

**Analytical method for indoxacarb and metabolites, IN-MP819 and IN-JT333, in soil/sediment**

- Reports:** ECM: EPA MRID No. 49599603. Henze, R.M. and J.J. Stry. 2014. Analytical Method for the Determination of Indoxacarb and Metabolites in Soil and Sediment Using LC/MS/MS. DuPont Study No.: DuPont-41157. Report prepared by E.I. du Pont de Nemours and Company, Newark, Delaware; sponsored and submitted by E.I. du Pont de Nemours and Company, Wilmington, Delaware; 78 pages. Final report issued June 19, 2014.
- ILV: EPA MRID No. 49623401. Shen, X. 2015. Independent Laboratory Validation of Analytical Method For The Determination of Indoxacarb and Metabolites In Soil And Sediment Using LC/MS/MS. DuPont Study Project ID: DuPont-42061. Report prepared by Primera Analytical Solutions Corp., Princeton, New Jersey; sponsored and submitted by E.I. du Pont de Nemours and Company, Wilmington, Delaware; 77 pages. Final report issued April 7, 2015.
- Document No.:** MRIDs 49599603 & 49623401
- Guideline:** 850.6100
- Statements:** ECM: The study was not conducted with the restriction of compliance with USEPA FIFRA (40 CFR, Part 160) or OECD Good Laboratory Practice (GLP) standards; however, work was done in a GLP facility following Standard Operating Procedures (p. 3 of MRID 49599603). Signed and dated Data Confidentiality, No Data Confidentiality, GLP, and Certification of Authenticity statements were provided (pp. 2-4). A Quality Assurance statement was not provided.
- ILV: The study was not conducted with the restriction of compliance with USEPA FIFRA (40 CFR, Part 160) or OECD Good Laboratory Practice (GLP) standards; however, work was done in a GLP facility following Standard Operating Procedures (p. 3 of MRID 49623401). Signed and dated Data Confidentiality, No Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-5).
- Classification:** With SAX Clean-up: This report is classified as **Unacceptable**. The method including the optional SAX clean-up was not attempted or validated by the ILV. In the ECM, the number of samples was insufficient for all analyses with SAX clean-up. No representative chromatograms were provided. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. This report is upgradeable with the submission of an ILV report.
- Without SAX Clean-up: This report is classified as **Unacceptable**. The communications between the ILV and ECM staff were not reported or discussed. Reagent blanks were not included in the ECM and ILV. The ECM contained deficiencies for supporting the method for indoxacarb: unacceptable recovery results at the LOQ in both soils; unsatisfactory linearity for the quantification ion; and unsupportive representative chromatograms for the specificity of the method in soil. This report is upgradeable with the submission of information on the communications

between the ILV and ECM staff sufficient to determine whether the ILV was independent.

**PC Code:** 067710  
**Reviewer:** Lewis Ross Brown, III  
 Environmental Biologist

**Signature:** *Lewis Ross Brown, III*  
**Date:** March 18, 2016

## Executive Summary

This analytical method, DuPont Study No. DuPont-41157, is designed for the quantitative determination of indoxacarb (DPX-KN128) and metabolites, IN-MP819 and IN-JT333 in soil and sediment at the LOQ (1.0 ppb; 0.001 mg/kg) using LC/MS/MS. The LOQ is less than the lowest known toxicological level of concern in soil/sediment for all analytes. The method contained an optional SAX clean-up procedure. The ECM validated the method without the optional SAX clean-up procedure using sandy loam and silt loam soils and clay loam sediment. The ECM validated the method with the optional SAX clean-up procedure using only clay loam sediment. The ILV only performed the method without the optional SAX clean-up; therefore, the method including the optional SAX clean-up was not validated by the ILV. The number of trials was not specified, but the reviewer assumed that the method (without the optional SAX clean-up) was validated by the ILV with the first trial for all analytes using loam soil and clay loam sediment. Several significant deficiencies were noted in the ECM data for the method with SAX clean-up, including an insufficient number of samples for all analyses and no representative chromatograms. For the method without SAX clean-up, the ECM contained several deficiencies for supporting the method for indoxacarb: unacceptable recovery results at the LOQ in soil; unsatisfactory linearity for the quantification ion; and unrepresentative chromatograms for the specificity of the method in soil. In the ILV, linearity was not satisfactory for the linear regressions of indoxacarb. The linearity was not satisfactory for the confirmation ion of IN-JT333 in the ECM and ILV. Representative chromatograms were not provided for both soil matrices in the ECM, and baseline interference was noted for IN-MP819. Reagent blanks were not included in the ECM and ILV. Additionally, the communications between the ILV and ECM were not reported or discussed.

**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									
Indoxacarb	49599603 <sup>1</sup>	49623401 <sup>2</sup>		Sediment & Soil	19/06/2014	E.I. du Pont de Nemours and Company	LC/MS/MS without SAX clean-up	0.001 mg/kg (1.0 ppb)			
IN-MP819											
IN-JT333											
Indoxacarb	49599603 <sup>1</sup>	None		Sediment Only						LC/MS/MS with SAX clean-up	
IN-MP819											
IN-JT333											

1 In the ECM, Sassafras soil (sandy loam; 8% clay, 1.7% organic matter), Drummer soil (silt loam; 18% clay, 3.9% organic matter) and Goose River Sediment (clay loam; 29% clay, 3.2% organic matter) were used (p. 13 of MRID 49599603). The sources were only identified as field test sites located in the USA.

