

**Test Material:** 2,4-DP-p and its transformation products: 2,4-DCP, 2,4-DCA and 2-EHE

**MRID:** 44243401

**Title:** Independent Laboratory Method Trials of a Residue Analytical Method for Dichlorprop-P (2,4-DP-p) and Metabolites in Soil.

**EPA PC Code:** 031402

**OCSPP Guideline:** 850.6100

**For CDM Smith**

**Primary Reviewer:** Lisa Muto

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**Analytical method for 2,4-DP-p and its transformation products, 2,4-DCP, 2,4-DCA and 2-EHE in soil**

**Reports:** MRID No. 44243401. Bruns, G., S. Nelson. 1997. Independent Laboratory Method Trials of a Residue Analytical Method for Dichlorprop-P (2,4-DP-p) and Metabolites in Soil. Report prepared by Enviro-Test Laboratories, Edmonton, Alberta, Canada; sponsored and submitted by 2,4-DP-p TASK FORCE, BASF Aktiengesellschaft, Limburgerhof, Germany. Protocol No.: 95ETL12.PRO. BASF Project No.: TSKF9301 (BASF/Adpen Labs). ETL Report No.: BSF06.REP. Final report issued January 3, 1997.

**Document No.:** MRID 44243401

**Guideline:** 850.6100

**Statements:** ECM: The study was conducted compliance with USEPA FIFRA GLP (40 CFR Part 160; Appendix 5, p. 123). Signed and dated GLP, Certification of Authenticity and Quality Assurance statements were provided (Appendix 5, pp. 123-124, 139). A No Data Confidentiality statement was not provided. ILV: The study was conducted in accordance with the USEPA FIFRA GLP (40 CFR Part 160; p. 3). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4, 21). A Certification of Authenticity was not included.

**Classification:** This analytical method study provides useful information and is classified as **supplemental**. However, performance data did not meet guideline requirements for multiple soil/analyte experiments in the ECM and ILV. Only three replicate samples were fortified at the LOQ and 10x LOQ for each test soil in the ECM. In the ILV, the fortification levels for 2,4-DP-p and 2-EHE differed from the nominal LOQ, 10xLOQ and 100xLOQ. The soil matrices were inadequately characterized in the ECM and ILV. GC/MS chromatogram sets were not provided for all test soils. Additional comments can be found in comments section.

**PC Code:** 031402

**Reviewer:** Faruque Khan, Ph.D.  
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**Signature:**  
**Date:** May 12, 2014

**Executive Summary**

This Analytical Method Number TSKF9301 (from Adpen Labs), is designed for the quantitative determination of 2,4-DP-p and its transformation products 2,4-DCP, 2,4-DCA and 2-EHE in soil using GC/MS. For the ECM, the reviewer could not adequately determine whether the method is quantitative for each analyte for each soil matrix because only three replicates were analyzed at the LOQ and 10x LOQ. However, the mean recovery ( $\pm$ SD) of 2,4-DP-p at the LOQ, across all five soils, was  $65.7 \pm 4.9\%$  (n=14), which is outside of the guideline requirement. Across all test soils, mean recoveries of 2,4-DCP, 2,4-DCA and 2-EHE at the LOQ and of all analytes at 10x and 200x LOQ were within the guideline requirement for mean recoveries. The lowest toxicological level of concern in soil was not reported; however, 2,4-DCP is listed in the EPA Regional Screening Levels with a screening level of 18 mg/kg in residential soil which is much

higher than the reported LOQ for this method (no other analyte was listed). In the ILV, performance data for all analytes were acceptable at both the LOQ and 10x LOQ in the California, New York and Washington soils (excluding high recovery of 2,4-DCP in California and New York soils at 10x LOQ and high RSD values for 2,4-DP-p in New York soil at the LOQ and 10x LOQ). Recoveries were low for multiple analytes in the Georgia and Indiana soils (see DER Table 3). No major modifications were made by the independent laboratory.

**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-DP-p and its transformation products 2,4-DCP, 2,4-DCA and 2-EHE	44243401	44243401		Soil	10/01/1994	BASF Aktiengesellschaft, Germany	GC/MS	0.01 mg/kg (0.01 ppm)

