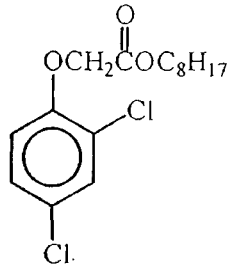
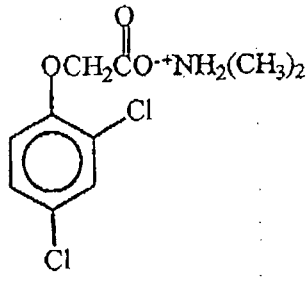
1. Scope

This method is applicable for the quantitative 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 acid equivalent, 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4-dichlorophenol (2,4-DCP), and 2,4-dichloroanisole (2,4-DCA) in soil at a validated Limit of Quantitation (LOQ) of 0.01 ppm (see Table 1 on Page 20).

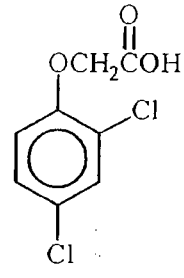
2. Structures



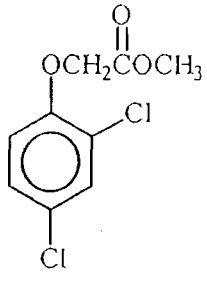
2,4-D 2-EHE



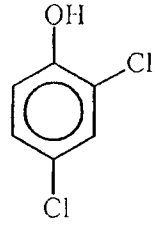
2,4-D DMAS



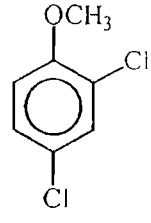
2,4-D



2,4-D ME



2,4-DCP



2,4-DCA

3. Principle

Residues of the above analytes are extracted from the soil by a combination of three solvent systems and sonication. The combined extracts are diluted with water and concentrated on a C18 solid phase extraction (SPE) cartridge. The analytes are eluted sequentially from the cartridge using two specific solvent systems which separate the analytes into two fractions. The first fraction contains 2,4-D 2-EHE, 2,4-DCP, and 2,4-DCA which are chromatographed without derivatization. The second fraction contains 2,4-D which is methylated using BF_3/methanol to form the methyl ester (2,4-D ME) then partitioned into hexane prior to chromatographic analysis. The first fraction and the hexane solution are combined into a single solution for injection. Quantitation is by gas chromatography/mass selective detection in the selected ion monitoring mode (SIM). The residue results are determined by comparing the peak areas of the test samples with the peak areas of a series of calibration standards prepared from known analytical standards.

4. Safety Precautions

4.1 Each analyst should be familiar with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. Sources of information include: MATERIAL SAFETY DATA SHEETS (MSDS), PRODUCT INFORMATION, and other related materials. Safety information on products should be requested from the supplier. Disposal of the reagents, reactants, and solvents must be in compliance with local, state, and federal laws and regulations.

4.2 Acetonitrile, acetone, hexane, and methanol are flammable and should be used in well ventilated areas away from ignition sources.

5. Equipment (Note 15.1)

5.1 Analytical Evaporator, Meyer N-evap, (Organomation, P.O. Box 159, South Berlin, MA, 01549), Model 111.

5.2 Automatic Sampler, Hewlett-Packard (HP, Route 41 and Starr Road, P.O. Box 900, Avondale, PA, 19311-9990), Model 7673.

5.3 Balance, Mettler Analytical, Model AE-240, analytical range 0-120 g, Fisher Scientific (711 Forbes Ave., Pittsburgh, PA, 15219-4785), cat. # 01-909-407.

5.4 Balance, Mettler Top Loading, Model PM2000, range 0-2100 g, Fisher Scientific (Pittsburgh, PA), cat. # 01-911-174.

5.5 Bath, Branson Ultrasonic, Model 2200, VWR (4717 Hinckley Indust. Pkwy., Cleveland, OH, 44109), cat. # 21812-323.

5.6 Bath, Buchi Water, Model B-461, VWR (Cleveland, OH), cat. # 27559-641.

5.7 Centrifuge, IEC Model HN-SII clinical, equipped with a rotor capable of holding eight 50 mL conical centrifuge tubes, Fisher Scientific (Pittsburgh, PA), cat. # 05-111.

