160; Figure 20, pp. 179-181; and Figure 27, pp. 200-202 of MRID 50932112.

11 Based on Figure 15, pp. 164-166; Figure 22, pp. 185-187; and Figure 29, pp. 206-208 of MRID 50932112.

#### IV. Method Deficiencies and Reviewer's Comments

1. The reviewer considered MRID 50932066 to be the primary ECM (ECM 1) since the ILV was reported performed to validate it (pp. 13, 40 of MRID 50932112). MRID 50932066 essentially contained the same information/performance data as ECM 2 MRID 50932065; however, both quantitation and confirmation ion transition chromatograms were included in ECM 1 MRID 50932066. MRID 50932065 essentially contained the same information as ECM 1 MRID 50932066; however, only quantitation ion transition chromatograms were included in ECM 2 MRID 50932065. Both MRIDs 50932066 and 50932065 referenced each other and had the same report date (pp. 1, 31 of MRID 50932066; pp. 1, 27 of MRID 50932065).
2. An updated ECM should be submitted incorporating the use of lower temperature for evaporation and solvent change to methanol since these modifications were necessary for the ILV to validate the method for the analytes, especially DPX-Q8U80, IN-VM862, and IN-RYC33 (pp. 26-29, 31, 39; Appendix 2, pp. 239-250 of MRID 50932112). In the first validation trial for the Sassafras Soil, the average recoveries of DPX-Q8U80, IN-VM862, and IN-RYC33 were lower than 70%. The ILV reported that, after communication with the Study Monitor, several minor method modifications were used for the second trial. The submitted ECMs incorporated the 0.45- $\mu$ m PTFE filter modification; however, the lower temperature and solvent change to methanol was not incorporated.
3. The specificity of the method for IN-VM862 was not supported by ILV and ECM representative chromatograms because the LOQ peak was very small compared to baseline noise and baseline noise significantly interfered with peak attenuation and integration (Figure 3, pp. 61-62; Figure 4, pp. 75-76, of MRID 50932066; Figure 13, pp. 158-160; Figure 20, pp. 179-181; and Figure 27, pp. 200-202 of MRID 50932112). The LOQ analyte peak for IN-VM862 was only slightly distinguishable from the baseline noise in all soil matrices. Additionally, in the ILV, matrix interferences were noted: matrix interferences were <15% of the LOQ (based on peak area; *ca.* 50% of LOD) and a nearby contaminant with peak height of *ca.* LOQ peak height.
4. The specificity of the method for IN-RYC33 was not supported by ILV representative chromatograms because the LOQ peak was small compared to baseline noise and baseline noise significantly interfered with peak attenuation and integration, as well as the fact that matrix interferences were <16% (Q) and <18% (C) of the LOQ (based on peak area; >50% of the LOD; Figure 15, pp. 164-166; Figure 22, pp. 185-187; and Figure 29, pp. 206-208 of MRID 50932112).
5. The ILV soil matrices, Drummer, Tama, and Sassafras soils, were not characterized or described (pp. 13, 25 of MRID 50932112). It could not be determined if the ILV was provided with the most difficult soil matrix with which to validate the method and if the ILV soil matrix covered the range of soils used in the terrestrial field dissipation (TFD) studies. The submitted fluazaindolizine TFD studies included Tulare, California (sandy loam/loamy sand; MRID 50932030), Fresno, California (loamy sand/sand; MRID 50932031 & MRID 50932331), Sutter, California (sandy loam/sandy clay loam; MRID 50932034), Willacy, Texas (sandy clay loam/clay loam/clay; MRID 50932035), Ontario, Canada (loam; MRID 50932332), Nambenheim, France (loam/silt loam; MRID 50932333), Thessaloniki, Greece (loam/sandy loam; MRID 50932334), Graffignana, Italy (loam/silt loam; MRID 50932335), and Catalunya, Spain (clay/silty clay; MRID 50932336). The two ECM soil matrices were obtained from fluazaindolizine terrestrial field dissipation studies from Seminole, Florida (MRID 50932032;



DuPont-36690) and Hunterdon, New Jersey (MRID 50932033; DuPont-36688; pp. 21, 31; Appendix 3, pp. 94-95 of MRID 50932066; pp. 17, 27; Appendix 4, pp. 66-67 of MRID 50932065). The reviewer noted that soils designated as Tama, and Sassafras soils were used in submitted fluazaindolizine aerobic soil metabolism studies (such as MRIDs 50923023 and 50923024).

