

Analytical method for 2,4-D [(2,4-dichlorophenoxy)acetic acid] and its transformation products, 2,4-dichlorophenol, 4-chlorophenol and 2,4-dichloroanisole, in surface, ground and drinking water

- Reports:** ECM: EPA MRID No.: 49709301. Gesell, J.T. 2012. Method Validation Study for the Determination of Residues of (2,4-dichlorophenoxy)acetic acid and its Metabolites in Surface Water, Ground Water and Drinking Water. Laboratory Study ID: 110504. Report prepared, Regulatory Sciences and Government Affairs, Dow AgroSciences LLC, Indianapolis, Indiana, and sponsored and submitted by European Union, 2,4-D Task Force 2012 c/o Dow AgroSciences LLC, Indianapolis, Indiana; 130 pages. Final report issued January 26, 2012.
- ILV: EPA MRID No. 49709302. Garcia-Alix, M. 2012. Independent Laboratory Validation of an Analytical Method for the Determination of Residues of (2,4-Dichlorophenoxy)acetic acid, 2,4-Dichlorophenol, 4-Chlorophenol and 2,4-Dichloroanisole in Water. CEMAS Study No.: CEMS-5324. Dow AgroSciences Study Reference No.: 110821. Report prepared by CEM Analytical Services (CEMAS), Berkshire, United Kingdom, and sponsored and submitted by European Union, 2,4-D Task Force 2012 c/o Dow AgroSciences LLC, Indianapolis, Indiana; 144 pages. Final report issued January 18, 2012.
- Document No.:** MRIDs 49709301 & 49709302
- Guideline:** 850.6100
- Statements:** ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practices (GLP) and OECD GLP (1998; p. 3 of MRID 49709301). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).
- ILV: The study was conducted in accordance with OECD GLP standards (1998), as well as the UK Department of Health (p. 3; Appendix C, p. 144 of MRID 49709302). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-4; Appendix C, p. 144). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).
- Classification:** This analytical method is classified as **Unacceptable**. The specificity of the method for 2,4-DCA could not be confirmed using the GC/MS chromatograms provided in the ECM and ILV. For 2,4-D, significant interferences in the matrix controls were observed at 20-30% of the LOQ in one matrix in the ECM and ILV. A reagent blank was not included in the ILV. Sample recoveries were corrected in the ECM and ILV. The ILV reported that communications between the ILV and the sponsor were unnecessary; however, a Study Plan Amendment was required to confirm the GC/MS instrument conditions.

PC Code: 030001**Reviewer:** Lewis Ross Brown, III**Signature:****Date:** Apr. 28, 2016**All page numbers refer to those listed in the upper right-hand corner of the MRIDs.****Executive Summary**

The analytical method, Method Validation No.110504, is designed for the quantitative determination of (2,4-dichlorophenoxy)acetic acid (2,4-D), 2,4-dichlorophenol and 4-chlorophenol in drinking, ground and surface water matrices at the LOQ of 0.10 µg/L using LC/MS/MS and 2,4-dichloroanisole in drinking, ground and surface water matrices at the LOQ of 0.10 µg/L using GC/MS. The LOQ is less than the lowest toxicological level of concern in water for all analytes. The specificity of the method for 2,4-DCA could not be confirmed using the very faint GC/MS chromatograms provided in the ECM and ILV. For 2,4-D, significant interferences in the matrix controls were observed at 25-30% of the LOQ in the ground water in the ECM and at 20-25% of the LOQ in the surface water in the ILV. In the ECM, the number of samples was insufficient for all analyses at 10×LOQ (n = 4), and mean recoveries of 2,4-DCP in tap water were <70% at 10×LOQ.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide ¹	MRID		EPA Review	Matrix	Method Date (dd/mm/ yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
2,4-D	49709301	49709302		Water ^{2,3}	26/01/2012	Dow AgroSciences LLC	LC/MS/MS	0.10 µg/L
2,4-DCP							GC/MS	
4-CP								
2,4-DCA								

1 2,4-D = (2,4-Dichlorophenoxy)acetic acid; 2,4-DCP = 2,4-Dichlorophenol; 4-CP = 4-Chlorophenol; 2,4-DCA = 2,4-Dichloroanisole.

2 For the ECM, drinking (tap) water (003; pH 8.6, dissolved organic carbon 2.6 ppm), ground (well) water (002; pH 8.2, dissolved organic carbon 2.2 ppm), and surface (pond) water (001; pH 8.0, dissolved organic carbon 6.9 ppm) were used in the study (p. 24 of MRID 49709301).

3 For the ILV, drinking (tap) water (CCON/039/001; pH 7.8, dissolved organic carbon 2.73 ppm), ground (well) water (CCON/038/001; pH 8.0, dissolved organic carbon 1.03 ppm), and surface (pond) water (CCON/037/001; pH 7.6, dissolved organic carbon 4.99 ppm) were used in the study (p. 13 of MRID 49709302).

I. Principle of the Method

2,4-D, 2,4-DCP and 4-CP

Samples (40 ± 0.4 mL) of water in 11 dram (45-mL) glass vials were fortified, as necessary, then acidified with 1.0 mL of 2 N hydrochloric acid (HCl) via shaking on a reciprocating shaker (minimum of 30 minutes at *ca.* 180 excursions/minute; pp. 25-26 of MRID 49709301). The sample was purified using an Oasis MCX solid phase extraction (SPE) cartridge (60-mg, 3-mL). The SPE column was pre-conditioned with methanol (1 mL) and 0.1 N HCl (2 x 1 mL) with full vacuum (*ca.* -25 in Hg). The sample was applied to the column (*ca.* 1 mL/min rate); the eluate was discarded. After drying the column with full vacuum for about 10 seconds, the column was washed with 1 mL of 0.1 N HCl. After drying the column with full vacuum for about 30 seconds, the analytes were eluted with two 0.5-mL aliquots of acetonitrile:methanol (80:20, v:v) containing 0.1% acetic acid (*ca.* 1 mL/min rate). Full vacuum was applied for about 10 seconds between solvent additions. The purified sample was evaporated to *ca.* 0.5 mL under nitrogen (*ca.* 500 mL/min) on a N-Evap evaporator at 40°C. The method noted that the 5.0 µg/L spiked samples should not be concentrated. The volume of the sample was adjusted to 1.0 mL using *ca.* 0.5 mL of water containing 0.1% acetic acid; for the 5.0 µg/L spiked samples, the volume of the sample was adjusted to 5.0 mL using 4.0 mL of water containing 0.1% acetic acid. After vortexing for 3-4 seconds, an aliquot of the sample was transferred to a LC/MS/MS vial for analysis.

Samples were analyzed for 2,4-D and its metabolite 2,4-DCP using an Applied Biosystems API5000 LC/MS/MS (pp. 17-19, 26 of MRID 49709301). The instrumental conditions consisted of a Synergi Hydro-RP column (4.6 x 75 mm, 4-µm; column temperature not reported), a mobile phase of (A) water containing 0.1% acetic acid and (B) acetonitrile:methanol (80:20, v:v) containing 0.1% acetic acid [45:55, v:v], MS/MS detection in negative-ion APCI tandem MS (MRM; temperature, 450°C), and injection volume 10 µL. Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): m/z 219 → 161 and m/z 221 → 163 for 2,4-D, and m/z 161 → 125 and m/z 163 → 127 for 2,4-DCP. Retention times were observed at *ca.* 2.5 and 3.2 min. for 2,4-D and 2,4-DCP, respectively (Figure 15, p. 91).

Samples were analyzed for 4-CP using an Applied Biosystems QTRAP 5500 LC/MS/MS (pp. 19-20, 26 of MRID 49709301). The instrumental conditions consisted of a Synergi Hydro-RP column (4.6 x 75 mm, 4-µm; column temperature not reported), a gradient mobile phase of (A) water containing 0.1% acetic acid and (B) acetonitrile:methanol (80:20, v:v) containing 0.1% acetic acid [percent A:B (v:v) at 0.01 min. 80:20, 4.50-7.50 min. 0:100, 8.00-12.50 min. 80:20], MS/MS detection in negative-ion electrospray MS (MRM; temperature, 500°C), and injection volume 10 µL. Two parent-daughter ion transitions were monitored (quantification and confirmation, respectively): m/z 127 → 91 and m/z 129 → 91 for 4-CP. Retention time was observed at *ca.* 3.0 min. for 4-CP-OH (Figure 19, pp. 106-107).

2,4-DCA

Samples (40 ± 0.4 mL) of water in 11 dram (45-mL) glass vials were fortified, as necessary, then acidified with 1.0 mL of 1 N hydrochloric acid (HCl; pp. 27-28 of MRID 49709301). For the 5.0

µg/L spiked samples, 5.0 mL of isooctane extraction solution was added; for all other samples, 1.0 mL of isooctane extraction solution was added. The samples were extracted via shaking on a reciprocating shaker (minimum of 30 minutes at *ca.* 180 excursions/minute). After centrifuging (5 minutes at 2000 rpm), the top layer was transferred for GC/MS analysis.

Samples were analyzed for 2,4-DCA using an Agilent Model 6890A gas chromatograph coupled to an Agilent Model 5973N mass spectrometer (pp. 20-22 of MRID 49709301). The instrumental conditions consisted of a Durabond-5MS capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness), helium carrier gas, oven method (80°C for 1.2 min., 80°C to 320°C at 20°C/min., 320°C for 2.0 min.), splitless injection and electron impact mode. Three ions were monitored (quantification, confirmation 1 and confirmation 2, respectively): m/z 176, 178 and m/z 161 for 2,4-DCA. Retention time was observed at *ca.* 8.00 min. for 2,4-DCA (Figure 25, pp. 118-121).

SPE Standardization

The ECM study author noted that variation in Oasis MCX SPE columns may influence that elution profile of 2,4-D and its metabolites (pp. 37-38; Figure 28, p. 130 of MRID 49709301). The study author presented a procedure using the mixed spiking solution and the typical elution profile which should be achieved. The study author also noted that instrument optimization was suggested for optimal chromatographic separation and sensitivity.

ILV

In the ILV, the ECM was performed exactly as written, except that a Zorbax SB-C8 column (4.6 x 7.5 mm, 3.5-µm) was used for 4-CP LC/MS/MS analysis and an Agilent Liner 1.8 mm ID PTV M was added to the operating conditions of the GC/MS (pp. 19-26 of MRID 49709302). LC/MS/MS mass transitions and GC/MS monitored ions were generally the same as those of the ECM. Retention times were observed at *ca.* 2.6, 3.2, 3.5 and 7.6 min. for 2,4-D, 2,4-DCP, 4-CP and 2,4-DCA, respectively Figure 13, p. 55; Figure 29, p. 71; Figure 45, p. 87; Figure 62, p. 104).

LOQ/LOD

The LOQ for all analytes was the same in the ECM and ILV at 0.10 µg/L (p. 37; Table 42, p. 73 of MRID 49709301; p. 11 of MRID 49709302). The LOD for all analytes was 0.03 µg/L in the ECM; the LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 49709301): Mean recoveries and relative standard deviations (RSDs) were within guidelines for analysis of (2,4-Dichlorophenoxy)acetic acid (2,4-D) and its metabolites, 2,4-dichlorophenol (2,4-DCP), 4-chlorophenol, 2,4-dichloroanisole (2,4-DCA), in drinking (tap), ground (well) and surface (pond) water matrices at fortification levels of 0.10 µg/L (LOQ), 1.00 µg/L (10×LOQ) and 5.00 µg/L (50×LOQ), except for analysis of 2,4-DCP at 10×LOQ in ground water [mean 64% (quantitation), 65% (confirmation); Tables 2-40, pp. 41-72]. The number of samples was sufficient for all analyses at the LOQ and 50×LOQ, but all analysis at 10×LOQ were only performed with four samples (n = 4). For 2,4-D, 2,4-DCP and 4-CP, two ion transitions were monitored using LC/MS/MS; performance data (recovery results) of the quantitative HPLC analysis and confirmatory HPLC analysis were comparable. For 2,4-DCA, three ions were monitored using GC/MS; performance data of the quantitation, confirmation 1 and confirmation 2 ions were comparable. The ECM calculations allowed for recovery data to be corrected for residues found in the control samples; residues were quantified in control samples of 2,4-D in well water and 2,4-DCP in tap water (GC/MS chromatograms for 2,4-DCA were extremely faint and difficult to interpret; pp. 28-30; Tables 2-28, pp. 41-67). Recoveries from samples fortified at 0.03 µg/L (LOD) ranged (ions/matrices combined) from 74-146% for 2,4-D, 47-108% for 2,4-DCP, 40-88% for 4-CP and 77-94% for 2,4-DCA (n = 2 for each matrix/analyte; DER Attachment 2). The water matrices were well characterized at and obtained from the Sample Management Group of Dow AgroSciences LLC (sources not further specified; p. 24). Drinking (tap) water (003; pH 8.6, dissolved organic carbon 2.6 ppm), ground (well) water (002; pH 8.2, dissolved organic carbon 2.2 ppm), and surface (pond) water (001; pH 8.0, dissolved organic carbon 6.9 ppm) were used in the study.

ILV (MRID 49709302): Mean recoveries and relative standard deviations (RSDs) were within guidelines for analysis of (2,4-Dichlorophenoxy)acetic acid (2,4-D) and its metabolites, 2,4-dichlorophenol (2,4-DCP), 4-chlorophenol, 2,4-dichloroanisole (2,4-DCA), in drinking (tap), ground (well) and surface (pond) water matrices at fortification levels of 0.10 µg/L (LOQ), 1.00 µg/L (10×LOQ) and 5.00 µg/L (50×LOQ); Tables 1-9, pp. 34-42; DER Attachment 2). Performance data (recovery results) of the quantitative HPLC analysis and confirmatory HPLC analysis were comparable for 2,4-D, 2,4-DCP and 4-CP. GC/MS performance data of the quantitation, confirmation 1 and confirmation 2 ions were comparable for 2,4-DCA. The ILV calculations allowed for recovery data to be corrected for residues found in the control samples; residues were observed in many of the chromatograms of control samples of the analytes/matrices (GC/MS chromatograms for 2,4-DCA were extremely faint and difficult to interpret; p. 27; Figures 11-73, pp. 53-115). Recoveries from samples fortified at 0.03 µg/L (LOD) were reported as not applicable by the study author (no raw recovery data was provided; Tables 1-9, pp. 34-42). The water matrices were well characterized at CEMAS (p. 13). Drinking (tap) water (CCON/039/001; pH 7.8, dissolved organic carbon 2.73 ppm), ground (well) water (CCON/038/001; pH 8.0, dissolved organic carbon 1.03 ppm), and surface (pond) water (CCON/037/001; pH 7.6, dissolved organic carbon 4.99 ppm) were used in the study. The drinking water was obtained from a “drinking water” tap at CEMAS (p. 13). The surface water was obtained from the River Lodden, Charvill, United Kingdom. The ground water was obtained from a well near Henley-on-Thames. The method was validated in the first trial for all analytes in drinking, surface and ground water matrices with insignificant modifications (pp. 29-30).

