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Page 1 of 48

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-DICHLOROPHENOXYACETIC 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-CHLOROPHENOXYACETIC ACID (4-CPA) IN SOIL SEDIMENT SAMPLES BY GAS CHROMATOGRAPHY WITH MASS SELECTIVE DETECTION.

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for the: INDUSTRY TASK FORCE ON 2,4-D RESEARCH DATA

Edited by

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METHOD NUMBER: QMAM94006

Page 2 of 48

## A. Scope

This method is applicable for the quantitative determination of 2,4-D 2-EHE, 2,4-D DMAS, 2,4-D, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA in soil sediment ranging in concentration from 0.01 to 1.0  $\mu\text{g/g}$  (Note O.1).

## B. Structures

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- (I) 2,4-dichlorophenoxyacetic acid 2-ethylhexyl ester (2,4-D 2-EHE)
  - (II) 2,4-dichlorophenoxyacetic acid dimethylamine salt (2,4-D DMAS)
  - (III) 2,4-dichlorophenoxyacetic acid (2,4-D)
  - (IV) 2,4-dichlorophenol (2,4-DCP)
  - (V) 2,4-dichloroanisole (2,4-DCA)
  - (VI) 4-chlorophenol (4 CP)
  - (VII) 4-chlorophenoxyacetic acid (4-CPA)
  - (VIII) 2,4-dichlorophenoxyacetic acid methyl ester (2,4-D ME)
  - (IX) 4-chlorophenoxyacetic acid methyl ester (4-CPA ME)

METHOD NUMBER: QMAM94006

Page 3 of 48

### C. Principle

A 10-g sample of soil sediment is vortexed and sonicated in 20 mL of 5% acetic acid in methanol, 20 mL of 50:50 5% acetic acid in methanol:5% acetic acid in deionized water and 20 mL of 5% acetic acid in deionized water for 20 minutes. The extracts are decanted, filtered after each extraction, combined, and brought to a known volume. An aliquot of extract (FRACTION A) is combined with hexane,  $\text{Na}_2\text{SO}_3$ , NaCl, 0.5 N NaOH (in that order exclusively) (Note O.2) and 2,4-D 2-EHE and 2,4-DC partitioned to hexane (FRACTION B). FRACTION B is concentrated to 5  $\mu\text{L}$ .

FRACTION A is acidified, and 2,4-D, 4-CPA, 2,4-DCP and 4-CP are partitioned to DCM (FRACTION C). The 2,4-D and 4-CPA are back-partitioned from FRACTION C to 1.0 N  $\text{NaHCO}_3$  (FRACTION D) and the 2,4-DCP and 4-CP are back-partitioned from FRACTION C to 0.5 N NaOH (FRACTION E).

FRACTION D is acidified, saturated with salt, and 2,4-D and 4-CPA are partitioned twice to ether. The ether is evaporated to incipient dryness and the 2,4-D and 4-CPA derivatized with  $\text{BF}_3$  to 2,4-D ME and 4-CPA ME, respectively. The reactants are swamped with water and combined with FRACTION B. The aqueous layer is discarded. The hexane layer is concentrated to 1 mL.

FRACTION E is acidified, saturated with salt, and 2,4-DCP and 4-CP are partitioned twice to ether. The ether is concentrated to 5 mL combined with FRACTION D, and concentrated to 1 mL. A 2- $\mu\text{L}$  aliquot is injected on the gas chromatograph for quantitation by mass selective detection (MSD).

### D. Safety Precautions

Each analyst should be acquainted with potential hazards of the reagents, products and solvents before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, PRODUCT LITERATURE, AND OTHER RELATED DATA. Safety information on products listed in this method should be requested from the supplier.

METHOD NUMBER: QMAM94006

Page 4 of 48

Disposal of reagents, solvents and reactants must be in compliance with the laboratory's Standard Operating Procedures (SOPs) and with local, state, and federal laws and regulations.

Exercise normal laboratory precautions when using laboratory reagents which are flammable and could be toxic. Flammable solvents must be used away from ignition sources and potential toxic materials should be used in a hood. Wear appropriate eye, hand, and clothing protection when working with the materials.

Concentrated acids and bases are corrosive and can cause severe burns. It is imperative that proper eye and personal protection equipment be worn when handling these reagents.

#### E. Equipment (Note O.3)

- E.1 Balance, analytical, Model AE 100, 0 to 109 g, Mettler Instrument Corporation, Princeton-Hightstown Road, Hightstown, NJ 08520.
- E.2 Balance, top-loading, Model TPK-4D, 0 to 4000 g, O'haus Corporation, P.O. Box 900, 29 Hanover Road, Florham Park, NJ 07932.
- E.3 Centrifuge, Model K, IEC, 300 2nd Ave., Needham Hts, MA 02194
- E.4 Crimper, Hewlett-Packard, catalog number 8710-0979, Hewlett Packard Company, 2850 Centerville Road, Wilmington, DE 19808.
- E.5 Gas chromatograph, Hewlett-Packard, Model Number 5890 Series II, equipped with an Mass Selective Detector, Hewlett-Packard Model Number 5972, Hewlett-Packard Company, 2025 West Larpenteur Avenue, St. Paul, MN 55113.
- E.6 Shaker, reciprocating, capable of achieving 180 excursions per minute (epm), Eberbach Corporation, Model Number 6000, Eberbach Corporation, 505 S. Maple Road, P.O. Box 1024, Ann Arbor, MI 48103.
- E.7 Shaker, vortex, Scientific Industries, Inc., Model number G-560, Scientific Industries, Inc. Bohemia, N.Y. 11716.

