Test Material: 2,4-D

MRID: 49314201

Title: Registration Review Data Call-in ID# GDCI-030001-1330 Soil 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:** Lynne Binari

Date: 10/08/14

Secondary Reviewer: Lisa Muto **Signature:** Lisa Muto

Date: 10/08/14

QC/QA Manager: Joan Gaidos **Signature:**

Date: 10/08/14

Analytical methods for 2,4-D in soil and sediment

Reports: ECM-1: EPA MRID No.: 49314201 (appended as RAM 8862-93-001). Steed, N., J. Chang, 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, AND 2,4-DICHLOROANISOLE IN SOIL BY GAS CHROMATOGRAPHY/MASS SELECTIVE DETECTION. 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; 40 pages. Method issued March 1, 1994. Revision 04, supersedes Rev. 03 (09/22/93).

ECM-2: EPA MRID No.: 49314201 (appended as QMAM94006). Sorenson, B., and R. McKellar. 1994. DETERMINATION OF 2,4-DICHLOROPHENOXYACETIC ACID 2-ETHYLHEXYL ESTER (2,4-D 2-EHE), 2,4-DICHLOROPHENOXYACETIC ACID DIMETHYLAMINE SALT (2,4-D DMAS) AS ITS 2,4-D ACID (2,4-D) EQUIVALENT, 2,4-D, 2,4-DICHLOROPHENOL (2,4-DCP), 2,4-DICHLOROANISOLE (2,4-DCA), 4-CHLOROPHENOL (4-CP), AND 4-CHLOROPHENOXYACETI ACID (4-CPA) IN SOIL SEDIMENT SAMPLES BY GAS CHROMATOGRAPHY WITH MASS SELECTIVE DETECTION. Report prepared by Quality Management and Analytical Services, Inc., and DowElanco, Indianapolis, Indiana, sponsor not specified, submitted by Industry Task Force II on 2,4-D Research Data Technical Committee; 49 pages. Method issued August 16, 1994.

ILV: None submitted.

Document No.: MRID 49314201

Guideline: 850.6100

Statements: ECM-1 and ECM-2: 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). A signature page was included in ECM-1 (p. 40). Quality Assurance and Authenticity Certification statements were not provided.

ILV: None submitted.

Classification: The analytical methods for soil and sediment are scientifically sound and classified as **“upgradeable”**. For the soil ECM (RAM 8862-93-001), it was not established that samples were fortified with 2,4-D and method recoveries at 10x LOQ did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy. For the sediment ECM (QMAM94006), samples were fortified with 2,4-D DMAS, rather than 2,4-D, and sufficient performance data at 10x LOQ were not provided. For both ECMs, ILVs were not submitted. The determination of the LOQ was not based on scientifically acceptable procedures, and the LOD was not reported. Sufficient chromatographic data were not provided to support validation of the ECMs. Soil and sediment matrices were not characterized.

PC Code: 030001

Reviewer: Faruque Khan
Senior Fate Scientist

Signature: Faruque A. Khan
Date: 06/08/2015

The Table of Contents of the 105-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 1-14), with all other citations referring to the page numbers located in the upper right corner of each appended method (RAM 8862-93-001 and QMAM94006).

Executive Summary

Analytical methods RAM 8862-93-001 and QMAM94006 are designed for the quantitative determination of 2,4-D in soil and sediment, respectively, using GC/MS. Both methods are quantitative for 2,4-D at the stated LOQ of 0.01 mg/kg (ppm). The LOQ is less than the lowest toxicological level of concern (200 µg/L for benthic organisms[1]) in sediment. However, the LOQ is greater than the lowest toxicological level of concern in soil based on terrestrial plant EC_{25} of 0.0038 lb a.e./A. An independent laboratory validation of each method was not submitted; the two methods are similar in extraction procedures and GC/MS analysis, but differ significantly in clean-up strategies.

Since the LOQ for soil (0.01 mg/kg) is greater than the lowest toxicological level of concern for terrestrial plants (0.0019 mg/kg), based on the lowest EC_{25} of 0.0038 lb a.e./A. Therefore, a new ECM for soil and an associated ILV are requested with an LOQ less than 0.0019 mg/kg, regardless of whether the submitted reports is upgraded to an acceptable classification.

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	49314201	Not submitted		Soil	01/03/1994	Industry Task Force II on 2,4-D Research Data	GC/MS (RAM 8862-93-001)	0.01 mg/kg
				Sediment	16/08/1994		GC/MS (QMAM94006)	

I. Principle of the Method

ECM-1 (RAM 8862-93-001) for soil:

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), and 2,4-dichloroanisole (2,4-DCA)] in addition to 2,4-D in soil (p. 2 of RAM 8862-93-001). Due to the procedures utilized, all analytes are included in this method summary.

Samples (10 g) of soil were sequentially extracted once with 5% acetic acid in methanol, followed by once with 5% acetic acid in methanol:5% acetic acid in water (50:50, v:v), and finally once with 5% acetic acid in water; extraction solvent volumes were 20 mL (p. 13). Each extraction was performed by vortexing for 30 seconds followed by sonication (ultrasonic water bath) for 20

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[1] http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm

minutes. Soil and extract were separated by centrifugation (*ca*. 2,000 rpm, 10 minutes). Extracts were combined, diluted with distilled water (430 mL) and acidified to <pH 2 with 85.5% phosphoric acid (2.5 mL; pp. 13-14). The acidified sample was loaded, under vacuum (<10" Hg, 5 mL/minute), onto a Bakerbond Octadecyl (C_{18}) silyl (1 g/6 mL) solid-phase extraction (SPE) cartridge pre-conditioned with methanol followed by 1.5% phosphoric acid in water (pp. 6, 14). The loaded cartridge was dried under vacuum (20" Hg) for ≥20 minutes. Residues were sequentially eluted with 6 mL of 2% acetone in hexane (Fraction A containing 2,4-D 2-EHE, 2,4-DCP, and 2,4-DCA), followed by 5 mL of 2% acetone in hexane [initial 2.5 mL Fraction A, then second 2.5 mL Fraction B containing 2,4-D], and finally with 5 mL of 20% methanol in acetone (Fraction B; pp. 3, 14-15). 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. 7, 15). 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 (*ca*. 20°C) to 1 mL, then brought to 2.0 mL with toluene for GC/MS analysis.

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. 11-12). 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. 12; Figure 1, p. 24; Figure 3, p. 26; Figure 14, p. 37).

<u>ECM-2 (QMAM94006) for sediment</u>:
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-chlorophenol (4-CP), and 4-chlorophenoxyacetic acid (4-CPA)] in addition to 2,4-D in sediment (pp. 1-3 of QMAM94006). Due to the procedures utilized, all analytes are included in this method summary.

Samples (10 g) of sediment were sequentially extracted as described above for method RAM 8862-93-001 (pp. 19-20 of QMAM94006). Sediment and extract were separated by centrifugation as described above, with the extracts then filtered (Whatman No. 3 filter paper), combined, and brought to 100 mL with water. An aliquot (50 mL) of the extract was partitioned twice with hexane (30 mL x 2) in combination with saturated sodium sulfite solution (2 mL), sodium chloride (20 g) and 0.5N sodium hydroxide (80 mL, pp. 20-21). Organic phases containing 2,4-D 2-EHE and 2,4-DCA were combined (Fraction A) and concentrated (pp. 21-22). The remaining aqueous phase containing 2,4-D (and 2,4-DCP, 4-CP and 4-CPA) was acidified with concentrated phosphoric acid (10 mL), then partitioned twice with methylene chloride (60 mL x 2). Organic phases were combined, then 2,4-D (and 4-CPA) was back-partitioned into 0.25N sodium bicarbonate (60 mL). The organic phase containing 2,4-DCP and 4-CP (Fraction B) was back-partitioned into 1N sodium hydroxide, salted, acidified, then re-partitioned into methylene chloride and concentrated (pp. 21-23). The aqueous phase containing 2,4-D (and 4-CPA) was partitioned twice with diethyl ether (30 mL x 2) in combination with sodium chloride (20 g) and concentrated phosphoric acid (10 mL, pp. 21-22). Organic phases containing 2,4-D (and 4-CPA) were combined, taken to dryness and derivatized (methylated) as described above. The methylated 2,4-D (2,4-D ME, plus 4-CPA ME) sample was combined with Fraction A (2,4-D 2-EHE and 2,4-DCA) and partitioned into the hexane phase, with the organic phase then combined with Fraction B (2,4-DCP and 4-CP) and concentrated under air (200 mL/minute, 30°C) for GC/MS analysis (p. 23).