## I. Principle of the Method

Samples (10.0 g) were measured into 50-mL screw-cap polypropylene centrifuge tubes (Appendix 5, pp. 128, 131-134). The samples were vortexed with 20 mL of 5% acetic acid in methanol at the highest speed for *ca.* 30 seconds. Cavitation of the samples was performed via sonication in an ultrasonic water bath at room temperature for 20 minutes. After centrifugation at *ca.* 2000 rpm for *ca.* 10 minutes, the supernatant was decanted into a 500 mL glass screw-cap bottle. The vortex/sonication/centrifuge/decant procedure was sequentially repeated with 20 mL of 5% acetic acid in methanol:water (1:1, v:v) then 20 mL of 10% acetone in 0.5 M KCl/0.1M NaOH. The combined supernatants were mixed with *ca.* 430 mL of deionized water then the sample was acidified with 2.5 mL of phosphoric acid (*ca.* 85%). After shaking by hand for *ca.* 30 seconds, the pH was measured to ensure that it was less than 2. The sample was purified using a preconditioned C<sub>18</sub> solid phase extraction (SPE) column (1 g, 6 mL; preconditioned with 10 mL methanol then 10 mL of 1.5% phosphoric acid in water). The sample was passed through the column under vacuum, and the eluates were discarded. The analytes were removed from the column via elution with 9 mL of 2% acetone in hexane (Fraction A, containing 2,4-DCP, 2,4-DCA and 2-EHE) and 9 mL of 50% methanol in acetone (Fraction B, containing 2,4-DP-p). Fraction B was mixed with 1.0 mL of hexane then reduced to 0.5-1.0 mL using a N-Evap and a room temperature water bath. The residue of Fraction B was derivitized by mixing with 1.0 mL of 14% BF<sub>3</sub>/methanol solution in a capped tube. The sample tube was transferred to a water bath at 70 ± 2°C for 30 minutes. After cooling, 8 mL of distilled water and 5 mL of hexane were added to the sample. After shaking for 10 minutes at high speed using a mechanical shaker, the aqueous layer was discarded. The remaining hexane layer of Fraction B and the rinse of the transfer pipet were combined with Fraction A. This mixture was reduced to 1 mL using a N-Evap and a room temperature water bath. The residue was vortexed then diluted as necessary for GC/MS analysis.

Samples were analyzed for 2,4-DP-p, 2,4-DCP, 2,4-DCA and 2-EHE by gas chromatography (HP-5 MS, 30 m x 0.25 mm, 0.25- $\mu$ m column) with mass spectrometry (two different MS conditions provided; Appendix 5, Tables IIa-IIb, pp. 142-143). Three or one qualifier ions were monitored for methylated 2,4-DP-p (164, 191 and 248 m/z or only 164 m/z) and 2-EHE (189, 234 and 346 m/z or only 189 m/z). One qualifier ion was monitored for 2,4-DCP (164 m/z) and 2,4-DCA (176 m/z). No confirmation method was used; however, the ECM study authors suggested that a GC column with a different polarity, such as a HP-1701, could be used for confirmation (Appendix 5, p. 135). Injection volume was 1 or 2  $\mu$ L (Appendix 5, p. 134).

In the ECM, the LOQ and LOD were 0.01 mg/kg and 0.005 mg/kg, respectively (Appendix 5, p. 138). In the ILV, the LOQ was reported as 0.01 ppm, but no LOD was reported (p. 20).

## II. Recovery Findings

ECM (MRID 44243401): It could not be adequately determined whether the method is quantitative for each analyte for each soil matrix because an insufficient number of replicates were analyzed at the LOQ and 10x LOQ (three replicates each). Based on only three replicates, the method was quantitative (mean 70-120%; RSD  $\leq$ 20%) for 2,4-DP-p at the LOQ in New York soil only and at 10x LOQ in California and New York soils only; for 2,4-DCP at the LOQ in Georgia and Indiana soils and at 10x LOQ in Georgia, Indiana and Washington soils; and for 2,4-DCA and 2-EHE in all soils at the LOQ and 10x LOQ (excluding 2,4-DCA at the LOQ in Indiana soil; Appendix 5, Tables IIIa-IIIc, pp. 144-146; DER Attachment 2). The mean recovery ( $\pm$  SD) of 2,4-DP-p at the LOQ, across all five soils, was  $65.7 \pm 4.9\%$  (n=14), which is outside of the guideline requirement. Across all test soils, mean recoveries of 2,4-DCP, 2,4-DCA and 2-EHE at the LOQ and of all analytes at 10x and 200x LOQ were within the guideline requirement for mean recoveries. Soil matrix characterization was not reported (Appendix 5, pp. 136-137). Analyte identification was based on the observation of the MS target ions and qualifier ions; no confirmation method was used (Appendix 5, pp. 135, 137).

ILV (MRID 44243401): For 2,4-DP-p, mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD  $\leq$ 20%) for analysis at 0.0093 mg/kg in the California, Indiana and Washington soils, at 0.093 mg/kg in the California and Washington soils, and at 0.93 mg/kg in the California, New York and Washington soils; however, recoveries at 0.0093 mg/kg (LOQ) in the Georgia and New York soils, at 0.093 mg/kg in the Georgia, Indiana and New York soils, and at 0.93 mg/kg in the Georgia and Indiana soils did not meet guideline requirements (Tables 3A-7B, pp. 22-31). For 2,4-DCP, mean recoveries and relative standard deviations (RSD) were within guideline requirements for analysis at 0.01 mg/kg (LOQ) in the California, Indiana, New York and Washington soils, at 0.10 mg/kg in the Georgia, Indiana and Washington soils, and at 1.0 mg/kg in the California and New York soils; however, recoveries at 0.01 mg/kg (LOQ) in the Georgia soil, at 0.10 mg/kg in the California and New York soils, and at 1.0 mg/kg in the Georgia, Indiana and Washington soils did not meet guideline requirements. For 2,4-DCA, mean recoveries and relative standard deviations (RSD) were within guideline requirements for analysis at 0.01 mg/kg (LOQ) in all five soils, at 0.10 mg/kg in the California, Indiana, New York and Washington soils, and at 1.0 mg/kg in the California, Georgia, New York and Washington soils; however, recoveries at 0.10 mg/kg in the Georgia soil and at 1.0 mg/kg in the Indiana soil did not meet guideline requirements. For 2-EHE, mean