METHOD NUMBER: QMAM94006

Page 5 of 48

- E.8 Evaporator, QMAS Model 100, Quality Management and Analytical Services, Inc., Hwy 32N, Walhalla, ND 58282.
- E.9 Evaporator, Rotavapor®, Rotary Vacuum Evaporators, Buchi, catalog number 270-623, Curtin Matheson Scientific, Inc., 7677 Equitable Dr. Eden Prairie, MN 55344
- E.10 Water bath, Equatherm, model number 295-729, Curtin Matheson Scientific, Inc.

## F. Glassware (Note O.3)

- F.1 Bottles, glass, 240-mL, with Teflon®-lined caps, catalog number 303-264, Curtin Matheson Scientific, Inc.
- F.2 Cylinders, mixing, graduated, glass, with ground glass stopper, to contain, 500-mL, Kimax, catalog number 075-390, Curtin Matheson Scientific.
- F.3 Centrifuge tubes, 15-mL, graduated, conical, Kimble, catalog number 253-822, Curtin Matheson Scientific, Inc. (Note G.2).
- F.4 Centrifuge tubes, 50-ml, glass, with teflon®-lined caps, Pyrex, catalog No. 054-171, Curtin Matheson Scientific, Inc.
- F.5 Centrifuge tubes, 200-ml, glass, with polypropylene®-lined caps, Pyrex, catalog No. 054-871, Curtin Matheson Scientific, Inc.
- F.6 Flasks, flat-bottom, boiling, 250-mL, with 24/40 joint, Pyrex, catalog number 095-869, Curtin Matheson Scientific, Inc.
- F.7 Flasks, volumetric, glass, 100-mL, Kimax with ground glass stopper, catalog number 104-323, Curtin Matheson Scientific, Inc.
- F.8 Flasks, volumetric, glass, 1000-mL, Kimax with ground glass stopper, catalog number 104-364, Curtin Matheson Scientific, Inc.
- F.9 Funnels, Buchner, 104-mm dia., Coors, catalog number 109-918, Curtin Matheson Scientific, Inc.

METHOD NUMBER: QMAM94005

Page 6 of 48

- F.10 Flasks, boiling, flat bottom, 250-mL, Pyrex, catalog number 095-943, Curtin Matheson Scientific, Inc.
- F.11 Pipets, pastuer, disposable, 5 3/4 inch, Kimax, catalog number 081-083, Curtin Matheson Scientific, Inc.
- F.12 Pipets, volumetric, to deliver 1.0 mL, Pyrex, catalog number 250-816, Curtin Matheson Scientific, Inc.
- F.13 Pipets, volumetric, to deliver 2.0 mL, Pyrex, catalog number 250-817, Curtin Matheson Scientific, Inc.
- F.14 Pipets, volumetric, to deliver 4.0 mL, Pyrex, catalog number 250-829, Curtin Matheson Scientific, Inc.
- F.15 Pipets, volumetric, to deliver 5.0 mL, Pyrex, catalog number 250-830, Curtin Matheson Scientific, Inc.
- F.16 Pipets, volumetric, to deliver 10.0 mL, Pyrex, catalog number 250-831, Curtin Matheson Scientific, Inc.
- F.17 Pipets, volumetric, to deliver 20.0 mL, Pyrex, catalog number 250-823, Curtin Matheson Scientific, Inc.
- F.18 Pipets, volumetric, to deliver 50.0 mL, Pyrex, catalog number 190-363, Curtin Matheson Scientific, Inc.
- F.19 Test tubes, 20-mL, threaded, disposable, Kimble, catalog number 254-268, Curtin Matheson Scientific, Inc.
- F.20 Stoppers, number 32, ground glass, Kimax, catalog number 850100-0032, to fit F.2. Kontes, 1022 Spruce St. Vineland, NJ 08360.
- F.21 Stoppers, glass, number 13, ground glass, Kimax, catalog number 219-121, to fit F.6, Curtin Matheson Scientific, Inc.
- F.22 Syringe, glass, 10- $\mu$ L, Hamilton, part number 9301-0725, Hewlett-Packard Company.

METHOD NUMBER: QMAM94006

Page 7 of 48

F.25 Vials, glass, 2-mL, part number 51811-3400, Hewlett-Packard Company.

G. Materials (Note O.3)

- G.1 Air, Compressed, catalog number UN1002, Genex, 706 2nd Avenue, Des Moines, Iowa 50302.
- G.2 Caps, plastic, with teflon® liners, size 24-400, catalog number 237-616, to fit F.1 above, Curtin Matheson Scientific, Inc.
- G.3 Caps, phenolic, with teflon® liners, size 15-415, catalog number 226-167, to fit F.3 and F.19 above, Curtin Matheson Scientific, Inc.
- G.4 Caps, plastic, with teflon® liners, size 24-410, catalog number 226-183, to fit F.4 above, Curtin Matheson Scientific, Inc.
- G.5 Caps, plastic, with teflon® liners, size 24-400, catalog number 237-616, to fit F.1 above, Curtin Matheson Scientific, Inc.
- G.6 Carrier gas, Helium, Ultra High Purity, Genex, 700 2nd Avenue, Des Moines, Iowa 50302.
- G.7 Column, Hewlett-Packard, HP-5MS, 0.25 mm X 30 meter, capillary, 0.25  $\mu$ m film thickness, part number 19091S-433 Hewlett-Packard Company.
- G.8 Filter paper, 9-cm, number 3, Whatman, catalog number 091-934, Curtin Matheson Scientific, Inc.
- G.9 Glass wool, unsilanized, fiber glass, Corning, part number 3950, catalog number 125-146, Curtin Matheson Scientific, Inc.
- G.10 Liner, Silanized, Injection, Hewlett-Packard, catalog number 5181-3316, Hewlett-Packard Company.
- G.11 Vial closures, 11-mm aluminum, Teflon®-lined, part number 5181-1210, Hewlett Packard Company.