Samples were analyzed for 2,4-D ME by GC/MS (Hewlett-Packard HP-5MS column, 0.25 mm x 30 m, 0.25 µm film thickness) using the following temperature program: hold at 50°C for 1 minute, 50-100°C at 5°C/min., 100-260°C at 10°C/min., hold at 260°C for 5 minutes, and SIM (pp. 10, 16-18). 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* 201 (qualifier ion 2; p. 17; Figure 2, p. 36; Figure 13, p. 47).

ECM-1 and ECM-2:
ILVs were not submitted for either ECM.

The LOQ for 2,4-D was 0.01 mg/kg (ppm; p. 2 of RAM 8862-93-001, p. 16 of QMAM94006). The LOD was not reported.

II. Recovery Findings

ECM-1 (RAM 8862-93-001): 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, and 2,4-DCA] in addition to 2,4-D in soil. The ECM study authors did not specify that soil 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 soil at fortification levels of 0.01 mg/kg (LOQ, n = 32), 1.0 mg/kg (100x LOQ, n = 2) and 10.0 mg/kg (1,000x LOQ, n = 8), but not at 0.10 mg/kg (10x LOQ, RSD 20.9%, n = 12; DER Attachment 2). 2,4-D ME was identified and quantified using GC/MS. The soil was not characterized.

ECM-2 (QMAM94006): 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-chlorophenol (4-CP), and 4-chlorophenoxyacetic acid (4-CPA)] in addition to 2,4-D in sediment. Sediment samples were fortified with 2,4-D DMAS, not 2,4-D (Table II, p. 30). 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 sediment at fortification levels of 0.01 mg/kg (LOQ, n = 10) and 0.10 mg/kg (10x LOQ, n = 2; DER Attachment 2). 2,4-D ME was identified and quantified using GC/MS. The sediment was not characterized.

ILV: None submitted.

Table 2. Initial Validation Method Recoveries for 2,4-D (as methyl ester) in Soil and Sediment[1]

Matrix[2]	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil (ECM-1)	0.01 (LOQ)	32	67-137	91	17.5	19.2
	0.10	12	67-124	88	18.5	**20.9**
	1.00	**2**	77, 78	78	0.7	0.9
	10.0	8	64-92	76	8.9	11.7
Sediment (ECM-2)	0.01 (LOQ)	10	80-128	106	16.0	15.1
	0.10	**2**	80, 83	82	2.1	2.6

Data were obtained from Table I, pp. 20-23 of RAM 8862-93-001 (ECM-1); Table II, p. 30 of QMAM94006 (ECM-2); and DER Attachment 2 (means, standard deviations, relative standard deviations, as needed). For ECM-1, example calculations allow for correction of recovery values for matrix controls, but matrix control results were reported as ≤0.01% (pp. 16-17; Table I, p. 20 of RAM 8862-9.3-001). For ECM-2, the reviewer could not determine whether or not recovery values were corrected.