2 In the ILV, 88 NJ 01 Nascna soil (loam; 25% clay, 2.0% organic matter) and Goose River Sediment (clay loam; 33% clay, 6.0% organic matter) were used (p. 15 of MRID 49623401). The sources were only identified as field test sites located in the USA.

## I. Principle of the Method

Samples ( $5 \text{ g} \pm 1\%$ ) were measured into 50-mL polypropylene centrifuge tubes and fortified, as necessary (pp. 11, 14 of MRID 49599603). The samples were allowed to dry in a fume hood for *ca.* 15 minutes. Two  $\frac{1}{4}$ " steel balls and 10 mL of acetonitrile:0.025% aqueous acetic acid (80:20, v:v) were added to each sample. After *ca.* 5 minutes of soak-time, the samples were placed on a genogrinder and homogenized for 3 minutes at *ca.* 1200 strokes per minutes. After centrifugation (5 minutes at *ca.* 3000 rpm), the supernatants were transferred to clean 50-mL centrifuge tubes. The extraction was repeated using 10 mL of acetonitrile:0.025% aqueous acetic acid (90:10, v:v) then 10 mL of acetonitrile. The volume of the combined extracts was adjusted to 30 mL using acetonitrile. After mixing the combined extracts (vortex mixer for *ca.* 30 seconds), a 10-mL aliquot was transferred to a centrifuge tube and combined with 10  $\mu\text{L}$  of concentrated acetic acid. The extract was evaporated to *ca.* 5 mL on an N-EVAP nitrogen evaporator set at  $40^\circ\text{C}$ . The volume of the residue was adjusted to 5 mL with acetonitrile. After sonication (*ca.* 5 minutes) and vortex mixing (*ca.* 30 seconds), a 300- $\mu\text{L}$  aliquot was transferred to an auto-sampler vial (optional clean-up procedure at this step, see below). After diluting the sample with 700  $\mu\text{L}$  of 0.01 M aqueous acetic acid, the sample was mixed via vortex mixer and analyzed by reversed-phase LC/MS/MS.

The ECM study authors noted that the extracts would be stable for *ca.* 72 hours if stored at  $4^\circ\text{C}$  (p. 14 of MRID 49599603). The study authors also noted that an optional clean-up procedure was recommended if poor recoveries occur due to LC/MS matrix effects or if poor separation of analyte peaks was observed. The analyte extraction procedure was the same as reported above. After sonication (*ca.* 5 minutes) and vortex mixing (*ca.* 30 seconds), 0.25 g of bulk Bondesal SAX material (PN 12213042, 40  $\mu\text{M}$ , 100 gram, Agilent, Wilmington, Do not substitute) was added to the 5-mL sample (Appendix 4, p. 76). The sample was hand-shaken for *ca.* 30 seconds then centrifuged for 10 minutes at 3000 rpm. A 300- $\mu\text{L}$  aliquot (of the supernatant) was transferred to an auto-sampler vial. After diluting the sample with 700  $\mu\text{L}$  of 0.01 M aqueous acetic acid, the sample was mixed via vortex mixer and analyzed by reversed-phase LC/MS/MS. The ECM study authors noted that the extracts would be stable for *ca.* 72 hours if stored at  $4^\circ\text{C}$ .

Samples were analyzed for indoxacarb and metabolites using an Agilent HP1290 HPLC coupled to an API 5000 triple quadrupole mass spectrometer using Turbo Ion Spray (pp. 9-10, 14-16; Appendix 3, pp. 66-75 of MRID 49599603). The reversed-phase HPLC/MS/MS conditions consisted of an Ace Excel 2 C18-AR column (3.0 x 50 mm, 2- $\mu\text{m}$ , column temperature  $40^\circ\text{C}$ ), a mobile phase gradient of (A) 0.01 M aqueous acetic acid and (B) acetonitrile [percent A:B (v:v) at 0.0-1.0 min. 50:50, 6.0-8.0 min. 1:99, 8.1-10.0 min. 50:50], and MS/MS detection in positive ion mode with Multiple Reaction Monitoring (MRM). Two parent-daughter ion transitions were monitored for each analyte (quantitative and confirmatory, respectively):  $m/z$  528.1  $\rightarrow$  203.0 and  $m/z$  528.1  $\rightarrow$  150.1 for indoxacarb;  $m/z$  470.2  $\rightarrow$  238.1 and  $m/z$  470.2  $\rightarrow$  205.9 for IN-MP819; and  $m/z$  470.2  $\rightarrow$  267.1 and  $m/z$  470.2  $\rightarrow$  207.1 for IN-JT333. Retention times were 2.95, 3.02 and 3.27 minutes for indoxacarb, IN-MP819 and IN-JT333, respectively. Injection volume was 0.020 mL.

The ECM study authors noted the following special precaution: analyte-contaminated glassware must be thoroughly rinsed with acetonitrile prior to normal glassware cleaning procedures due to the tendency of indoxacarb and IN-JT333 to adhere to surfaces when in water (p. 11 of MRID 49599603).

In the ILV, DuPont-41157 was performed as written, except that a Shimadzu LC-10ADVP coupled with an API 4000 triple quadrupole mass spectrometer using Turbo Ion Spray was used as the analytical instrument, and a Multi-Tube Vortexer was used instead of a genogrinder (pp. 15-18 of MRID 49623401). The injection volume was increased to 0.050 mL. The two monitored parent-daughter ion transitions were the same as those of the ECM. Retention times were 3.26, 3.30 and 3.62 minutes for indoxacarb, IN-MP819 and IN-JT333, respectively.

In both the ECM and ILV, the LOQ for all analytes was reported as 0.0010 mg/kg (1.0 ppb; 1.0 µg/kg; pp. 8-9, 19 of MRID 49599603; pp. 9, 11, 21 of MRID 49623401). The LOD for all analytes was *ca.* 0.3 ppb and *ca.* 0.5 ppb in the ECM and ILV, respectively.

## II. Recovery Findings

ECM (MRID 49599603): Without optional SAX clean-up procedure, mean recoveries and relative standard deviations (RSD) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of indoxacarb, IN-MP819 and IN-JT333 in sandy loam and silt loam soils and clay loam sediment at the LOQ (1.0 ppb) and 10×LOQ (10 ppb), except for LOQ recovery results of indoxacarb in Sassafras soil (216-224% mean, 121-125% RSD) and Drummer soil (126-132% mean, 57-60% RSD; uncorrected recovery results; p. 17; Tables 1-2, pp. 22-27; DER Attachment 2). All results were calculated by the study authors, except those for LOQ recovery results of indoxacarb in Sassafras and Drummer soils. The study authors calculated the statistics for the LOQ recovery results of indoxacarb in Sassafras and Drummer soils with the exclusion of one outlier per sample set (n = 4). The recovery statistics (n = 4) which were calculated by the study authors were as follows (quantitation ion and confirmation ion, respectively): 103 ± 8.8% (RSD 8.5%) and 96 ± 8.3% (RSD 8.6%) for Sassafras soil; and 98 ± 21.1% (RSD 21.7%) and 94 ± 12.1% (RSD 12.9%) for Drummer soil (these recovery results were within the acceptable ranges of the guidelines). The results for these analyses which were presented in the study report were reviewer-calculated using all reported values. With optional SAX clean-up procedure, mean recoveries and relative standard deviations (RSD) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of indoxacarb, IN-MP819 and IN-JT333 in sandy loam and silt loam soils and clay loam sediment at the LOQ (1.0 ppb) and 10×LOQ (10 ppb); however, the number of samples was insufficient for all analyses (n = 3 for LOQ; n = 2 for 10×LOQ; Appendix 4, pp. 77-78). For all analyses with or without the optional SAX clean-up, two parent-daughter ion transitions were monitored (Tables 1-2, pp. 22-27; Appendix 4, pp. 77-78). The results of both ion transitions were reported for all analytes; the results were comparable. The soil/sediment matrices were well characterized (p. 13). Sassafras soil (sandy loam; 64% sand, 28% silt, 8% clay; 1.7% organic matter), Drummer soil (silt loam; 23% sand, 59% silt, 18% clay; 3.9% organic matter) and Goose River Sediment (clay loam; 49% sand, 22% silt, 29% clay; 3.2% organic matter) were used. The sources were only identified as field test sites located in the USA.

ILV (MRID 49623401): Without optional SAX clean-up procedure, mean recoveries and RSDs were within guidelines (mean 70-120%; RSD ≤20%) for analysis of indoxacarb, IN-MP819 and IN-JT333 in loam soil and clay loam sediment at the LOQ (1.0 ppb) and 10×LOQ (10 ppb; uncorrected recovery results; pp. 19-20; Tables 1-2, pp. 24-31). Two parent-daughter ion transitions were monitored. The results of both ion transitions were reported for all analytes; the results were comparable. The soil/sediment matrices were well characterized by Agvise Laboratories, Northwood, North Dakota (p. 15). 88 NJ 01 Nascna soil (loam; 28% sand, 47% silt, 25% clay; 2.0% organic matter) and Goose River Sediment (clay loam; 28% sand, 39% silt, 33% clay; 6.0% organic

matter) were used. The sources were only identified as field test sites located in the USA. The number of trials was not specified, but the reviewer assumed that the method was validated with the first trial for all analytes (pp. 9-10, 23). Only the method without the optional SAX clean-up was performed (pp. 15-16).

**Table 2. Initial Validation Method Recoveries for Indoxacarb (DPX-KN128) and Metabolites, IN-MP819 and IN-JT333, in Soil and Sediment<sup>1,2</sup>**

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Method Without SAX Clean-up</b>						
<b>Sassafras Soil – Sandy Loam</b>						
Quantitation ion						
Indoxacarb (DPX-KN128)	1.0 (LOQ)	5 <sup>3</sup>	94-710	<b>224</b>	272	<b>121</b>
	10	5	79-93	85	5.3	6.2
IN-MP819	1.0 (LOQ)	5	82-110	95	13.4	14.0
	10	5	81-91	84	4.1	4.8
IN-JT333	1.0 (LOQ)	5	89-98	95	3.6	3.7
	10	5	87-92	90	2.3	2.6
Confirmation ion						
Indoxacarb (DPX-KN128)	1.0 (LOQ)	5 <sup>3</sup>	90-698	<b>216</b>	269	<b>125</b>
	10	5	82-102	91	7.8	8.6
IN-MP819	1.0 (LOQ)	5	84-112	100	11.3	11.3
	10	5	75-82	79	2.6	3.3
IN-JT333	1.0 (LOQ)	5	89-99	93	3.8	4.1
	10	5	87-91	89	1.6	1.9
<b>Drummer Soil – Silt Loam</b>						
Quantitation ion						
Indoxacarb (DPX-KN128)	1.0 (LOQ)	5 <sup>3</sup>	80-268	<b>132</b>	78	<b>60</b>
	10	5	87-94	91	2.4	2.6
IN-MP819	1.0 (LOQ)	5	87-102	94	7.3	7.7
	10	5	80-89	83	3.8	4.6
IN-JT333	1.0 (LOQ)	5	87-103	95	7.1	7.4
	10	5	85-91	88	2.3	2.6
Confirmation ion						
Indoxacarb (DPX-KN128)	1.0 (LOQ)	5 <sup>3</sup>	80-253	<b>126</b>	72	<b>57</b>
	10	5	85-93	89	3.2	3.6
IN-MP819	1.0 (LOQ)	5	81-102	91	10.0	11.0
	10	5	76-89	82	4.8	5.8
IN-JT333	1.0 (LOQ)	5	90-96	93	2.4	2.6
	10	5	88-94	90	2.5	2.8
<b>Goose River Sediment – Clay Loam</b>						
Quantitation ion						
Indoxacarb	1.0 (LOQ)	5	86-99	93	4.9	5.3

(DPX-KN128)	10	5	79-97	89	7.0	7.9
IN-MP819	1.0 (LOQ)	5	83-93	88	3.8	4.4
	10	5	80-91	85	4.0	4.7
IN-JT333	1.0 (LOQ)	5	93-105	97	4.8	5.0
	10	5	81-93	88	5.0	5.7
Confirmation ion						
Indoxacarb (DPX-KN128)	1.0 (LOQ)	5	86-103	97	7.6	7.8
	10	5	87-101	93	5.7	6.1
IN-MP819	1.0 (LOQ)	5	82-106	94	9.2	9.9
	10	5	72-79	75	2.6	3.4
IN-JT333	1.0 (LOQ)	5	88-105	97	6.5	6.8
	10	5	84-96	89	5.6	6.3
<b>Method With SAX Clean-up</b>						
<b>Goose River Sediment – Clay Loam</b>						
Quantitation ion						
Indoxacarb (DPX-KN128)	1.0 (LOQ)	<b>3</b>	87-112	99	12.5	12.6
	10	<b>2</b>	84, 96	90	8.5	9.4
IN-MP819	1.0 (LOQ)	<b>3</b>	93-104	97	6.1	6.3
	10	<b>2</b>	84, 86	85	1.4	1.7
IN-JT333	1.0 (LOQ)	<b>3</b>	88-96	93	4.4	4.7
	10	<b>2</b>	87, 90	89	2.1	2.4
Confirmation ion						
Indoxacarb (DPX-KN128)	1.0 (LOQ)	<b>3</b>	104-124	111	11.5	10.4
	10	<b>2</b>	91	91	0.0	0.0
IN-MP819	1.0 (LOQ)	<b>3</b>	80-103	92	11.6	12.6
	10	<b>2</b>	77, 78	78	0.7	0.9
IN-JT333	1.0 (LOQ)	<b>3</b>	88-95	92	3.8	4.1
	10	<b>2</b>	90, 92	91	1.4	1.6

Data (recovery results) were obtained from Tables 1-2, pp. 22-27 of MRID 49599603. All results were calculated by the study authors, except those of indoxacarb in the two soils at the LOQ (see DER Attachment 2; see Footnote #3).

Reported sample recoveries in the study report were corrected for any residues found in the matrix blanks; however, no residues were detected in the controls (p. 17; Tables 1-2, pp. 22-27).

1 Soil/sediment matrices were well characterized (p. 13). Sassafras soil (sandy loam; 64% sand, 28% silt, 8% clay; 1.7% organic matter), Drummer soil (silt loam; 23% sand, 59% silt, 18% clay; 3.9% organic matter) and Goose River Sediment (clay loam; 49% sand, 22% silt, 29% clay; 3.2% organic matter) were used. The sources were only identified as field test sites located in the USA.

2 Two parent-daughter ion transitions were monitored for each analyte (quantitative and confirmatory, respectively):  $m/z$  528.1  $\rightarrow$  203.0 and  $m/z$  528.1  $\rightarrow$  150.1 for indoxacarb;  $m/z$  470.2  $\rightarrow$  238.1 and  $m/z$  470.2  $\rightarrow$  205.9 for IN-MP819; and  $m/z$  470.2  $\rightarrow$  267.1 and  $m/z$  470.2  $\rightarrow$  207.1 for IN-JT333 (pp. 15-16).

3 The study authors calculated the statistics for the LOQ recovery results of indoxacarb in Sassafras and Drummer soils with the exclusion of one outlier per sample set. The excluded recovery values were 710% (Q) and 698% (C) for the Sassafras soil and 268% (Q) and 253% (C) for the Drummer soil (Tables 1-2, pp. 22, 25). The recovery statistics ( $n = 4$ ) which were calculated by the study authors were as follows (quantitation ion and confirmation ion, respectively):  $103 \pm 8.8\%$  (RSD 8.5%) and  $96 \pm 8.3\%$  (RSD 8.6%) for Sassafras soil; and  $98 \pm 21.1\%$  (RSD 21.7%) and  $94 \pm 12.1\%$  (RSD 12.9%) for Drummer soil.

**Table 3. Independent Validation Method Recoveries for Indoxacarb (DPX-KN128) and Metabolites, IN-MP819 and IN-JT333, in Soil and Sediment<sup>1,2</sup>**

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Method Without SAX Clean-up</b>						
<b>88 NJ 01 Nascna Soil - Loam</b>						
Quantitation ion						
Indoxacarb (DPX-KN128)	1.0 (LOQ)	5	72.6-106	88.6	11.7	13.2
	10	5	77.2-115	89.1	16.1	18.0
IN-MP819	1.0 (LOQ)	5	71.0-98.6	85.0	10.4	12.2
	10	5	81.0-117	91.4	14.8	16.2
IN-JT333	1.0 (LOQ)	5	84.8-102	91.3	6.55	7.18
	10	5	84.8-114	93.8	12.3	13.1
Confirmation ion						
Indoxacarb (DPX-KN128)	1.0 (LOQ)	5	75.7-107	90.4	11.7	13.0
	10	5	71.3-110	83.7	15.5	18.5
IN-MP819	1.0 (LOQ)	5	70.1-93.9	81.0	10.9	13.5
	10	5	79.5-121	91.9	17.3	18.8
IN-JT333	1.0 (LOQ)	5	77.5-107	89.2	11.2	12.6
	10	5	81.3-117	91.8	14.5	15.8
<b>Goose River Sediment – Clay Loam</b>						
Quantitation ion						
Indoxacarb (DPX-KN128)	1.0 (LOQ)	5	85.4-99.4	95.0	5.60	5.89
	10	5	77.0-87.4	80.9	3.93	4.86
IN-MP819	1.0 (LOQ)	5	88.5-101	95.4	4.88	5.11
	10	5	80.5-89.6	83.7	3.46	4.13
IN-JT333	1.0 (LOQ)	5	93.0-106	97.8	4.88	4.99
	10	5	84.1-92.6	88.2	3.11	3.52
Confirmation ion						
Indoxacarb (DPX-KN128)	1.0 (LOQ)	5	84.9-103	94.0	6.47	6.89
	10	5	77.2-88.0	82.1	4.39	5.34
IN-MP819	1.0 (LOQ)	5	91.5-99.5	96.1	3.72	3.87
	10	5	81.2-92.7	86.6	4.52	5.22
IN-JT333	1.0 (LOQ)	5	92.4-105	98.0	5.06	5.16
	10	5	85.4-93.9	88.0	3.53	4.01

Data (recovery results) were obtained from Tables 1-2, pp. 24-31 of MRID 49623401. Reported sample recoveries in the study report were corrected for any residues found in the matrix blanks; however, no residues were detected in the controls (pp. 19-20; Tables 1-2, pp. 24-31).

1 The soil/sediment matrices were well characterized by Agvise Laboratories, Northwood, North Dakota (p. 15). 88 NJ 01 Nascna soil (loam; 28% sand, 47% silt, 25% clay; 2.0% organic matter) and Goose River Sediment (clay loam; 28% sand, 39% silt, 33% clay; 6.0% organic matter) were used. The sources were only identified as field test sites located in the USA.

2 Two parent-daughter ion transitions were monitored for each analyte (quantitative and confirmatory, respectively):  $m/z$  528.1  $\rightarrow$  203.0 and  $m/z$  528.1  $\rightarrow$  150.1 for indoxacarb;  $m/z$  470.2  $\rightarrow$  238.1 and  $m/z$  470.2  $\rightarrow$  205.9 for IN-MP819; and  $m/z$  470.2  $\rightarrow$  267.1 and  $m/z$  470.2  $\rightarrow$  207.1 for IN-JT333 (pp. 17-18 of MRID 49623401).

### III. Method Characteristics

In both the ECM and ILV, the LOQ for all analytes was reported as 0.0010 mg/kg (1.0 ppb; 1.0 µg/kg; pp. 8-9, 19 of MRID 49599603; pp. 9, 11, 21 of MRID 49623401). In the ECM and ILV, the LOQ was defined as the lowest fortification level which obtained average recoveries of 70-120% and a RSD <20%. The LOQ also corresponded to the fortification in which analyte peak heights were consistently *ca.* 10-20 times the signal in the control at the retention time of the analyte for the lowest responding analyte. The LOD for all analytes was *ca.* 0.3 ppb and *ca.* 0.5 ppb in the ECM and ILV, respectively. In the ECM and ILV, the LOD was estimated for each analyte based on signal-to-noise. The LOD was defined as the concentration of IN-MP819, the least responsive analyte, at which analyte peaks were approximately three times the chromatographic baseline noise observed near the retention time or approximately 1/3 the concentration of the LOQ

