

Analytical method for 2,4-D Acid, 2,4-D 2-EHE, 2,4-D DMAS, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA in soil and sediment

ECM: EPA MRID No. 50454301. Swaim, L. 2017. Analytical Method Validation for 2,4-D Acid; 2,4-D 2-EHE; 2,4-D DMAS; 2,4-DCP; 2,4-DCA; 4-CP; and 4-CPA in Soil and/or Sediment Matrices. EAG Study No.: 83640. Report prepared by Analytical Bio-Chemistry Laboratories, Inc., a wholly owned subsidiary of EAG, Inc., Columbia, Missouri; sponsored and submitted by Industrial Task Force II on 2,4-D Research Data c/o Dentons US LLP, Washington, D.C.; 327 pages (including 2 unintentional blank pages). Final report issued January 9, 2017.

ILV: EPA MRID No. 50454302. Claussen, F. 2017. 2,4-D: Independent Laboratory Validation of EAG Method No. 83640-M for the Determination of 2,4-D Acid and Metabolites in Soil and Sediment. EPL BAS Study No.: 445G1444. Report prepared by EPL Bio Analytical Services (EPL), Niantic, Illinois; sponsored and submitted by Industrial Task Force II on 2,4-D Research Data, Raleigh, North Carolina; 244 pages. Final report issued November 15, 2017.

Document No.: MRIDs 50454301 & 50454302


Guideline: 850.6100

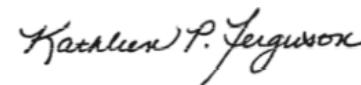
Statements: ECM: The study was conducted in accordance with the USEPA FIFRA Good Laboratory Practices (GLP; 40 CFR Part 160; p. 3 of MRID 50454301). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A statement on the certification of the authenticity of the report was included (p. 5).
ILV: The study was conducted in accordance with the USEPA FIFRA GLP (40 CFR Part 160; p. 3 of MRID 50454302). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A statement on the certification of the authenticity of the report was included in the Quality Assurance statement.

Classification: This analytical method is classified as **Supplemental**. The specificity of the method for 2,4-D acid was not well-supported by ECM and ILV representative chromatograms; consistent quantification could not be achieved due to the poorly defined analyte peaks versus the baseline noise. The LOD was not reported in the ILV. The LOQ exceeds the lowest toxicological level of concern for 2,4-D salts, esters, and degradates (*i.e.*, all analytes except 2,4-D acid).

PC Code: 030001/030019/030063

EFED Final Reviewer: A'ja Duncan, M.S., Ph.D.,
Chemist
Signature:
Date: 9/26/2018

CDM/CSS-Dynamac JV Reviewers: Lisa Muto, M.S.,
Environmental Scientist
Signature: 
Date: 4/12/18

Reviewers: Kathleen Ferguson, Ph.D.,
Environmental Scientist
Signature: 
Date: 4/12/18

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

This analytical method, EAG Method No. 83640-M, is designed for the quantitative determination of 2,4-D Acid and its transformation products 2,4-D 2-EHE, 2,4-D DMAS, and 2,4-DCP in soil and sediment using LC/MS/MS, for the quantitative determination of 2,4-D Acid transformation products 4-CP and 4-CPA in sediment using LC/MS/MS, and for the quantitative determination of 2,4-D Acid transformation product 2,4-DCA in soil and sediment using GC/MS. The method is quantitative for 2,4-D Acid at the stated LOQ of 0.0019 mg/kg and for 2,4-D 2-EHE, 2,4-D DMAS, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA at the stated LOQ of 0.010 mg/kg. The LOQ for 2,4-D Acid is equal to the lowest toxicological level of concern and LOQs for 2,4-D DMAS, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA are greater than the lowest toxicological level of concern (0.0019 mg/kg; USEPA 2013) in soil/sediment for all analytes. The LOQ for 2,4-D 2-EHE is greater than the lowest toxicological level of concern for 2,4-D esters (0.004 mg/kg; USEPA 2013). The ECM used two characterized soil matrices and two characterized sediment matrices. The ILV validated the ECM in the first trial with only insignificant modifications to the analytical instrument or parameters and the identification of the optimization of the APCI probe as a critical step to reduce background signal during 2,4-D Acid analysis. All ECM and ILV data was satisfactory regarding accuracy and precision for all analytes, except for 2,4-D DMAS in one of the ECM soil matrices. All ECM and ILV data was satisfactory regarding linearity and specificity for all analytes, except 2,4-D Acid. The specificity of the method for 2,4-D Acid was not well-supported by ECM and ILV representative chromatograms since the analyte peak was poorly defined versus the baseline noise, which did not allow consistent quantification. The LOD was not reported in the ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
2,4-D Acid	50454301	50454302	Yes	Soil & Sediment	09/01/2017	Industrial Task Force II on 2,4-D Research Data	LC/MS/MS	0.0019 mg/kg
2,4-D 2-EHE								0.010 mg/kg
2,4-D DMAS								
2,4-DCP								
4-CP								
4-CPA								
2,4-DCA							GC/MS	

2 In the ECM, silt loam soil (M983; 35% sand 59% silt 6% clay, pH 5.4, 4.6% organic carbon) obtained from Brierlow, Derbyshire, United Kingdom, Sandy loam soil (M986; 65% sand 28% silt 7% clay, pH 6.0, 0.5% organic carbon) obtained from Hanford, Hickman, California, loamy sand sediment (M940; 85% sand 10% silt 5% clay, pH 8.2, 0.9% organic carbon) obtained from Golden Lake, Steele, North Dakota, and clay loam sediment (M947; 26% sand 38% silt 36% clay, pH 7.8, 3.3% organic carbon) obtained from Goose River, Grand Forks, North Dakota, were used (USDA soil texture classification; p. 24; Table 2, p. 36 of MRID 50454301). The pH values were based on 1:1 soil:water ratio results. All soil/sediment samples were provided by the Sponsor.

3 In the ILV, silt loam soil (M983; 53° 13' 9.4"N, 1° 51' 32.4"W; 35% sand 59% silt 6% clay, pH 5.4, 4.6% organic carbon) obtained from Brierlow, Peak District National Park, United Kingdom, and clay loam sediment (M947; 47° 43'

779°N, W097137.312; 26% sand 38% silt 36% clay, pH 7.8, 3.3% organic carbon) obtained from Goose River, Grand Forks, North Dakota, were used (USDA soil texture classification; pp. 16-17; Appendix C, pp. 232-237 of MRID 50454302). The pH values were based on 1:1 soil:water ratio results. All soil/sediment samples were provided by Dow AgroSciences, LLC; soil classification performed by Agvise Laboratories, Northwood, North Dakota. The ILV soil and sediment matrices matched two of the four matrices used in the ECM.

I. Principle of the Method

Samples (10 g) were weighed into 25 x 150 mm glass culture tubes and fortified as necessary (pp. 21-22, 25-26 of MRID 50454301). Soil samples were fortified with either a mixed fortification solution containing 2,4-D acid, 2,4-DCP and 2,4-DCA in acetone, an individual fortification solution of 2,4-D 2-EHE in acetone, or an individual fortification solution of 2,4-D DMAS in acetone. Sediment samples were fortified with either a mixed fortification solution containing 2,4-D acid, 2,4-DCP, 2,4-DCA, 4-CP and 4-CPA in acetone, an individual fortification solution of 2,4-D 2-EHE in acetone, or an individual fortification solution of 2,4-D DMAS in acetone. The soil/sediment samples were extracted sequentially with 20 mL each of 5% acetic acid in methanol, 5% acetic acid in methanol:5% acetic acid in water (50:50, v:v), and 5% acetic acid in water. For each extraction, the mixture was vortexed for *ca.* 30 seconds, sonicated for *ca.* 20 minutes, centrifuged for *ca.* 10 minutes at *ca.* 2000 rpm. All supernatants were combined, and the volume was adjusted to 100 mL with water.