1 Sediment samples were fortified with 2,4-D DMAS (Table II, p. 30 of QMAM94006). For ECM-1, the study authors did not specify if soil 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 soil (Table I, p. 23 of RAM8862-93-001).

2 Matrices were not characterized.

Table 3. Independent Validation Method Recoveries for 2,4-D (as methyl ester) in Soil and Sediment

Matrix	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil (ECM-1)	0.01 (LOQ)					
	0.1		ILVs were not submitted.			
Sediment (ECM-2)	0.01 (LOQ)					
	0.1					

III. Method Characteristics

In both ECMs, the LOQ for 2,4-D in soil and sediment was 0.01 mg/kg (ppm, p. 2 of RAM 8862-93-001, p. 16 of QMAM94006). The LOQ was established by the lowest fortification level. The LOD was not reported.

Table 4. Method Characteristics for 2,4-D (as methyl ester) in Soil and Sediment

	ECM-1 (RAM 8862-93-001)/Soil	ECM-2 (QMAM94006)/Sediment
Limit of Quantitation (LOQ)	0.01 mg/kg	0.01 mg/kg
Limit of Detection (LOD)	Not reported.	Not reported.
Linearity (calibration curve correlation coefficient and concentration range)[1]	Correlation coefficient = 1.000 (0.04-0.40 µg/mL)	Not reported.
Repeatable	Yes at LOQ and 1,000x LOQ Yes at 100x LOQ, but n = 2 No at 10x LOQ	Yes at LOQ Yes at 10x LOQ, but n = 2
Reproducible	No ILV	No ILV
Specific	Undetermined. Not specified that soil was fortified with 2,4-D. Insufficient chromatographic data were provided to establish that are no	Undetermined. Sediment fortified with 2,4-D DMAS, rather than 2,4-D. Insufficient chromatographic data were provided to establish that are no known

	known interferences from matrices, reagents, solvents, or equipment.[2]	interferences from matrices, reagents, solvents, or equipment.[2]

Data were obtained from pp. 2, 10; Figure 8, p. 31; Figures 11-12, pp. 34-35 of RAM 8862-93-001; p. 16; Figure 8, p. 42; Figures 14-15, pp. 48-49 of QMAM94006; DER Attachment 2.

1 Correlation coefficient was not specified as r or r^2 (Figure 8, p. 31 of RAM 8862-93-001); linearity could not be verified by the reviewer.

2 Only one chromatogram of an uncharacterized matrix control and one chromatogram of a LOQ fortified sample were provided (Figures 11-12, pp. 34-35 of RAM 8862-93-001; Figures 14-15, pp. 48-49 of QMAM94006).

IV. Method Deficiencies and Reviewer's Comments

1. For the sediment ECM (ECM-2, QMAM94006), the summary ECM "Validation Data" (p. 9, fortification range 0.50-5.00 µg/g) provided by the registrant did not originate from the ECM and does not appear valid for 2,4-D based on the fortification range. Performance data were provided with the ECM, but sediment samples were fortified with 2,4-D DMAS (PC 030019), rather than 2,4-D (Table II, p. 30 of QMAM94006). In addition, only two samples were fortified at 10x LOQ. A minimum of five spiked replicates should be analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.

   For the soil ECM (ECM-1, RAM 8862-93-001), the study authors did not specify that samples were fortified with 2,4-D, rather than 2,4-D DMAS. In addition, method recoveries at 10x LOQ did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy (mean 70-120%; RSD ≤20%) with a RSD of 20.9%.

   For both ECMs, the analytical purity of the 2,4-D standards were not reported (p. 7 of RAM 8862-93-001; p. 12 of QMAM94006).

2. ILVs were not submitted for either ECM (soil or sediment). Extraction of soil and sediment and GC/MS analysis of final samples were similar between the two ECMs; however, clean-up strategies differed significantly. Soil extracts were cleaned-up using solid-phase extraction, whereas sediment extracts were cleaned up using multiple phase/phase partitioning steps.