METHOD NUMBER: QMAM94006

Page 8 of 48

## H. Chemicals (Note O.3)

- H.1 Ammonium Hydroxide Solution, 28%, EM Science, catalog number MAX 1303-3, Curtin Matheson Scientific, Inc.
- H.2 Acetic Acid, ACS, EM Science, catalog number MAX0073-9, Curtin Matheson Scientific, Inc.
- H.3 Boron trifluoride/methanol, 12%, catalog number 26412-1, Aldrich Chemical Company.
- H.4 Dichloromethane, Omnisolv, Residue Grade, E.M. Science, catalog number MDX0831-6, Curtin Matheson Scientific, Inc.
- H.5 Ethyl Ether, Anhydrous, E.M. Science, catalog number MEX0190-3, Curtin Matheson Scientific, Inc.
- H.6 Hexane, OmniSolv, Residue grade, E. M. Science, catalog number MTX0737-1, Curtin Matheson Scientific, Inc.
- H.7 Methanol, OmniSolv, Pesticide Residue Quality, E.M. Science, catalog number MMX0484-1, Curtin Matheson Scientific, Inc.
- H.8 Phosphoric Acid, 85%, ACS, Chempure, catalog number 831-622, Curtin Matheson Scientific, Inc.
- H.9 Potassium Permanganate, ACS, E.M. Science, catalog number MPX1550-1, Curtin Matheson Scientific, Inc.
- H.10 Sodium Bicarbonate, ACS, E.M. Science, 12 kg, catalog number MSX0325-5, Curtin Matheson Scientific, Inc.
- H.11 Sodium Chloride, ACS, E.M. Science, 12 kg, catalog number MSX0420-5, Curtin Matheson Scientific, Inc.
- H.12 Sodium Hydroxide, ACS, E.M. Science, catalog number MSXC600-1, Curtin Matheson Scientific, Inc.
- H.13 Sodium Sulfit, ACS, E.M. Science, catalog number MSX0785-1, Curtin Matheson Scientific, Inc.



METHOD NUMBER: QMAM94006

Page 9 of 48

## H.14 Standards, analytical: (Note O.4)

- H.14.1 2,4-dichlorophenoxyacetic acid 2-ethylhexyl ester (2,4-D 2-EHE)
- H.14.2 2,4-dichlorophenoxyacetic acid dimethylamine salt (2,4-D DMAS)
- H.14.3 2,4-dichlorophenoxyacetic acid (2,4-D)
- H.14.4 2,4-dichlorophenol (2,4-DCP)
- H.14.5 2,4-dichloroanisole (2,4-DCA)
- H.14.6 4-chlorophenol (4-CP)
- H.14.7 4-chlorophenoxyacetic acid (4-CPA)
- H.14.8 2,4-dichlorophenoxyacetic acid methyl ester (2,4-D ME)
- H.14.9 4-chlorophenoxyacetic acid methyl ester (4-CPA ME)

H.15 Sulfuric Acid, 96%, ACS, Chempure, catalog number 832-547, Curtin Matheson Scientific, Inc.

H.16 Water, deionized, Culligan, reverse osmosis, activated charcoal filter, and deionizer resin tanks, Culligan Water Conditioning, 416 Gateway Dr., Grand Forks, ND 58201

## I. Reagents (Note O.3)

- I.1 5 % ammonium hydroxide in methanol solution, dilute 5 mL of 28 %  $\text{NH}_4\text{OH}$  solution in 100 mL methanol.
- I.2 5 % acetic acid in methanol: dilute 5.0 mL of 99 % acetic acid in 95 mL of methanol.
- I.3 5 % acetic acid in water: dilute 5.0 mL of 99 % acetic acid in 95 mL of water.
- I.4 5 % phosphoric acid solution: dilute 50 mL of 85 % phosphoric acid to one liter with methanol and mix.
- I.5 8 % phosphoric acid solution: dilute 80 mL of 85 % phosphoric acid to one liter with methanol and mix.

METHOD NUMBER: QMAM94006

Page 10 of 48

- I.6 35 % phosphoric acid solution: dilute 350 mL of 85% phosphoric acid to 500 mL of water and dilute to one liter in a 1-L volumetric flask with water, cap, and mix by inverting 20 times.
- I.7 Saturated potassium permanganate solution: add 200 g  $\text{KMnO}_4$  to 1000 mL of water, cap, and mix for 20 min at ambient temperature.
- I.8 0.25 N sodium bicarbonate solution: dissolve 21 g of  $\text{NaHCO}_3$  in DI water and dilute to 1 L in a 1-L volumetric flask (Note O.5).
- I.9 0.1 N sodium hydroxide solution: dissolve 4.0 g of sodium hydroxide pellets in DI water, bring to 1 L in a 1-L volumetric flask with water, cap and mix by inverting 20 times.
- I.10 0.5 N sodium hydroxide solution: dissolve 20.0 g of sodium hydroxide pellets in DI water, bring to 1 L in a 1-L volumetric flask with water, cap and mix by inverting 20 times.
- I.11 1.0 N sodium hydroxide solution: dissolve 40.0 g of sodium hydroxide pellets in DI water, bring to 1 L in a 1-L volumetric flask with water, cap and mix by inverting 20 times.
- I.12 Saturated sodium sulfite solution: dissolve 130 g  $\text{Na}_2\text{SO}_3$  in 1000 mL of DI water and mix for 20 min.
- I.13 50 % sulfuric acid solution: add 250 mL conc.  $\text{H}_2\text{SO}_4$  to 250 mL of water. (Note: Add the acid to the water carefully.)

### J. Preparation of Standards

#### Fortification standards:

- J.1 2,4-D 2-EHE analytical standard. Weigh out 0.1000 g 2,4-D 2-EHE analytical standard. Place it in a 100-mL volumetric flask. Rinse the weighing vessel with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1000  $\mu\text{g}/\text{mL}$  stock solution.

METHOD NUMBER: QMAM94006

Page 11 of 48

- J.2 2,4-D DMAS analytical standard. Weigh out 0.1205 g 2,4-D DMAS analytical standard. Place it in a 100-mL volumetric flask. Rinse the weighing vessel with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1000  $\mu\text{g}/\text{mL}$  stock solution 2-4,D (Note O.6).
- J.3 2,4-D analytical standard. Weigh out 0.1000 g 2,4-D analytical standard. Place it in a 100-mL volumetric flask. Rinse the weighing vessel with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1000  $\mu\text{g}/\text{L}$  stock solution.
- J.4 2,4-DCP analytical standard. Weigh out 0.1000 g 2,4-DCP analytical standard. Place it in a 100-mL volumetric flask. Rinse the weighing vessel with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1000  $\mu\text{g}/\text{mL}$  stock solution.
- J.5 2,4-DCA analytical standard. Weigh out 0.1000  $\mu\text{g}$  2,4-DCA analytical standard. Place it in a 100-mL volumetric flask. Rinse the weighing vessel with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1000  $\mu\text{g}/\text{mL}$  stock solution.
- J.6 4-CP analytical standard. Weigh out 0.1000 g 4-CPA analytical standard. Place it in a 100-mL volumetric flask. Rinse the weighing paper with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1000  $\mu\text{g}/\text{mL}$  stock solution.
- J.7 4-CPA analytical standard. Weigh out 0.1000 g 2,4-DCP analytical standard. Place it in a 100-mL volumetric flask. Rinse the weighing vessel with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1000  $\mu\text{g}/\text{mL}$  stock solution.

Take the 1000  $\mu\text{g}/\text{mL}$  solutions from J.1 to J.7 and serially dilute in methanol as given in J.8. Each standard can be prepared separately or as combination by combining 1 mL of each 1000  $\mu\text{g}/\text{mL}$  solution in a 100-mL volumetric flask and bringing it to volume with methanol.

METHOD NUMBER: QMAM94006

Page 12 of 48

J.8

Conc. of Initial Solution $\mu\text{g/mL}$	Aliquot of Initial Solution mL	Final Volume of Diluted Solution mL	Conc. of Final Solution $\mu\text{g/mL}$
1000	1.0	100	10.0
10.0	10.0	100	1.0 (a)
10.0	4.0	100	0.40(a)
10.0	1.0	100	0.10(a)

(a) these are the series of standards that make up the fortification solutions.

J.9

Conc. of Initial Solution $\mu\text{g/mL}$	Aliquot of Initial Solution mL	Mass of Soil Sediment $\mu$	Fort. Conc. on Soil Sediment $\mu\text{g/g}$
1.0	1.0	10.0	0.1
0.40	1.0	10.0	0.04
0.10	1.0	10.0	0.01

Calibration standards:

- J.10 2,4-D methyl ester analytical standard. Weigh out 0.1063 g 2,4-D ME analytical standard. Place it in a 100-mL volumetric flask. Rinse the weighing vessel with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1000  $\mu\text{g/mL}$  stock solution 2,4-D (Note O.7).
- J.11 4-CPA methyl ester analytical standard. Weigh out 0.1075 g 4-CPA ME analytical standard. Place it in a 100-mL volumetric flask. Rinse the weighing vessel with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1000  $\mu\text{g/mL}$  stock solution 4-CPA (Note O.8).

Dilute the 1000  $\mu\text{g/mL}$  stock solution of J.1, J.4, J.5, J.6, J.10, and J.11 with hexane in the following manner to obtain a series of calibration standards from one half the Limit of Quantitation(LOQ) to 10 times the LOQ as given in J.12.

METHOD NUMBER: QMAM94006

Page 13 of 48

Each standard can be prepared separately or as a combination by combining 1 mL of each 10  $\mu\text{g/mL}$  solution for compounds from J.1, J.4, J.5, J.6, J.10, and J.11 to a 100-mL flask and bringing it to volume with hexane.

J.12

Conc. of Initial Solution $\mu\text{g/mL}$	Aliquot of Initial Solution mL	Final Volume of Diluted Solution mL	Conc. of Final Solution (1) $\mu\text{g/mL}$
1000	1.0	100	10.0
10	5.0	100	0.50 (a)
10	2.0	100	0.20 (a)
10	1.0	100	0.10 (a)
0.50	10.0	100	0.050(a)
0.50	5.0	100	0.025(a)

- 1) Concentrations of J.10 and J.11 are based on 2,4-D and 4-CPA equivalence, respectively.
- (a) these are the series of standards that make up the calibration curve.

#### 1K. Instrument Operating Conditions

- K.1 Inlet liner, septum and columns should be installed according to manufacturers specifications using lint-free gloves.
- K.2 To obtain optimum performance for the instrument, an autotune is conducted before the analysis of a set of samples. The autotune should be done at 170  $^{\circ}\text{C}$  which is mid-range on the GC temperature program where most of the analytes will be eluting. The ions at  $m/z$  69, 219, and 502 from perfluorotributylamine (PFTBA) are used to autotune the instrument. The autotune adjusts MS parameters and calibrates the mass axis so that the instrument will achieve maximum performance. Results from the autotune report should be compared on a daily basis to point out drifts or the need for ion-source cleaning.

METHOD NUMBER: QMAM94006

Page 14 of 48

K.3 The analysis of the target analytes will be performed in the selected ion monitoring (SIM) mode. The ions to be monitored for each analyte are shown below:

Analyte	Quantitation Ion	Qualifier Ion 1	Qualifier Ion 2
2,4-D 2-EHE	220	222	332
2,4-D ME	234	236	201
4-CPA ME	200	202	141
2,4-DCP	162	164	166
4-CP	128	130	100
2,4-DCA	176	178	163

K.4 Typical operating conditions for the analysis of 2,4-D 2-EHE, 2,4-D ME, 2,4-DCP, and 2,4-DCA are summarized in the table below:

Instrumentation	Hewlett-Packard model 5890 Series II Gas Chromatograph/model 5972 mass selective detector
Column	HP-5MS, (0.25 mm i.d. x 30-m, 0.25 $\mu$ m film thickness)
Oven temperature	Hold at 50 °C for 1 min, then 50 to 100 at 5°C/min, then 100 to 260°C at 10°C/min then hold 5 min.
Injector temperature	240 °C
Transfer line temperature	280 °C
Carrier Gas	Helium
Carrier Gas Flow Rate	1 mL/min
Head Pressure	12 psi
Injection Mode	Splitless

METHOD NUMBER: QMAM94006

Page 15 of 48

Injection Liner	Silanized single taper
Injector Purge Delay	1.5 min
Septum Purge	50 mL/min
Injection volume	2 $\mu$ L
Ionization Potential	70 eV
Electron Multiplier Voltage	1400 to 1900 V (typical)
Dwell Time	100 msec

K.5 A mass spectrum for each analyte is shown in Figures 1 through 6.

K.6 Confirmation

K.6.1 Inject the series of calibration standards described in Section J.6 and determine the peak area/height for the quantitation and qualifier ion for each analyte (e.g. 2,4-D ME ( $m/z$  234, 236)).

K.6.2 For each standard of each analyte (Section K.3), calculate the confirmation ratio. The average confirmation ratio for the each analyte will be used to confirm the presence of each analyte in the soil sediment samples.

i.e. 2,4-D ME

$$\text{Confirmation Ratio} = \frac{\text{Peak Area of Confirmation Ion}}{\text{Peak Area of Quantitation Ion}}$$

$$\text{Confirmation Ratio} = \frac{\text{Peak Area/Height at } m/z \text{ 236}}{\text{Peak Area/Height at } m/z \text{ 234}}$$

$$\text{Confirmation Ratio} = \frac{289}{426}$$

$$\text{Confirmation Ratio} = 0.68$$

METHOD NUMBER: QMAM94006

Page 16 of 48

Positive confirmation of the presence of each analyte is indicated when the confirmation ratio for the sample is within  $\pm 20\%$  of the average found for the standards.

Any of the three ions listed in K.3 for each analyte can be used as the quantitation or confirmation ion in the event that interference is observed in the quantitation or qualifier ions.

- L. Recovery of 2,4-D, 2,4-D 2-EHE, 2,4-DCP, 2,4-DCA, 4-CP and 4-CPA from soil sediment.
- L.1 Place a 10-g aliquot of soil sediment into a series of 50-mL centrifuge tubes.
- L.2 Retain one as a control and fortify the remaining with the appropriate aliquot of standard solution as shown in the table in Section J.1.

Treat each sample as follows:

- L.3 Add 20 mL of 5 % acetic acid in methanol and cap.
- L.4 Vortex for 30 seconds on high speed.
- L.5 Suspend the samples in an ultrasonic bath and sonicate for 20 minutes.
- L.6 Centrifuge at 2000 rpm for 10 min.
- L.7 Decant supernatant and vacuum filter through an 10-cm Buchner funnel fitted with 9-cm Whatman Number 3 filter paper. Collect filtrate in a 100-mL graduated, mixing cylinder.
- L.8 Add 20 mL of 50:50 5 % acetic acid in methanol:5 % acetic acid in DI water and cap.
- L.9 Repeat L.4 to L.7. and combine filtrate.
- L.10 Add 20 mL of 5 % acetic acid in water and cap.
- L.11 Repeat L.4 to L.7.
- L.12 Transfer the soil sediment to the funnel in L.7 using two 10-mL aliquots of deionized water, vacuum filter, and combine filtrates.



METHOD NUMBER: QMAM94006

Page 17 of 48

- L.13 Bring to 100-mL volume with DI water and stopper.
- L.14 Mix by inverting several times and remove stopper.
- L.15 Take a 50-mL aliquot of extract and transfer to a 200-mL centrifuge bottle (FRACTION A).
- L.16 To FRACTION A add:(Note G.2)
  - L.16.1 30 mL of hexane.
  - L.16.2 2 mL of saturated  $\text{Na}_2\text{SO}_3$  solution.
  - L.16.3 20 g NaCl.
- L.17 Mix vigorously by hand for 1 min.
- L.18 Add 80 mL of 0.5 N NaOH (Note: make sure the pH of the aqueous layer is greater than 12).
- L.19 Shake on a reciprocating shaker for 5 min.
- L.20 Centrifuge the sample at 2000 rpm for 2 min.
- L.21 Transfer the hexane layer to a 250-mL flat-bottom boiling flask (FRACTION B).
- L.22 Repeat L.16.1, L.19 and L.20.
- L.23 Transfer the hexane layer and combine with FRACTION B.
- L.24 To Fraction A add:
  - L.24.1 10 mL of conc.  $\text{H}_3\text{PO}_4$
  - L.24.2 60 mL of dichloromethane (DCM)
- L.25 Mix for 5 min on a reciprocating shaker at 180 rpm.
- L.26 Centrifuge the sample at 2000 rpm for 2 min.
- L.27 Transfer the DCM layer to a 200-mL centrifuge tube.

METHOD NUMBER: QMAM94005

Page 18 of 48

- L.28 Repeat L.24.2 to L.26.
- L.29 Transfer the DCM layer and combine with L.27 (FRACTION C).
- L.30 Discard FRACTION A.
- L.31 Add 60 mL of 0.25 N NaHCO<sub>3</sub> to FRACTION C.
- L.32 Mix for 5 min on a reciprocating shaker at 180 rpm.
- L.33 Allow the phases to separate completely, centrifuge if necessary.
- L.34 Transfer the NaHCO<sub>3</sub> layer to a 200-mL centrifuge bottle using a 50-mL pipet (FRACTION D).
- L.35 Add 50 mL of 1.0 N NaOH to FRACTION C (pH of the aqueous fraction must be greater than 12).
- L.36 Mix for 5 min on a reciprocating shaker at 180 rpm.
- L.37 Centrifuge at 2000 rpm for 2 min.
- L.38 Transfer the NaOH layer to a 200-mL centrifuge tube using a 50-mL pipet (FRACTION E).
- L.39 Discard FRACTION C.

## FRACTION B

- L.40 Evaporate the hexane to approximately 10 mL using a rotovap with the water bath set at 50°C.
- L.41 Transfer the hexane to a 15-mL graduated conical tube using two 2-mL hexane rinses.
- L.42 Evaporate FRACTION B to 5 mL at 40°C with 200 mL/min compressed air delivered through capillary stainless-steel tubing (Note O.10)
- L.43 Hold FRACTION B for step L.60.

METHOD NUMBER: QMAM94006

Page 19 of 48

## FRACTION D

L.44 To FRACTION D add:

L.44.1 20 g NaCl.

L.44.2 10 mL of conc.  $H_3PO_4$  dropwise (Note: pH of the aqueous layer must be  $< 2$ ).

L.44.3 30 mL of ether.

L.45 Shake the sample on a reciprocating shaker at 180 rpm for 5 min.

L.46 Allow phases to separate and remove cap.

L.47 Remove the ether layer and transfer to a 250-mL flat-bottom boiling flask.

L.48 Repeat steps L.44.3 and L.46.

L.49 Remove the ether layer and combine with L.47 (FRACTION D).

L.50 Evaporate FRACTION D to approximately 5 mL using a rotovap with a water bath temperature set at 40°C.

L.51 Transfer FRACTION D to a 20-mL test tube using two 2-mL ether washes.

L.52 Add 1 mL of 1%  $NH_4OH$  in methanol.

L.53 Evaporate to incipient dryness at 50 °C with 400 mL/min compressed air delivered through capillary stainless-steel tubing (Note O.10) (Note: Add 0.5 mL of methanol to azeotrope off water if evaporation slows as solution volume gets below 1.0 mL).

L.54 Add 0.2 mL of 3%  $H_3PO_4$  in methanol.L.55 Add 1 mL of 12%  $BF_3$ /methanol and cap tightly.

L.56 Vortex for 1 min.

L.57 Incubate in a water bath at 70 °C for 30 min (Note O.12). Make sure the caps remain tight.

L.58 Remove from water bath and allow to cool.

METHOD NUMBER: QMAM94006

Page 20 of 48

- L.59 Remove cap and add 5 mL of DI water.
- L.60 Transfer FRACTION B to FRACTION D using two 0.5-mL hexane washes and cap (FRACTION D).
- L.61 Shake for 5 min on a vortex shaker at high speed (Note O.11).
- L.62 Let phases separate and remove cap.
- L.63 Remove the aqueous layer and discard.
- L.64 Evaporate the hexane layer to 1 mL at 40 °C with 200 mL/min compressed air delivered through capillary stainless-steel tubing (Note O.10).
- L.65 Hold for FRACTION D for L.73.

## FRACTION E.

- L.66 To FRACTION E add:
  - L.66.1 20 g NaCl.
  - L.66.2 10 mL of conc.  $H_3PO_4$  (Note: pH of the aqueous layer must be < 2).
  - L.66.3 30 mL of ether and cap.
- L.67 Shake for 5 min on a reciprocating shaker at 180 rpm.
- L.68 Transfer the ether layer to a 250-mL flat-bottom boiling flask (FRACTION E).
- L.69 Repeat L.66.3 and L.67.
- L.70 Remove the ether layer and combine with L.68. Discard the aqueous layer.
- L.71 Evaporate the ether layer to 10 mL using a rotovap with a water bath set at 25°C.
- L.72 Transfer FRACTION E to FRACTION D.
- L.73 Rinse FRACTION E vial with 2 1-mL ether washes.

METHOD NUMBER: QMAM94006

Page 21 of 48

- L.74 Evaporate to 1 mL at 30 °C using 200 mL/min compressed air delivered through stainless-steel capillary tubing.
- L.75 Inject a 2- $\mu$ L aliquot on the gas chromatograph for quantitation using a mass selective detector.
- L.76 Use the peak height/area from the series of calibration standards injected to generate a regression curve. (Note O.13)
- L.77 Determine the concentration of the final solution for each fortified sample from the regression curve and record as  $\mu$ g/mL.
- L.78 Multiply the  $\mu$ g/mL found times a method factor of one times any additional dilution factors to obtain  $\mu$ g/g in the soil.
- L.79 Subtract any contribution of the control from the fortified sample.
- L.80 Divide the  $\mu$ g/g found by the  $\mu$ g/g added and multiply by 100 to obtain percent recovery.

$$\text{Percent Recovery} = \frac{\text{total } \mu\text{g/g found (fortified sample)} - \text{total } \mu\text{g/g found (control)}}{\text{total } \mu\text{g added}}$$

- M. Determination of 2,4-D, 2,4-D 2-EHE, 2,4-DCA, 2,4-DCP, 4-CP and 4-CPA in soil sediment.
- M.1 Begin the analysis with Step L.3, the addition of the first extracting solution through Step L.60.
- M.2 Determine the concentration of the sample in  $\mu$ g/mL from the standard curve. A typical standard curve is shown in Figure 7 to 12.

METHOD NUMBER: QMAM94006

Page 22 of 48

- M.3 Calculate the  $\mu\text{g/g}$  by multiplying  $\mu\text{g/mL}$  times 0.40 times  $\mu\text{g/mL}$  found in the final solution times any additional dilution factor. The entire formula is provided below:

$$\mu\text{g/g} = \mu\text{g/mL} \times 0.4$$

- M.4 Typical chromatograms of standard, control and recovery are shown in Figures 13 through 15.

#### Miscellaneous

- N.1 A suggested analytical set is as follows:

- lowest standard (0.025  $\mu\text{g/mL}$ )
- one reagent blank
- one control
- one recovery at the LOQ
- one recovery at the LOQ
- 0.05  $\mu\text{g/mL}$  standard
- field sample
- field sample
- field sample
- field sample
- 0.10  $\mu\text{g/mL}$  standard
- field sample
- field sample
- field sample
- field sample
- 0.20  $\mu\text{g/mL}$  standard
- field sample
- field sample
- field sample
- field sample
- 0.50  $\mu\text{g/mL}$  standard

- N.2 A typical analytical set could consist of twelve analyses made up of any combination of reagent blank(s), controls, fortified controls, and field samples. These twelve analytical samples can be carried through to derivatization in one eight-hour day. The set can be completed to encapsulation in four hours on the second day.

METHOD NUMBER: QMAM94006

Page 23 of 48

## O. Notes

- O.1 When 2,4-D DMAS is shaken for 20 min in the presence of acetic acid in methanol or acetic acid in DI water it is quantitatively dissociated to 2,4-D.
- O.2 The order of addition must be completed in the order given or complete loss of 2,4-DCP and 4-CP will occur. If this order is not maintained start the analysis over at step L.15.
- O.3 Equipment, glassware, materials, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests.
- O.4 Obtain from Sampling Coordinator, Formulations, DowElanco, P.O. Box 63689, Indianapolis, Indiana 46268-1053.
- O.5 Make sure the pH of the sodium bicarbonate solution is not greater than 8.3. Prepare new solution if pH is greater than 8.3.
- O.6 The molecular weight of 2,4-D DMAS is 267. The molecular weight of 2,4-D is 222. The ratio of 2,4-D ME to 2,4-D is 1.203. When 0.1203 g of 2,4-D ME is weighed out it is equivalent to 0.100 g of 2,4-D. This weighing out the standards in this manner saves having to make a molecular weight correction for every analytical sample.
- O.7 The molecular weight of 2,4-D ME is 234. The molecular weight of 2,4-D is 222. The ratio of 2,4-D ME to 2,4-D is 1.063. When 0.1063 g of 2,4-D ME is weighed out it is equivalent to 0.100 g of 2,4-D. This weighing out the standards in this manner saves having to make a molecular weight correction for every analytical sample.
- O.8 The molecular weight of 4-CPA ME is 200. The molecular weight of 4-CPA is 186. The ratio of 4-CPA ME to 4-CPA is 1.075. When 0.1075 g of 4-CPA ME is weighed out it is equivalent to 0.100 g of 2,4-D. This weighing out the standards in this manner saves having to make a molecular weight correction for every analytical sample.

METHOD NUMBER: QMAM94006

Page 24 of 48

- O.9 The initial dilution of the methanolic solution should be no greater than 1 mL methanol diluted to 100 mL with toluene to overcome any solvent immiscibility problems.
- O.10 The evaporation apparatus must be set up in the same fashion each time with conditions carefully controlled.
- O.11 The samples are shaken vertically by attaching the vials to the platform with rubber bands.
- O.12 Immerse the tube into the water to the same depth as the liquid in the vial.
- O.13 A standard should be injected at the beginning and end of each sample run and at least every four samples throughout the run.



SOIL/SEDIMENT METHOD MODIFICATIONS  
(QMAM94006, Revision 8/16/94)

Number	Step(s) Modified	Description	Reason	Impact on Study
1	J.	The 4-CP analytical standard was prepared by weighing out 4-CP, rather than 4-CPA. The 4-CPA standard was prepared by weigh out 4-CPA, rather than 2,4-DCP.	The method listed the incorrect analytical standard.	Samples were fortified with the correct analytes.
2	L.7	Up to 3/15/95, soil sediment was filtered through a glass fritted funnel (150 mL coarse) lined with Whatman No. 4 filter paper.	A glass fritted filter allowed for vacuum to be applied easiest with available equipment.	None.
3	L.20 L.26 L.46 L.67	After these steps, the entire contents of the 200 centrifuge bottle was transferred into a separatory funnel.	Made transfer of the layer to be saved easier.	Improved accuracy and precision.
4	L.26 L.37	These steps were omitted.	These steps were unnecessary because the contents were transferred over to the separatory funnel and adequate separation occurs in the funnel.	Improved efficiency.
5	L.42 L.53 L.64 L.74	The flowrate of the n-evap was adjusted as follows. L.42 50-60 mL/min L.53 100 mL/min L.64 50-60 mL/min L.74 50-60 mL/min	Low recoveries resulted using higher flow rates because analytes were being evaporated.	Improved recoveries.

## SOIL/SEDIMENT METHOD MODIFICATIONS (Continued)

Number	Step(s) Modified	Description	Reason	Impact on Study
6	L.71	After this step, ether was transferred to a 15 mL test tube with two 1 mL rinses. However, after the $H_3PO_4$ was added and shaken, it was not removed with a pipette. Instead, the ether layer was removed and transferred to Fraction D. The 15 mL centrifuge tube was rinsed with two 1 mL rinses of ether and transferred to Fraction D as well. (See method modification 11, number 8.)	To make less surface area available for the partitioning step with $NaHCO_3$ and $H_3PO_4$ . $H_3PO_4$ not removed because the top layer was the one of interest.	Improved accuracy and precision.
7	L.7	From 3/15/95 on, this modification replaces modification number 2. Instead of filtering through a glass fritted funnel, the sediment soil was filtered through a 63 mm Buchner Funnel, which was lined with Whatman 5.5 cm diameter filter paper into a 250 mL side armed flask. The flask was rinsed with approximately 10 mL DI $H_2O$ . The rinse was then transferred to the 100 mL graduated cylinder.	The glass fritted funnel started clogging with soil particles, which resulted in a slower flow rate. This modification improves the flow rate through the funnel.	None.

## SOIL/SEDIMENT METHOD MODIFICATIONS (Continued)

Number	Step(s) Modified	Description	Reason	Impact on Study
8	L.63	From 8/3/95 on, a second partition of aqueous fraction D from step L.59 was added in order to assure complete partition of both 2,4-DME and 4-CPA ME. The method was adjusted as follows.  L.63 Remove hexane layer and transfer to 20 mL test tube. L.63.1 Add 2 mL of hexane. L.63.2 Repeat steps L.61, L.62, and L.63	To assure the complete partition of both 2,4-DME and 4-CPA ME material from aqueous fraction D to the combined fraction B/D.	None
9	L.43; L.60 to L.73	Fraction D and B/E were not combined for the final blow down. See below for details. Note: This modification only affected set 17.	An attempt to obtain increased recoveries for laboratory fortification samples.	None
10	L.60 to L.74	Fraction B, D and E were not combined for final blowdown. See below for details. Note: This modification only affected set 8C.	An attempt to obtain improved recoveries for laboratory fortified samples.	None
11	See below.	Method modifications developed by Centre Analytical Laboratories in their method validation study were used. See below.	To clean up samples further and eliminate unnecessary steps.	None

Explanation of Method Modification 9

**Fraction D.** Add 2 mL of petroleum ether, shake for 3 min. on a vortex shaker and transfer the organic layer to a graduated test tube. Repeat the partition step twice, initially using petroleum ether, then hexane. Evaporate the combined organics to 1 mL at room temperature with 30 to 40 mL/min compressed helium delivered through a capillary stainless steel tubing.

**Fraction B/E.** Transfer fraction B at L.43/L.60 to a graduated test tube using two 1 mL hexane rinses. Transfer fraction E at step L.72/73 to the graduated test tube containing fraction B. Evaporate to 1 mL at 30°C using 30-40 mL/min compressed helium delivered through stainless steel capillary tubing.

#### Explanation of Method Modification 10

**Fraction B.** The method was followed through step L.42 (not including Note 0.10). The hexane was evaporated to 1 mL at room temperature with 30-40 mL/min of compressed nitrogen delivered through capillary stainless steel tubing.

**Fraction D.** The method was followed through L.59. Add 2 mL of petroleum ether, shake for 3 minutes on a vortex shaker and transfer organic layer to a graduated test tube. Repeat the partition step twice; initially using petroleum ether, finally using hexane. Evaporate the combined organics to 1 mL at room temperature with 30-40 mL/min of compressed nitrogen delivered through capillary stainless-steel tubing.

**Fraction E.** The method was followed through L.71. Transfer the ether layer from a 250 mL flat bottom boiling flask to a graduated test tube. Rinse the flask with two 1 mL ether washes, making certain to avoid transferring any residual water into the test tube. Evaporate the ether to 1 mL at room temperature with 30-40 mL/min of compressed nitrogen delivered through capillary stainless-steel tubing.

#### Explanation of Method Modification 11

1. In steps L.5, L.6, L.9, and L.11, samples were sonicated for 10 minutes instead of 20 minutes and centrifuged for 5 minutes instead of 10 minutes.
2. In steps L.7, L.9, L.11, and L.12, samples were centrifuged and the supernatant was transferred directly to a 100 mL volumetric flask instead of first filtering the supernatant.
3. In step L.21, the hexane layer was passed through a  $\text{Na}_2\text{SO}_4$  column (approximately 10 g) pre-washed with hexane. The column was rinsed with 10 mL of hexane after the final hexane layer was passed through the column.
4. The  $\text{NaHCO}_3$  and  $\text{NaOH}$  from steps L.34 and L.38 were transferred to the centrifuge tube from a separatory funnel instead of using a 50 mL pipette.
5. The n-evap used  $\text{N}_2$  gas instead of compressed air to reduce the sample volume.

6. After fraction B was evaporated to 5 mL in step L.42, 5 mL of 0.1 N NaOH was added and the sample was placed on a vortex shaker for approximately 3 minutes. The NaOH was removed with a pipette.
7. In step L.64, the aqueous layer was removed and 5 mL of 1 N NaHCO<sub>3</sub> was added. The sample was placed on a vortex shaker for 3 minutes before removing the NaHCO<sub>3</sub> with a pipette.
8. In step L.71, after the sample was evaporated to 10 mL using a rotovap, 5 mL of NaHCO<sub>3</sub> was added and the sample was shaken on a vortex shaker for approximately 3 minutes. The NaHCO<sub>3</sub> was removed with a pipette and 5 mL of 8% H<sub>3</sub>PO<sub>4</sub> was added. The sample was shaken on a vortex shaker for approximately 3 minutes and the H<sub>3</sub>PO<sub>4</sub> was removed with a pipette.