recoveries and relative standard deviations (RSD) were within guideline requirements for analysis at 0.012 mg/kg (LOQ) in the California, Indiana, New York and Washington soils, at 0.12 mg/kg in the California, New York and Washington soils, and at 1.2 mg/kg in the New York soil; however, recoveries at 0.012 mg/kg (LOQ) in the Georgia soil, at 0.12 mg/kg in the Georgia and Indiana soils, and at 1.2 mg/kg in the California, Georgia, Indiana and Washington soils did not meet guideline requirements.

Soil matrices were characterized as follows: loam soil from New York, sand soil from Washington, loamy sand soil from Georgia, sandy loam/loamy sand soil from California and loam/clay loam soil from Indiana; all soils were collected from the 0-12" soil layer from 2,4-DP-p terrestrial field dissipation sites (p. 14). The method was validated with the first trial for the California and New York soils, the second trial for the Georgia and Washington soils and the third trial for the Indiana soil; data from several unacceptable calibration curves was also provided (p. 20; Appendix 6, pp. 164-202).

**Table 2. Initial Validation Method Recoveries for 2,4-DP-p and Its Transformation products in Soil\***

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>California soil</b>						
Methylated 2,4-DP-p	0.01 (LOQ)	3	60.9-66.6	<b>63.9</b>	2.9	4.5
	0.10	3	69.9-90.2	83.1	11.4	13.8
	2.0	2	82.6-92.6	87.6	7.1	8.1
2,4-DCP	0.01 (LOQ)	3	122.0-134.0	<b>129.8</b>	6.8	5.2
	0.10	3	129.0-131.0	<b>130.3</b>	1.2	0.9
	2.0	2	68.8-72.5	70.7	2.6	3.7
2,4-DCA	0.01 (LOQ)	3	89.0-96.5	92.0	4.0	4.3
	0.10	3	100.5-118.5	111.0	9.4	8.4
	2.0	2	99.4-111.9	105.7	8.8	8.4
2-EHE (2,4-DP-p 2-EHE)	0.01 (LOQ)	3	87.5-106.0	93.8	10.5	11.2
	0.10	3	86.5-105.0	97.3	9.6	9.9
	2.0	2	89.4-99.4	94.4	7.1	7.5
<b>Georgia soil</b>						
Methylated 2,4-DP-p	0.01 (LOQ)	2 <sup>1</sup>	59.9-61.4	<b>60.7</b>	1.1	1.7
	0.10	3	62.8-70.3	<b>66.4</b>	3.8	5.7
	2.0	2	62.5-81.4	72.0	13.4	18.6
2,4-DCP	0.01 (LOQ)	3	94.5-98.5	95.8	2.3	2.4
	0.10	3	95.0-109.5	101.8	7.3	7.2
	2.0	1 <sup>1</sup>	91.9	91.9	--	--
2,4-DCA	0.01 (LOQ)	3	79.0-88.0	84.3	4.7	5.6
	0.10	3	84.5-97.0	92.5	6.9	7.5
	2.0	2	73.1-96.9	85.0	16.8	19.8
2-EHE (2,4-DP-p 2-EHE)	0.01 (LOQ)	3	76.5-93.5	84.8	8.5	10.0
	0.10	3	83.5-113.0	96.0	15.3	15.9
	2.0	2	78.8-91.9	85.4	9.3	10.9
<b>Indiana soil</b>						
Methylated 2,4-DP-p	0.01 (LOQ)	3	60.4-64.7	<b>63.1</b>	2.4	3.7
	0.10	3	62.3-67.5	<b>65.8</b>	3.0	4.6