Fraction A: For 2,4-DCA, a 20-mL aliquot of the final extract from above was transferred into 25 x 150 mm glass culture tubes (pp. 26-27 of MRID 50454301). The sample was extracted twice with iso-octane (2 x 5 mL) via hand-shaking for *ca.* 30 seconds, shaking on a platform shaker for *ca.* 5 minutes, centrifugation (*ca.* 1000 rpm for *ca.* 30 seconds) and decanting. All supernatants were combined, and the volume was reduced to ≤ 1 mL via N-evap set to *ca.* 40°C (not allowing to go to dryness). The residue was reconstituted with 1 mL of iso-octane then diluted to 2 mL with 0.2% peanut oil in iso-octane. Further dilution with 0.2% peanut oil in iso-octane was performed, if necessary, to achieve a level within the standard calibration range. 2,4-DCA was identified and quantified with GC/MS analysis.

Fraction B: For 2,4-D 2-EHE and 2,4-DMAS, a 2-mL aliquot of the final extract from above was transferred into 25 x 150 mm glass culture tubes (pp. 26-27 of MRID 50454301). The sample was hydrolyzed with 10 mL of 1 N NaOH via vortex-mixing then incubating for *ca.* 30 minutes at 40°C (water bath). After cooling to room temperature, 2 mL of 1:1 HCl:water was added with mixing. More 1:1 HCl:water was added if necessary to achieve pH <2. An HLB solid phase extraction (SPE) column was pre-conditioned with one column volumes of methanol then water. The sample was loaded onto the column via gravity. The sample tube was washed with 2 mL water which was added to the column as before. The analytes were collected by washing the sample tube with 2 mL of acetonitrile and applying it to the column. The eluates were collected under strong vacuum. The eluate was diluted to 10 mL with 0.1% aqueous formic acid. Further dilutions were performed with acetonitrile:0.1% formic acid, if necessary, to achieve a level within the standard calibration range. 2,4-D 2-EHE and 2,4-DMAS were identified and quantified via LC/MS/MS analysis.

Fraction C: For 2,4-D Acid, 2,4-DCP, 4-CP and 4-CPA, a 2-mL aliquot of the final extract from above was transferred into 16 x 125 mm glass culture tubes (pp. 27-28 of MRID 50454301). The sample was processed with 12 mL of 0.1 N HCl via vortex-mixing. An HLB solid phase extraction (SPE) column was pre-conditioned with one column volumes of methanol then water. The sample

was loaded onto the column via 0.5-1 mL/min. flow rate. The sample tube was washed with 2 mL water which was added to the column as before. The analytes were collected by washing the sample tube with 2 mL of acetonitrile and applying it to the column. The eluates were collected under strong vacuum. The eluate was reduced to ≤ 1 mL via N-evap set to *ca.* 40°C (not allowing to go to dryness). The residue was reconstituted with 1 mL of acetonitrile then diluted to 5 mL with 0.1% aqueous formic acid. Further dilutions were performed with acetonitrile:0.1% formic acid, if necessary, to achieve a level within the standard calibration range. 2,4-D Acid, 2,4-DCP, 4-CP and 4-CPA were identified and quantified via LC/MS/MS analysis.

Flowcharts: Soil Analysis Flowcharts were provided (Appendix A, pp. 211-212 of MRID 50454301).

GC/MS: Samples were analyzed by an Agilent 7890 Gas Chromatograph [HP-5MS column (30 m x 0.25 mm, 0.25 μ m) using helium carrier gas, injector temperature of 275°C, and oven temperature program of 80°C for 3 min., 80 to 150°C at 10°C/min., 150 to 310°C at 40°C/min., and hold for 5 min.] coupled to an 5975C inert XL EI/CI MSD with triple-axis detection and Multiple Reaction Monitoring (MRM; pp. 18-19 of MRID 50454301). Injection volume was 2.0 μ L. The monitored ions were m/z 178 (quantitation), m/z 161 (confirmation 1), and m/z 163 (confirmation 2) for 2,4-DCA.

LC/MS/MS: Samples were analyzed by an AB-Sciex Q-Trap API 6500 LC/MS/MS system (Phenomenex Synergi Hydro-RP column, 4.6 mm x 75 mm, 4 μ m column; column temperature 40°C) using a mobile phase gradient of (A) 5 mM ammonium acetate in water and (B) methanol [percent A:B at 6.00-7.50 min. 10:90, 7.60-9.00 min. 70:30] with MS/MS detection (APCI) in negative ion mode and Multiple Reaction Monitoring (MRM; pp. 19-21 of MRID 50454301). Two ion transitions were monitored, as follows (quantitation and confirmation, respectively): m/z 219 \rightarrow 161 and m/z 221 \rightarrow 163 for 2,4-D Acid, m/z 161 \rightarrow 125 and m/z 163 \rightarrow 127 for 2,4-DCP, and m/z 185 \rightarrow 127 and m/z 187 \rightarrow 129 for 4-CPA. 2,4-D DMAS and 2,4-D 2-EHE are detected as 2,4-D Acid. Injection volume was 25 μ L.

LC/MS/MS for 4-CP: Samples were analyzed by an AB-Sciex Q-Trap API 6500 LC/MS/MS system (Phenomenex Synergi Hydro-RP column, 4.6 mm x 75 mm, 4 μ m column; column temperature 40°C) using a mobile phase gradient of (A) 5 mM ammonium acetate in water and (B) methanol [percent A:B at 3.00-4.50 min. 10:90, 4.60-6.00 min. 60:40] with MS/MS detection (Electrospray) in negative ion mode and Multiple Reaction Monitoring (MRM; pp. 19-21 of MRID 50454301). Two ion transitions were monitored, as follows (quantitation and confirmation, respectively): m/z 127 \rightarrow 91 and m/z 127 \rightarrow 35 for 4-CP. Injection volume was 35 μ L.

ILV: The ECM was performed as written, except for the following insignificant modifications to the analytical instrument or parameters: DB-5MS column (30 m x 0.25 mm, 0.25 μ m) for GC/MS analysis; Agilent 1290 LC coupled to API 6500 Q-Trap MS/MS for all LC/MS/MS analyses; and injection volume of 20 μ L, instead of 35 μ L for 4-CP (pp. 19-26 of MRID 50454302). All monitored ion transitions were the same as those of the ECM. The ILV noted the following critical step: optimization of the APCI probe in order to reduce the background signal during 2,4-D Acid analysis, which was found to be significant (p. 31).

LOQ/LOD: In the ECM, the method Limit of Quantification (LOQ) and Limit of Detection (LOD) were 0.0019 mg/kg and 0.00057 mg/kg, respectively, for 2,4-D in soil and sediment (pp. 13, 31; Table 29, p. 92 of MRID 50454301). The method LOQ and LOD were 0.010 mg/kg and 0.003

mg/kg, respectively, for all metabolites, 2,4-D 2-EHE, 2,4-D DMAS, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA. In the ILV, the method LOQs were the same as those of the ECM; the LODs were not reported (pp. 15, 30-31 of MRID 50454302). In the ECM and ILV, calculated LODs and/or LOQs generally supported the method LOQs and LODs.