**Table 4. Method Characteristics**

		Indoxacarb (DPX-KN128)	IN-MP819	IN-JT333
Limit of Quantitation (LOQ)		1.0 ppb (0.0010 mg/kg; 1.0 µg/kg)		
Limit of Detection (LOD)	ECM	<i>ca.</i> 0.3 ppb		
	ILV	<i>ca.</i> 0.5 ppb		
Linearity (calibration curve $r^2$ and concentration range)	ECM	$r^2 = 0.9911$ (Q) $r^2 = 0.9981$ (C)	$r^2 = 0.9997$ (Q) $r^2 = 0.9979$ (C)	$r^2 = 0.9961$ (Q) $r^2 = 0.9865$ (C)
	ILV <sup>1</sup>	$r^2 = 0.9904$ (Q) $r^2 = 0.9898$ (C)	$r^2 = 0.9962$ (Q) $r^2 = 0.9950$ (C)	$r^2 = 0.9952$ (Q) $r^2 = 0.9936$ (C)
	Conc. Range	0.050-5.0 ng/mL		
Repeatable	ECM <sup>2</sup>	<b>Method Without SAX Clean-up</b>		
		No at the LOQ (n=5) in soil matrices [Sassafras soil ( <b>216-224%</b> mean, <b>121-125%</b> RSD) and Drummer soil ( <b>126-132%</b> mean, <b>57-60%</b> RSD)]. <sup>3</sup>	Yes at the LOQ and 10×LOQ (n=5) in soil and sediment matrices.	
		Yes at 10×LOQ (n=5) in soil matrices.		
	Yes at the LOQ and 10×LOQ (n=5) in sediment matrix.	<b>Method With SAX Clean-up</b>		
	Yes at the LOQ and 10×LOQ in sediment matrix (only matrix used); however, n = <b>3</b> at LOQ and n = <b>2</b> at 10×LOQ.			
ILV <sup>4</sup>	<b>Method Without SAX Clean-up<sup>5</sup></b>			
Yes at the LOQ and 10×LOQ (n=5) in soil and sediment matrices.				
Reproducible	Yes at the LOQ and 10×LOQ in soil and sediment matrices.			
Specific	ECM	Only chromatograms of Sassafras soil and Goose River sediment were provided. Only chromatograms of the method without SAX clean-up were provided.		
		Matrix interferences were <i>ca.</i> 17% and <i>ca.</i> 7% of the	Yes, no matrix interferences were observed in soil and	Yes, no matrix interferences were observed.



		LOQ in soil and sediment, respectively. <sup>6</sup>  Baseline noise was <b>extremely significant</b> in soil, causing the analyte peak to be raised twice its height in the LOQ chromatogram.	sediment; however, baseline noise interfered with peak integration at the LOQ, more extreme interference with the confirmation ion in which interference was also observed in the 10×LOQ chromatogram.	
	ILV	Yes, no matrix interferences were observed; however, minor baseline noise interfered with peak integration at the LOQ, more extreme interference with the confirmation ion.	Yes, no matrix interferences were observed; however, minor baseline noise interfered with peak integration at the LOQ, more extreme interference with the confirmation ion. Also, a couple of contaminant peaks with retention times near that of the analyte were observed in the confirmation ion chromatogram.	Yes, no matrix interferences were observed; however, minor baseline noise interfered with peak integration at the LOQ, more extreme interference with the confirmation ion.

Data were obtained from pp. 8-9, 19; Tables 1-2, pp. 22-27; Figure 3, pp. 32-33; Figure 5, pp. 43-60; Appendix 4, pp. 77-78 of MRID 49599603; pp. 9, 11, 21; Tables 1-2, pp. 24-31; Figure 3, pp. 36-38; Figure 5, pp. 48-65 of MRID 49623401. Q = quantitative ion; C = confirmatory ion.

- In the ILV, standard curves were weighted 1/x. ILV  $r^2$  values are reviewer-generated for all analytes from reported  $r$  values of 0.9952-0.9981 (Q) and 0.9949-0.9975 (C; calculated from data in Figure 3, pp. 36-38 of MRID 49623401; see DER Attachment 2).
- In the ECM, Sassafras soil (sandy loam; 8% clay, 1.7% organic matter), Drummer soil (silt loam; 18% clay, 3.9% organic matter) and Goose River Sediment (clay loam; 29% clay, 3.2% organic matter) were used (p. 13 of MRID 49599603). The sources were only identified as field test sites located in the USA.
- The reviewer calculated the recovery results based on all results provided in the study report (Tables 1-2, pp. 22-27 of MRID 49599603). The study authors calculated the statistics for the LOQ recovery results of indoxacarb in Sassafras and Drummer soils with the exclusion of one outlier per sample set. The excluded recovery values were 710% (Q) and 698% (C) for the Sassafras soil and 268% (Q) and 253% (C) for the Drummer soil. The recovery statistics ( $n = 4$ ) which were calculated by the study authors were as follows (quantitation ion and confirmation ion, respectively):  $103 \pm 8.8\%$  (RSD 8.5%) and  $96 \pm 8.3\%$  (RSD 8.6%) for Sassafras soil; and  $98 \pm 21.1\%$  (RSD 21.7%) and  $94 \pm 12.1\%$  (RSD 12.9%) for Drummer soil.
- In the ILV, 88 NJ 01 Nascna soil (loam; 25% clay; 2.0% organic matter) and Goose River Sediment (clay loam; 33% clay, 6.0% organic matter) were used (p. 15 of MRID 49623401). The sources were only identified as field test sites located in the USA.
- The ILV only performed the method without the optional SAX clean-up (pp. 15-16 of MRID 49623401).
- Based on peak area counts reported in the indoxacarb representative chromatograms (Figure 5, pp. 43, 46, 52, 55 of MRID 49599603).  
Linearity is satisfactory when  $r^2 \geq 0.995$ .

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

1. The analytical method, DuPont Study No. DuPont-41157, contained an optional SAX clean-up (pp. 11, 14; Appendix 4, p. 76 of MRID 49599603). Results were provided for the method with and without the optional SAX clean-up. The method without the optional SAX clean-up was the main method presented in the ECM. The ILV only performed the method without the optional SAX clean-up (pp. 15-16 of MRID 49623401). Therefore, the method including the optional SAX clean-up was not validated by the ILV.

For the ECM method including the optional SAX clean-up, the number of samples was insufficient for all analytes at the LOQ ( $n = 3$ ) and  $10\times$ LOQ ( $n = 2$ ), and no representative chromatograms were provided (Appendix 4, pp. 76-78 of MRID 49599603). OCSPP guidelines recommend a minimum of five spiked replicates to be analyzed at each concentration (*i.e.*, minimally, the LOQ and  $10\times$  LOQ) for each analyte. OCSPP guidelines also recommend that representative chromatograms are provided for all analytes/matrices/methods.

2. The validation of analytical method, DuPont Study No. DuPont-41157 without the optional SAX clean-up, by the originating laboratory contained several deficiencies for supporting the method for indoxacarb in soil and sediment: 1) recovery results at the LOQ in both soils were unacceptable; 2) linearity was not satisfactory for the quantification ion; and 3) the representative chromatograms did not support the specificity of the method for analyses in soil (Tables 1-2, pp. 22-27; Figure 3, pp. 32-33; Figure 5, pp. 43-60; Appendix 4, pp. 77-78 of MRID 49599603)

The LOQ recovery results of indoxacarb in Sassafras (216-224% mean, 121-125% RSD) and Drummer soils (126-132% mean, 57-60% RSD) did not meet OCSPP guideline recommendations (mean 70-120%; RSD  $\leq 20\%$ ; reviewer-calculated, uncorrected recovery results; p. 17; Tables 1-2, pp. 22-27 of MRID 49599603; DER Attachment 2). These results were reviewer-calculated using all reported values ( $n = 5$ ). The study authors calculated the statistics for the LOQ recovery results of indoxacarb in Sassafras and Drummer soils with the exclusion of one outlier per sample set. The excluded recovery values were 710% (Q) and 698% (C) for the Sassafras soil and 268% (Q) and 253% (C) for the Drummer soil. The recovery statistics ( $n = 4$ ) which were calculated by the study authors were as follows (quantitation ion and confirmation ion, respectively):  $103 \pm 8.8\%$  (RSD 8.5%) and  $96 \pm 8.3\%$  (RSD 8.6%) for Sassafras soil; and  $98 \pm 21.1\%$  (RSD 21.7%) and  $94 \pm 12.1\%$  (RSD 12.9%) for Drummer soil (these recovery results were within the acceptable ranges of the guidelines).

Linearity was not satisfactory for the quantification ion of indoxacarb ( $r^2 = 0.9911$ ; Figure 3, pp. 32-33 of MRID 49599603). Linearity is satisfactory when  $r^2 \geq 0.995$ . Linear regressions were not matrix-matched.

The representative chromatograms of indoxacarb in soil did not support the specificity of the method for the soil matrix (Figure 5, pp. 43-60 of MRID 49599603). The baseline noise was extremely significant in soil, causing the analyte peak to be raised twice its height in the LOQ chromatogram (Figure 5, p. 46). Matrix interferences were *ca.* 17% and *ca.* 7% of the LOQ in soil and sediment, respectively (% recovery was reported as “-“ in Tables 1-2, pp.

22-27). In soil, the matrix interferences were slightly greater than 50% of the LOD (*ca.* 0.3 ppb; *ca.* 30% of the LOQ). Additionally, chromatograms of the reagent blank and all analyses in Drummer soil were not provided.

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. The LOQ was defined as the lowest fortification level which obtained average recoveries of 70-120% and a RSD <20% (pp. 8-9, 19 of MRID 49599603; pp. 9, 11, 21 of MRID 49623401). The LOQ also corresponded to the fortification in which analyte peak heights were consistently *ca.* 10-20 times the signal in the control at the retention time of the analyte for the lowest responding analyte. The LOD was estimated for each analyte based on signal-to-noise. The LOD was defined as the concentration of IN-MP819, the least responsive analyte, at which analyte peaks were approximately three times the chromatographic baseline noise observed near the retention time or approximately 1/3 the concentration of the LOQ. The LOQ and LOD were not adequately supported by calculations. The LOD of the ECM differed from that of the ILV.

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

4. In the ILV, linearity was not satisfactory for the linear regressions of indoxacarb and the confirmation ion of IN-JT333 (see above). In the ECM, linearity was also not satisfactory for the linear regression of the confirmation ion of IN-JT333 (see above). Linearity is satisfactory when  $r^2 \geq 0.995$ . Linear regressions were not matrix-matched.
5. In the ILV, the representative chromatograms showed minor baseline noise which interfered with peak integration at the LOQ; this interference was more extreme in the confirmation ion chromatograms (Figure 5, pp. 48-65 of MRID 49623401). In confirmation ion chromatograms of IN-MP819 in soil and sediment, a couple of contaminant peaks were observed at retention times near the retention time of the analyte. Also, a reagent blank was not included.
6. In the ECM, representative chromatograms were not provided for the Drummer soil or the extract from method with optional SAX clean-up (Figure 5, pp. 43-60 of MRID 49599603). OCSPP guidelines recommend that representative chromatograms are provided for all analytes/matrices/methods. In provided chromatograms of IN-MP819 in soil and sediment, baseline noise interfered with peak integration at the LOQ; this interference was more extreme in the confirmation ion chromatograms in which the baseline noise also interfered with the peak integration at 10×LOQ. Issues in provided chromatograms of indoxacarb were discussed above. Also, a reagent blank was not included.
7. The communications between the ILV study author and ECM study monitor were not reported or discussed.
8. The soil sources were only identified as field test sites located in the USA in the ECM and ILV (p. 13 of MRID 49599603; p. 15 of MRID 49623401).