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	2.0	2	63.1-70.2	<b>66.7</b>	5.0	7.5
2,4-DCP	0.01 (LOQ)	3	111.5-121.5	115.7	5.2	4.5
	0.10	3	99.0-107.5	103.7	4.3	4.2
	2.0	2	81.3-88.1	84.7	4.8	5.7
2,4-DCA	0.01 (LOQ)	3	65.0-72.0	<b>69.3</b>	3.8	5.5
	0.10	3	79.1-84.6	82.1	2.8	3.4
	2.0	2	83.1-84.3	83.7	0.8	1.0
2-EHE (2,4-DP-p 2-EHE)	0.01 (LOQ)	3	85.0-105.0	95.8	10.1	10.5
	0.10	3	78.5-86.5	82.5	4.0	4.8
	2.0	2	72.5-74.4	73.5	1.3	1.8
<b>New York soil</b>						
Methylated 2,4-DP-p	0.01 (LOQ)	3	64.7-75.0	70.3	5.2	7.4
	0.10	3	72.7-76.9	74.6	2.1	2.9
	2.0	2	82.6-84.4	83.5	1.3	1.5
2,4-DCP	0.01 (LOQ)	3	124.0-135.0	<b>131.3</b>	6.4	4.8
	0.10	3	106.5-122.0	115.7	8.1	7.0
	2.0	2	92.5-101.9	97.2	6.6	6.8
2,4-DCA	0.01 (LOQ)	3	89.0-107.5	100.7	10.2	10.1
	0.10	3	91.5-106.0	99.2	7.3	7.3
	2.0	2	92.5-96.3	94.4	2.7	2.8
2-EHE (2,4-DP-p 2-EHE)	0.01 (LOQ)	3	93.5-105.0	100.8	6.4	6.3
	0.10	3	80.5-93.5	87.8	6.7	7.6
	2.0	2	82.5-87.5	85.0	3.5	4.2
<b>Washington soil</b>						
Methylated 2,4-DP-p	0.01 (LOQ)	3	63.2-73.6	<b>68.9</b>	5.3	7.7
	0.10	3	63.2-65.1	<b>64.2</b>	1.0	1.5
	2.0	2	73.8-77.3	75.6	2.5	3.3
2,4-DCP	0.01 (LOQ)	3	116.5-127.0	<b>121.3</b>	5.3	4.4
	0.10	3	117.3-122.3	119.5	2.6	2.1
	2.0	2	89.9-119.3	104.6	20.8	19.9
2,4-DCA	0.01 (LOQ)	3	92.0-98.0	96.0	3.5	3.6
	0.10	3	92.5-99.5	96.7	3.7	3.8
	2.0	2	95.0-101.3	98.2	4.5	4.5
2-EHE (2,4-DP-p 2-EHE)	0.01 (LOQ)	1 <sup>1</sup>	107.5	107.5	--	--
	0.10	3	86.0-91.5	89.5	3.0	3.4
	2.0	2	94.4-96.9	95.7	1.8	1.8

\* Data were obtained from Appendix 5, Tables IIIa-IIIc, pp. 144-146 in the study report. Reported values for mean recovery, standard deviation and relative standard deviation were reviewer-calculated because the study authors only provided these values for the entire data set at each fortification level. The values which were outside of guideline requirements are **bolded**.

<sup>1</sup> The number of tests equalled 1 or 2, instead of 3, because 1 or 2 procedural recoveries were not included in the data set in the study report due to matrix interferences.

**Table 3. Independent Validation Method Recoveries for 2,4-DP-p and Its Transformation products in Soil\***

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Sandy loam/loamy sand soil (California; 0-12"; GR9442)</b>						
2,4-DP-p	0.0093 (LOQ <sup>1</sup> )	6	81-118	101	16	16
	0.093	6	73-105	90	10	11
	0.93	6	86-118	97	12	12
2,4-DCP	0.010 (LOQ)	6	100-130	117	10	8.5
	0.10	6	120-150	<b>135</b>	14	10
	1.0	6	88-130	108	14	13
2,4-DCA	0.010 (LOQ)	6	78-96	89	7.3	8.2
	0.10	6	67-100	81	11	14
	1.0	6	70-100	80	11	14
2-EHE (2,4-DP-p 2-EHE)	0.012 (LOQ <sup>1</sup> )	6	76-83	80	2.4	3.0
	0.12	6	65-92	76	9.0	12
	1.2	6	63-72	<b>68</b>	3.2	4.7
<b>Loamy sand soil (Georgia; 0-12"; GR9481)</b>						
2,4-DP-p	0.0093 (LOQ <sup>1</sup> )	6	55-118	83	24	<b>29</b>
	0.093	6	58-73	<b>65</b>	5.3	8.2
	0.93	6	60-75	<b>69</b>	5.4	7.8
2,4-DCP	0.010 (LOQ)	6	47-120	93	32	<b>39</b>
	0.10	6	57-88	79	11	14
	1.0	6	52-75	<b>60</b>	9.2	15
2,4-DCA	0.010 (LOQ)	4 <sup>2</sup>	79-90	84	5.2	6.2
	0.10	5 <sup>3</sup>	64-72	<b>60</b>	19	<b>32</b>
	1.0	6	68-81	75	4.9	7
2-EHE (2,4-DP-p 2-EHE)	0.012 (LOQ <sup>1</sup> )	6	42-92	<b>69</b>	20	<b>29</b>
	0.12	6	46-83	<b>64</b>	17	<b>27</b>
	1.2	6	45-92	<b>65</b>	19	<b>29</b>
<b>Loam/clay loam soil (Indiana; 0-12"; GR9452)</b>						
2,4-DP-p	0.0093 (LOQ <sup>1</sup> )	5 <sup>4</sup>	62-95	77	12	16
	0.093	6	52-67	<b>59</b>	4.9	8.3
	0.93	6	44-71	<b>61</b>	9.6	16
2,4-DCP	0.010 (LOQ)	6	75-87	79	4.9	6.2
	0.10	6	71-80	73	3.5	4.8
	1.0	6	63-74	<b>69</b>	4.4	6.4
2,4-DCA	0.010 (LOQ)	6	68-81	73	4.9	6.7
	0.10	6	58-77	71	6.6	9.3
	1.0	6	59-76	<b>67</b>	6.5	9.7
2-EHE (2,4-DP-p 2-EHE)	0.012 (LOQ <sup>1</sup> )	6	49-83	72	13	18
	0.12	6	37-83	<b>68</b>	19	<b>28</b>
	1.2	6	41-108	80	22	<b>28</b>
<b>Loam soil (New York; 0-12"; GR9445)</b>						
2,4-DP-p	0.0093 (LOQ <sup>1</sup> )	6	68-118	90	23	<b>26</b>
	0.093	6	51-96	<b>69</b>	15	<b>22</b>
	0.93	6	47-83	72	13	18

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
2,4-DCP	0.010 (LOQ)	6	70-130	113	23	20
	0.10	6	110-130	<b>123</b>	8.2	6.7
	1.0	6	78-110	92	13	14
2,4-DCA	0.010 (LOQ)	6	75-110	87	14	16
	0.10	6	63-83	73	8.0	11
	1.0	6	68-97	80	12	15
2-EHE (2,4-DP-p 2-EHE)	0.012 (LOQ <sup>1</sup> )	6	62-73	70	6.2	8.9
	0.12	6	64-83	73	6.4	8.8
	1.2	6	72-88	78	6.4	8.2
<b>Sand soil (Washington; 0-12"; GR9451)</b>						
2,4-DP-p	0.0093 (LOQ <sup>1</sup> )	6	74-129	106	20	19
	0.093	6	66-77	71	4.1	5.8
	0.93	6	66-84	71	6.6	9.3
2,4-DCP	0.010 (LOQ)	6	71-100	83	13	16
	0.10	6	68-95	82	12	15
	1.0	6	50-71	<b>61</b>	8.6	14
2,4-DCA	0.010 (LOQ)	6	70-85	78	6.1	7.8
	0.10	6	67-82	74	5.9	8.0
	1.0	6	62-82	71	6.8	9.6
2-EHE (2,4-DP-p 2-EHE)	0.012 (LOQ <sup>1</sup> )	6	74-108	94	16	17
	0.12	6	66-100	82	13	16
	1.2	6	61-117	77	20	<b>26</b>

\* Data were obtained from p. 14 and Tables 3A-7B, pp. 22-31 of the study report. The values which were outside of guideline requirements are **bolded**. 2,4-DP-p was reported in the tables of the study report, as opposed to methylated 2,4-DP-p, since a conversion factor was used.

1 Fortification levels for 2,4-DP-p and 2-EHE differed from the nominal fortification levels of 0.010 (LOQ), 0.1 and 1.0.

2 Outliers of 20% and 24% recovery not included in data set calculations.

3 Outlier of 22% recovery not included in data set calculations.

4 Outlier of 28% recovery not included in data set calculations.

### III. Method Characteristics

The LOQ was the same in the ECM and ILV, 0.01 ppm (0.01 mg/kg). No comparison to baseline noise, other justification or calculation was provided. The ILV study authors noted that the LOQ was an approximate value since five different soil types were investigated (p. 20). The ILV study authors proposed LOQ S/N ranging *ca.* 2-10 for 2,4-DCP and *ca.* 5-20 for the other analytes, depending on instrument sensitivity and column performance. The LOD of 0.005 ppm (0.005 mg/kg) was based on the calibration standards injected at half of the LOQ (Appendix 5, p. 138). No other justification or calculation was provided.