II. Recovery Findings

ECM (MRID 50454301): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of 2,4-D Acid at 0.0019 mg/kg (LOQ), 0.019 mg/kg (10 \times LOQ), and 0.19 mg/kg (100 \times LOQ) in soil and sediment matrices (Tables 3-28, pp. 37-91; DER Attachment 2). Mean recoveries and RSDs were within guideline requirements for analysis of 2,4-D Acid transformation products 2,4-D 2-EHE, 2,4-D DMAS, 2,4-DCP, and 2,4-DCA at 0.010 mg/kg (LOQ), 0.10 mg/kg (10 \times LOQ), and 1.0 mg/kg (100 \times LOQ) in soil and sediment matrices, except for the LOQ quantitation analysis of 2,4-D DMAS in Brierlow silt loam soil (mean 69%). Mean recoveries and RSDs were within guideline requirements for analysis of 2,4-D Acid transformation products 4-CP and 4-CPA at 0.010 mg/kg (LOQ), 0.10 mg/kg (10 \times LOQ), and 1.0 mg/kg (100 \times LOQ) in sediment matrices. LC/MS/MS analysis was used for all analytes, except 2,4-DCA. The metabolite 2,4-DCA was analyzed by GC/MS Analysis. Analytes were identified using two ion transitions via LC/MS/MS or three ions via GC/MS; however, only two ions were quantified for GC/MS analysis. Recoveries of the quantitation and confirmation analyses were comparable. Recoveries for the 0.00057 mg/kg and 0.003 mg/kg LOD samples (n = 1 for each analyte/matrix) were reviewer-calculated since the study author did not calculate these recoveries (see DER Attachment 2). LOD recoveries ranged 0-148% for 2,4-D Acid, 72-112% for 2,4-D 2-EHE, 51-93% for 2,4-D DMAS, 57-71% for 2,4-DCP, 70-94% for 2,4-DCA, 57-89% for 4-CP, and 43-56% of 4-CPA (ions/matrices combined). Silt loam soil (M983; 35% sand 59% silt 6% clay, pH 5.4, 4.6% organic carbon) obtained from Brierlow, Derbyshire, United Kingdom, Sandy loam soil (M986; 65% sand 28% silt 7% clay, pH 6.0, 0.5% organic carbon) obtained from Hanford, Hickman, California, loamy sand sediment (M940; 85% sand 10% silt 5% clay, pH 8.2, 0.9% organic carbon) obtained from Golden Lake, Steele, North Dakota, and clay loam sediment (M947; 26% sand 38% silt 36% clay, pH 7.8, 3.3% organic carbon) obtained from Goose River, Grand Forks, North Dakota, were used (USDA soil texture classification; p. 24; Table 2, p. 36). The pH values were based on 1:1 soil:water ratio results. All soil/sediment samples were provided by the Sponsor.

ILV (MRID 50454302): Mean recoveries and RSDs were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of 2,4-D Acid at 0.0019 mg/kg (LOQ) and 0.019 mg/kg (10 \times LOQ) in soil and sediment matrices (Tables 1-38, pp. 35-72; DER Attachment 2). Mean recoveries and RSDs were within guideline requirements for analysis of 2,4-D Acid transformation products 2,4-D 2-EHE, 2,4-D DMAS, 2,4-DCP, and 2,4-DCA at 0.010 mg/kg (LOQ) and 0.10 mg/kg (10 \times LOQ) in soil and sediment matrices. Mean recoveries and RSDs were within guideline requirements for analysis of 2,4-D Acid transformation products 4-CP and 4-CPA at 0.010 mg/kg (LOQ) and 0.10 mg/kg (10 \times LOQ) in sediment matrices. LC/MS/MS Analysis was used for all analytes, except 2,4-DCA; GC/MS Analysis was used for 2,4-DCA. Analytes were identified using two ion transitions via LC/MS/MS or three ions via GC/MS. Recoveries of the quantitation and confirmation analyses were comparable. Recoveries were corrected when residues were quantified in the controls (pp. 26-28). Residues were quantified in the controls for the following sample sets: 2,4-DCA in sediment (C 2; 0.0001 mg/kg); 2,4-D Acid in soil (Q; 0.0004 mg/kg); and 2,4-DCP in soil (C; 0.0007 mg/kg) in sediment (Q; 0.0003 mg/kg). Recoveries for the 0.00057 mg/kg and 0.003 mg/kg LOD samples (n =

1 for each analyte/matrix) were reviewer-calculated since the study author did not calculate these recoveries (see DER Attachment 2). LOD recoveries ranged 53-246% for 2,4-D Acid, 56-69% for 2,4-D 2-EHE, 81-123% for 2,4-D DMAS, 71-113% for 2,4-DCP, 77-103% for 2,4-DCA, 0-65% for 4-CP, and 83-93% of 4-CPA (ions/matrices combined). Silt loam soil (M983; 53° 13' 9.4"N, 1° 51' 32.4"W; 35% sand 59% silt 6% clay, pH 5.4, 4.6% organic carbon) obtained from Brierlow, Peak District National Park, United Kingdom, and clay loam sediment (M947; 47° 43' 779"N, W097137.312; 26% sand 38% silt 36% clay, pH 7.8, 3.3% organic carbon) obtained from Goose River, Grand Forks, North Dakota, were used (USDA soil texture classification; pp. 16-17; Appendix C, pp. 232-237). The pH values were based on 1:1 soil:water ratio results. All soil/sediment samples were provided by Dow AgroSciences, LLC; soil classification performed by Agvise Laboratories, Northwood, North Dakota. The ILV soil and sediment matrices matched two of the four matrices used in the ECM. The ILV validated the ECM in the first trial with only insignificant modifications to the analytical instrument or parameters and the identification of the optimization of the APCI probe as a critical step to reduce background signal during 2,4-D Acid analysis (pp. 31-32; Tables 1-26, pp. 35-60).

Table 2. Initial Validation Method Recoveries for 2,4-D Acid and Its Transformation Products 2,4-D 2-EHE, 2,4-D DMAS, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA in Soil and Sediment^{1,2,3,4}

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Brierlow Silt Loam Soil						
Quantitation Ion Transition/Quantitation Ion						
2,4-D Acid	0.00057 (LOD)	1	78	--	--	--
	0.0019 (LOQ)	5	65-75	72	4.2	5.8
	0.019	5	73-76	74	1.2	1.7
	0.19	5	72-77	74	1.9	2.5
2,4-D 2-EHE	0.003 (LOD)	1	112	--	--	--
	0.010 (LOQ)	5	83-96	88	5.1	5.7
	0.10	5	77-83	80	2.4	3.0
	1.0	5	80-88	84	3.0	3.6
2,4-D DMAS	0.003 (LOD)	1	62	--	--	--
	0.010 (LOQ)	5	68-72	69	1.9	2.8
	0.10	5	70-83	78	5.7	7.4
	1.0	5	80-88	83	3.3	3.9
2,4-DCP	0.003 (LOD)	1	62	--	--	--
	0.010 (LOQ)	5	69-74	71	2.2	3.0
	0.10	5	76-82	79	2.4	3.0
	1.0	5	79-81	80	0.84	1.0
2,4-DCA	0.003 (LOD)	1	86	--	--	--
	0.010 (LOQ)	5	73-87	81	6.0	7.4
	0.10	5	74-83	77	3.6	4.7
	1.0	5	71-81	75	3.8	5.1
Confirmation Ion Transition/Confirmation Ion						
2,4-D Acid	0.00057 (LOD)	1	78	--	--	--
	0.0019 (LOQ)	5	68-83	75	6.5	8.6
	0.019	5	70-77	74	2.9	3.9
	0.19	5	71-77	74	2.4	3.3
2,4-D 2-EHE	0.003 (LOD)	1	89	--	--	--

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.010 (LOQ)	5	72-90	82	6.9	8.4
	0.10	5	78-87	81	3.5	4.3
	1.0	5	81-89	85	3.6	4.3
2,4-D DMAS	0.003 (LOD)	1	59	--	--	--
	0.010 (LOQ)	5	65-76	72	4.3	6.0
	0.10	5	71-84	78	5.9	7.5
	1.0	5	76-89	81	5.2	6.5
2,4-DCP	0.003 (LOD)	1	68	--	--	--
	0.010 (LOQ)	5	66-75	72	3.4	4.8
	0.10	5	76-84	80	3.4	4.2
	1.0	5	80-82	81	0.84	1.0
2,4-DCA	0.003 (LOD)	1	79	--	--	--
	0.010 (LOQ)	5	74-85	80	5.0	6.3
	0.10	5	74-83	77	3.6	4.7
	1.0	5	71-8	75	3.8	5.1
Hanford Sandy Loam Soil						
Quantitation Ion Transition/Quantitation Ion						
2,4-D Acid	0.00057 (LOD)	1	72	--	--	--
	0.0019 (LOQ)	5	83-89	85	2.4	2.8
	0.019	5	81-89	85	3.4	3.9
	0.19	5	86-89	88	1.3	1.5
2,4-D 2-EHE	0.003 (LOD)	1	101	--	--	--
	0.010 (LOQ)	5	81-97	92	6.9	7.5
	0.10	5	85-91	87	2.5	2.9
	1.0	5	82-92	86	3.8	4.5
2,4-D DMAS	0.003 (LOD)	1	83	--	--	--
	0.010 (LOQ)	5	82-90	85	3.3	3.9
	0.10	5	89-97	92	3.0	3.3
	1.0	5	87-92	89	2.0	2.2
2,4-DCP	0.003 (LOD)	1	70	--	--	--
	0.010 (LOQ)	5	77-86	83	3.6	4.3
	0.10	5	81-86	83	1.9	2.3
	1.0	5	80-84	83	1.5	1.8
2,4-DCA	0.003 (LOD)	1	94	--	--	--
	0.010 (LOQ)	5	84-92	88	3.8	4.3
	0.10	5	77-87	84	4.0	4.7
	1.0	5	78-85	83	2.9	3.5
Confirmation Ion Transition/Confirmation Ion						
2,4-D Acid	0.00057 (LOD)	1	49	--	--	--
	0.0019 (LOQ)	5	73-119	93	17	18
	0.019	5	82-89	86	2.8	3.2
	0.19	5	83-89	86	2.6	3.0
2,4-D 2-EHE	0.003 (LOD)	1	83	--	--	--
	0.010 (LOQ)	5	81-97	91	6.0	6.6
	0.10	5	84-95	89	4.5	5.1
	1.0	5	78-95	86	6.4	7.4
2,4-D DMAS	0.003 (LOD)	1	51	--	--	--

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.010 (LOQ)	5	80-87	84	2.8	3.3
	0.10	5	85-94	89	3.2	3.6
	1.0	5	85-90	88	2.0	2.3
2,4-DCP	0.003 (LOD)	1	57	--	--	--
	0.010 (LOQ)	5	73-84	79	4.9	6.2
	0.10	5	81-86	83	1.9	2.3
	1.0	5	81-83	82	0.89	1.1
2,4-DCA	0.003 (LOD)	1	83	--	--	--
	0.010 (LOQ)	5	83-91	86	3.4	4.0
	0.10	5	77-87	83	3.9	4.7
	1.0	5	78-85	82	2.7	3.3
Golden Lake Loamy Sand Sediment						
Quantitation Ion Transition/Quantitation Ion						
2,4-D Acid	0.00057 (LOD)	1	42	--	--	--
	0.0019 (LOQ)	5	75-101	88	10	12
	0.019	5	81-86	84	1.9	2.3
	0.19	5	74-79	78	22	2.8
2,4-D 2-EHE	0.003 (LOD)	1	72	--	--	--
	0.010 (LOQ)	5	90-95	92	1.9	2.1
	0.10	5	88-93	90	2.1	2.4
	1.0	5	84-96	89	5.4	6.1
2,4-D DMAS	0.003 (LOD)	1	76	--	--	--
	0.010 (LOQ)	5	82-92	87	3.6	4.1
	0.10	5	91-100	95	3.3	3.5
	1.0	5	75-85	81	4.3	5.3
2,4-DCP	0.003 (LOD)	1	65	--	--	--
	0.010 (LOQ)	5	75-85	80	4.1	5.2
	0.10	5	81-86	84	2.1	2.5
	1.0	5	76-80	78	1.4	1.8
2,4-DCA	0.003 (LOD)	1	82	--	--	--
	0.010 (LOQ)	5	84-94	89	3.8	4.3
	0.10	5	76-85	80	3.4	4.2
	1.0	5	69-73	71	1.5	2.1
4-CP	0.003 (LOD)	1	71	--	--	--
	0.010 (LOQ)	5	82-97	91	6.5	7.2
	0.10	5	84-93	89	4.3	4.8
	1.0	5	81-86	82	2.2	2.7
4-CPA	0.003 (LOD)	1	45	--	--	--
	0.010 (LOQ)	5	63-78	71	6.1	8.5
	0.10	5	85-91	88	2.4	2.7
	1.0	5	79-84	81	2.4	2.5
Confirmation Ion Transition/Confirmation Ion						
2,4-D Acid	0.00057 (LOD)	1	148	--	--	--
	0.0019 (LOQ)	5	85-115	99	13	13
	0.019	5	80-88	83	3.1	3.8

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.19	5	74-78	76	1.7	2.2
2,4-D 2-EHE	0.003 (LOD)	1	96	--	--	--
	0.010 (LOQ)	5	77-96	84	7.4	8.8
	0.10	5	85-96	89	3.4	3.9
	1.0	5	87-96	90	3.4	3.8
2,4-D DMAS	0.003 (LOD)	1	58	--	--	--
	0.010 (LOQ)	5	82-88	86	2.7	3.1
	0.10	5	92-100	96	3.5	3.6
	1.0	5	77-89	84	4.7	5.6
2,4-DCP	0.003 (LOD)	1	65	--	--	--
	0.010 (LOQ)	5	77-84	82	2.9	3.5
	0.10	5	81-87	85	2.3	2.7
	1.0	5	78-80	79	0.84	1.1
2,4-DCA	0.003 (LOD)	1	70	--	--	--
	0.010 (LOQ)	5	81-90	85	4.2	4.9
	0.10	5	76-86	80	3.9	4.9
	1.0	5	70-72	71	0.84	1.2
4-CP	0.003 (LOD)	1	82	--	--	--
	0.010 (LOQ)	5	77-103	93	9.9	11
	0.10	5	88-95	91	2.7	3.0
	1.0	5	73-85	80	5.5	7.0
4-CPA	0.003 (LOD)	1	43	--	--	--
	0.010 (LOQ)	5	65-81	74	6.4	8.7
	0.10	5	85-90	88	2.0	2.3
	1.0	5	79-85	81	2.5	3.1
Goose River Clay Loam Sediment						
Quantitation Ion Transition/Quantitation Ion						
2,4-D Acid	0.00057 (LOD)	1	76	--	--	--
	0.0019 (LOQ)	5	84-103	93	8.6	9.3
	0.019	5	85-98	92	5.9	6.4
	0.19	5	87-93	89	2.7	3.0
2,4-D 2-EHE	0.003 (LOD)	1	86	--	--	--
	0.010 (LOQ)	5	69-94	84	9.4	11
	0.10	5	85-98	91	5.2	5.7
	1.0	5	79-106	93	11	11
2,4-D DMAS	0.003 (LOD)	1	93	--	--	--
	0.010 (LOQ)	5	76-83	79	3.0	3.7
	0.10	5	80-100	92	9.2	10
	1.0	5	99-108	103	4.0	3.9
2,4-DCP	0.003 (LOD)	1	67	--	--	--
	0.010 (LOQ)	5	76-86	81	4.1	5.0
	0.10	5	84-95	90	4.7	5.3
	1.0	5	88-91	90	1.3	1.4
2,4-DCA	0.003 (LOD)	1	75	--	--	--
	0.010 (LOQ)	5	84-96	89	5.8	6.5
	0.10	5	76-90	81	5.4	6.7
	1.0	5	78-82	80	1.5	1.9

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
4-CP	0.003 (LOD)	1	57	--	--	--
	0.010 (LOQ)	5	75-95	88	8.0	9.1
	0.10	5	89-96	92	2.8	3.0
	1.0	5	89-97	92	3.0	3.2
4-CPA	0.003 (LOD)	1	56	--	--	--
	0.010 (LOQ)	5	71-81	77	5.5	7.1
	0.10	5	92-98	95	2.4	2.5
	1.0	5	88-94	91	2.4	2.6
Confirmation Ion Transition/Confirmation Ion						
2,4-D Acid	0.00057 (LOD)	1	0	--	--	--
	0.0019 (LOQ)	5	71-98	85	10	12
	0.019	5	88-102	96	6.0	6.2
	0.19	5	74-78	76	1.7	2.2
2,4-D 2-EHE	0.003 (LOD)	1	90	--	--	--
	0.010 (LOQ)	5	61-83	72	9.8	14
	0.10	5	85-96	91	4.2	4.6
	1.0	5	82-102	94	7.4	7.8
2,4-D DMAS	0.003 (LOD)	1	80	--	--	--
	0.010 (LOQ)	5	69-72	71	1.3	1.8
	0.10	5	81-102	92	8.7	9.5
	1.0	5	97-107	102	4.2	4.1
2,4-DCP	0.003 (LOD)	1	71	--	--	--
	0.010 (LOQ)	5	76-85	81	3.4	4.2
	0.10	5	85-94	90	4.4	4.9
	1.0	5	88-92	90	1.6	1.8
2,4-DCA	0.003 (LOD)	1	81	--	--	--
	0.010 (LOQ)	5	83-97	90	5.7	6.4
	0.10	5	76-91	81	5.8	7.1
	1.0	5	76-81	80	1.1	1.4
4-CP	0.003 (LOD)	1	89	--	--	--
	0.010 (LOQ)	5	70-90	78	8.9	11
	0.10	5	86-95	91	3.5	3.9
	1.0	5	83-94	89	4.5	5.0
4-CPA	0.003 (LOD)	1	52	--	--	--
	0.010 (LOQ)	5	70-82	77	5.4	7.1
	0.10	5	93-97	95	1.8	1.9
	1.0	5	87-92	90	2.3	2.6

Data (uncorrected recovery results, pp. 28-20) were obtained from Tables 3-28, pp. 37-91 of MRID 50454301 and DER Attachment 2.

1 LC/MS/MS Analysis for all analytes, except 2,4-DCA: Analytes were identified using two ion transitions (quantitation and confirmation, respectively): m/z 219→161 and m/z 221→163 for 2,4-D Acid, m/z 161→125 and m/z 163→127 for 2,4-DCP, m/z 127→91 and m/z 127→35 for 4-CP, and m/z 185→127 and m/z 187→129 for 4-CPA. 2,4-D DMAS and 2,4-D 2-EHE are detected as 2,4-D Acid.

2 GC/MS Analysis for 2,4-DCA: Monitored ions were m/z 178 (quantitation), m/z 161 (confirmation 1), and m/z 163 (confirmation 2). Only the quantitation and confirmation 1 ions were quantified.

3 Silt loam soil (M983; 35% sand 59% silt 6% clay, pH 5.4, 4.6% organic carbon) obtained from Brierlow, Derbyshire, United Kingdom, Sandy loam soil (M986; 65% sand 28% silt 7% clay, pH 6.0, 0.5% organic carbon) obtained from Hanford, Hickman, California, loamy sand sediment (M940; 85% sand 10% silt 5% clay, pH 8.2, 0.9% organic carbon) obtained from Golden Lake, Steele, North Dakota, and clay loam sediment (M947; 26% sand 38% silt 36% clay, pH

7.8, 3.3% organic carbon) obtained from Goose River, Grand Forks, North Dakota, were used in this study (USDA soil texture classification; p. 24; Table 2, p. 36). The pH values were based on 1:1 soil:water ratio results. All soil/sediment samples were provided by the Sponsor.

4 Recoveries for the 0.00057 mg/kg and 0.003 mg/kg LOD samples were reviewer-calculated based on data from Tables 3-26, pp. 37-84 since the study author did not calculate these recoveries (DER Attachment 2). Mean recovery, s.d. and RSDs could not be determined since n = 1.

Table 3. Independent Validation Method Recoveries for 2,4-D Acid and Its Transformation Products 2,4-D 2-EHE, 2,4-D DMAS, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA in Soil and Sediment^{1,2,3,4}

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Brierlow Silt Loam Soil						
Quantitation Ion Transition/Quantitation Ion						
2,4-D Acid	0.00057 (LOD)	1	246	--	--	--
	0.0019 (LOQ)	5	79-107	91	12	13
	0.019	5	97-109	104	5	5
2,4-D 2-EHE	0.003 (LOD)	1	59	--	--	--
	0.010 (LOQ)	5	82-88	86	2	3
	0.10	5	71-88	82	6	8
2,4-D DMAS	0.003 (LOD)	1	81	--	--	--
	0.010 (LOQ)	5	92-114	101	8	8
	0.10	5	100-105	102	2	2
2,4-DCP	0.003 (LOD)	1	71	--	--	--
	0.010 (LOQ)	5	89-98	93	4	4
	0.10	5	105-110	108	2	2
2,4-DCA	0.003 (LOD)	1	77	--	--	--
	0.010 (LOQ)	5	81-93	87	6	7
	0.10	5	78-85	82	3	4
Confirmation Ion Transition/Confirmation Ion						
2,4-D Acid	0.00057 (LOD)	1	53	--	--	--
	0.0019 (LOQ)	5	75-115	96	15	16
	0.019	5	96-100	98	2	2
2,4-D 2-EHE	0.003 (LOD)	1	59	--	--	--
	0.010 (LOQ)	5	81-87	85	3	3
	0.10	5	72-89	82	7	8
2,4-D DMAS	0.003 (LOD)	1	87	--	--	--
	0.010 (LOQ)	5	97-107	100	4	4
	0.10	5	100-105	103	2	2
2,4-DCP	0.003 (LOD)	1	103	--	--	--
	0.010 (LOQ)	5	82-108	95	10	11
	0.10	5	104-111	108	3	3
2,4-DCA	0.003 (LOD)	1	87	--	--	--
	0.010 (LOQ)	5	81-92	86	5	6
	0.10	5	78-84	82	3	3
Confirmation Ion 2						
2,4-DCA	0.003 (LOD)	1	81	--	--	--
	0.010 (LOQ)	5	83-93	88	5	6
	0.10	5	79-85	82	3	4

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Goose River Clay Loam Sediment						
Quantitation Ion Transition/Quantitation Ion						
2,4-D Acid	0.00057 (LOD)	1	88	--	--	--
	0.0019 (LOQ)	5	75-88	80	5	7
	0.019	5	75-100	88	10	12
2,4-D 2-EHE	0.003 (LOD)	1	56	--	--	--
	0.010 (LOQ)	5	82-102	94	9	9
	0.10	5	86-88	87	1	1
2,4-D DMAS	0.003 (LOD)	1	119	--	--	--
	0.010 (LOQ)	5	69-111	94	16	17
	0.10	5	95-99	96	2	2
2,4-DCP	0.003 (LOD)	1	90	--	--	--
	0.010 (LOQ)	5	66-78	72	4	6
	0.10	5	86-104	91	7	8
2,4-DCA	0.003 (LOD)	1	103	--	--	--
	0.010 (LOQ)	5	85-98	92	5	5
	0.10	5	80-90	84	4	5
4-CP	0.003 (LOD)	1	65	--	--	--
	0.010 (LOQ)	5	77-88	84	5	6
	0.10	5	97-101	99	1	1
4-CPA	0.003 (LOD)	1	93	--	--	--
	0.010 (LOQ)	5	64-83	77	8	10
	0.10	5	87-104	94	6	7
Confirmation Ion Transition/Confirmation Ion						
2,4-D Acid	0.00057 (LOD)	1	105	--	--	--
	0.0019 (LOQ)	5	74-91	82	7	8
	0.019	5	81-103	92	9	10
2,4-D 2-EHE	0.003 (LOD)	1	69	--	--	--
	0.010 (LOQ)	5	96-104	100	3	3
	0.10	5	84-89	86	2	3
2,4-D DMAS	0.003 (LOD)	1	123	--	--	--
	0.010 (LOQ)	5	71-115	99	17	17
	0.10	5	98-101	100	1	1
2,4-DCP	0.003 (LOD)	1	113	--	--	--
	0.010 (LOQ)	5	65-83	76	8	10
	0.10	5	89-108	96	7	7
2,4-DCA	0.003 (LOD)	1	93	--	--	--
	0.010 (LOQ)	5	83-93	89	4	4
	0.10	5	79-89	84	4	5
4-CP	0.003 (LOD)	1	0	--	--	--
	0.010 (LOQ)	5	70-85	78	7	9
	0.10	5	95-102	98	3	3
4-CPA	0.003 (LOD)	1	83	--	--	--
	0.010 (LOQ)	5	64-84	79	8	11
	0.10	5	91-108	97	7	7
Confirmation Ion 2						

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
4-CPA	0.003 (LOD)	1	93	--	--	--
	0.010 (LOQ)	5	88-95	91	3	3
	0.10	5	80-89	84	4	5

Data (recovery results were corrected for residues quantified in the controls, pp. 26-28) were obtained from Tables 1-38, pp. 35-72 of MRID 50454302 and DER Attachment 2.

- 1 LC/MS/MS Analysis for all analytes, except 2,4-DCA: Analytes were identified using two ion transitions (quantitation and confirmation, respectively): m/z 219→161 and m/z 221→163 for 2,4-D Acid, m/z 161→125 and m/z 163→127 for 2,4-DCP, m/z 127→91 and m/z 127→35 for 4-CP, and m/z 185→127 and m/z 187→129 for 4-CPA. 2,4-D DMAS and 2,4-D 2-EHE are detected as 2,4-D Acid.
- 2 GC/MS Analysis for 2,4-DCA: Monitored ions were m/z 178 (quantitation), m/z 161 (confirmation 1), and m/z 163 (confirmation 2). All three ions were quantified.
- 3 Silt loam soil (M983; 53° 13' 9.4"N, 1° 51' 32.4"W; 35% sand 59% silt 6% clay, pH 5.4, 4.6% organic carbon) obtained from Brierlow, Peak District National Park, United Kingdom, and clay loam sediment (M947; 47° 43' 779"N, W097137.312; 26% sand 38% silt 36% clay, pH 7.8, 3.3% organic carbon) obtained from Goose River, Grand Forks, North Dakota, were used in this study (USDA soil texture classification; pp. 16-17; Appendix C, pp. 232-237). The pH values were based on 1:1 soil:water ratio results. All soil/sediment samples were provided by Dow AgroSciences, LLC; soil classification performed by Agvise Laboratories, Northwood, North Dakota. The ILV soil and sediment matrices matched two of the four matrices used in the ECM.
- 4 Recoveries for the 0.00057 mg/kg and 0.003 mg/kg LOD samples were reviewer-calculated based on data from Tables 1-26, pp. 35-60 since the study author did not calculate these recoveries (DER Attachment 2). Mean recovery, s.d. and RSDs could not be determined since $n = 1$. The LOD fortification for 0.003 mg/kg samples varied from 0.003-0.0032 mg/kg.

III. Method Characteristics

In the ECM, the method LOQ and LOD were 0.0019 mg/kg and 0.00057 mg/kg, respectively, for 2,4-D in soil and sediment (pp. 13, 31; Table 29, p. 92 of MRID 50454301). The method LOQ and LOD were 0.010 mg/kg and 0.003 mg/kg, respectively, for all metabolites, 2,4-D 2-EHE, 2,4-D DMAS, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA. The LODs were reported as 30% of the respectively LOQ. In the ILV, the method LOQs were the same as those of the ECM; the LODs were not reported (pp. 15, 30-31 of MRID 50454302). Following the method of Keith, L. H., *et al.* (not specifically referenced in the ECM or ILV), the LOD and LOQ for determination of 2,4-D and its metabolites were calculated using the standard deviation from the 0.0019 mg/kg recovery results for 2,4-D and from the 0.010 mg/kg recovery results for all metabolites. The LOD was calculated as three times the standard deviation ($3s$), and the LOQ was calculated as ten times the standard deviation ($10s$) of the recovery results. The LOQ was calculated in the ECM and ILV; the LOD was only calculated in the ECM. The calculated values generally supported the LOQ and LOD established for the study (see Table 4); however, several of the LOQ and LOD values for 2,4-D Acid were significantly greater than the established LOQ and LOD values.

Table 4. Method Characteristics for 2,4-D Acid, 2,4-D 2-EHE, 2,4-D DMAS, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA in Soil and Sediment

Matrix			Soil and Sediment				Sediment		
Analysis			LC/MS/MS			GC/MS	LC/MS/MS		
Analyte			2,4-D ACID	2,4-D 2-EHE ¹	2,4-D DMAS ¹	2,4-DCP	2,4-DCA ²	4-CP	4-CPA
Limit of Quantitation (LOQ)	ECM	Nominal	0.0019 mg/kg	0.010 mg/kg					
		Calculated ³	0.00046-0.0020 mg/kg (Q) 0.0012-0.0031 mg/kg (C)	0.0019-0.0098 mg/kg	0.0013-0.0044 mg/kg	0.0021-0.0051 mg/kg	0.0033-0.0059 mg/kg	0.0064-0.0097 mg/kg	0.0052-0.0063 mg/kg
	ILV	Nominal	0.0019 mg/kg	0.010 mg/kg					
		Calculated ³	0.00101-0.00282 mg/kg	0.00232-0.00913 mg/kg	0.00430-0.0177 mg/kg	0.00371-0.0107 mg/kg	0.00272-0.00598 mg/kg	0.00484-0.00703 mg/kg	0.00787-0.00857 mg/kg
Limit of Detection (LOD)	ECM	Nominal	0.00057 mg/kg	0.003 mg/kg					
		Calculated ³	0.00014-0.00059 mg/kg (Q) 0.00037-0.00094 mg/kg (C)	0.00058-0.0029 mg/kg	0.00039-0.0013 mg/kg	0.00062-0.0015 mg/kg	0.0010-0.0018 mg/kg	0.0019-0.0029 mg/kg	0.0016-0.0019 mg/kg
	ILV	Nominal	Not reported						
		Calculated	Not calculated						
Linearity (calibration curve r ² and concentration range)	ECM ⁴	Soil	r ² = 0.9992-0.9999 (Q) r ² = 0.9982-0.9997 (C)	r ² = 0.9976-0.9995 (Q) ⁵ r ² = 0.9969-0.9993 (C) ⁵		r ² = 0.9995-0.9999 (Q) r ² = 0.9976-0.9998 (C)	r ² = 0.9997-0.9999 (Q) r ² = 0.9993-0.9999 (C)	Not analyzed	
		Sediment	r ² = 0.9958-0.9999 (Q) r ² = 0.9941 -0.9991 (C) ⁶	r ² = 0.9986-0.9995 (Q) ⁵ r ² = 0.9970-0.9999 (C) ⁵		r ² = 0.9995-1.0000 (Q) r ² = 0.9997-0.9999 (C)	r ² = 0.9991-0.9999 (Q) r ² = 0.9992-1.0000 (C)	r ² = 0.9978-0.9991 (Q) r ² = 0.9985-0.9992 (C)	r ² = 0.9990-1.0000 (Q) r ² = 0.9994-0.9999 (C)
	Range	0.0228-1.14 ng/mL			0.12-6.0 ng/mL	3-100 ng/mL	0.12-6.0 ng/mL		

Matrix			Soil and Sediment				Sediment		
Analysis			LC/MS/MS			GC/MS	LC/MS/MS		
Analyte			2,4-D ACID	2,4-D 2-EHE ¹	2,4-D DMAS ¹	2,4-DCP	2,4-DCA ²	4-CP	4-CPA
Linearity (calibration curve r ² and concentration range)	ILV ⁷	Soil/Sediment	r ² = 0.99335 (Q) r ² = 0.99778 (C)	r ² = 0.99896 (Q) ⁵ r ² = 0.99529 (C) ⁵		r ² = 0.99824 (Q) r ² = 0.99952 (C)	r ² = 1.000 (Q) r ² = 0.999 (C1) r ² = 1.000 (C2)	r ² = 0.99964 (Q) r ² = 0.99956 (C)	r ² = 0.99772 (Q) r ² = 0.99800 (C)
		Range	0.0228-1.14 ng/mL	0.0381-3.808 ng/mL		0.126-6.276 ng/mL	3.1-102 ng/mL	0.122-6.108 ng/mL	0.126-6.300 ng/mL
Repeatable	ECM ⁸		Yes at LOQ and 10×LOQ in two soil and two sediment matrices (characterized).		Yes at LOQ and 10×LOQ in one soil and two sediment matrices (characterized); Yes at 10×LOQ in silt loam soil, but no at LOQ (Q mean 69%).	Yes at LOQ and 10×LOQ in two soil and two sediment matrices (characterized).			
	ILV ^{9,10}		Yes at LOQ and 10×LOQ in one soil and one sediment matrix (characterized).				Yes at LOQ and 10×LOQ in one sediment matrix (characterized).		
Reproducible			Yes at LOQ and 10×LOQ in soil and sediment matrices				Yes at LOQ and 10×LOQ in sediment matrices		
Specific	ECM		Yes, no matrix interferences were observed; however, LOQ peak definition was very poor. Some minor baseline noise interfered with peak integration.	Yes, no matrix interferences were observed. Some minor baseline noise interfered with peak integration.	Yes, no matrix interferences were observed. Non-uniform peak attenuation was noted for the LOQ peak in soil.	Yes, no matrix interferences were observed in soil, and matrix interferences were <i>ca.</i> 1% in sediment (based on peak area). LOQ peak tailing was noted in sediment.	Yes, no matrix interferences were observed.		

Matrix		Soil and Sediment				Sediment		
Analysis		LC/MS/MS				GC/MS	LC/MS/MS	
Analyte		2,4-D ACID	2,4-D 2-EHE ¹	2,4-D DMAS ¹	2,4-DCP	2,4-DCA ²	4-CP	4-CPA
	ILV ¹¹	Yes, matrix interferences were <i>ca.</i> 21% of the LOQ in soil (based on quantified residues) and <9% of the LOQ in sediment (based on quantified residues). Baseline noise surrounded the analyte peak and interfered with peak integration.	Yes, matrix interferences were <i>ca.</i> 10-13% of the LOQ (based on peak area). Some minor baseline noise interfered with peak integration.	Yes, matrix interferences were <i>ca.</i> 6-8% of the LOQ (based on quantified residues; in reagent blank).	Yes, matrix interferences were <i>ca.</i> 3-7% of the LOQ (based on quantified residues).	Yes, matrix interferences were <2% of the LOQ (based on quantified residues).	Yes, matrix interferences were <i>ca.</i> 9% of the LOQ (C; based on peak area; in reagent blank).	Yes, matrix interferences were <1% of the LOQ in sediment (based on peak area).

Data were obtained from pp. 13, 31; Table 29, p. 92 (LOD/LOQ); Tables 3-28, pp. 37-91 (recovery results); Figure 1, pp. 93-98 (calibration curves); Figures 5-20, pp. 118-209 (chromatograms); Appendix D, pp. 244-325 (correlation coefficients) of MRID 50454301; pp. 15, 30-31 (LOQ); Tables 1-38, pp. 35-72 (recovery results); Figures 1-13, pp. 76-88 (calibration curves); Figures 16-106, pp. 93-196 (chromatograms) of MRID 50454302; DER Attachment 2. Q = Quantitation ion transition/Quantitation ion; C = Confirmation ion transition/Confirmation ion; C1 = Confirmation ion 1; C2 = Confirmation ion 2.

1 2,4-D DMAS and 2,4-D 2-EHE were detected as 2,4-D Acid.

2 2,4-DCA was quantified using two ions in the ECM and three ions in the ILV; therefore, C1 and C2 were only applicable to the ILV data.

3 Ions/matrices combined, except where noted otherwise.

4 ECM coefficient of determination (r²) values are reviewer-generated from reported correlation coefficient (r) values (1/x weighting; Appendix D, pp. 245-326 of MRID 50454301; DER Attachment 2). Solvent-based calibration standards were used, but calibration curves were provided for all sample sets. The reviewer limited the calculated r² to 4 significant figures although 5 significant figures were reported in the ECM for r.

5 2,4-D DMAS and 2,4-D 2-EHE were analyzed in the same sample set with the same calibration curves.

6 Deviation of linearity does not affect the validity of the method since a confirmation method is usually not required when LC/MS or GC/MS is used as the primary method for quantifying residues.

7 Solvent-based calibration standards were used in the ILV.

8 In the ECM, the ECM, silt loam soil (M983; 35% sand 59% silt 6% clay, pH 5.4, 4.6% organic carbon) obtained from Brierlow, Derbyshire, United Kingdom, Sandy loam soil (M986; 65% sand 28% silt 7% clay, pH 6.0, 0.5% organic carbon) obtained from Hanford, Hickman, California, loamy sand sediment (M940; 85% sand 10% silt 5% clay, pH 8.2, 0.9% organic carbon) obtained from Golden Lake, Steele, North Dakota, and clay loam sediment (M947; 26% sand 38%

- silt 36% clay, pH 7.8, 3.3% organic carbon) obtained from Goose River, Grand Forks, North Dakota, were used (USDA soil texture classification; p. 24; Table 2, p. 36 of MRID 50454301). The pH values were based on 1:1 soil:water ratio results. All soil/sediment samples were provided by the Sponsor.
- 9 In the ILV, silt loam soil (M983; 53° 13' 9.4"N, 1° 51' 32.4"W; 35% sand 59% silt 6% clay, pH 5.4, 4.6% organic carbon) obtained from Brierlow, Peak District National Park, United Kingdom, and clay loam sediment (M947; 47° 43' 779"N, W097137.312; 26% sand 38% silt 36% clay, pH 7.8, 3.3% organic carbon) obtained from Goose River, Grand Forks, North Dakota, were used (USDA soil texture classification; pp. 16-17; Appendix C, pp. 232-237 of MRID 50454302). The pH values were based on 1:1 soil:water ratio results. All soil/sediment samples were provided by Dow AgroSciences, LLC; soil classification performed by Agvise Laboratories, Northwood, North Dakota. The ILV soil and sediment matrices matched two of the four matrices used in the ECM.
- 10 The ILV validated the ECM in the first trial with only insignificant modifications to the analytical instrument or parameters and the identification of the optimization of the APCI probe as a critical step to reduce background signal during 2,4-D Acid analysis (pp. 31-32; Tables 1-26, pp. 35-60 of MRID 50454302).
- 11 Interferences identified in the samples were primarily evaluated by their quantification in ng/mL or mg/kg, but peak areas were used, if no quantification was reported.
- Linearity is satisfactory when $r^2 \geq 0.995$.

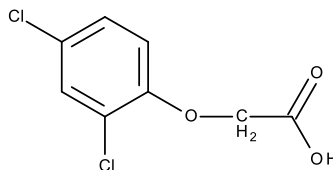
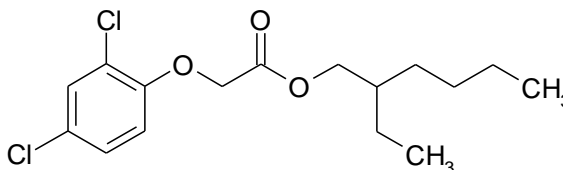
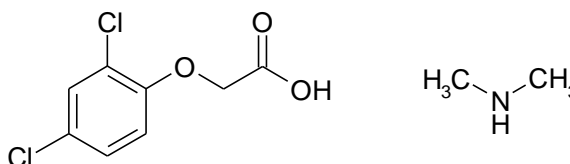
IV. Method Deficiencies and Reviewer's Comments

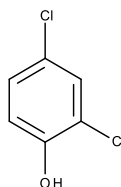
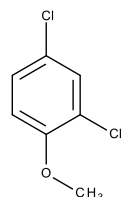
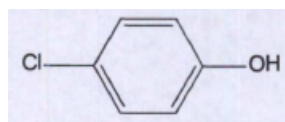
1. The analytical method meets the LOQ target identified in a previous review (0.0019 mg/kg; USEPA, 2015) only for 2,4-D acid. For 2,4-D salts, esters, and degradates, the LOQ exceeds the lowest toxicological levels of concern, which are 0.0004 mg/kg for 2,4-D esters and 0.0019 mg/kg for all other analytes; USEPA, 2013.
2. The specificity of the method for 2,4-D Acid was not well-supported by ECM and ILV representative chromatograms (Figures 5-20, pp. 118-209 of MRID 50454301; Figures 16-106, pp. 93-196 of MRID 50454302). In the ECM, LOQ peak definition was poor. The LOQ peak appeared to be multiple peaks and involved some of the baseline noise. In the ILV, baseline noise surrounded the analyte peak and interfered with peak integration. Overall, in all LOQ chromatograms, the 2,4-D Acid analyte peak was not well-distinguished from the baseline/baseline noise to allow consistent quantification. The ILV identified the optimization of the APCI probe as a critical step to reduce background signal during 2,4-D Acid analysis (pp. 31-32 of MRID 50454302). The reviewer noted that the method uses the same sample processing for 2,4-D Acid and 2,4-DCP, 4-CP and 4-CPA, even though the LOQ of 2,4-D Acid is 19% of the LOQ of the other analytes.
3. The LODs of the method were not reported or calculated by the ILV.
4. Performance data was not satisfactory for the ECM quantitation ion analysis of 2,4-D DMAS in Brierlow silt loam soil (mean 69%; Tables 3-28, pp. 37-91; DER Attachment 2). The OCSPP guideline requirements state that the mean recovery is 70-120% and the RSD is $\leq 20\%$. This guideline deviation is not considered significant since the ECM performance data deviation occurred in only one of the four matrices tested.
5. ECM linearity was not satisfactory for 2,4-D Acid in one of the sediment confirmation calibration curves, $r^2 = 0.9941$ (Appendix D, pp. 244-325 of MRID 50454301; DER Attachment 2). Linearity is satisfactory when $r^2 \geq 0.995$, but this deviation occurred in the confirmation ion analysis which is not necessary when LC/MS is used as the primary method to generate study data.
6. The two ILV matrices were the same as two of the four matrices used in the ECM. Additionally, it could not be determined if the ILV was provided with the most difficult matrices with which to validate the method and that the ILV soil and sediment matrix covered the range of soils used in the terrestrial field dissipation studies.
7. The matrix effects were found to be insignificant ($< 20\%$) for the analytes in the test matrices in the ILV (quantitation and confirmatory transitions; p. 30; Tables 39-40, pp. 73-74 of MRID 50454302). Solvent standards were used in the ECM and ILV.
8. The reviewer noted that the data in the ECM representative chromatograms was very difficult to interpret since the data of the chromatograms appeared to have been copied upside-down and backwards (Figures 5-20, pp. 118-209 of MRID 50454301).

9. In the ECM, the Sponsor reported that the fortification standard and calibration solutions were reported to be stable for at least 11 days when stored refrigerated (p. 31 of MRID 50454301). In the ILV, the Study Monitor reported that the stock solutions would be stable for the duration of the experiment (Appendix B, p. 230 of MRID 50454302).
10. The ILV detailed the communications between the Study Director, Sponsor, and the Study Monitor (p. 32; Appendix B, pp. 229-230 of MRID 50454302). The communication mainly involved protocol clarification and discussion regarding an ILV method deviation from the ECM. However, there was no impact on the ILV method validation.
11. It was reported for the ILV that one sample set (14 samples: 1 reagent blank, 2 control samples, 1 LOD sample, 5 LOQ samples, and 5 10×LOQ samples) required *ca.* 2-4 person-hours each for the initial extraction and each Fraction processing (p. 32 of MRID 50454302). LC/MS/MS was performed unattended overnight; evaluation of the LC/MS/MS results for each Fraction was completed in *ca.* 2-4 hours.

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.
- U.S. Environmental Protection Agency. 2013. Registration Review Problem Formulation for 2,4-D-Revised. DP Barcode 402410. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Environmental Fate and Effects Division. Memorandum to the Pesticide Re-Evaluation Division. April 2, 2013.
- U.S. Environmental Protection Agency. 2015. Reviews of Environmental Chemistry Methods for 2,4-D in Soil, Sediment and Water. DP 419836. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Environmental Fate and Effects Division. Memorandum to the Pesticide Re-Registration Division. May 8, 2015.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

DER Attachment 1: Chemical Names and Structures**2,4-D Acid****IUPAC Name:** 2-(2,4-Dichlorophenoxy)acetic acid**CAS Name:** 2,4-Dichlorophenoxy acetic acid**CAS Number:** 94-75-7**SMILES String:** O=C(O)COc(c(cc(c1)Cl)Cl)c1**2,4-D 2-EHE [2,4-D Ethylhexyl ester; 2,4-D-2-ethylhexyl ester; TSN102005]****IUPAC Name:** (RS)-2-Ethylhexyl (2,4-dichlorophenoxy)acetate**CAS Name:** 2-Ethylhexyl (2,4-dichlorophenoxy)acetate
(2,4-Dichlorophenoxy) acetic acid 2-ethylhexyl ester**CAS Number:** 1928-43-4**SMILES String:** Clc1cc(Cl)ccc1OCC(=O)OCC(CC)CCCC (EPI Suite, v3.12 SMILES String)**2,4-D DMAS [2,4-D Dimethylamine salt; 2,4-D DMA salt; 2,4-D-dimethylammonium; TSN100485]****IUPAC Name:** (2,4-Dichlorophenoxy)acetic acid: dimethylamine (1:1)
Dimethylammonium (2,4-dichlorophenoxy)acetate**CAS Name:** (2,4-Dichlorophenoxy)acetic acid dimethylamine salt
(2,4-Dichlorophenoxy)acetic acid compound with N-methylmethanamine (1:1)**CAS Number:** 2008-39-1**SMILES String:** CN(C)(H)(H)OC(=O)COc1c(Cl)cc(Cl)cc1 (EPI Suite, v3.12 SMILES String)

2,4-DCP**IUPAC Name:** 2,4-Dichlorophenol**CAS Name:** 2,4-Dichlorophenol**CAS Number:** 120-83-2**SMILES String:** Oc1cc(Cl)c(Cl)cc1**2,4-DCA****IUPAC Name:** 2,4-Dichloro-1-methoxy-benzene**CAS Name:** 2,4-Dichloroanisole**CAS Number:** 553-82-2**SMILES String:** COc1cc(Cl)c(Cl)cc1**4-CP****IUPAC Name:** Not reported**CAS Name:** 4-Chlorophenol**CAS Number:** 106-48-9**SMILES String:** Not reported**4-CPA****IUPAC Name:** Not reported**CAS Name:** 4-Chlorophenoxyacetic acid**CAS Number:** 122-88-3**SMILES String:** Not reported