

Test Material: 2,4-D

MRID: 49314202

Title: Registration Review Data Call-in ID# GDCI-030001-1330 Water  
Environmental Chemistry Method for 2,4-Dichlorophenoxyacetic Acid  
(2,4-D)

EPA PC Code: 030001

OCSPP Guideline: 850.6100

**For CDM Smith**

**Primary Reviewer:** Lynne Binari

**Signature:** 

**Date:** 10/08/14

**Secondary Reviewer:** Lisa Muto

**Signature:** 

**Date:** 10/08/14

**QC/QA Manager:** Joan Gaidos

**Signature:** 

**Date:** 10/08/14

**Analytical method for 2,4-D in water**

**Reports:** ECM: EPA MRID No.: 49314202 (appended as RAM 8862-93-002). Steed, N., J. Chang, M. Bauer, and R. McKellar. 1994. DETERMINATION OF 2,4-DICHLOROPHENOXYACETIC ACID 2-ETHYLHEXYL ESTER, 2,4-DICHLOROPHENOXYACETIC ACID DIMETHYLAMINE SALT AS ITS 2,4-D ACID EQUIVALENT, 2,4-DICHLOROPHENOXYACETIC ACID, 2,4-DICHLOROPHENOL, 2,4-DICHLOROANISOLE, 4-CHLOROPHENOL, AND 4-CHLORPHENOXYACETIC ACID IN WATER BY GAS CHROMATOGRAPHY/MASS SELECTIVE DETECTION (p. 1 of RAM 8862-93-002). Report prepared by Battelle, Columbus, Ohio, and DowElanco, Indianapolis, Indiana, sponsor not specified, submitted by Industry Task Force II on 2,4-D Research Data Technical Committee; 43 pages. Method issued March 15, 1994. Revision 2, supersedes Rev. 01 (3/1/93).  
ILV: None submitted.

**Document No.:** MRID 49314202

**Guideline:** 850.6100

**Statements:** ECM: The registrant specified that the study was "not subject to" Good Laboratory Practice (GLP) standards and that information contained in the study report was "not specifically reviewed or audited in a GLP context." Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3 of Overview). Quality Assurance and Authenticity Certification statements were not provided. A signature page was included (p. 43 of RAM 8862-93-002).  
ILV: None submitted.

**Classification:** This analytical method is classified as **"Upgradeable"**. Insufficient performance data were provided to validate the ECM at the LOQ. It was not established that samples were fortified with 2,4-D. Sufficient chromatographic data were not provided to support validation of the ECM. The water matrix was not characterized.

**PC Code:** 030001

**Reviewer:** Faruque Khan  
Senior Scientist

**Signature:**  
**Date:** 06/08/2015

The Table of Contents of the 56-page MRID indicate overall pagination, but the pagination was not apparent in the provided document. Page citations for the Overview section of the document refer to the Table of Contents pagination (pages 1-12), with all other citations referring to the page numbers located in the upper right corner of the appended method (RAM 8862-93-002).

## Executive Summary

This analytical method, RAM 8862-93-002, is designed for the quantitative determination of 2,4-D (as the methyl ester) in water using GC/MS. Insufficient performance data were provided to validate the ECM for quantitative analysis of 2,4-D at the stated LOQ of 0.001 mg/L (ppm). The LOQ is less than the lowest toxicological level of concern (13.2 µg/L, aquatic life benchmark criteria<sup>1</sup>) in water. An independent laboratory validation of the method was not submitted.

**Table 1. Analytical Method Summary**

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
2,4-D	49314202	Not submitted		Water	15/03/1994	Industry Task Force II on 2,4-D Research Data	GC/MS	0.001 mg/L

## I. Principle of the Method

This analytical method is designed for the quantitative determination of other analytes [2,4-dichlorophenoxyacetic acid 2-ethylhexylester (2,4-D 2-EHE), 2,4-dichlorophenoxyacetic acid dimethylamine salt (2,4-D DMAS) as its acid equivalent (2,4-D), 2,4-dichlorophenol (2,4-DCP), 2,4-dichloroanisole (2,4-DCA), 4-chlorophenol (4-CP), and 4-chlorophenoxyacetic acid (4-CPA)] in addition to 2,4-D in water (p. 2 of RAM 8862-93-002). Due to the procedures utilized, all analytes are included in this method summary.