9. The ILV study author noted that the chance of carryover in auto-samplers can be minimized by using needle washes and injecting blank samples after injecting high concentration standards (p. 22 of MRID 49623401). No method modifications were recommended by the ILV; the method was performed without any significant modifications (p. 11).
10. The reviewer noted a significant typographical error in ILV MRID 49623401: the LOD was reported as “approximately 0.05 ppb” (p. 21), instead of approximately 0.5 ppb (pp. 9, 11).
11. It was reported for the ILV that 10-12 samples were processed in an 8-hour workday (p. 21 of MRID 49623401). The LC/MS/MS analysis was performed unattended overnight.

## 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.

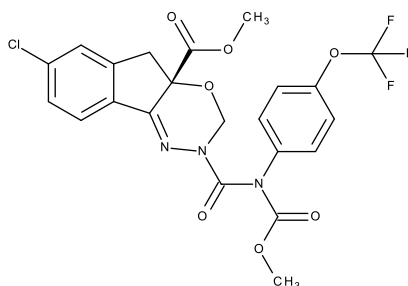
**Attachment 1: Chemical Names and Structures****Indoxacarb (DPX-KN128)**

**IUPAC Name:** Methyl (S)-N-[7-chloro-2,3,4a,5-tetrahydro-4a-(methoxycarbonyl)indeno[1,2-e][1,3,4]oxadiazin-2-ylcarbonyl]-4-(trifluoromethoxy)carbanilate.

**CAS Name:** Methyl (4aS)-7-chloro-2,5-dihydro-2-[[[(methoxycarbonyl)[4-(trifluoromethoxy)phenyl]amino]carbonyl]indeno[1,2-e][1,3,4]oxadiazine-4a(3H)-carboxylate.  
(S)-methyl 7-chloro-2,5-dihydro-2-[[[(methoxycarbonyl)[4-(trifluoromethoxy)phenyl]amino]carbonyl]=indeno[1,2-e][1,3,4]oxadiazine-4a(3H)-carboxylate.

**CAS Number:** 173584-44-6

**SMILES String:** COC(=O)[C@]12Cc3cc(ccc3C1=NN(CO2)C(=O)N(c4ccc(cc4)OC(F)(F)F)C(=O)OC)Cl

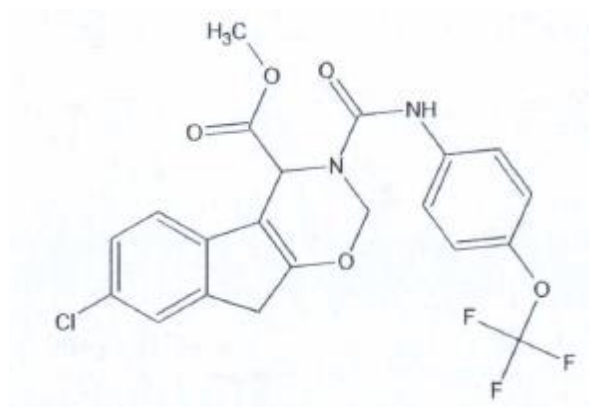
**IN-MP819**

**IUPAC Name:** Not reported

**CAS Name:** Methyl ester 7-chloro-3,5-dihydro-2-[[[4-(trifluoromethoxy)phenyl]amino]carbonyl]-indeno[1.2-e][1,3,4]oxadiazine-1(2H)-carboxylic acid.

**CAS Number:** Not reported

**SMILES String:** Not found



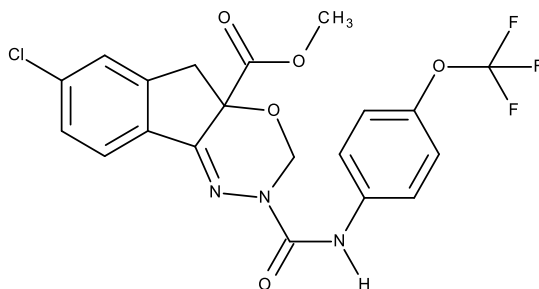
**IN-JT333**

**IUPAC Name:** Methyl 7-chloro-2-[[4-(trifluoromethoxy)phenyl]carbamoyl]-3,5-dihydroindeno[1,2-e][1,3,4]oxadiazine-4a-carboxylate.

**CAS Name:** Methyl 7-chloro-2,5-dihydro-2-[[[4-(trifluoromethoxy)phenyl]amino)carbonyl]indeno[1,2-e][1,3,4]oxadiazine-4a(3H)-carboxylate.

**CAS Number:** 144171-39-1

**SMILES String:** [H]N(c1ccc(cc1)OC(F)(F)F)C(=O)N2COC3(Cc4cc(ccc4C3=N2)Cl)C(=O)OC



**Test Material:** Indoxacarb

**MRID:** 49599603

**Title:** Analytical Method for the Determination of Indoxacarb and Metabolites in Soil and Sediment Using LC/MS/MS

**MRID:** 49623401

**Title:** Independent Laboratory Validation of Analytical Method For The Determination of Indoxacarb and Metabolites In Soil And Sediment Using LC/MS/MS

**EPA PC Code:** 067710

**OCSPP Guideline:** 850.6100

**For CDM Smith**

**Primary Reviewer:** Lisa Muto

**Signature:** 

**Date:** 12/1/15

**Secondary Reviewer:** Kathleen Ferguson

**Signature:** 

**Date:** 12/1/15

**QC/QA Manager:** Joan Gaidos

**Signature:** 

**Date:** 12/1/15