

Analytical method for dicloran in soil

Reports: ECM: EPA MRID No.: 51056602. Smith, R.J. 2020. Environmental Chemistry Method: Validation of the Analytical Method for the Determination of Dicloran in Soil by LC-MS/MS. Report prepared by Smithers (formerly Smithers Viscient), Wareham, Massachusetts, and sponsored and submitted by Gowan Company, Yuma, Arizona; 61 pages. Smithers Viscient Study No.: 12791.6320. Sponsor Protocol/Project No.: EXG-2019-20. Final report issued January 14, 2020.

ILV: EPA MRID No.: 51254805. Cashmore, A., and O. Idialu. 2020. Independent Laboratory Validation of Analytical Method 12791.6320 for the Determination of Dicloran in Soil. Report prepared by Smithers ERS Limited, North Yorkshire, United Kingdom, and sponsored and submitted by Gowan Company, Yuma, Arizona; 71 pages. Study No.: 3202456. Final report issued July 8, 2020.

Document No.: MRIDs 51056602 & 51254805

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160), as accepted by OECD GLP (1998), with the following exception: the reference substance, dicloran Pestanal, was non-GLP characterized prior to use in the study (p. 3 of MRID 51056602). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). An Authenticity statement was included with the Quality Assurance statement.

ILV: The study was conducted in compliance with United Kingdom (1999) GLP standards, as amended by GLP (2004), and OECD GLP (1998), as well as the United Kingdom Department of Health, with the following exception: some characterization of the sandy loam test soil by Fraunhofer IME (a GLP facility; p. 3; Appendix 6, p. 71 of MRID 51254805). The study was suitable for submission to US FDA, USEPA, and Japanese regulatory authorities. Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5). An Authenticity statement was also included with the GLP and Quality Assurance statements (pp. 3-4).

Classification: This analytical method is classified as supplemental. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method.


PC Code: 031301

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**CDM/CSS-
Dynamac JV**

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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 JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Smithers Viscient Study No. 12791.6320, is designed for the quantitative determination of dicloran in soil at the stated LOQ of 0.05 mg/kg using LC-MS/MS. The LOQ is less than the lowest toxicological level of concern in soil for dicloran. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for dicloran in the tested soil matrices (0.05 mg/kg).

The ECM validated the method using characterized sandy loam and loamy sand soil matrices; the ILV validated the method using characterized sandy loam and silt loam soil matrices. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method and if the ILV soil matrices covered the range of soils used in the terrestrial field dissipation studies since no dicloran terrestrial field dissipation studies were submitted. The ILV validated the method for dicloran in the first trial for both soils with insignificant modifications to the analytical parameters. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for dicloran in test soil matrices. Matrix-matched calibration standards were used when matrix effects were determined to be close to $\pm 20\%$ (i.e., $> -18\%$) in the ECM and ILV for loamy sand and silt loam soils, respectively. Representative chromatograms of these two matrices showed significant contaminants and/or baseline noise around the analyte peak.

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						
Dicloran	51056602 ¹	51254805 ²		Soil	14/01/2020	Gowan Company	LC- MS/MS	0.05 mg/kg

1 In the ECM, sandy loam soil (SMV Batch No. 24Oct18Soil-A; 64% sand, 17% silt, 19% clay; pH 6.6 in 1:1 soil:water ratio; 3.7% organic matter) collected from Grand Forks, North Dakota, and loamy sand soil (SMV Batch No. 041917B; 83% sand, 16% silt, 1% clay; pH 6.6 in 1:1 soil:water ratio; 13.5% organic matter) collected from Rochester, Massachusetts, were used in the study (USDA soil texture classification; p. 13 of MRID 51056602). The soils were characterized by Agvise Laboratories, Northwood, North Dakota.

2 In the ILV, sandy loam soil (Refe Sol 01-A; ID: CS 30/18; 74% sand, 20% silt, 6% clay; pH 6.4 in water; pH 5.3 in 0.01M CaCl₂; 0.9% organic carbon) from Schmallenberg, Northrhine-Westphalia, Germany, and silt loam soil (Newhaven; ID: CS 17/18; 25% sand, 51% silt, 24% clay; pH 6.0 in water; pH 5.4 in 0.01M CaCl₂; 3.2% organic carbon) from Newhaven, Derbyshire, United Kingdom, were sourced by Smithers ERS and used in the study (USDA soil texture classification; p. 12; Appendix 2, pp. 55-56 of MRID 51254805). The soils were characterized by Fraunhofer IME and Smithers Visient (ERS) Ltd., United Kingdom.