Samples (100 mL) of water were acidified to <pH 2 with 15% phosphoric acid (1 mL), then loaded, under vacuum (5 mL/minute), onto a Bakerbond Octadecyl (C<sub>18</sub>) silyl (1 g/6 mL) solid-phase extraction (SPE) cartridge pre-conditioned with methanol followed by 0.15% phosphoric acid in water (pp. 8, 16-17 of RAM 8862-93-002). The loaded cartridge was dried under vacuum (20" Hg) for ≥20 minutes. Residues were sequentially eluted with 11 mL of 4% acetone in hexane (Fraction A containing 2,4-D 2-EHE, 2,4-DCP, 2,4-DCA, and 4-CP), followed by 5 mL of 10% methanol in acetone (Fraction B containing 2,4-D and 4-CPA; pp. 3, 17 of RAM 8862-93-002). Fraction B was concentrated under nitrogen to 0.5-1.0 mL, then derivatized (methylated) with boron trifluoride-methanol (12% w/w, 1 mL) solution for *ca.* 30 minutes at *ca.* 70°C (pp. 8, 17-18 of RAM 8862-93-002). The reaction mixture was cooled, diluted with distilled water (8 mL), then partitioned with hexane (5 mL). The resulting organic phase was combined with Fraction A, treated with toluene (1 mL), concentrated under nitrogen (20°C) to *ca.* 1 mL, then brought to 2.0 mL with toluene for GC/MS analysis.

<sup>1</sup> [http://www.epa.gov/oppefed1/ecorisk\\_ders/aquatic\\_life\\_benchmark.htm](http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm)

Samples were analyzed for 2,4-D methyl ester (2,4-D ME) by GC/MS (Durabond-1 column, 0.25 mm x 15 m, 0.25 µm DF film thickness; Stabilwax pre-column, 0.25 mm x 1 m, 0.25 µm DF film thickness) using the following temperature program: hold at 60°C for 2 minutes, 60-150°C at 10°C/min., 150-200°C at 45°C/min., 200-240°C at 10°C/min., hold at 240°C for 2 minutes, and selected ion monitoring (SIM, pp. 13-15 of RAM 8862-93-002). Injection volume was 2 µL. 2,4-D ME was identified and quantified by monitoring three ions: *m/z* 234 (quantitation ion), *m/z* 236 (qualifier ion 1), and *m/z* 199 (qualifier ion 2; p. 14; Figure 1, p. 25; Figure 3, p. 27; Figure 18, p. 42 of RAM 8862-93-002).

An ILV of the method was not submitted.

The LOQ for 2,4-D was 0.001 mg/L (ppm; p. 2 of RAM 8862-93-002). The LOD was not reported.

## II. Recovery Findings

This analytical method is designed for the quantitative determination of other analytes [2,4-D 2-EHE, 2,4-D DMAS as its acid equivalent (2,4-D), 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA] in addition to 2,4-D in water. The ECM study authors did not specify that water samples were fortified with 2,4-D (rather than 2,4-D DMAS). Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of 2,4-D (as the methyl ester) in water at fortification levels of 0.001 mg/L (LOQ, n = 2), 0.01 mg/L (10x LOQ, n = 6), 0.1 mg/L (100x LOQ, n = 2), and 1.0 mg/L (1,000x LOQ, n = 2; DER Attachment 2). 2,4-D ME was identified and quantified using GC/MS. The water was not characterized.

ILV: None submitted.

**Table 2. Initial Validation Method Recoveries for 2,4-D (as methyl ester) in Water<sup>1</sup>**

Matrix <sup>2</sup>	Fortification Level (mg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Water	0.001 (LOQ)	2	94, 97	96	2.1	2.2
	0.01	6	108-118	115	3.6	3.1
	0.1	2	91, 93	92	1.4	1.5
	1.0	2	96	96	0.0	0.0

Data were obtained from Table I, p. 24 of RAM 8862-93-002 and DER Attachment 2 (means, standard deviations, relative standard deviations, as needed). Example calculations allow for correction of recovery values for matrix controls, but matrix control results were reported as 0% (pp. 19-20; Table I, p. 24 of RAM 8862-93-002).

1 The study authors did not specify if water samples were fortified with 2,4-D or 2,4-D DMAS; 2,4-D DMAS converts to 2,4-D rapidly on contact with water (Table I, p. 24 of RAM8862-93-002).

2 The water matrix was not characterized.

**Table 3. Independent Validation Method Recoveries for 2,4-D (as methyl ester) in Water**

Matrix	Fortification Level (mg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Water	0.001 (LOQ)		ILV was not submitted.			
	0.01					

### III. Method Characteristics

The LOQ for 2,4-D in water was 0.001 mg/L (ppm, p. 2 of RAM 8862-93-002). The LOQ was established by the lowest fortification level (p. 7 of Overview). The LOD was not reported.

**Table 4. Method Characteristics for 2,4-D (as methyl ester) in Water**

	<b>2,4-D (as methyl ester)</b>
Limit of Quantitation (LOQ)	0.001 mg/L
Limit of Detection (LOD)	Not reported.
Linearity (calibration curve correlation coefficient and concentration range) <sup>1</sup>	Correlation coefficient = 0.999 (0.1-2, units not reported) <sup>2</sup>
Repeatable	Yes at LOQ, 10x LOQ (0.01 mg/L), 100x LOQ (0.1 mg/L), and 1,000x LOQ (1.0 mg/L), but only n = 2 at LOQ, 0.1 mg/L, and 1.0 mg/L
Reproducible	No ILV
Specific	Undetermined. Not specified that water was fortified with 2,4-D. Insufficient chromatographic data were provided to establish that there are no known interferences from matrices, reagents, solvents, or equipment. <sup>3</sup>

Data were obtained from pp. 2, 10; Figure 10, p. 34 of RAM 8862-93-002; DER Attachment 2.