5.8 Data System, Hewlett Packard Model 59970 mass spectrometer (HP, 1601 California Ave., Palo Alto, CA, 94304).

5.9 Gas Chromatograph, Hewlett-Packard (Avondale, PA) Model 5890 Series II.

5.10 Mass Selective Detector, Hewlett-Packard (Palo Alto, CA) Model 5971A.

5.11 Mechanical Shaker - New Brunswick Scientific (New Brunswick, NJ).

5.12 Personal Computer, Hewlett-Packard ChemStation (DOS series) Software (Palo Alto, CA) operating on a Hewlett-Packard Vectra Personal Computer Model QS/120(386/25).

5.13 Vacuum Manifold, Supelco (Supelco Park, Bellefonte, PA, 16823-0048), Visiprep, cat. # 5-7030.

5.14 Vacuum Pump, Sargeant-Welch Model 8803 capable of maintaining a minimum vacuum of 25 mm Hg, VWR (Cleveland, OH), cat. # 54969-828.

5.15 Vial Crimper, Hewlett-Packard (Avondale, PA), cat. # 8710-0979.

5.16 Vortex Mixer, Genie 2, Fisher Scientific (Pittsburgh, PA), cat. # 12-812.

6. Glassware (Note 15.1)

6.1 Bottle, glass, 500 mL (16 oz.) screw cap, cat. # B7451-11, Baxter (1430 Waukegan Road, McGaw Park, IL, 60085-9988).

6.2 Column, capillary gas chromatography, Durabond-1 (DB-1) bonded phase, 15 m x 0.25 mm i.d., 0.25 μm DF film thickness, Alltech (2051 Waukegan Road, Deerfield, IL, 60015-1899), cat. # 122-1012.

6.3 Column, pre-column, Stabilwax bonded phase, 1 m x 0.25 mm i.d., 0.25 μm DF film thickness, Restek (110 Benner Circle, Bellefonte, PA, 16823-8812), cat. # 10623.

6.4 Inlet Liner, silanized dual tapered, splitless, Hewlett-Packard (Avondale, PA), cat. # 5181-3315.

6.5 Cylinder, graduated, 100 mL with pour spout, Baxter (McGaw Park, IL), cat. # C9085-100.

6.6 Flask, 2000 mL Pyrex side-arm Erlenmeyer, to collect loading solutions. Attaches to Supelco Vacuum Manifold, Fisher Scientific (Pittsburgh, PA), cat. # 10-180G.

6.7 Flask, volumetric, 10 mL, Baxter (McGraw Park, IL), cat. # F4663-10A.

6.8 Flask, volumetric, 50 mL, Baxter (McGraw Park, IL), cat. # F4663-50A.

6.9 Flask, volumetric, 100 mL, Baxter (McGraw Park, IL), cat. F4663-100A.

6.10 Pipet, Pasteur, 2 mL, Fisher Scientific (Pittsburgh, PA), cat. # 13-678-20C.

6.11 Pipet, Glass disposable, 1 mL, Fisher Scientific (Pittsburgh, PA), cat. # 13-678-25B.

6.12 Pipet, Glass disposable, 5 mL, Fisher Scientific (Pittsburgh, PA), cat. # 13-678-25D.

6.13 Pipet, Glass disposable, 10 mL, Fisher Scientific (Pittsburgh, PA), cat. # 13-678-25E.

6.14 Pipet, Glass, volumetric, 20 mL, Fisher Scientific (Pittsburgh, PA), cat. # 13-650N

6.15 Pipet, Glass disposable, 25 mL, Fisher Scientific (Pittsburgh, PA), cat. # 13-676-29D.

6.16 Pipet, motorized, 2.5 mL, Rainin (Mack Road, Woburn, MA, 01801-4628), cat. # E2-2500.

6.17 Pipet, motorized, 1.0 mL, Rainin (Woburn, MA), cat. # E2-1000.

6.18 Pipet, motorized, 100 μL, Rainin (Woburn, MA), cat. # E2-100.

6.19 Tube, 15 mL conical, graduated in 0.1 mL increments, Pyrex # 8082, Baxter (McGaw Park, IL), cat. # C3980-15.

6.20 Vials, autosampler, 2 mL, Hewlett-Packard, Sunbroker (P.O. Box 2230, Wilmington, NC, 28402), cat. # 1100.

6.21 Vials, 20 mL, with Teflon®/silica septa caps (included), Industrial Glassware (P.O. Box 5, Millville, NJ) cat. # 2757FL.

7. Materials (Note 15.1)

7.1 Adapters, SPE, Bakerbond, VWR (Cleveland, OH), cat. # 7122-00.

7.2 Caps, Teflon®/Silicon/Teflon® septum, crimptop, to fit autosampler vials, National Scientific (975 Progress Circle, Lawrenceville, GA, 30243), cat. # C4011-2A.

7.3 Caps, Teflon®-lined screw cap to fit 15 mL conical tubes, 17 mm, Baxter (McGaw Park, IL), cat. # C3980-15.

7.4 Caps, Teflon®-lined screw cap to fit 500 mL bottles, 28 mm, Baxter (McGaw Park, IL), cat. # B7503-7.

7.5 Cartridges, Bakerbond solid phase extraction (SPE), Octadecyl silyl, 1 g/6 mL size, VWR (Cleveland, OH), cat. # 7020-07.

7.6 Gas, Helium, 99.999%, Air Products (7201 Hamilton Blvd., Allentown, PA, 18195), cat. # 801-M-26513.

7.7 Gas, Nitrogen, 99.998%, Air Products (Allentown, PA), cat. # K01-3-39017.

7.8 pH paper, Whatman CF strips, 0-14 pH range, Fisher Scientific (Pittsburgh, PA), cat. # 09-876-17.

7.9 Rack, Test Tube, S/P brand heavy duty polypropylene, 30 mm, Baxter (McGaw Park, IL), cat. # S9262.

7.10 Tubes, Polypropylene, 50 mL printed, nonsterile, Baxter (McGaw Park, IL), cat. # C3903-13.

7.11 Tubing, Teflon®, 1/8" O.D., 2.1 mm I.D., Supelco (Bellefonte, PA), cat. # 2-0532M.

8. Chemicals (Note 15.1)

8.1 Acetone, Burdick and Jackson, HPLC grade, Baxter (McGaw Park, IL), cat. # 015-1DK.

8.2 Acetonitrile, Burdick and Jackson, HPLC grade, Baxter (McGaw Park, IL), cat. # 015-1DK.

8.3 Acid, acetic, Baker-Analyzed Reagent grade, concentration ≥99.7% J.T. Baker, VWR (Cleveland, OH), cat. # 9508-01.

8.4 Acid, phosphoric, 85.0-87.0%, Baker, VWR (Cleveland, OH), Baker-Analyzed Reagent grade, cat. # 0260-01.

8.5 BF_3-Methanol (12% w/w), Supelco (Bellefonte, PA), cat. # 3-3040M.

8.6 2,4-Dichlorophenoxyacetic acid 2-ethylhexyl ester, analytical standard, DowElanco (9330 Zionsville Road, Indianapolis, IN, 46268-1053).

8.7 2,4-Dichlorophenoxyacetic acid dimethylamine salt, analytical standard, DowElanco (Indianapolis, IN).

8.8 2,4-Dichlorophenoxyacetic acid, analytical standard, DowElanco (Indianapolis, IN).

8.9 2,4-Dichlorophenol, analytical standard, DowElanco (Indianapolis, IN).

8.10 2,4-Dichloroanisole, analytical standard, DowElanco (Indianapolis, IN).

8.11 2,4-Dichlorophenoxyacetic acid methyl ester, analytical standard, DowElanco (Indianapolis, IN).

8.12 Hexane, Burdick and Jackson, HPLC grade, Baxter (McGaw Park, IL), cat. # 230-1DK.

8.13 Methanol, Burdick and Jackson, HPLC grade, Baxter (McGaw Park, IL), cat. # 230-1DK.

8.14 PFTBA, 99.9%, for tuning the mass spectrometer, Hewlett-Packard (Avondale, PA), cat. # 8500-0656.

8.15 Water, Distilled, Magnetic Springs (1801 Lone Eagle Street, Columbus, OH, 43228).

9. Reagents and Solutions

9.1 2% (v:v) acetone in hexane. Prepared by combining 2 mL acetone with 98 mL hexane.

9.2 20% (v:v) methanol in acetone. Prepared by combining 20 mL methanol with 80 mL acetone

9.3 1.5% phosphoric acid in water. Prepared by combining 3 mL concentrated (85-87%) phosphoric acid with 170 mL distilled water.

9.4 5% (v:v) acetic acid in methanol. Prepared by combining 5 mL acetic acid with 95 mL methanol.

9.5 5% (v:v) acetic acid in water. Prepared by combining 5 mL acetic acid with 95 mL distilled water.

9.6 Aqueous methanol/acetic acid mixture (50:50, v:v). Prepared by combining 50 mL of 9.4 with 50 mL 9.5.

10. Preparation of Standards

10.1 Weigh out 0.1000 g 2,4-D 2-EHE analytical standard. Place it into a 100 mL volumetric flask. Rinse the weighing vessel with acetonitrile and transfer the rinsate to the volumetric flask. Dilute to the mark with acetonitrile to prepare an approximate 1000 μg/mL stock solution.

10.2 Weigh out 0.1000 g 2,4-D analytical standard. Place it into a 100 mL volumetric flask. Rinse the weighing vessel with acetonitrile and transfer the rinsate to the volumetric flask. Dilute to the mark with acetonitrile to prepare an approximate 1000 μg/mL stock solution.

10.3 Weigh out 0.1000 g 2,4-DCP analytical standard. Place it into a 100 mL volumetric flask. Rinse the weighing vessel with acetonitrile and transfer the rinsate to the volumetric flask. Dilute to the mark with acetonitrile to prepare an approximate 1000 μg/mL stock solution.

10.4 Weigh out 0.1000 g 2,4-DCA analytical standard. Place it into a 100 mL volumetric flask. Rinse the weighing vessel with acetonitrile and transfer the rinsate to the volumetric flask. Dilute to the mark with acetonitrile to prepare an approximate 1000 μg/mL stock solution.

10.5 Weigh out 0.1123 g 2,4-D DMAS analytical standard. Place it into a 100 mL volumetric flask. Rinse the weighing vessel with acetonitrile and transfer the rinsate to the volumetric flask. Dilute to the mark with acetonitrile to prepare an approximate 1000 μg/mL acid equivalent stock solution when measured against the 2,4-D methyl ester standard as prepared in 10.6.

10.6 Weigh out 0.1050 g 2,4-D methyl ester analytical standard. Place it into a 100 mL volumetric flask. Rinse the weighing vessel with acetonitrile and transfer the rinsate to the volumetric flask. Dilute to the mark with acetonitrile to prepare an approximate 1000 μg/mL acid equivalent stock solution.

10.7 Fortification Standards

10.7.1 Pipet 20 mL each of the stock standards 10.1-10.4 into a 100 mL volumetric flask and dilute to the mark with acetone to give a mixture containing 200 μg/mL of each standard.

10.7.2 Pipet 5 mL of solution 10.7.1 into a 100 mL volumetric flask and dilute to the mark with acetone to give a mixture containing 10 μg/mL of each standard.

10.7.3 Pipet 1 mL of solution 10.7.2 into a 10 mL volumetric flask and dilute to the mark with acetone to give a mixture containing 1 μg/mL of each standard.

10.7.4 Repeat steps 10.7.1 through 10.7.3 using stock standards 10.3-10.5.

10.7.5 Fortify 10 g portions of soil with the appropriate analytes (see Note 15.2) by utilizing the appropriate aliquot (Column 2) of the appropriate standard solutions (Column 1) in the table below to obtain fortification concentrations from 0.01-10 ppm (Column 3).

Column 1	Column 2	Column 3
Concentration of the Initial Solution	Aliquot of the Initial Solution	Fortification Level
μg/mL	μL	ppm
1.0	100	0.01
10.0	100	0.10
200	500	10.0

10.8 Calibration Standards

10.8.1 Pipet 1 mL each of 10.1, 10.3, 10.4, and 10.6 into a 100 mL volumetric flask, add 10 μL acetone, and dilute to volume using hexane to give a solution concentration of 10 μg/mL for each analyte.

10.8.2 Serially dilute the solution from 10.8.1 with hexane to obtain GC/MSD standards for 2,4-D 2-EHE, 2,4-DCA, 2,4-DCP, and 2,4-DME as shown in the following table:

Concentration of Initial Solution	Aliquot of Initial Solution	Volume of Final Solution	Concentration of Final Solution
μg/mL	mL	mL	μg/mL
10.0	40	100	4.0
4.0	10	100	0.40*
0.4	25	50	0.20*
0.2	25	50	0.10*
0.4	10	100	0.04*

*Use for GC standards (10 fold concentration range).

11. Gas Chromatography/Mass Spectrometry

11.1 General

11.1.1 Install the splitless liner and the capillary column and pre-column on the split/splitless port of the GC/MSD following the manufacturer's recommended procedure.

11.2 Operating Conditions

11.2.1 Typical operating conditions for the analysis of 2,4-D 2-EHE, 2,4-D as the Methyl Ester, 2,4-DCA, and 2,4-DCP are summarized in the table below:

Instrumentation	Hewlett-Packard model 5890 Series II gas chromatograph/model 5971A mass selective detector
Column	Durabond-1 (DB-1), 15 m x 0.25 mm i.d., 0.25 μm DF film thickness
Pre-Column	Stabilwax, 1 m x 0.25 mm i.d., 0.25 μm DF film thickness
Oven Temperature	Hold at 60° for 2 minutes, then 60-150°C at a rate of 10°C/minute, then, 150-200°C at a rate of 45°C/minute, then, 200-240°C at a rate of 10°C/minute, then, hold at 240°C for 2 minutes
Injector Temperature	250°C
Transfer Line Temperature	280°C
Carrier Gas	Helium
Carrier Gas Flow Rate	~40-60 cm/second (internal flow sensor)
Head Pressure	5 psi
Injection Mode	splitless
Injection Liner	Silanized dual taper
Injector Purge Delay	1.5 minutes

Septum Purge	~7.5 mL/minute
Injection Volume	2 μL
Ionization Potential	70 eV
Electron Multiplier Voltage	1400-1900 V (typical)
Dwell Time	50-200 msec

11.2.2 To obtain optimum performance for the instrument, an autotune is conducted before analysis of a set of samples. The autotune should be done at 170°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.

11.2.3 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	222	332	220
2,4-D (Methyl ester)	234	236	199
2,4-DCP	162	164	98
2,4-DCA	176	161	178

*see 13.1.6.

11.2.4 A typical total ion chromatogram of the four analytes is shown in Figure 1.

11.2.5 A mass spectrum for each analyte is shown in Figures 2-5.

12. Recovery of 2,4-D 2-EHE, 2,4-D DMAS as its Acid Equivalent, 2,4-D, 2,4-DCP, and 2,4-DCA

12.1 Weigh 10 g of control soil into a series of 50 mL screw-cap polypropylene centrifuge tubes.

12.2 Use part of the samples as the controls and fortify the remaining samples by adding 100 μL to 500 μL of the appropriate fortification solutions to obtain soil concentrations ranging from 0.01-10.0 ppm.

Treat each sample as follows

12.3 Add 20 mL of 5% acetic acid in methanol to each sample.

12.4 Cap tubes with a polyethylene 28 mm screw cap lid.

12.5 Vortex to mix at the highest speed for approximately 30 seconds.

12.6 Place one tier of the polypropylene test tube rack over the ultrasonic bath. The sample tubes are inserted through the openings in the tier so that the tubes are suspended by the caps.

12.7 Immerse the tubes into the ultrasonic water bath (Note 15.3).

12.8 Turn on the ultrasonic bath power and sonicate for 20 minutes. Cavitation should be evident.

12.9 After sonication, centrifuge at approximately 2000 rpm for approximately 10 minutes.

12.10 Decant each supernatant into a labeled 500 mL glass screw-cap bottle.

12.11 Repeat steps 12.3 through 12.10 using 50:50, 5% acetic acid in methanol:5% acetic acid in water (20 mL) as the extraction solvent and combine the supernatant with the supernatant from 12.10.

12.12 Repeat steps 12.3 through 12.10 using 5% acetic acid in water (20 mL) as the extraction solvent and combine the supernatant with those from 12.10 and 12.11.

12.13 Add 430 mL of distilled water to the combined extracts.

12.14 Acidify the sample by adding 2.5 mL of phosphoric acid (85.5%) to each bottle.

12.15 Cap the bottle with a Teflon®-lined screw cap.

12.16 Shake the bottles by hand thoroughly by inversion for approximately 30 seconds.

12.17 Using pH paper, ensure that the pH of each sample is less than 2.

12.18 Condition 1 g, 6 mL, C_{18} Solid Phase Extraction cartridges by pulling through under vacuum, 10 mL of methanol, followed immediately by 10 mL of 1.5% phosphoric acid in water (see Note 15.4). Do not allow cartridge to dry before loading sample.

12.19 Connect the SPE cartridge to the sample bottle using a SPE adapter. Using a pasteur pipet, transfer a portion of each sample to its corresponding cartridge. Run the Teflon® tubing from the SPE adapter to the sample bottle. The sample bottles must be raised above the level of the vacuum manifold in order to more efficiently pull the entire sample through the SPE cartridge (see Figure 6).

12.20 Charge the diluted solutions onto the SPE cartridges at a flow rate of approximately 5 mL/min. using a vacuum manifold. Vacuum should be set at <10" Hg. All of the SPE eluate may be discarded at this point.

12.21 When the solution has completely passed through the SPE cartridge, allow each cartridge to dry under a vacuum of 20" Hg for a minimum of 20 minutes to remove excess water. Remove any remaining droplets adhered to the sides of the cartridge with a cotton swab or clean tissue. <u>It is crucial that the water is removed or problems will occur during methylation.</u>

12.22 Remove the cartridge set-up from the manifold and rinse the manifold ports with acetone to remove water. Replace the SPE cartridges after removing the SPE adapters and tubing.

12.23 Elute the SPE cartridge under vacuum (Note 15.4) into a 15 mL conical centrifuge tube as follows:

12.23.1 6 mL of 2% acetone in hexane (FRACTION A).

12.23.2 5 mL of 2% acetone in hexane. Collect the first 2.5 mL into the same conical tube with FRACTION A (see Note 15.5). Change to 20 mL vial and collect the second 2.5 mL (FRACTION B).

12.23.3 5 mL of 20% methanol in acetone. Collect into the same vial with FRACTION B (Note 15.6).

12.24 Under a gentle stream of nitrogen in an N-evap (see Note 15.7), evaporate FRACTION B to a final volume of 0.5 -1.0 mL.

12.25 Add 1 mL of BF_3-methanol solution to each sample.

12.26 Cap tightly with a Teflon®-lined screw cap. Shake side-to-side once or twice to ensure complete mixing.

12.27 Immerse so that the water level is above the level of the sample in the tube in an approximately 70°C water bath for approximately 30 minutes (see Note 15.8). Ensure that the caps remain tight.

12.28 Cool the reaction mixture.

12.29 Add 8 mL of distilled water to each sample tube.

12.30 Add 5 mL of hexane to each sample tube.

12.31 Shake for 10 minutes at high speed on a mechanical shaker.

12.32 Let the sample stand until the layers in the tubes separate (centrifuge if necessary).

12.33 Using a pasteur pipet, remove the water layer and discard.

12.34 Using a pasteur pipet, combine the methylated FRACTION B (hexane layer, see Note 15.9) with the pooled FRACTION A into one solution.

12.35 Add 1 mL of toluene to the combined solution and shake briefely to mix.

12.36 Evaporate the solvents under a gentle nitrogen stream in an N-evap at a flow rate of approximately 25 mL/minute distributed through the stainless steel capillary tubes. Set the tubes so that the nitrogen initially impinges the surface of the solvent from a height of approximately 2 mm. Maintain the water bath at approximately room temperature to 20°C. As the solvent evaporates, lower

the tubes accordingly. Evaporate to a volume of 1 mL and then bring to a final volume of 2.0 mL with toluene.

12.37 Vortex the sample on medium speed for 10 seconds to mix.

12.38 Transfer the concentrate to a GC vial and cap using a crimper (see Note 15.10).

12.39 Inject 2 μL of the sample onto the GC/MSD to quantitate for the appropriate ions (see Note 15.11).

12.40 Using toluene as the solvent, dilute any samples that are outside the 8 - 10 fold range of the standard curve.

13. Determination/Calculation of Percent Recovery

13.1 For analytes 2,4-D 2-EHE, 2,4-D, 2,4-DCP, and 2,4-DCA:

13.1.1 Inject the calibration standards described in section 10.8.2 Determine the peak areas of the appropriate quantitation ions.