   In lieu of ILVs the registrant presented summary validation data from terrestrial field dissipation studies (MRIDs 43500301, 43514601, 43592801, 43470401, 43533401) for the soil ECM (ECM-1, RAM 8862-93-001) and an aquatic field dissipation study (MRID 43908302) for the sediment ECM (ECM-2, QMAM94006; pp. 10-14 of Overview). Methods, individual recovery results, calibration curves, and chromatograms were not provided. Soil/sediment matrices were not characterized.

Table 5. Summary Validation Method Recoveries for 2,4-D in Soil/Sediment

Matrix	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil (ECM-1)	0.01 (LOQ)	10	70-130	101	--[1]	18.4
	0.025	6	72-92	81	--	8.5
	0.050	35	56-124	83	--	**20.6**
	0.100	34	60-129	85	--	18.7
	0.150	6	55-87	70	--	16.0
	0.200	8	72-94	79	--	9.7
	0.250	**4**	54-72	**63**	--	12.5
	0.500	19	54-165	80	--	**29.4**
	1.00	**3**	73-92	82	--	11.8
Soil sediment (ECM-2)	0.01 (LOQ)	**4**	100	100	--	0.0
	0.020	6	70-85	80	--	7.0
	0.040	13	68-90	77	--	10.3
	0.050	5	70-92	82	--	9.9
	0.080	**2**	99-103	101	--	2.9
	0.100	9	68-89	79	--	9.2
	0.500	**3**	66-77	71	--	7.4
	1.00	6	69-104	78	--	17.6

Data were obtained from pp. 12, 14 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.

3. The determination of the LOQ was not based on scientifically acceptable procedures. The LOQ (0.01 mg/kg) for both ECMs was established by the lowest fortification level (p. 2 of RAM 8862-93-001, p. 16 of QMAM94006). 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 soil was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

4. Sufficient chromatographic data were not provided to support validation of the ECMs. For ECM-1, one total ion chromatogram of a matrix blank and a 0.01 mg/kg (LOQ) fortification were provided (Figures 11-12, pp. 34-35 of RAM 8862-93-001). One SIM chromatogram

for 2,4-D ME was provided, but not further identified (analytical standard or fortified sample; Figure 14, p. 37). A standard curve was provided, but individual peak height data were not reported (Figure 8, p. 31); chromatograms of calibration standards were not provided. The total ion chromatograms indicate there were interferences at/near the retention time of 2,4-D ME (Figures 11-12, pp. 34-35 of RAM 8862-93-001), and SIM chromatograms of matrix blank and LOQ fortified samples were not provided.

For ECM-2, one chromatogram of a matrix blank, a 0.01 mg/kg (LOQ) fortification (of 2,4-D DMAS), and a 0.05 µg/mL (equivalent to 0.01 mg/kg) calibration standard were provided (Figures 13-15, pp. 47-19 of QMAM94006). A standard curve was provided, but linearity of the curve and individual peak height data were not reported (Figure 8, p. 42); chromatograms of calibration standards other than the 0.05 µg/mL standard were not provided.

For both ECMs, no chromatograms of reagents blanks or spiked samples at 10x LOQ were provided.

5. For ECM-1, example calculations allow for correction of recovery values for matrix controls, but matrix control results were reported as ≤0.01% (pp. 16-17; Table I, p. 20 of RAM 8862-9.3-001). For ECM-2, the reviewer could not determine whether or not recovery values were corrected.

6. The soil and sediment matrices were not characterized.

7. Both ECMs were used in submitted field (terrestrial and aquatic) dissipation studies (p. 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

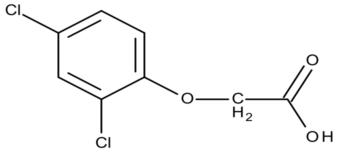
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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



Calculations

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50 6100_calculations