6. The specificity of the method for IN-QEK31 was not well-supported by ECM representative chromatograms because the LOQ peak was relatively small compared to baseline noise (Figure 3, pp. 63-64; Figure 4, pp. 77-78, of MRID 50932066). Additionally, in ILV chromatograms, a nearby minor contaminant (peak height *ca.* 5% of LOQ peak height) noted which interfered with peak attenuation and integration (Figure 14, pp. 161-163; Figure 21, pp. 182-184; and Figure 28, pp. 203-205 of MRID 50932112).
7. Communication (emails) between the ILV personnel (Meng Fang) and the DuPont Study Monitor for the ILV (Joseph Klems) was provided; however, the communication was either incomplete or not all questions between the parties were answered in the form of communication provided [see ILV questions in email on January 31, 2017 at 3:06 pm (Appendix 2, p. 240); pp. 6, 39; Appendix 2, pp. 239-250 of MRID 50932112]. Joseph Klems was not listed as a laboratory personnel of either ECM 1 or ECM 2 (p. 10 of MRID 50932066; p. 1 of MRID 50932065). The provided communication between the ILV and ILV Study Monitor involved technical advice regarding ILV validation issues, modifications to the ECM, RT shifts of analytes, data transfer, and approval between soil matrices. The reviewer noted two things regarding the ILV/Study Monitor communication: 1) the ILV personnel involved in the communication was Meng Fang, the ILV Study Director, and not Yixiao Shen, the ILV analyst and study author; and 2) all modifications of the ECM which were incorporated by the ILV originated with the Study Monitor and not the ILV lab personnel.
8. A nearby contaminant (peak height *ca.* LOQ peak height) was noted in C ion analysis of IN-REG72 which co-eluted with analyte peak (Figure 3, p. 60; Figure 4, p. 74, of MRID 50932066). The reviewer noted that deviations of acceptability in the confirmation ion analysis did not affect the validity of the method since a confirmation method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.
9. The determinations of LOD and LOQ in the ECM 1, ECM 2, and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 11, 29 of MRID 50932066; pp. 10, 25; Appendix 2, p. 64 of MRID 50932065; p. 13 of MRID 50932112). In the ECMs 1 and 2, the LOQ was defined as the lowest fortification level evaluated at which acceptable average recoveries (70-120%, RSD <20%) were obtained, as well as the fortification level at which analyte peaks are consistently generated at a level of *ca.* 3-20xs the signal at the analytes in the untreated controls. The ECM 1, the method LOD was 1/3 of the LOQ. In the ECM 2, the LOD was defined as the concentration of the least responsive analyte (IN-VM862) at which analyte peaks are *ca.* 3x the chromatographic baseline noise observed near the retention time or *ca.* one-third the concentration of the LOQ. In ECM 2, the LOD was set as *ca.* 0.3 µg/kg for all analytes in soil matrices, based on the signal-to-noise ratio of 3.5 in the IN-VM862 chromatogram at the LOQ. The LOD was not reported in the ILV. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM 1, ECM 2 or ILV. Detection limits should not be based on arbitrary values.

10. In the ECMs 1 and 2, the stabilities of the stock solutions, fortification stands, calibration standards, sample extracts, and final sample volumes were reported as at least 6 months, 6 months, 1 week, 1 week, and 2 days, respectively, under frozen conditions (p. 29 of MRID 50932066; p. 26 of MRID 50932065).
11. In the ECMs 1 and 2, the extraction efficiency of acidic acetonitrile solution was reportedly studied in extraction efficiency study, DuPont-34707 (p. 29 of MRID 50932066; p. 25 of MRID 50932065). Additionally, extraction with acidic acetonitrile solution was reportedly used in aerobic soil metabolism study, DuPont-35135.
12. In the ECM 2, the matrix effects were reportedly insignificant for each matrix (p. 26 of MRID 50932065).
13. In the ECMs 1 and 2, the time requirement for a set of at least 13 samples was an eight-hour day for preparation with LC/MS/MS analysis run overnight (p. 29 of MRID 50932066; p. 25 of MRID 50932065). In the ILV, one set of 12 samples required *ca.* 2 working days, including LC/MS/MS instrument run-time (p. 39 of MRID 50932112).

## 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.

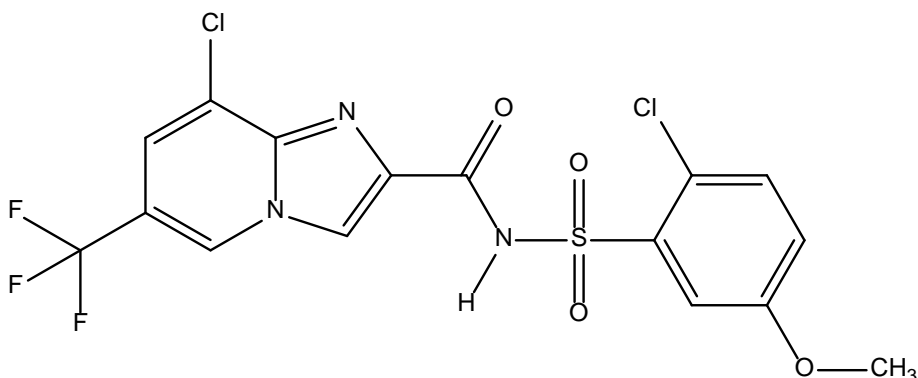
**Attachment 1: Chemical Names and Structures****Fluazaindolizine (DPX-Q8U80)**

**IUPAC Name:** 8-Chloro-N-[(2-chloro-5-methoxyphenyl)sulfonyl]-6-(trifluoromethyl)imidazo[1,2-a]pyridine-2-carboxamide

**CAS Name:** 8-Chloro-N-[(2-chloro-5-methoxyphenyl)sulfonyl]-6-(trifluoromethyl)imidazo[1,2-a]pyridine-2-carboxamide

**CAS Number:** 1254304-22-7

**SMILES String:** C1C=CC(C(F)(F)F)=CN2C1=NC(C(N(S(C3=CC(OC)=CC=C3Cl))=O)=O)[H]=O)=C2

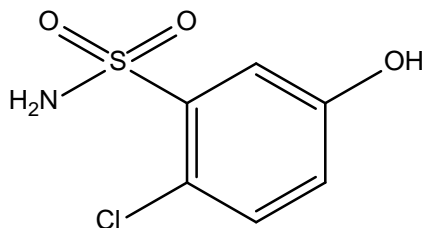
**IN-A5760**

**IUPAC Name:** 2-Chloro-5-hydroxybenzenesulfonamide

**CAS Name:** Not reported

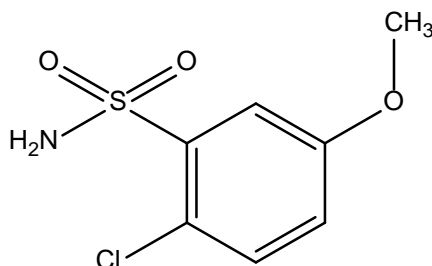
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**SMILES String:** OC1=CC(S(N)=O)=C(Cl)C=C1

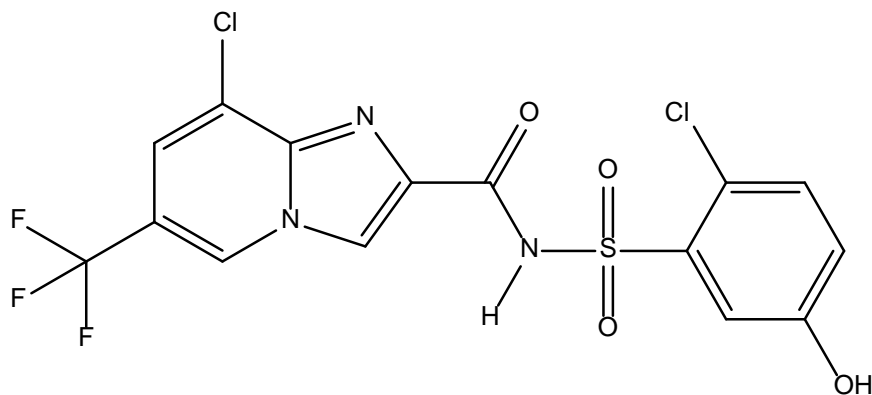


**IN-F4106**

**IUPAC Name:** 2-Chloro-5-methoxybenzenesulfonamide  
**CAS Name:** Not reported  
**CAS Number:** 502187-53-3  
**SMILES String:** COC1=CC(S(N)(=O)=O)=C(Cl)C=C1

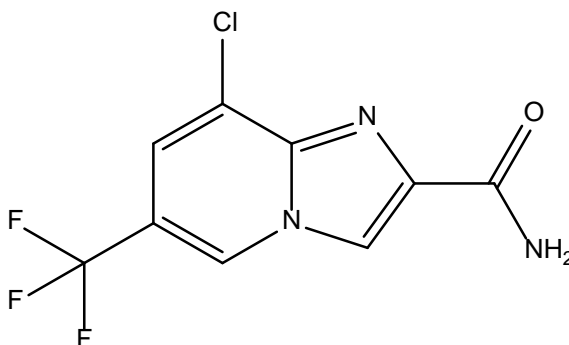
**IN-REG72**

**IUPAC Name:** 8-Chloro-N-((2-chloro-5-hydroxyphenyl)sulfonyl)-6-(trifluoromethyl)imidazo[1,2-a]pyridine-2-carboxamide  
**CAS Name:** Not reported  
**CAS Number:** Not reported  
**SMILES String:** ClC1=CC(C(F)(F)F)=CN2C1=NC(C(N(S(C3=CC(O)=CC=C3Cl))(=O)=O)[H])=O)=C2

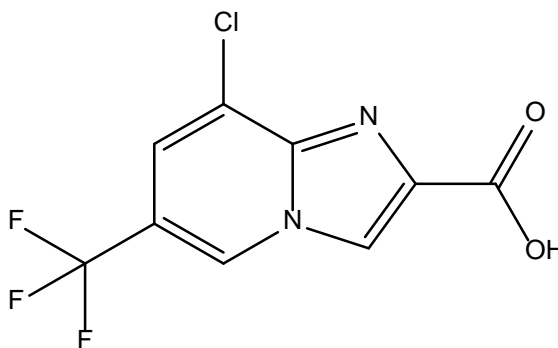


**IN-RYC33**

**IUPAC Name:** 8-Chloro-6-(trifluoromethyl)imidazo[1,2-a]pyridine-2-carboxamide  
**CAS Name:** Not reported  
**CAS Number:** 1228376-01-9  
**SMILES String:** ClC1=CC(C(F)(F)F)=CN2C1=NC(C(N)=O)=C2

**IN-QEK31**

**IUPAC Name:** 8-Chloro-6-(trifluoromethyl)imidazo[1,2-a]pyridine-2-carboxylic acid  
**CAS Name:** Not reported  
**CAS Number:** 353258-35-2  
**SMILES String:** ClC1=CC(C(F)(F)F)=CN2C1=NC(C(=O)O)=C2



**IN-VM862****IUPAC Name:** 3-Chloro-5-(trifluoromethyl)pyridin-2-amine**CAS Name:** Not reported**CAS Number:** 79456-26-1**SMILES String:** ClC1=CC(C(F)(F)F)=CN=C1N