I. Principle of the Method

Soil samples (5 g dry wt.) were fortified (0.25 mL of 1.00 or 10.0 mg/L fortification solution) and extracted twice with 20 mL with acetonitrile via sonication for 10 minutes, shaking on a shaker table for 30 minutes (at 250 rpm), and centrifugation at 3000 rpm for 10 minutes (pp. 16-17 of MRID 51056602). The volume of the combined supernatants was adjusted to 50 mL with acetonitrile. The LOQ samples (2.00 mL aliquot) were diluted 50xs with acetonitrile:purified reagent water (20:80, v:v). The 10×LOQ samples (0.4 mL aliquot) of the sandy loam soil samples were diluted 250xs with acetonitrile:purified reagent water (20:80, v:v). The 10×LOQ samples (0.2 mL aliquot) of the loamy sand soil samples were diluted 250xs with the matrix-matched control loamy sand diluent [control final fraction which had been diluted with acetonitrile:purified reagent water (20:80, v:v)]. The diluted sample was centrifuged at 13,000 rpm for 5 minutes, then an aliquot was taken for LC-MS/MS analysis.

Samples were analyzed for dicloran using a Shimadzu LC-20ADXR HPLC coupled with an AB MDS Sciex 5000 MS with an ESI Turbo V ion source operated in the positive ion mode with multiple reaction monitoring (MRM; pp. 12, 18 of MRID 51056602). The following LC conditions were used: Phenomenex Kinetex, 2.6 μ m phenyl-hexyl column (3 x 50 mm, 2.6 μ m; column temperature 40°C), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.01-0.50 min. 80:20, 3.00-4.00 min. 0.00:100, 4.10-5.50 min. 80:20] and injection volume of 100 μ L. MS source temperature was 550°C. Two ion pair transitions were monitored for dicloran (quantitation and confirmation, respectively): m/z 207.1→190.0 and m/z 207.1→160.0. Reported retention time was *ca.* 3.0 minutes for dicloran.

The ILV performed the ECM method (Smithers Viscient Method No. 12791.6320) as written, except for insignificant modifications to the analytical parameters (pp. 13-17; Appendix 3, pp. 58-68 of MRID 51254805). Samples were analyzed for dicloran using Shimadzu Nexera series HPLC coupled with an AB Sciex API 5000 Triple Quadrupole LC-MS/MS. The LC-MS/MS parameters were the same as those of the ECM, with the exception that the injection volume was 50 μ L (10 μ L for repeat matrix assessment) and some minor MS parameters. Two ion pair transitions were monitored for dicloran (quantitation and confirmation, respectively): m/z 207→190 and m/z 207→160. These ion transitions were similar to those of the ECM. Reported retention time was *ca.* 2.5 minutes for dicloran. The ILV noted that the LC column and mobile phase solvents could not be modified. The ILV modifications did not warrant an updated ECM.

The Limit of Quantification (LOQ) for dicloran in soil was 0.05 mg/kg in the ECM and ILV (pp. 10, 20-25 of MRID 51056602; pp. 10, 21-24 of MRID 51254805). In the ECM, the Limit of Detection (LOD) for dicloran was calculated as 0.0003-0.0006 mg/kg for sandy loam soil and 0.001 mg/kg for loamy sand soil. In the ILV, the LOD for dicloran was calculated as 0.00426-0.00511 mg/kg for sandy loam soil and 0.00560-0.00714 mg/kg for silt loam soil. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

II. Recovery Findings

ECM (MRID 51056602): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of dicloran at fortification levels of 0.05 mg/kg (LOQ) and 0.5 mg/kg (10 \times LOQ) in two soil matrices (Tables 1-4, pp. 28-31). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses. The reviewer noted that all results were comparable, except for the results of the LOQ analyses in sandy loam soil which were significantly less than all other results. The sandy loam soil (SMV Batch No. 24Oct18Soil-A; 64% sand, 17% silt, 19% clay; pH 6.6 in 1:1 soil:water ratio; 3.7% organic matter) collected from Grand Forks, North Dakota, and loamy sand soil (SMV Batch No. 041917B; 83% sand, 16% silt, 1% clay; pH 6.6 in 1:1 soil:water ratio; 13.5% organic matter) collected from Rochester, Massachusetts, were used in the study (USDA soil texture classification; p. 13). The soils were characterized by Agvise Laboratories, Northwood, North Dakota.

ILV (MRID 51254805): Mean recoveries and RSDs were within guidelines for analysis of dicloran at fortification levels of 0.05 mg/kg (LOQ) and 0.5 mg/kg (10 \times LOQ) in one soil matrix (Tables 1-4, pp. 27-30). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses. The sandy loam soil (Refe Sol 01-A; ID: CS 30/18; 74% sand, 20% silt, 6% clay; pH 6.4 in water; pH 5.3 in 0.01M CaCl₂; 0.9% organic carbon) from Schmalleberg, Northrhine-Westphalia, Germany, and silt loam soil (Newhaven; ID: CS 17/18; 25% sand, 51% silt, 24% clay; pH 6.0 in water; pH 5.4 in 0.01M CaCl₂; 3.2% organic carbon) from Newhaven, Derbyshire, United Kingdom, were sourced by Smithers ERS and used in the study (USDA soil texture classification; p. 12; Appendix 2, pp. 55-56). The soils were characterized by Fraunhofer IME and Smithers Viscient (ERS) Ltd., United Kingdom. The method for dicloran in soil was validated in the first trial with insignificant modifications to the analytical parameters (pp. 10, 21-24; Appendix 4, p. 69).

Table 2. Initial Validation Method Recoveries for Dicloran in Soil^{1,2}

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
Sandy Loam Soil						
Quantitation ion transition						
Dicloran	0.05 (LOQ)	5	82.0-88.2	84.6	2.76	3.27
	0.5	5	95.2-119	105	10.0	9.59
Confirmation ion transition						
Dicloran	0.05 (LOQ)	5	84.2-87.1	85.8	1.33	1.55
	0.5	5	96.6-116	105	7.82	7.45
Loamy Sand Soil						
Quantitation ion transition						
Dicloran	0.05 (LOQ)	5	94.5-111	105	6.58	6.28
	0.5	5	97.7-109	105	4.30	4.11
Confirmation ion transition						
Dicloran	0.05 (LOQ)	5	101-107	104	2.50	2.40
	0.5	5	98.5-106	103	3.13	3.04

Data (uncorrected recovery results; p. 20) were obtained from Tables 1-4, pp. 28-31 of MRID 51056602.

1 The sandy loam soil (SMV Batch No. 24Oct18Soil-A; 64% sand, 17% silt, 19% clay; pH 6.6 in 1:1 soil:water ratio; 3.7% organic matter) collected from Grand Forks, North Dakota, and loamy sand soil (SMV Batch No. 041917B; 83% sand, 16% silt, 1% clay; pH 6.6 in 1:1 soil:water ratio; 13.5% organic matter) collected from Rochester, Massachusetts, were used in the study (USDA soil texture classification; p. 13). The soils were characterized by Agvise Laboratories, Northwood, North Dakota. The the soil texture was verified by the reviewer using USDA-NRCS technical support tools.

2 Two ion pair transitions were monitored for dicloran (quantitation and confirmation, respectively): m/z 207.1→190.0 and m/z 207.1→160.0.

Table 3. Independent Validation Method Recoveries for Dicloran in Soil^{1,2,3}

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam Soil						
Quantitation ion transition						
Dicloran	0.05 (LOQ)	5	92.4-113	98.8	8.48	8.58
	0.5	5	104-114	109	3.98	3.65
Confirmation ion transition						
Dicloran	0.05 (LOQ)	5	92.4-110	101	6.91	6.85
	0.5	5	96.8-114	106	6.70	6.35
Silt Loam Soil						
Quantitation ion transition						
Dicloran	0.05 (LOQ)	5	79.8-95.4	87.3	7.59	8.69
	0.5	5	104-113	108	3.95	3.66
Confirmation ion transition						
Dicloran	0.05 (LOQ)	5	91.6-108	99.0	7.15	7.22
	0.5	5	91.0-106	98.2	5.56	5.66

Data (uncorrected recovery results; p. 18) were obtained from Tables 1-4, pp. 27-30 of MRID 51254805.

1 The sandy loam soil (Refe Sol 01-A; ID: CS 30/18; 74% sand, 20% silt, 6% clay; pH 6.4 in water; pH 5.3 in 0.01M CaCl₂; 0.9% organic carbon) from Schmallenberg, Northrhine-Westphalia, Germany, and silt loam soil (Newhaven; ID: CS 17/18; 25% sand, 51% silt, 24% clay; pH 6.0 in water; pH 5.4 in 0.01M CaCl₂; 3.2% organic carbon) from Newhaven, Derbyshire, United Kingdom, were sourced by Smithers ERS and used in the study (USDA soil texture classification; p. 12; Appendix 2, pp. 55-56). The soils were characterized by Fraunhofer IME and Smithers Viscient (ERS) Ltd., United Kingdom. The the soil texture was verified by the reviewer using USDA-NRCS technical support tools.

2 Two ion pair transitions were monitored for dicloran (quantitation and confirmation, respectively): m/z 207→190 and m/z 207→160. These ion transitions were similar to those of the ECM.

III. Method Characteristics

The LOQ for dicloran in soil was 0.05 mg/kg in the ECM and ILV (pp. 10, 20-25 of MRID 51056602; pp. 10, 21-24 of MRID 51254805). In the ECM and ILV, the LOQ was defined as the lowest fortification level validated. Also, in the ECM, the LOQ was defined as the level which the blank values did not exceed 30% of the LOQ. In the ECM, the LOD was calculated as 0.0003-0.0006 mg/kg for sandy loam soil and 0.001 mg/kg for loamy sand soil from the signal-to-noise response of each analyte in matrix at the LOQ level using the following equation:

$$\text{LOD} = (3 \times N_{\text{ctl}}) / (\text{Resp}_{\text{LS}}) \times \text{Conc}_{\text{LS}} \times \text{DF}_{\text{CNTL}}$$

Where, LOD is the limit of detection of the analysis, N_{ctl} is the mean signal to noise in height of the control samples (or blanks), Resp_{LS} is the mean response in height of the two low calibration standards, Conc_{LS} is the concentration of the low calibration standard, and DF_{CNTL} is the dilution factor of the control samples (smallest dilution factor used, i.e., 50.0 mL/g).

The LOD for dicloran in soil was estimated in the ILV as 0.00426-0.00511 mg/kg for sandy loam soil and 0.00560-0.00714 mg/kg for silt loam soil at 3 x baseline noise for the primary and confirmatory transitions.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

Table 4. Method Characteristics in Soil

		Dicloran
Limit of Quantitation (LOQ)*	ECM	0.05 mg/kg
	ILV	
Limit of Detection (LOD)	ECM (calc)	0.0003-0.0006 mg/kg (SL) 0.001 mg/kg (LS)
	ILV (calc)	0.00426-0.00511 mg/kg (SL) 0.00560-0.00714 mg/kg (SIL)
Linearity (calibration curve r and concentration range)	ECM ¹	r = 0.9955 (Q & C, SL) r = 0.9990 (Q, LS) r = 0.9985 (C, LS)
	ILV	r = 0.9954 (Q, SL) r = 0.9956 (C, SL) r = 0.9950 (Q & C, SIL)
	Range	0.5-5 µg/L
Repeatable	ECM ²	Yes at LOQ (0.05 mg/kg) and 10×LOQ (0.50 mg/kg) (two characterized soil matrices)
	ILV ^{3,4}	
Reproducible		Yes for 0.05 mg/kg (LLMV)* and 0.50 mg/kg in soil matrices
Specific	ECM	Yes, matrix interferences were <1% of the LOQ (based on peak area). Significant baseline noise was observed in the loamy sand soil, but the interference was not near the RT of the analyte. ⁵
	ILV	Yes, no matrix interferences were observed at the RT of the analyte (<i>ca.</i> 2.5 min.); however, a nearby significant contaminant (RT <i>ca.</i> 2.18 min.; peak height <i>ca.</i> 150% of LOQ peak height) was observed in the silt loam soil. ⁶

Data were obtained from pp. 10, 20-25 (LOQ/LOD); Tables 1-4, pp. 28-31 (recovery results); p. 22; Figures 11-14, pp. 46-49 (calibration curves); Figures 1-10, pp. 36-45 (chromatograms) of MRID 51056602; pp. 10, 21-24 (LOQ/LOD); Tables 1-4, pp. 27-30 (recovery results); Figures 1-2, pp. 36-37; Figures 15-16, pp. 44-45 (calibration curves); Figures 3-28, pp. 38-51 (chromatograms) of MRID 51254805; DER Excel Attachment. Q = quantitation ion transition; C = confirmation ion transition; SL = Sandy Loam Soil; LS = Loamy Sand Soil; SIL = Silt Loam Soil.

* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 ECM correlation coefficients (r) were reviewer-calculated based on r² values reported in the study report (p. 22; Figures 11-14, pp. 46-49 of MRID 51056602; DER Excel Attachment). In the ECM, matrix effects were insignificant (<±20%) for both soils; however, solvent-based calibration standards were used for sandy loam soil and matrix-matched calibration standards were used for loamy sand soil (p. 21; Tables 5-8, pp. 32-35 of MRID 51056602). In the ILV, matrix effects were insignificant (<±20%) for both soils; however, solvent-based calibration standards were used for sandy loam soil and matrix-matched calibration standards were used for silt loam soil (pp. 21-24 of MRID 51254805).

2 In the ECM, sandy loam soil (SMV Batch No. 24Oct18Soil-A; 64% sand, 17% silt, 19% clay; pH 6.6 in 1:1 soil:water ratio; 3.7% organic matter) collected from Grand Forks, North Dakota, and loamy sand soil (SMV Batch No. 041917B; 83% sand, 16% silt, 1% clay; pH 6.6 in 1:1 soil:water ratio; 13.5% organic matter) collected from Rochester, Massachusetts, were used in the study (USDA soil texture classification; p. 13 of MRID 51056602). The soils were characterized by Agvise Laboratories, Northwood, North Dakota. The the soil texture was verified by the reviewer using USDA-NRCS technical support tools.

3 In the ILV, sandy loam soil (Refe Sol 01-A; ID: CS 30/18; 74% sand, 20% silt, 6% clay; pH 6.4 in water; pH 5.3 in 0.01M CaCl₂; 0.9% organic carbon) from Schmallenberg, Northrhine-Westphalia, Germany, and silt loam soil (Newhaven; ID: CS 17/18; 25% sand, 51% silt, 24% clay; pH 6.0 in water; pH 5.4 in 0.01M CaCl₂; 3.2% organic carbon) from Newhaven, Derbyshire, United Kingdom, were sourced by Smithers ERS and used in the study (USDA soil texture classification; p. 12; Appendix 2, pp. 55-56 of MRID 51254805). The soils were characterized by Fraunhofer IME and Smithers Viscient (ERS) Ltd., United Kingdom.

4 The ILV validated the method for dicloran in the first trial with insignificant modifications to the analytical parameters (pp. 10, 21-24; Appendix 4, p. 69 of MRID 51254805). The ILV modifications did not warrant an updated ECM.

5 See Figure 8, p. 43 of MRID 51056602.

6 See Figure 25, p. 50 of MRID 51254805.

IV. Method Deficiencies and Reviewer's Comments

1. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ (pp. 10, 20-25 of MRID 51056602; pp. 10, 21-24 of MRID 51254805). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for dicloran in the tested soil matrices (0.05 mg/kg).
2. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method. In the ILV, sandy loam soil (6% clay; 0.9% organic carbon) and silt loam soil (24% clay; 3.2% organic carbon) were used in the study (p. 12; Appendix 2, pp. 55-56 of MRID 51254805). In the ECM, sandy loam soil (19% clay; 3.7% organic matter) and loamy sand soil (1% clay; 13.5% organic matter) were used in the study (p. 13 of MRID 51056602). OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs. Additionally, it could not be determined if the ILV soil matrices covered the range of soils used in the terrestrial field dissipation studies since no dicloran terrestrial field dissipation studies were submitted. Relevant submitted dicloran studies included the following: MRID 51056601 (aerobic soil metabolism; loam, 22% clay, 6.2% organic carbon; loam, 22% clay, 0.84% organic carbon; Tables 1-2, pp. 36-37 of MRID 51056601); MRID 51254801 (anaerobic soil metabolism, loam, 22% clay, 6.2% organic carbon; loam, 22% clay, 0.84% organic carbon; Table 1, p. 42 of MRID 51254801); and MRID 51254802 (aerobic aquatic metabolism, silt loam, 13% clay, 4.2% organic carbon; sand, 0% clay, 0.61% organic carbon; p. 15; Tables 1-3, pp. 42-44 of MRID 51254802).
3. Even though matrix effects were insignificant ($\leq \pm 20\%$) for all test soils in the ECM and ILV, matrix-matched calibration standards were used for loamy sand soil in the ECM and for silt loam soil in the ILV (p. 21; Tables 5-8, pp. 32-35 of MRID 51056602; pp. 21-24 of MRID 51254805). The reason for the use of matrix-matched calibration standards was reported as a "precaution" since the matrix effect was close to 20% (p. 21; Table 7, p. 34 of MRID 51056602; pp. 21, 23 of MRID 51254805). The reviewer believed that the ECM should consider an update with the standard use of matrix-matched solvents.
4. The specificity of the method at the LOQ was not well-supported by ILV silt loam representative chromatograms and ECM loamy sand representative chromatograms (Figure 8, p. 43 of MRID 51056602; Figure 25, p. 50 of MRID 51254805). Significant contaminants and/or baseline noise were observed around the analyte peak. Matrix-matched calibration standards were used for these matrices.
5. The communications between the ILV study authors (Angela Cashmore and Ofure Idialu, Smithers ERS Limited) and ILV Study Monitor (James T. Cole, Gowan Company) were not

summarized (p. 1; Appendix 5, p. 70 of MRID 51254805). Reported communications included: protocol issue and the results of the first attempt of the ILV.

6. The reviewer noted that the ECM and ILV laboratories were part of the same company, Smithers (formerly Smithers Viscient) and Smithers ERS Limited, respectively (pp. 1, 5-6 of MRID 51056602; pp. 1, 6 of MRID 51254805). The laboratory location, personnel and equipment differed between the two laboratories. The only exchange of information was the ECM Method/Protocol provided to the ILV via the Sponsor Representative (Appendix 3, pp. 57-68; Appendix 5, p. 70 of MRID 51254805).
7. The reviewer noted that all ECM performance data results were comparable, except for the results of the LOQ analyses in sandy loam soil which were significantly less than all other results (Tables 1-4, pp. 28-31 of MRID 51056602).
8. The determinations of the LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 10, 20-25 of MRID 51056602; pp. 10, 21-24 of MRID 51254805). In the ECM and ILV, the LOQ was defined as the lowest fortification level validated. Also, in the ECM, the LOQ was defined as the level which the blank values did not exceed 30% of the LOQ. No further justification of the LOQ was reported in the ECM or ILV. The LOD was calculated in the ECM using the following equation: $LOD = (3 \times N_{ctl}) / (Res_{PLS}) \times Con_{CLS} \times DF_{CNTL}$, where, LOD is the limit of detection of the analysis, N_{ctl} is the mean noise in height of the control samples (or blanks), Res_{PLS} is the mean response in height of the two low calibration standards, Con_{CLS} is the concentration of the low calibration standard, and DF_{CNTL} is the dilution factor of the control samples (smallest dilution factor used, i.e., 50.0 mL/g). The LOD was estimated in the ILV using the following equation: 3 x baseline noise for the primary and confirmatory transitions. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

The method detection limit (MDL) was calculated in the ECM and ILV as dependent upon the lowest concentration calibration standard and the dilution factor of the controls (p. 21 of MRID 51056602; p. 21 of MRID 51254805). In the ECM, the MDL was equivalent to $0.500 \mu\text{g/L} \times 50.0 \text{ mL/g} \times (1 \text{ L}/1000 \text{ mL}) = 0.0250 \text{ mg/kg}$; in the ILV, the MDL was equivalent to $25 \mu\text{g/kg}$ for dicloran ($0.5 \mu\text{g/L} \times 50 \text{ mL/g}$). This MDL calculation was not in accordance with the EPA Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 (2016).

9. The total time required to complete one set of samples was not reported in the ECM or ILV.

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.
- USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at: <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides>.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 344-347, and Revision 2; 2015 and 2016.

Attachment 1: Chemical Names and Structures**Dicloran**

IUPAC Name: 2,6-Dichloro-4-nitroaniline
CAS Name: 2,6-Dichloro-4-nitrobenzenamine
CAS Number: 99-30-9
SMILES String: ClC1=CC([N+](=[O-])=O)=CC(Cl)=C1N