1 Correlation coefficient was not specified as  $r$  or  $r^2$  (Figure 10, p. 34 of RAM 8862-93-002); linearity could not be verified by the reviewer.

2 The ECM recommended a calibration standard range of 0.02-0.40  $\mu\text{g/mL}$  (p. 13 of RAM 8862-93-002). The provided standard curve also covered a 20-fold concentration range at 0.1-2, but units were not reported (Figure 10, p. 34 of RAM 8862-93-002).

3 Only one total ion chromatogram of an uncharacterized matrix control and one total ion chromatogram of a LOQ fortified sample were provided (Figures 15-16, pp. 39-40 of RAM 8862-93-002). SIM chromatograms were not provided.

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

1. Insufficient performance data were provided to validate the ECM at the LOQ ( $n = 2$ ). A minimum of five spiked replicates should be analyzed at each concentration (*i.e.*, minimally, the LOQ and  $10\times$  LOQ) for each analyte.

The study authors did not specify that samples were fortified with 2,4-D, and the Table I footnote (p. 24 of RAM 8862-93-002) implies samples were fortified with 2,4-D DMAS. The analytical purity of the 2,4-D standard was not reported (p. 9 of RAM 8862-93-002).

2. An ILV was not submitted for the ECM. In lieu of an ILV the registrant presented summary validation data from aquatic field dissipation studies (MRIDs 43954701, 43908302, 43491601; pp. 9-11 of Overview). Methods, individual recovery results, calibration curves, and chromatograms were not provided. Water matrices were not characterized.

**Table 5. Summary Validation Method Recoveries for 2,4-D in Water**

Matrix	Fortification Level (mg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Water	0.001 (LOQ)	6	90-133	110	-- <sup>1</sup>	14.8
	0.0083	5	96-157	<b>124</b>	--	<b>23.0</b>
	0.010	6	75-141	101	--	<b>26.6</b>
	0.083	5	76-95	87	--	9.0
	0.100	10	71-108	90	--	13.2
	0.200	37	59-104	85	--	15.4
	1.00	16	72-105	88	--	11.0
	3.00	<b>4</b>	92-107	98	--	6.7

Data were obtained from p. 12 of Overview. The study authors did not specify if samples were fortified with 2,4-D or 2,4-D DMAS.

1 Not reported and could not be determined; individual recovery results were not reported.

- The determination of the LOQ was not based on scientifically acceptable procedures. The LOQ (0.001 mg/L) was established by the lowest fortification level (p. 7 of Overview; p. 2 of RAM 8862-93-002). The LOD was not reported.

Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in water was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

- Sufficient chromatographic data were not provided to support validation of the ECM. One total ion chromatogram of a matrix blank, a 0.001 mg/L (LOQ) fortification, and a 0.05 µg/mL standard were provided (Figure 1, p. 25; Figures 15-16, pp. 39-40 of RAM 8862-93-002). One SIM chromatogram for 2,4-D ME was provided, but not further identified (analytical standard or fortified sample; Figure 18, p. 42). A standard curve was provided, but individual peak height data were not reported (Figure 10, p. 34); chromatograms of calibration standards used for the standard curve were not provided. No chromatograms of reagents blanks or spiked samples at 10x LOQ were provided.

The total ion chromatograms indicate there were no significant interferences at/near the retention time of 2,4-D ME (Figures 15-16, pp. 39-40 of RAM 8862-93-002), but SIM chromatograms of matrix blank and LOQ fortified samples were not provided.

- Example calculations allow for correction of recovery values for matrix controls, but matrix control results were reported as 0% (pp. 19-20; Table I, p. 24 of RAM 8862-9.3-002).
- The water matrix was not characterized.
- This ECM was used in submitted aquatic field dissipation studies (pp. 5-6 of Overview). However, insufficient information was provided to determine if the LOQ is less than 10% of the expected or actual peak concentration of the test compound in the field.

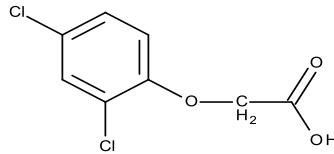
8. A confirmatory method was not used. However, OCSPP 850.6100 guidelines specify that a confirmatory procedure is not typically necessary where GC/MS and LC/MS methods are used as the primary method(s) to generate study data.

## **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-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

**Calculation**

030001\_49314202\_8  
50 6100\_calculation: