# Analytical method for dicloran in water

**Reports:** ECM: EPA MRID No.: 51018204. Masse, A.M. 2019. Environmental Chemistry Method: Validation of the Analytical Method for the Determination of Dicloran in Ground Water and Surface Water by LC-MS/MS. Report prepared by Smithers (formerly Smithers Viscient), Wareham, Massachusetts, and sponsored and submitted by Gowan Company, Yuma, Arizona; 67 pages. Smithers Viscient Study No.: 12791.6319. Sponsor Protocol/Project No.: EXG-2019-21. Final report issued September 26, 2019.

ILV: EPA MRID No.: 51254804. Cashmore, A., and O. Idialu. 2020. Independent Laboratory Validation of Analytical Method 12791.6319 for the Determination of Dicloran in Ground Water and Surface Water by LC-MS/MS. Report prepared by Smithers ERS Limited, North Yorkshire, United Kingdom, and sponsored and submitted by Gowan Company, Yuma, Arizona; 89 pages. Study No.: 3202455. Final report issued August 4, 2020.

**Document No.:** MRIDs 51018204 & 51254804

**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 51018204). 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 (p. 3; Appendix 6, p. 88 of MRID 51254804). 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. The ILV linearity was not acceptable for surface water. The ECM groundwater matrix was not characterized.

**PC Code:** 031301

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| **EFED Final Reviewer:** | Iwona L. Maher<br>Chemist | Signature: IWONA MAHER Digitally signed by IWONA MAHER Date: 2021.06.10 15:20:24 -04'00'<br>Date: 06/10/2021 |
| **CDM/CSS-Dynamac JV Reviewers:** | Lisa Muto, M.S.,<br>Environmental Scientist | Signature: Lisa Muto<br>Date: 01/15/2021 |
| | Mary Samuel, M.S.,<br>Environmental Scientist | Signature: Mary Samuel<br>Date: 01/15/2021 |

*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. 12791.6319, is designed for the quantitative determination of dicloran in water at the stated LOQ of 0.100 µg/L using LC-MS/MS. The LOQ is less than the lowest toxicological level of concern in water 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 water matrices (0.100 µg/L).

The ECM and ILV validated the method using different groundwater and surface water matrices; only the ECM groundwater matrix was not characterized. The ILV validated the method for dicloran in water was validated with insignificant modifications to the analytical parameters. The ILV modifications did not warrant an updated ECM. The validation for ground water analysis was acceptable after three trials for both fortifications. The validation for surface water was acceptable in the first trial at the LOQ fortification and after the second trial for the 10×LOQ fortification. The failures of other validation trials were reportedly due to a missing reagent blank or incorrect fortification concentrations and not issues with the reproducibility of the method.

All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for dicloran in test water matrices, except for ILV linearity for surface water analysis. Representative chromatograms of ILV ground water (trial 3) and ECM ground and surface water showed significant contaminants and/or baseline noise around the analyte peak. Of these matrices, matrix-matched calibration standards were only used for ECM surface water.

**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 | 51018204[1] | 51254804[2] | | Water | 26/09/2019 | Gowan Company | LC-MS/MS | 0.100 µg/L |

[1] In the ECM, ground water (uncharacterized, unadulterated water from a 100-meter bedrock well) and surface water (SMV Lot No. 05Feb19Wat-A; pH 6.57, dissolved oxygen concentration 10.22 mg/L) were used in the study (p. 14 of MRID 51018204). The surface water was collected from Taunton River, Bridgewater, Massachusetts, and was characterized by Smithers Viscient.

[2] In the ILV, ground water (CS 13/18 Borehole; pH 8.0, conductivity 436 µS/cm, dissolved organic carbon 0.00 mg/L; hardness 349 mg/L $CaCO_3$) and surface water (CS 01/20 Fountains Abbey; pH 7.51, conductivity 140 µS/cm, dissolved organic carbon 8.53 mg/L; hardness 132 mg/L $CaCO_3$) were collected by Smithers Viscient ERS (p. 13; Appendix 2, pp. 72-74 of MRID 51254804). The surface water was collected from The Lake, Studley Royal, Ripon, United Kingdom. Water characterization was performed at the ILV.

## I. Principle of the Method

Water samples (100 mL final volume) were fortified (0.100 mL of 0.100 or 1.00 mg/L fortification solution) and adjusted to pH 2 (measured with pH paper) with 0.0200 mL of phosphoric acid (pp. 18-20 of MRID 51018204). Samples were extracted twice with 100 mL of dichloromethane in a separatory funnel. After the extractions were completed and the aqueous layer was removed, the separatory funnel was rinsed with 50 mL of dichloromethane. The volume of the combined extracts and rinsate was reduced to *ca*. 2.00 mL by rotary evaporation at <35°C. To remove excess water from the sample, 100 mL of acetone was added, then the volume was reduced to *ca*. 5.00 mL by rotary evaporation at <35°C. The sample was transferred to a glass centrifuge tube, and the volume was reduced to almost dryness (*ca*. 100 µL). The method noted that the samples should not be reduced to dryness. All samples were reconstituted to 10.0 mL using acetonitrile:purified reagent water (20:80, v:v). The high fortification ground water samples were further diluted by taking 3.00 mL of the sample and diluting to a final volume of 10.0 mL with acetonitrile:purified reagent water (20:80, v:v). The high fortification surface water samples were further diluted by taking 0.300 mL of the sample and diluting to a final volume of 1.00 mL with the matrix blank final extract. An aliquot was taken for LC-MS/MS analysis.

Samples were analyzed for dicloran using a Shimadzu LC-20AD HPLC (surface water) or Shimadzu LC-20ADXR HPLC (ground water) 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. 13, 20-21 of MRID 51018204). The following LC conditions were used: Phenomenex Kinetex, 2.6 µm phenyl-hexyl column (3 mm x 50 mm, 2.6 µm; column temperature 40°C), mobile phase of (A) 0.1% formic acid in reagent grade 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 µL. 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*. 3.1 minutes (ground water) and *ca*. 3.0 minutes (surface water) for dicloran.

The ILV performed the ECM method (Smithers Viscient Method No. 12791.6319) as written, except for insignificant modifications to the analytical parameters (pp. 14-20; Appendix 3, pp. 75-85 of MRID 51254804). 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 10 µL 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 water was 0.100 µg/L in the ECM and ILV (pp. 10, 23-28 of MRID 51018204; pp. 11, 22-28 of MRID 51254804). In the ECM, the Limit of Detection (LOD) for dicloran was calculated as 0.005-0.01 µg/L for ground water and 0.003-0.01 µg/L for surface water. In the ILV, the LOD for dicloran was calculated as 0.0189-0.0346 µg/L for surface water and 0.0200-0.0677 µg/L for ground water. 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 51018204): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of dicloran at fortification levels of 0.100 µg/L (LOQ) and 1.00 µg/L (10×LOQ) in two water matrices (Tables 1-4, pp. 31-34). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses. The groundwater (uncharacterized, unadulterated water from a 100-meter bedrock well) and surface water (SMV Lot No. 05Feb19Wat-A; pH 6.57, dissolved oxygen concentration 10.22 mg/L) were used in the study (p. 14). The surface water was collected from Taunton River, Bridgewater, Massachusetts, and was characterized by Smithers Viscient.

ILV (MRID 51254804): Mean recoveries and RSDs were within guidelines for analysis of dicloran at fortification levels of 0.1 µg/L (LOQ) and 1.0 µg/L (10×LOQ) in two water matrices (Tables 1-6, pp. 31-36). Two ion pair transitions were monitored. For the ground water analysis, performance data from the first and third validation attempts was reported for both fortifications. For the surface water analysis, LOQ performance data was reported from the first validation attempt and 10×LOQ performance data was reported from the second validation attempt. Performance data was comparable between the quantitation and confirmation analyses, although all LOQ recoveries were lower in the quantitation analysis than the confirmation analysis. The groundwater (CS 13/18 Borehole; pH 8.0, conductivity 436 µS/cm, dissolved organic carbon 0.00 mg/L; hardness 349 mg/L $CaCO_3$) and surface water (CS 01/20 Fountains Abbey; pH 7.51, conductivity 140 µS/cm, dissolved organic carbon 8.53 mg/L; hardness 132 mg/L $CaCO_3$) were collected by Smithers Viscient ERS (p. 13; Appendix 2, pp. 72-74). The surface water was collected from The Lake, Studley Royal, Ripon, United Kingdom. Water characterization was performed at the ILV. The method for dicloran in water was validated with insignificant modifications to the analytical parameters (pp. 14-20). The ILV modifications did not warrant an updated ECM. The validation for ground water analysis was acceptable after three trials for both fortifications (pp. 23, 25; Appendix 4, p. 86). The first trial was not considered valid because a reagent blank was not included; however, the reviewer noted that the performance data was acceptable. The second trial was not acceptable because the stock solution was prepared at an incorrect concentration. The validation for surface water was acceptable in the first trial at the LOQ fortification and after the second trial for the 10×LOQ fortification. The first trial for the 10×LOQ fortification was not acceptable because the stock solution was prepared at an incorrect concentration, resulting in a 100×LOQ fortification.

## Table 2. Initial Validation Method Recoveries for Dicloran in Water[1,2]

| Analyte | Fortification Level (µg/L) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%)[3] | Relative Standard Deviation (%) |
|---|---|---|---|---|---|---|
| | **Ground Water** | | | | | |
| | Quantitation ion transition | | | | | |
| Dicloran | 0.100 (LOQ) | 5 | 87.5-109 | 98.7 | 9.28 | 9.40 |
| | 1.00 | 5 | 86.8-91.8 | 89.3 | 1.82 | 2.04 |
| | Confirmation ion transition | | | | | |
| Dicloran | 0.100 (LOQ) | 5 | 92.5-106 | 97.7 | 5.20 | 5.32 |
| | 1.00 | 5 | 86.3-96.0 | 92.2 | 4.05 | 4.40 |
| | **Surface Water** | | | | | |
| | Quantitation ion transition | | | | | |
| Dicloran | 0.100 (LOQ) | 5 | 91.3-123 | 107 | 13.1 | 12.2 |
| | 1.00 | 5 | 76.5-89.0 | 82.9 | 4.50 | 5.43 |
| | Confirmation ion transition | | | | | |
| Dicloran | 0.100 (LOQ) | 5 | 70.5-112 | 90.8 | 15.7 | 17.3 |
| | 1.00 | 5 | 75.2-90.7 | 83.9 | 5.96 | 7.10 |

Data (uncorrected recovery results; pp. 22-23) were obtained from Tables 1-4, pp. 31-34 of MRID 51018204.

1 The ground water (uncharacterized, unadulterated water from a 100-meter bedrock well) and surface water (SMV Lot No. 05Feb19Wat-A; pH 6.57, dissolved oxygen concentration 10.22 mg/L) were used in the study (p. 14). The surface water was collected from Taunton River, Bridgewater, Massachusetts, and was characterized by Smithers Viscient.

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 Water[1,2,3]

| Analyte | Fortification Level (µg/L) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|---|---|---|---|---|---|---|
| | **Borehole Ground Water** | | | | | |
| | First Validation – No reagent blank | | | | | |
| | Quantitation ion transition | | | | | |
| Dicloran | 0.1 (LOQ) | 5 | 63.1-88.1 | 78.7 | 9.49 | 12.1 |
| | 1.0 | 5 | 79.8-84.0 | 81.9 | 1.54 | 1.88 |
| | Confirmation ion transition | | | | | |
| Dicloran | 0.1 (LOQ) | 5 | 80.7-103 | 91.7 | 9.24 | 10.1 |
| | 1.0 | 5 | 74.0-95.5 | 86.2 | 8.30 | 9.63 |
| | Third Validation – Reagent blank included | | | | | |
| | Quantitation ion transition | | | | | |
| Dicloran | 0.1 (LOQ) | 5 | 54.7-89.0 | 75.7 | 13.0 | 17.2 |
| | 1.0 | 5 | 80.5-112 | 96.2 | 13.8 | 14.3 |
| | Confirmation ion transition | | | | | |
| Dicloran | 0.1 (LOQ) | 5 | 76.3-93.0 | 85.9 | 8.47 | 9.86 |
| | 1.0 | 5 | 77.9-112 | 93.6 | 13.2 | 14.1 |
| | | | | | | |
| | **Fountains Abbey Surface Water[3]** | | | | | |
| | Quantitation ion transition | | | | | |
| Dicloran | 0.1 (LOQ) | 5 | 74.6-101 | 86.1 | 9.85 | 11.4 |
| | 1.0 | 5 | 82.4-94.6 | 87.4 | 4.43 | 5.07 |
| | Confirmation ion transition | | | | | |
| Dicloran | 0.1 (LOQ) | 5 | 72.1-89.2 | 81.7 | 6.71 | 8.21 |
| | 1.0 | 5 | 81.1-106 | 96.4 | 9.45 | 9.80 |

Data (uncorrected recovery results; p. 21) were obtained from Tables 1-6, pp. 31-36 of MRID 51254804.

1 The ground water (CS 13/18 Borehole; pH 8.0, conductivity 436 µS/cm, dissolved organic carbon 0.00 mg/L; hardness 349 mg/L $CaCO_3$) and surface water (CS 01/20 Fountains Abbey; pH 7.51, conductivity 140 µS/cm, dissolved organic carbon 8.53 mg/L; hardness 132 mg/L $CaCO_3$) were collected by Smithers Viscient ERS (p. 13; Appendix 2, pp. 72-74). The surface water was collected from The Lake, Studley Royal, Ripon, United Kingdom. Water characterization was performed at the ILV.

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.

3 Data in the study report provided for the first validation attempt at the LOQ and for the second validation attempt for the 10×LOQ.

## III. Method Characteristics

The LOQ for dicloran in water was 0.100 µg/L in the ECM and ILV (pp. 10, 23-28 of MRID 51018204; pp. 11, 22-28 of MRID 51254804). 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 0.005-0.01 µg/L for ground water and 0.003-0.01 µg/L for surface water from the signal-to-noise response of each analyte in matrix at the LOQ level using the following equation:

$$\mathrm{LOD} = (3\mathrm{x}(\mathrm{N}_{\mathrm{ctl}})/(\mathrm{Resp}_{\mathrm{LS}}) \text{ x } \mathrm{Conc}_{\mathrm{LS}} \text{ x } \mathrm{DF}_{\mathrm{CNTL}}$$

Where, LOD is the limit of detection of the analysis, $\mathrm{N}_{\mathrm{ctl}}$ is the mean signal to noise in height of the control samples (or blanks), $\mathrm{Resp}_{\mathrm{LS}}$ is the mean response in height of the two low calibration standards, $\mathrm{Conc}_{\mathrm{LS}}$ is the concentration of the low calibration standard, and $\mathrm{DF}_{\mathrm{CNTL}}$ is the dilution factor of the control samples (smallest dilution factor used, i.e., 0.100).

The LOD for dicloran in water was estimated in the ILV as 0.0189-0.0346 µg/L for surface water and 0.0200-0.0677 µg/L for ground water at 3 x baseline noise for the primary and confirmatory transitions. ILV calculated LODs were reported for the reported data: surface water validation trials 1 (LOQ) and 2 (10×LOQ); and ground water validation trials 1 and 3. The reviewer noted that several calculated LODs were >30% of the LOQ.

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 Water

| | | Dicloran |
|---|---|---|
| Limit of Quantitation (LOQ)* | ECM | 0.100 µg/L |
| | ILV | |
| Limit of Detection (LOD) | ECM (calc) | 0.005 µg/L (Q, GW)<br>0.01 µg/L (C, GW)<br>0.01 µg/L (Q, SW)<br>0.003 µg/L (C, SW) |
| | ILV (calc)[1] | 0.0539[2] µg/L (Q, GW, trial 1)<br>0.0677[3] µg/L (C, GW, trial 1)<br>0.0200 µg/L (Q, GW, trial 3)<br>0.0353[3] µg/L (C, GW, trial 3)<br>0.0314[2] µg/L (Q, SW, LOQ)<br>0.0189 µg/L (C, SW, LOQ)<br>0.0218 µg/L (Q, SW, 10×LOQ)<br>0.0346[3] µg/L (C, SW, 10×LOQ) |
| Linearity (calibration curve r and concentration range) | ECM[4] | r = 0.9990 (Q, GW)<br>r = 0.9985 (C, GW)<br>r = 0.9985 (Q & C, SW) |
| | ILV | r = 0.9967/0.9966 (Q, GW)<br>r = 0.9959/0.9979 (C, GW)<br>r = **0.9946**/**0.9945** (Q, SW)<br>r = 0.9976/0.9961 (C, SW) |
| | Range | 0.5-5.0 µg/L |
| Repeatable | ECM[5] | Yes at LOQ (0.100 µg/L) and 10×LOQ (1.00 µg/L)<br>(one uncharacterized ground water and one characterized surface water) |
| | ILV[6,7,8] | Yes at LOQ (0.1 µg/L) and 10×LOQ (1.0 µg/L)<br>(one characterized ground water and one characterized surface water) |
| Reproducible | | Yes for 0.100 µg/L (LLMV)* and 1.00 µg/L in water matrices |
| Specific | ECM | Yes, no matrix interferences were observed. Significant baseline noise was observed in both waters, but the interference was not near the RT of the analyte.[9] |
| | ILV | Yes, no matrix interferences were observed at the RT of the analyte (*ca.* 2.6 min.); however, nearby significant contaminants (RTs *ca.* 2.18 min. and 2.28 min.; peak height *ca.* 50% of LOQ peak height) were observed in the ground water (third trial).[10] |

Data were obtained from pp. 10, 23-28 (LOQ/LOD); Tables 1-4, pp. 31-34 (recovery results); p. 25; Figures 11-14, pp. 49-52 (calibration curves); Figures 1-10, pp. 39-48 (chromatograms) of MRID 51018204; pp. 11, 22-28 (LOQ/LOD); Tables 1-6, pp. 31-36 (recovery results); Figures 1-4, pp. 40-43; Figures 25-28, pp. 54-57 (calibration curves); Figures 5-50, pp. 44-68 (chromatograms) of MRID 51254804. Q = quantitation ion transition; C = confirmation ion transition; GW = Groundwater; SW = Surface Water.

* 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] ILV calculated LODs were reported for the reported data: surface water validation trials 1 (LOQ) and 2 (10×LOQ); and ground water validation trials 1 and 3.

[2] The calculated LODs were >30% of the LOQ.

[3] The calculated LOD was >30% of the LOQ; however, a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.

[4] ECM correlation coefficients (r) were reviewer-calculated based on $r^2$ values reported in the study report (p. 25; Figures 11-14, pp. 49-52 of MRID 51018204; DER Attachment 2). In the ECM, matrix effects were insignificant (<±20%) for ground water and significant (>±20%) for surface water, so solvent-based calibration standards were used for ground water and matrix-matched calibration standards were used for surface water (p. 24; Tables 5-8, pp. 35-38 of MRID 51018204). In the ILV, matrix effects were insignificant (<±20%) for both water matrices; however,

solvent-based calibration standards were used for ground water and surface water trial 2 (10×LOQ) while matrix-matched calibration standards were used for surface water trial 1 (LOQ; pp. 22, 24-25, 28; Tables 7-8, pp. 37-38 of MRID 51254804).

5 In the ECM, ground water (uncharacterized, unadulterated water from a 100-meter bedrock well) and surface water (SMV Lot No. 05Feb19Wat-A; pH 6.57, dissolved oxygen concentration 10.22 mg/L) were used in the study (p. 14 of MRID 51018204). The surface water was collected from Taunton River, Bridgewater, Massachusetts, and was characterized by Smithers Viscient.

6 In the ILV, ground water (CS 13/18 Borehole; pH 8.0, conductivity 436 µS/cm, dissolved organic carbon 0.00 mg/L; hardness 349 mg/L $CaCO_3$) and surface water (CS 01/20 Fountains Abbey; pH 7.51, conductivity 140 µS/cm, dissolved organic carbon 8.53 mg/L; hardness 132 mg/L $CaCO_3$) were collected by Smithers Viscient ERS (p. 13; Appendix 2, pp. 72-74 of MRID 51254804). The surface water was collected from The Lake, Studley Royal, Ripon, United Kingdom. Water characterization was performed at the ILV.

7 For the ground water analysis, performance data from the first and third validation attempts was reported for both fortifications. For the surface water analysis, LOQ performance data was reported from the first validation attempt and 10×LOQ performance data was reported from the second validation attempt.

8 The ILV validated the method for dicloran in water with insignificant modifications to the analytical parameters (pp. 14-20 of MRID 51254804). The ILV modifications did not warrant an updated ECM. The validation for ground water analysis was acceptable after three trials for both fortifications. The first trial was not considered valid because a reagent blank was not included; however, the reviewer noted that the performance data was acceptable. The second trial was not acceptable because the stock solution was prepared at an incorrect concentration. The validation for surface water was acceptable in the first trial at the LOQ fortification and after the second trial for the 10×LOQ fortification (p. 25; Appendix 4, p. 86). The first trial for the 10×LOQ fortification was not acceptable because the stock solution was prepared at an incorrect concentration, resulting in a 100×LOQ fortification.

9 See Figures 3-4, pp. 41-42 and Figures 8-9, pp. 46-47 of MRID 51018204. The reviewer noted that contaminants were observed near the analyte in the C ion in surface water at the LOQ, but deficiencies in the confirmatory method do not affect the validity of the method since a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.

10 See Figure 45, p. 66 of MRID 51254804.

Linearity is acceptable when r ≥0.995.

## 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, 23-28 of MRID 51018204; pp. 11, 22-28 of MRID 51254804). 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 water matrices (0.100 µg/L).

2. The ILV performed the ground water validation three times and the surface water validation two times; however, in the second surface water validation, only 10×LOQ samples were included (pp. 23, 25; Appendix 4, p. 86 of MRID 51254804). Reported ILV performance data was included for all validations which yielded acceptable results at the LOQ and 10×LOQ. No data from failed validation trials was reported for review. The reasons which the ILV reported for the failure of these validation trials were either a missing reagent blank or incorrect fortification concentrations. The reviewer noted that neither of these reasons were based on the reproducibility of the method; they were due to ILV laboratory errors. No ILV suggestions or modifications were generated in response to the failed validation trials.

3. The ILV linearity was not acceptable for the quantitation ion analysis of surface water in both validation 1 (r = 09946; LOQ; Sequence Val 2; matrix-matched calibrants) and validation 2 (r = 0.9945; 10×LOQ; Sequence Val 3; solvent-based calibrants; Figure 1, p. 40; Figure 3, p. 42 of MRID 51254804). Linearity is acceptable when r ≥0.995. The reviewer noted that the correlation coefficients were rounded to three significant figures in the results summary of the study report (pp. 26-27).

4. The ECM groundwater matrix was not characterized (p. 14 of MRID 51018204). The reviewer noted that ILV surface water matrix (CS 14/18 Fountains Abbey) was only used for matrix assessment; therefore, this surface water matrix was not included in the ILV validation results.

5. In the ECM, matrix effects were insignificant (<±20%) for ground water and significant (>±20%) for surface water, so solvent-based calibration standards were used for ground water and matrix-matched calibration standards were used for surface water (p. 24; Tables 5-8, pp. 35-38 of MRID 51018204). In the ILV, matrix effects were insignificant (<±20%) for both water matrices; however, solvent-based calibration standards were used for ground water and surface water trial 2 (10×LOQ) while matrix-matched calibration standards were used for surface water trial 1 (LOQ; pp. 22, 24-25, 28; Tables 7-8, pp. 37-38 of MRID 51254804). The reason for the use of matrix-matched calibration standards for surface water trial 1 in the ILV was reported as a "precaution" since the matrix-matched calibration standards were used for surface water in the ECM (p. 25 of MRID 51254804). The reviewer believed that the ECM should consider an update with the standard use of matrix-matched solvents for the surface water matrices.

6. The specificity of the method at the LOQ was not well-supported by ILV ground water (trial 3) representative chromatograms and ECM ground and surface water representative chromatograms (Figures 3-4, pp. 41-42; Figures 8-9, pp. 46-47 of MRID 51018204; Figure 45, p. 66 of MRID 51254804). Significant contaminants and/or baseline noise were observed around the analyte peak. Matrix-matched calibration standards were only used for the ECM surface water matrix.

7. 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. 87 of MRID 51254804). Reported communications included**:** protocol issue, the relaying of ILV instrument response issues and resolutions to the Sponsor, and the results of the ILV validations in surface and ground water.

8. 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 51018204; pp. 1, 6 of MRID 51254804). 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. 75-85; Appendix 5, p. 87 of MRID 51254804).

9. The reviewer noted that nearby significant contaminants (RTs *ca*. 2.18 min. and 2.28 min.; peak height *ca*. 50% of LOQ peak height) were observed in ILV LOQ representative chromatograms of the third trial of the ground water (RT of the analyte, *ca*. 2.6 min.; Figure

45, p. 66 of MRID 51254804).

10. 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, 23-28 of MRID 51018204; pp. 11, 22-28 of MRID 51254804). 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 = $(3x(N_{ctl})/(Resp_{LS})$ x $Conc_{LS}$ x $DF_{CNTL}$, where, LOD is the limit of detection of the analysis, $N_{ctl}$ is the mean noise in height of the control samples (or blanks), $Resp_{LS}$ 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., 0.100). 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 reviewer noted that the following calculated ILV quantitation analysis LODs were >30% of the LOQ: validation trial 1 in ground water and the validation trial 1 (LOQ) in surface water (p. 23 of MRID 51254804).

    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 (pp. 23-24 of MRID 51018204; p. 24 of MRID 51254804). In the ECM, the MDL was equivalent to 0.500 µg/L × 0.100 = 0.0500 µg/L; in the ILV, the MDL was also equivalent to 0.05 µg/L for dicloran (0.5 µg/L × 0.100). This MDL calculation was not in accordance with the EPA Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 (2016).

11. 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 |