Table 2. Initial Validation Method Recoveries for 2,4-D and Its Metabolites, 2,4-DCP, 4-CP and 2,4-DCA, in Drinking, Ground and Surface Water^{1,2,3}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface (Pond) Water						
Quantitation ion transition or Quantitation ion						
2,4-D	0.03 (LOD)	2	74, 122	--	--	--
	0.1 (LOQ)	5	87-104	95	6.9	7.3
	1.0	4	90-115	104	11.4	10.9
	5.0	5	101-107	104	2.6	2.5
2,4-DCP	0.03 (LOD)	2	62, 73	--	--	--
	0.1 (LOQ)	5	67-75	71	3.2	4.5
	1.0	4	69-82	74	5.5	7.4
	5.0	5	67-93	79	9.5	12.1
4-CP	0.03 (LOD)	2	47, 65	--	--	--
	0.1 (LOQ)	5	65-78	71	4.7	6.5
	1.0	4	70-83	78	5.4	6.9
	5.0	5	95-102	97	2.6	2.7
2,4-DCA	0.03 (LOD)	2	83, 91	--	--	--
	0.1 (LOQ)	5	86-93	89	3.0	3.4
	1.0	4	101-104	102	1.4	1.3
	5.0	5	91-94	92	1.1	1.2
Confirmation ion transition or Confirmation ion 1						
2,4-D	0.03 (LOD)	2	115, 118	--	--	--
	0.1 (LOQ)	5	93-107	98	6.4	6.5
	1.0	4	94-120	105	12.1	11.5
	5.0	5	101-107	104	2.6	2.5
2,4-DCP	0.03 (LOD)	2	57, 77	--	--	--
	0.1 (LOQ)	5	63-75	71	4.4	6.3
	1.0	4	71-89	78	8.8	11.2
	5.0	5	89-101	93	4.5	4.8
4-CP	0.03 (LOD)	2	40, 74	--	--	--
	0.1 (LOQ)	5	64-75	70	3.9	5.5
	1.0	4	69-79	75	4.1	5.5
	5.0	5	90-100	95	3.9	4.1
2,4-DCA	0.03 (LOD)	2	82, 87	--	--	--
	0.1 (LOQ)	5	87-97	92	3.9	4.2
	1.0	4	101-105	102	1.6	1.6
	5.0	5	91-95	94	1.6	1.7
Confirmation ion 2						
2,4-DCA	0.03 (LOD)	2	87, 92	--	--	--
	0.1 (LOQ)	5	85-91	89	2.6	3.0
	1.0	4	101-104	102	1.3	1.3
	5.0	5	91-94	92	1.5	1.7
Ground (Well) Water						

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Quantitation ion transition or Quantitation ion						
2,4-D	0.03 (LOD)	2	100, 130	--	--	--
	0.1 (LOQ)	5	93-103	97	4.0	4.2
	1.0	4	92-110	99	7.8	7.9
	5.0	5	97-109	103	6.0	5.8
2,4-DCP	0.03 (LOD)	2	55, 71	--	--	--
	0.1 (LOQ)	5	72-86	78	5.6	7.2
	1.0	4	70-86	75	7.9	10.6
	5.0	5	90-94	92	1.5	1.6
4-CP	0.03 (LOD)	2	49, 81	--	--	--
	0.1 (LOQ)	5	72-83	78	5.0	6.5
	1.0	4	75-91	81	7.0	8.6
	5.0	5	95-102	98	3.2	3.3
2,4-DCA	0.03 (LOD)	2	79, 85	--	--	--
	0.1 (LOQ)	5	85-92	90	2.7	3.0
	1.0	4	100-106	104	2.7	2.6
	5.0	5	89-94	92	2.3	2.5
Confirmation ion transition or Confirmation ion 1						
2,4-D	0.03 (LOD)	2	81, 146	--	--	--
	0.1 (LOQ)	5	89-103	95	6.5	6.8
	1.0	4	98-115	103	8.2	8.0
	5.0	5	98-107	103	4.2	4.1
2,4-DCP	0.03 (LOD)	2	66, 78	--	--	--
	0.1 (LOQ)	5	73-87	79	7.0	8.8
	1.0	4	70-86	74	8.3	11.2
	5.0	5	89-94	91	2.0	2.2
4-CP	0.03 (LOD)	2	43, 88	--	--	--
	0.1 (LOQ)	5	67-99	80	14.2	17.8
	1.0	4	73-92	81	7.9	9.8
	5.0	5	92-101	96	4.1	4.3
2,4-DCA	0.03 (LOD)	2	77, 85	--	--	--
	0.1 (LOQ)	5	88-97	92	3.1	3.4
	1.0	4	100-105	102	2.4	2.3
	5.0	5	88-95	92	2.5	2.8
Confirmation ion 2						
2,4-DCA	0.03 (LOD)	2	81, 87	--	--	--
	0.1 (LOQ)	5	87-93	90	2.4	2.6
	1.0	4	101-106	104	2.4	2.3
	5.0	5	89-95	91	2.9	3.1
Drinking (Tap) Water						
Quantitation ion transition or Quantitation ion						
2,4-D	0.03 (LOD)	2	81, 91	--	--	--
	0.1 (LOQ)	5	81-100	89	7.6	8.5
	1.0	4	82-103	95	9.0	9.5

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	5.0	5	98-109	102	4.5	4.4
2,4-DCP	0.03 (LOD)	2	47, 98	--	--	--
	0.1 (LOQ)	5	63-77	70	5.2	7.5
	1.0	4	54-74	64	8.8	13.7
	5.0	5	87-92	90	2.4	2.7
4-CP	0.03 (LOD)	2	53, 82	--	--	--
	0.1 (LOQ)	5	65-75	72	4.4	6.2
	1.0	4	68-81	71	6.4	9.0
	5.0	5	97-107	102	4.1	4.0
2,4-DCA	0.03 (LOD)	2	85, 94	--	--	--
	0.1 (LOQ)	5	87-93	90	2.0	2.2
	1.0	4	101-105	103	1.9	1.8
	5.0	5	85-98	94	5.2	5.5
Confirmation ion transition or Confirmation ion 1						
2,4-D	0.03 (LOD)	2	80, 81	--	--	--
	0.1 (LOQ)	5	76-104	91	10.1	11.2
	1.0	4	86-103	96	7.0	7.3
	5.0	5	101-105	104	2.0	1.9
2,4-DCP	0.03 (LOD)	2	91, 108	--	--	--
	0.1 (LOQ)	5	75-77	76	0.9	1.2
	1.0	4	56-77	65	9.1	14.0
	5.0	5	88-92	89	1.9	2.1
4-CP	0.03 (LOD)	2	68, 76	--	--	--
	0.1 (LOQ)	5	67-74	71	2.9	4.1
	1.0	4	67-79	71	5.2	7.3
	5.0	5	98-107	102	3.4	3.3
2,4-DCA	0.03 (LOD)	2	80, 93	--	--	--
	0.1 (LOQ)	5	89-96	92	2.8	3.0
	1.0	4	100-104	102	2.0	1.9
	5.0	5	86-98	94	4.4	4.7
Confirmation ion 2						
2,4-DCA	0.03 (LOD)	2	91, 93	--	--	--
	0.1 (LOQ)	5	85-96	89	4.5	5.1
	1.0	4	103-105	104	1.3	1.2
	5.0	5	88-100	95	4.5	4.7

Data (recovery results corrected for residues found in the controls; pp. 28-30) were obtained from Tables 2-28, pp. 41-67 (LOD results) and Tables 29-40, pp. 68-72 of MRID 49709301 and DER Attachment 2 (LOD calculations).

1 2,4-D = (2,4-Dichlorophenoxy)acetic acid; 2,4-DCP = 2,4-Dichlorophenol; 4-CP = 4-Chlorophenol; 2,4-DCA = 2,4-Dichloroanisole.

2 The water matrices were well characterized (p. 24). Drinking (tap) water (003; pH 8.6, dissolved organic carbon 2.6 ppm), ground (well) water (002; pH 8.2, dissolved organic carbon 2.2 ppm), and surface (pond) water (001; pH 8.0, dissolved organic carbon 6.9 ppm) were used in the study.

3 For 2,4-D, 2,4-DCP and 4-CP, two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): m/z 219 → 161 and m/z 221 → 163 for 2,4-D; m/z 161 → 125 and m/z 163 → 127 for 2,4-DCP; and m/z 127 → 91 and m/z 129 → 91 for 4-CP (pp. 18-22). For 2,4-DCA, three ions were monitored (quantification, confirmation 1 and confirmation 2, respectively): m/z 176, 178 and m/z 161.

Table 3. Independent Validation Method Recoveries for 2,4-D and Its Metabolites, 2,4-DCP, 4-CP and 2,4-DCA, in Drinking, Ground and Surface Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Tap Water						
Quantitation ion transition or Quantitation ion						
2,4-D	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	103-116	109	5	4.6
	1.0	5	85-105	95	8	8.3
	5.0	5	97-111	101	6	5.8
2,4-DCP	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	98-117	109	8	7.5
	1.0	5	72-84	78	5	6.7
	5.0	5	82-92	87	4	4.4
4-CP	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	94-118	104	10	9.5
	1.0	5	84-101	93	7	7.7
	5.0	5	94-108	99	5	5.3
2,4-DCA	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	94-104	99	4	3.6
	1.0	5	101-102	102	1	0.5
	5.0	5	86-116	98	11	11.3
Confirmation ion transition or Confirmation ion 1						
2,4-D	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	100-118	110	7	6.6
	1.0	5	84-105	95	8	8.7
	5.0	5	96-110	101	6	5.5
2,4-DCP	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	95-119	105	10	9.3
	1.0	5	74-86	80	6	7.0
	5.0	5	77-90	84	5	6.5
4-CP	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	94-118	103	10	9.4
	1.0	5	83-101	92	7	8.1
	5.0	5	93-104	97	4	4.4
2,4-DCA	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	91-104	98	6	5.7
	1.0	5	100-103	102	1	1.1
	5.0	5	86-120	99	13	12.8
Confirmation ion 2						
2,4-DCA	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	90-103	98	5	5.3
	1.0	5	100-102	101	1	0.8
	5.0	5	86-119	99	12	12.6
Surface Water						
Quantitation ion transition or Quantitation ion						

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
2,4-D	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	101-120	109	7	6.4
	1.0	5	97-105	101	4	4.0
	5.0	5	97-102	101	2	2.1
2,4-DCP	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	89-103	96	5	5.7
	1.0	5	83-96	91	5	5.8
	5.0	5	96-103	99	3	2.9
4-CP	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	82-97	91	6	6.4
	1.0	5	82-91	87	4	4.7
	5.0	5	97-107	101	4	3.7
2,4-DCA	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	102-110	106	3	2.8
	1.0	5	102-111	105	4	3.6
	5.0	5	83-95	91	6	6.3
Confirmation ion transition or Confirmation ion 1						
2,4-D	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	102-117	110	6	5.1
	1.0	5	97-107	102	4	4.4
	5.0	5	97-102	101	2	2.2
2,4-DCP	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	94-108	101	7	6.8
	1.0	5	84-97	92	5	5.9
	5.0	5	95-103	99	3	3.4
4-CP	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	81-92	88	4	5.1
	1.0	5	81-91	85	4	5.1
	5.0	5	94-104	99	4	3.8
2,4-DCA	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	102-111	106	3	3.2
	1.0	5	102-111	105	4	3.6
	5.0	5	83-96	90	6	7.0
Confirmation ion 2						
2,4-DCA	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	102-107	105	2	2.1
	1.0	5	103-111	105	3	3.2
	5.0	5	83-96	97	6	6.7
Ground Water						
Quantitation ion transition or Quantitation ion						
2,4-D	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	100-120	105	8	8.0
	1.0	5	84-102	92	8	8.8
	5.0	5	90-97	92	3	3.3

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
2,4-DCP	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	86-101	92	6	6.8
	1.0	5	75-95	82	8	10.6
	5.0	5	74-89	80	6	7.2
4-CP	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	90-103	98	5	4.9
	1.0	5	84-99	93	7	8.0
	5.0	5	90-101	95	4	4.3
2,4-DCA	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	108-114	110	2	2.3
	1.0	5	100-102	101	1	0.9
	5.0	5	98-108	101	4	3.9
Confirmation ion transition or Confirmation ion 1						
2,4-D	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	98-118	104	8	7.8
	1.0	5	85-101	92	7	7.6
	5.0	5	88-98	91	4	4.5
2,4-DCP	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	86-101	93	7	7.2
	1.0	5	75-95	82	8	9.9
	5.0	5	74-89	80	6	7.2
4-CP	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	94-109	101	7	6.8
	1.0	5	84-100	91	7	7.4
	5.0	5	87-100	93	5	5.0
2,4-DCA	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	100-113	108	5	4.7
	1.0	5	100-102	101	1	0.9
	5.0	5	99-109	102	4	4.0
Confirmation ion 2						
2,4-DCA	0.03 (LOD)	1	NA	--	--	--
	0.1 (LOQ)	5	102-113	107	4	3.9
	1.0	5	100-102	101	1	0.9
	5.0	5	99-109	102	4	4.0

Data (recovery results corrected for residues found in the controls; p. 27) were obtained from Tables 1-9, pp. 34-42 of MRID 49709302 and DER Attachment 2 (s.d. at LOQ, 10×LOQ and 50×LOQ). NA = Not Applicable; % recoveries at the LOD were not calculated by the study author, and raw data was not reported so that the values could be reviewer-calculated.

1 2,4-D = (2,4-Dichlorophenoxy)acetic acid; 2,4-DCP = 2,4-Dichlorophenol; 4-CP = 4-Chlorophenol; 2,4-DCA = 2,4-Dichloroanisole.

2 The water matrices were well characterized (p. 13). Drinking (tap) water (CCON/039/001; pH 7.8, dissolved organic carbon 2.73 ppm), ground (well) water (CCON/038/001; pH 8.0, dissolved organic carbon 1.03 ppm), and surface (pond) water (CCON/037/001; pH 7.6, dissolved organic carbon 4.99 ppm) were used in the study.

3 For 2,4-D, 2,4-DCP and 4-CP, two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): m/z 219 → 161 and m/z 221 → 163 for 2,4-D; m/z 161 → 125 and m/z 163 → 127 for 2,4-DCP; and m/z 127 → 91 and m/z 129 → 91 for 4-CP (pp. 24-26). For 2,4-DCA, three ions were monitored (quantification, confirmation 1 and confirmation 2, respectively): m/z 176, 178 and m/z 161.

III. Method Characteristics

In the ECM and ILV, the LOQ value for 2,4-D and its metabolites, 2,4-DCP, 4-CP and 2,4-DCA, was established at 0.10 µg/L (pp. 37, 39; Table 42, p. 73 of MRID 49709301; p. 11 of MRID 49709302). The LOD for all analytes was 0.03 µg/L in the ECM. The LOD was not reported in the ILV. Following the method of Keith, L. H., *et al.* (see section **V. References** below), the LOD and LOQ for determination of 2,4-D and its metabolites in water were calculated in the ECM using the standard deviation from the 0.10 µg/L recovery results. 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 calculated values support the LOQ and LOD established for the study and are presented in **Table 4** below.

Table 4. Method Characteristics

		HPLC/MS/MS Analysis			GC/MS Analysis	
		2,4-D	2,4-DCP	4-CP	2,4-DCA	
Limit of Quantitation (LOQ)	Established	0.10 µg/L				
	Calculated (ECM)	0.0691-0.0791 µg/L	0.0574-0.0576 µg/L	0.0532-0.0930 µg/L	0.0245-0.0312 µg/L	
Limit of Detection (LOD)	Established	0.03 µg/L				
	Calculated (ECM)	0.0207-0.0237 µg/L	0.0172-0.0173 µg/L	0.0160-0.0279 µg/L	0.0074-0.0094 µg/L	
Linearity (Least squares calibration curve r and concentration range)	ECM ¹	r ² = 0.999378 (Q) r ² = 0.999697 (C)	r ² = 0.998871 (Q) r ² = 0.999182 (C)	r ² = 0.999641 (Q) r ² = 0.999463 (C)	r ² = 0.9996 (Q) r ² = 0.9997 (C1) r ² = 0.9995 (C2)	
		1.2-50 ng/mL				
	ILV	r ² = 0.9999 (Q) r ² = 0.9999 (C)	r ² = 0.9998 (Q) r ² = 0.9998 (C)	r ² = 0.9999 (Q) r ² = 0.9999 (C)	r ² = 0.9985 (Q) r ² = 0.9987 (C1) r ² = 0.9986 (C2)	
Repeatable	ECM ²	Yes at LOQ and 50×LOQ. Yes at 10×LOQ, but n = 4.	Yes at LOQ and 50×LOQ. Yes at 10×LOQ in surface and ground water, but n = 4. No at 10×LOQ in tap water: mean 64-65% and n = 4.	Yes at LOQ and 50×LOQ. Yes at 10×LOQ, but n = 4.		
	ILV ³	Yes at LOQ, 10×LOQ and 50×LOQ (n = 5).				
Reproducible		Yes at LOQ, 10×LOQ and 50×LOQ.				
Specific	ECM	Residues in the matrix controls were quantified as <LOQ.				
		Tap	Yes, no interferences were observed in the matrix control.	Yes, interferences (<10% of the LOQ) were observed in the matrix control.	Yes, no interferences were observed in the matrix control.	GC/MS chromatograms for 2,4-DCA were extremely faint and difficult to interpret.
		Surface	Interferences (25-30% of the LOQ were observed in the matrix control.	Yes, no interferences were observed in the matrix control.		
	Ground					
	ILV	Interferences were reported as <30% of the LOQ.				
		Tap	Yes, interferences (ca.10% of the LOQ) were observed in the matrix control.	Yes, no interferences were observed in the matrix control.	Yes, only minor residues (<5% to ca. 5% of the LOQ) in the matrix control.	GC/MS chromatograms for 2,4-DCA were extremely faint and difficult to interpret.
Surface		Interferences (20-25% of the LOQ were observed in the matrix control.	Yes, only minor residues (<5% of the LOQ) in the matrix control.			
Ground	Yes, only minor residues (<5% of the LOQ) in the matrix control.	Yes, no interferences were observed in the matrix control.				

Data were obtained from pp. 37, 39; Tables 2-40, pp. 41-72; Table 42, p. 73; Figures 10-13, pp. 84-89 (calibration curves); Figures 14-22, pp. 90-113 (HPLC chromatograms); Figures 23-27, pp. 114-129 (GC chromatograms) of MRID 49709301; pp. 11, 29-30; Tables 1-9, pp. 34-42; Figures 1-9, pp. 43-51 (calibration curves); Figures 11-57, pp. 53-99 (HPLC chromatograms); Figures 58-73, pp. 100-115 (GC chromatograms) of MRID 49709302. Q = Quantitative HPLC or GC/MS analysis; C = Confirmatory HPLC analysis; C1 = Confirmation 1 GC/MS analysis; C2 = Confirmation 2 GC/MS analysis.

- 1 ECM standard curves were weighted $1/x$ for 2,4-D, 2,4-DCP and 4-CP. ECM r^2 values are reviewer-generated for those analytes from reported r values of 0.9994352-0.9998204 (Q) and 0.999591-0.9998483 (C; calculated from data in Figures 10-12, pp. 84-86 of MRID 49709301; see DER Attachment 2).
- 2 For the ECM, drinking (tap) water (003; pH 8.6, dissolved organic carbon 2.6 ppm), ground (well) water (002; pH 8.2, dissolved organic carbon 2.2 ppm), and surface (pond) water (001; pH 8.0, dissolved organic carbon 6.9 ppm) were used in the study (p. 24 of MRID 49709301).
- 3 For the ILV, drinking (tap) water (CCON/039/001; pH 7.8, dissolved organic carbon 2.73 ppm), ground (well) water (CCON/038/001; pH 8.0, dissolved organic carbon 1.03 ppm), and surface (pond) water (CCON/037/001; pH 7.6, dissolved organic carbon 4.99 ppm) were used in the study (p. 13 of MRID 49709302). Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. In the ILV, a reagent blank was not included (p. 18 of MRID 49709302).
2. The results were corrected for residues quantified in the controls in the ECM and ILV (pp. 28-30 of MRID 49709301; p. 27 of MRID 49709302).
3. The ILV reported that communications between the ILV and the sponsor were unnecessary; however, a Study Plan Amendment was required to confirm the GC/MS instrument conditions (p. 30 of MRID 49709302).
4. The specificity of the method for 2,4-DCA could not be confirmed using the GC/MS chromatograms provided in the ECM and ILV (Figures 23-27, pp. 114-129 of MRID 49709301; Figures 58-73, pp. 100-115 of MRID 49709302). The baseline and peaks were extremely faint and difficult to interpret.
5. For 2,4-D, significant interferences in the matrix controls were observed at 25-30% of the LOQ in the ground water in the ECM and at 20-25% of the LOQ in the surface water in the ILV (Figure 17, pp. 98-99 of MRID 49709301; Figure 16, p. 58; Figure 18, p. 60 of MRID 49709302). OCSPP Guidelines recommend that interferences with peak areas are less than 50% at the LOD (15% of the LOQ).
6. In the ECM, the number of samples was insufficient for all analyses at $10 \times \text{LOQ}$ ($n = 4$; Tables 2-40, pp. 41-72 of MRID 49709301). OCSPP guidelines recommend a minimum of five samples spiked at each fortification level (*i.e.*, minimally, the LOQ and $10 \times \text{LOQ}$) for each analyte.

In the ECM, method recoveries of 2,4-DCP in tap water did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy (mean recoveries for replicates at each spiking level between 70% and 120% and relative standard deviations (RSD) $\leq 20\%$) at

the 10×LOQ fortification level [mean 64% (quantitation), 65% (confirmation); Tables 2-40, pp. 41-72 of MRID 49709301].

7. In the ILV, minor residues (<10% of the LOQ) were observed in the control samples of 2,4-D in tap and ground water, 2,4-DCP in surface water and 4-CP in all water matrices (Figures 11-57, pp. 53-99 of MRID 49709302).

In the ECM, minor residues (<10% of the LOQ) were observed in the control samples of 2,4-DCP in tap water (Figure 18, p. 102 of MRID 49709301).

8. The LOD was not reported in the ILV.
9. The reviewer noted the following significant typographical error: the titling of the individual recovery data tables for the quantitation ion of “2,4-DCA” were entitled “2,4-D” (Tables 20-22, pp. 59-61 of MRID 49709301).
10. In the ECM, the calibration standards of 2,4-D, 2,4-DCP and 4-CP [in 50% acetonitrile:methanol (80:20) containing 0.1% acetic acid and 50% water containing 0.1% acetic acid] were stable for at least 3 days when refrigerated (p. 34 of MRID 49709301). These results were established in DAS Study No. 110503. The study author concluded that the corresponding sample extracts were stable for up to 3 days under refrigeration storage. The calibration standard of 2,4-DCA in isooctane were stable for at least 2 days under ambient conditions. These results were established in DAS Study No. 110503. The study author concluded that the corresponding sample extracts were stable for up to 2 days under ambient conditions.

In the ECM, matrix effects were also studied (p. 34 of MRID 49709302). Matrix effects were insignificant ($\pm 16\%$) for all matrices.

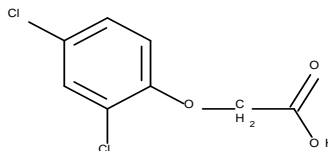
11. It was reported for the ILV that the analytical procedure for one set of 18 samples required approximately 8 person hours for preparation (p. 230 of MRID 49709302). The LC/MS/MS or GC/MS was conducted overnight unattended. The interpretation of data required approximately 2 hours. The overall time to complete a set of samples was 1.5 calendar days.

V. References

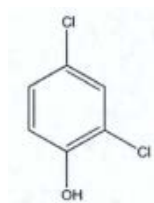
- Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* 1983, 55, 2210-2218 (p. 39 of MRID 49709301).
- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures**2,4-D**

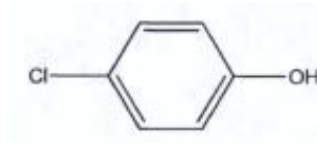
IUPAC Name: (2,4-Dichlorophenoxy)acetic acid
CAS Name: 2-(2,4-Dichlorophenoxy)acetic acid
CAS Number: 94-75-7
SMILES String: O=C(O)COc(c(cc(c1)Cl)Cl)c1

**2,4-DCP**

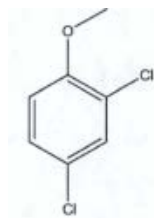
IUPAC Name: 2,4-Dichlorophenol
CAS Name: Not reported
CAS Number: 120-83-2
SMILES String: Not found

**4-Chlorophenol**

IUPAC Name: 4-Chlorophenol
CAS Name: Not reported
CAS Number: 106-48-9
SMILES String: Not found

**2,4-DCA**

IUPAC Name: 2,4-Dichloroanisole
CAS Name: Not reported
CAS Number: 553-82-3
SMILES String: Not found



Test Material: 2,4-D

MRID: 49709301

Title: Method Validation Study for the Determination of Residues of (2,4-dichlorophenoxy)acetic acid and its Metabolites in Surface Water, Ground Water and Drinking Water

MRID: 49709302

Title: Independent Laboratory Validation of an Analytical Method for the Determination of Residues of (2,4-Dichlorophenoxy)acetic acid, 2,4-Dichlorophenol, 4-Chlorophenol and 2,4-Dichloroanisole in Water

EPA PC Code: 030001

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto

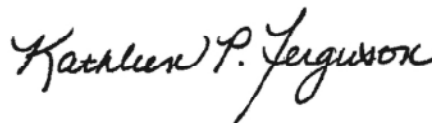
Signature:



Date: 1/6/16

Secondary Reviewer: Kathleen Ferguson

Signature:



Date: 1/6/16

QC/QA Manager: Joan Gaidos

Signature:



Date: 1/6/16