**Table 4. Method Characteristics**

	<b>2,4-DP-p</b>				
	<b>California</b>	<b>Georgia</b>	<b>Indiana</b>	<b>New York</b>	<b>Washington</b>
Limit of Quantitation (LOQ)	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg
Limit of Detection (LOD)	0.005 mg/kg	0.005 mg/kg	0.005 mg/kg	0.005 mg/kg	0.005 mg/kg
Linearity (calibration curve $r^2$ and concentration range)	Quadratic equations reported by ECM and ILV				
Repeatable	No	No	No	Yes <sup>4</sup>	No
Reproducible <sup>1</sup>	Yes <sup>2</sup>	No <sup>2</sup>	No <sup>2,3</sup>	No <sup>2</sup>	Yes <sup>2</sup>
Specific	Yes	Yes	Yes	Yes	Yes
	<b>2,4-DCP</b>				
	<b>California</b>	<b>Georgia</b>	<b>Indiana</b>	<b>New York</b>	<b>Washington</b>
Limit of Quantitation (LOQ)	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg
Limit of Detection (LOD)	0.005 mg/kg	0.005 mg/kg	0.005 mg/kg	0.005 mg/kg	0.005 mg/kg
Linearity (calibration curve $r^2$ and concentration range)	Quadratic equations reported by ECM and ILV				
Repeatable	No	Yes <sup>4</sup>	Yes <sup>4</sup>	No	No
Reproducible <sup>1</sup>	No <sup>3</sup>	No	Yes <sup>5</sup>	No <sup>3</sup>	Yes <sup>5</sup>
Specific	Yes	Yes	Yes	Yes	Yes
	<b>2,4-DCA</b>				
	<b>California</b>	<b>Georgia</b>	<b>Indiana</b>	<b>New York</b>	<b>Washington</b>
Limit of Quantitation (LOQ)	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg
Limit of Detection (LOD)	0.005 mg/kg	0.005 mg/kg	0.005 mg/kg	0.005 mg/kg	0.005 mg/kg
Linearity (calibration curve $r^2$ and concentration range)	Quadratic equations reported by ECM and ILV				
Repeatable	Yes <sup>4</sup>	Yes <sup>4</sup>	No	Yes <sup>4</sup>	Yes <sup>4</sup>
Reproducible <sup>1</sup>	Yes	No <sup>3,6</sup>	Yes <sup>5</sup>	Yes	Yes
Specific	Yes	Yes	Yes	Yes	Yes
	<b>2-EHE</b>				
	<b>California</b>	<b>Georgia</b>	<b>Indiana</b>	<b>New York</b>	<b>Washington</b>
Limit of Quantitation (LOQ)	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg
Limit of Detection (LOD)	0.005 mg/kg	0.005 mg/kg	0.005 mg/kg	0.005 mg/kg	0.005 mg/kg
Linearity (calibration curve $r^2$ and concentration range)	Quadratic equations reported by ECM and ILV				
Repeatable	Yes <sup>4</sup>	Yes <sup>4</sup>	Yes <sup>4</sup>	Yes <sup>4</sup>	Yes <sup>4, 7</sup>
Reproducible <sup>1</sup>	Yes <sup>5,8</sup>	No <sup>8</sup>	No <sup>3,8</sup>	Yes <sup>8</sup>	Yes <sup>5,8</sup>
Specific	Yes	Yes	Yes	Yes	Yes

Data were obtained from pp. 8-9, 20; Appendix 2, pp. 85-105; Appendix 5, pp. 134-135, 138; Appendix 5, Table IV, pp. 147-149; Appendix 5, Figure 3, p. 151 of the study report.

1 Although it was not reported as such in the study report, the reviewer compiled this table with the assumption that the five soils of the ECM were the same as the five soils of the ILV (see Comment#5).

2 2,4-DP-p Test samples were fortified at 0.0093, 0.093 and 0.93 mg/kg, instead of 0.01, 0.1 and 1.0 mg/kg.

3 Recoveries at LOQ were acceptable, but recoveries at 10×LOQ did not meet guideline requirements.

4 Based on only three replicates fortified.

5 Recoveries at LOQ and 10×LOQ were acceptable, but recoveries at 100×LOQ did not meet guideline requirements.

6 n = 4 at LOQ fortification level.

7 n = 1 at LOQ fortification level (n = 3 at 10×LOQ).

8 2-EHE Test samples were fortified at 0.012, 0.12 and 1.2 mg/kg, instead of 0.01, 0.1 and 1.0 mg/kg.

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

1. Method recoveries did not meet guideline criteria for precision and accuracy (mean 70-120%; RSD  $\leq$ 20%) for multiple soil/analyte experiments in both the ECM and ILV (see DER Table 4). In the ILV, mean recoveries or RSDs at the LOQ or 10 $\times$ LOQ were not within guideline requirements for analysis of 2,4-DP-p in the Georgia, Indiana and New York test soils, 2,4-DCP in the California, Georgia and New York test soils, 2,4-DCA in the Georgia test soil and 2-EHE in the Georgia and Indiana test soils. The ILV study authors proposed that microbial degradation prior to the extraction step was the cause of the low recoveries in the Indiana and Georgia test soils (p. 20). In the ECM, only three samples were spiked at the LOQ for each soil/analyte (see Comment #2), however, the mean recovery ( $\pm$ SD) of 2,4-DP-p at the LOQ, across all five soils, was  $65.7 \pm 4.9\%$ , which is outside of the guideline requirement (Appendix 5, Table IIIa, p. 144).
2. A minimum of five spiked samples were not analyzed at each concentration for each soil matrix in the ECM. Only three samples were considered for each test soil at a fortification level of LOQ and 10x LOQ (Appendix 5, Tables IIIa-IIIc, pp. 144-146). The OCSPP Guideline 850.6100 criteria requires a minimum of five samples. Therefore, the reviewer could not adequately determine whether the method is quantitative for each analyte for each soil matrix. Across all test soils, mean recoveries of 2,4-DCP, 2,4-DCA and 2-EHE at the LOQ and of all analytes at 10x and 200x LOQ were within the guideline requirement (Appendix 5, Table IIIa-IIIb, pp. 143-145).
3. The estimations of the LOQ and LOD in the ECM were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. No calculations were reported; no comparison was made to chromatogram background levels. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in soil was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification. 2,4-DP-p, 2,4-DCA and 2-EHE are not listed in the EPA Regional Screening Levels (<http://www.epa.gov/region9/superfund/prg/>) for residential soil; however, 2,4-DCP is listed with a screening level of 18 mg/kg in residential soil which is much higher than the reported LOQ for this method.
4. In the ILV, measured fortification levels differed from the nominal levels of 0.01 mg/kg, 0.10 mg/kg and 1.0 mg/kg. The reported fortification levels were 0.0093 mg/kg, 0.093 mg/kg and 0.93 mg/kg for 2,4-DP-p and 0.012 mg/kg, 0.12 mg/kg and 1.2 mg/kg for 2-EHE. The reviewer evaluated the ILV results based on the nominal levels, but it is noted that the 2-EHE testing was not performed at  $\leq$ LOQ.
5. The five test soils used in the ECM were not characterized and were only identified by the state in which they were collected (Appendix 5, pp. 136-137). Test soils used in the ILV were characterized by texture and depth of collection; however, percentages of sand, silt, clay, moisture and organic matter were not reported (p. 14). Although the states in which the five soils were collected were the same, there was no statement in either the

ECM or ILV which clarified whether the soils used in the ILV were the same as those used in the ECM.

6. In the ECM, the GC/MS chromatograms are provided for reagent blanks, the matrix blank for the New York soil and spiked samples at the LOQ, 10× LOQ and 200× LOQ for all analytes in the New York soil (Appendix 5, Figures 3-6d, pp. 151-163). Chromatograms were not included for matrix blanks of the California, Indiana, Georgia and Washington soils and spiked samples at the LOQ, 10× LOQ and 200× LOQ for all analytes in the California, Indiana, Georgia and Washington soils. It is preferred that chromatograms are provided for all test soils.
7. In the ILV, GC/MS Chromatograms were provided for only three of the five test soils: New York, Indiana and California (Appendix 1, pp. 32-84). The study authors noted that the Indiana soil was the “worst” soil type, the California soil was the “cleanest” soil type and the New York soil was the “average” soil type (p. 8). It is preferred that chromatograms are provided for all test soils.
8. The communication between the ILV and ECM was documented (Appendix 3, pp. 106-108). Only two minor modifications of the ECM were performed by the ILV authors: silanized glass wool was not included in the deactivated glass injection port liner and a deactivated fused silica pre-column was installed in front of the analytical capillary column (pp. 10-11). The documentation of approval of these modifications by the ECM authors, ADPEN Labs, was provided (Appendix 3, pp. 106-108). Neither of these modifications required an internal validation to be conducted by the ECM authors or the sponsor. The ILV authors also reported a few critical steps regarding column maintenance (pp. 19-20).
9. Calibration curves were calculated by the ECM and ILV study authors using quadratic equations (Appendix 2, pp. 85-105; Appendix 5, Table IV, pp. 147-149; Appendix 5, Figure 3, p. 151). The reviewer did not perform linear regression analysis for the raw data sets.
10. It was stated in the ILV that initially there were several trials that were unsuccessful (p. 20). One trial was done on the Washington and Georgia soil types that gave variable recoveries and were unacceptable and two other trials were done on the Indiana soil that were also unacceptable.
11. It was reported for the ILV that a single analyst completed a sample set consisting of 12 samples in 14-19 hours (p. 17). The ECM reported that a single analyst completed a sample set consisting of 12 soil samples in *ca.* 12.0 hours (Appendix 5, p. 134).

## **V. References**

U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

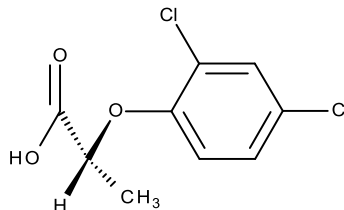
**Attachment 1: Chemical Names and Structures****2,4-DP-p; Dichlorprop-p; 2,4-Dichlorprop-p**

**IUPAC Name:** (2R)-2-(2,4-dichlorophenoxy)propanoic acid  
(+)-(R)-2-(2,4-dichlorophenoxy)propionic acid (“chemical name” in MRID p. 11-12)

**CAS Name:** Not reported

**CAS Number:** 120-36-5

**SMILES String:** O=C(O)C(Oc1ccc(Cl)cc1)C

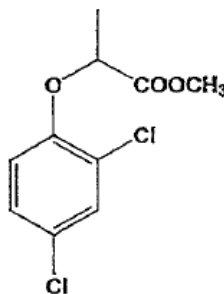
**Methylated 2,4-DP-p; 2,4-DP-p methyl ester**

**IUPAC Name:** (+)-(R)-2-(2,4-dichlorophenoxy)propionic acid methyl ester (“chemical name” in MRID p. 11-12)

**CAS Name:** Not reported

**CAS Number:** Not reported

**SMILES String:** Not reported



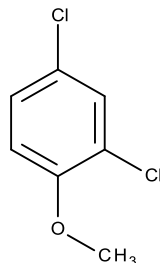
**2,4-DCA; 2,4-Dichloroanisole**

**IUPAC Name:** 2,4-Dichloro-1-methoxy-benzene  
2,4-Dichloroanisole (“chemical name” in MRID p. 11-12)

**CAS Name:** Not reported

**CAS Number:** 553-82-2

**SMILES String:** Oc(c(cc(c1)Cl)Cl)c1C

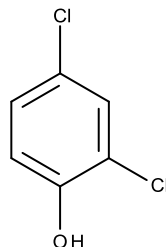
**2,4-DCP; 2,4-Dichlorophenol**

**IUPAC Name:** 2,4-Dichlorophenol

**CAS Name:** Not reported

**CAS Number:** 120-83-2

**SMILES String:** Oc(c(cc(c1)Cl)Cl)c1

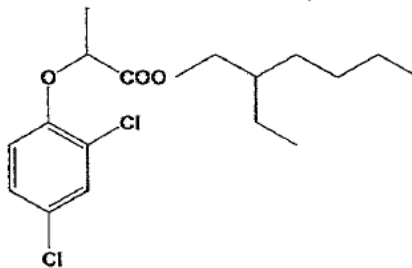
**2-EHE; 2,4-DP-p 2-EHE; 2,4-DP-p 2-Ethylhexyl ester**

**IUPAC Name:** (+)-(R)-2-(2,4-dichlorophenoxy)propionic acid 2-ethylhexyl ester  
 (“chemical name” in MRID p. 11-12)

**CAS Name:** Not reported

**CAS Number:** Not reported

**SMILES String:** Not reported



Chemical: 2,4-DP-p

PC: 031402

MRID: 44243401

Guideline: 850.6100

ECM Validation for Determination of 2,4-DP-p in Soil

Fortified (mg a.i./kg)	2,4-DP-p (Methylated)						
	Recovery (%)	Mean (%)	SD <sup>1</sup> (%)	RSD <sup>2</sup> (%)	Max	Min	n =
<b>California</b>							
0.01 LOQ	64.2 66.6 60.9	63.9	2.9	4.5	66.6	60.9	3
0.10	90.2 89.2 69.9	83.1	11.4	13.8	90.2	69.9	3
2.0	82.6 92.6	87.6	7.1	8.1	92.6	82.6	2
<b>Georgia</b>							
0.01 LOQ	61.4 59.9	60.7	1.1	1.7	61.4	59.9	2
0.10	66.1 62.8 70.3	66.4	3.8	5.7	70.3	62.8	3
2.0	62.5 81.4	72.0	13.4	18.6	81.4	62.5	2
<b>Indiana</b>							
0.01 LOQ	60.4 64.7 64.2	63.1	2.4	3.7	64.7	60.4	3
0.10	67.5 62.3 67.5	65.8	3.0	4.6	67.5	62.3	3
2.0	70.2 63.1	66.7	5.0	7.5	70.2	63.1	2
<b>New York</b>							
0.01 LOQ	71.3 75.0 64.7	70.3	5.2	7.4	75.0	64.7	3
0.10	76.9 74.1 72.7	74.6	2.1	2.9	76.9	72.7	3
2.0	84.4 82.6	83.5	1.3	1.5	84.4	82.6	2
<b>Washington</b>							
0.01 LOQ	63.2 69.9 73.6	68.9	5.3	7.7	73.6	63.2	3
0.10	65.1 63.2 64.2	64.2	1.0	1.5	65.1	63.2	3
2.0	77.3 73.8	75.6	2.5	3.3	77.3	73.8	2

Results from Appendix 5, Tables IIIa-IIIc, pp. 144-146 of MRID 44243401.

Means and standard deviations calculated using Microsoft program functions =AVERAGE(A1:A2) and =STDEV(A1:A2)

Any discrepancies between reviewer calculated values and reported results most likely due to rounding.

1 SD = Standard Deviation; determined using the "unbiased" or "n-1" method.

2 RSD = Relative Standard Deviation; calculated as (SD/mean) x 100.