13.1.2 Prepare a standard curve by plotting the quantitation peak area versus the calibration standard concentrations.

13.1.3 Determine the concentration in μg/mL of the analytes found in the recovery samples by comparing their peak areas to the standard curve.

13.1.4 Calculate the concentration in the final solution as follows:

$$\mu g/mL = \frac{peak\ area - C}{m}$$

where, m is the slope and C is the y-intercept of the regression line obtained from a plot of the peak area versus the nominal standard concentration.

13.1.5 The analyte concentration in the original soil sample is calculated using the equation shown below:

$$ppm = \frac{\mu g/mL\ found\ final\ solution\ x\ 2\ x\ any\ additional\ dilution}{sample\ weight}$$

13.1.6 Positive confirmation of the presence of each analyte is indicated when the confirmation ion ratio is in the range of ±20% of the average ion ratio found for the standards.

13.1.7 Subtract any contribution from the control sample, then, calculate the percent recovery as shown below:

$$Recovery(\%) = \frac{Corrected\ ppm}{ppm\ added}\ x\ 100$$

13.1.8 Calculate the confirmation ion ratio using the equation shown below:

$$Confirmation\ ratio = \frac{peak\ area\ Qualifier\ 1}{peak\ area\ Quantitation\ Ion}$$

14. Determination of 2,4-D 2-EHE, 2,4-D, 2,4-DCP, and 2,4-DCA in Soil

14.1 Prepare control, recovery (using the fortification standards appropriate for the analytes under investigation), and treated samples as described in Section 12.

14.2 Determine the concentration in μg/mL from the standard curves. Typical standard curves are shown in Figures 7-10.

14.3 Calculate the μg/mL as described in section 13.1.4.

14.4 Calculate the original soil concentration using the equations shown below:

14.4.1 Soil Residue Concentration (ppm) - 2,4-D 2-EHE, 2,4-DCP, 2,4-DCA, and 2,4-D.

$$\text{Analyte soil conc., ppm} = \frac{\text{Observed analyte conc. X Final volume}}{\text{sample weight}}$$

14.5 Typical total ion chromatograms of a control soil and control soil fortified at 0.01 ppm are shown in Figures 11 and 12. Typical Single Ion Monitoring chromatograms for each analyte are shown in Figures 13-16.

14.6 Recoveries for each analyte over the concentration range of 0.01 to 10 ppm averaged 136, 91, 97, and 110% for 2,4-D 2-EHE, 2,4-D, 2,4-DCP and 2,4-DCA, respectively. The results are summarized in Table 1.

15. Notes

15.1 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.

15.2 The fortification solution containing 2,4-D 2-EHE is used for sample sets in which 2,4-D 2-EHE is under investigation. The solution containing 2,4-D DMAS is used for sample sets in which 2,4-D DMAS is under investigation

15.3 The sonication bath is filled with distilled water to a depth equal to the level of the slurries in the sample tubes. Maintain the sonication bath at room temperature.

15.4 Condition cartridges at a vacuum level less than 5 in. Hg or a flow rate of 1 mL/minute. Do not allow the vacuum to exceed 5 in. Hg or a flow rate of approximately 1-2 mL/minute.

15.5 This solution contains 2,4-DCP, and 2,4-DCA, and if appropriate 2,4-D 2-EHE. These analytes are chromatographed without derivitization.

15.6 This solution contains 2,4-D.

15.7 Use Meyer N-Evap with capillary stainless steel tubing and a flow rate of 10-25 mL/minute.

15.8 The water bath should be set at a temperature of 70 $\pm$ 2°C for the methylation reaction.

15.9 This solution contains the methyl ester of 2,4-D.

15.10 All analytes are chromatographed on the same gas chromatography system using the same conditions.

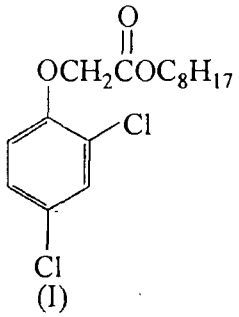
15.11 2,4-D 2-EHE is only monitored for if it is one of the analytes under investigation. 2,4-D DMAS is detected as its methyl ester.

15.12 2,4-D concentrations can be determined directly from the quantities of 2,4-D methyl ester detected because the calibration solutions used to generate the 2,4-D methyl ester calibration curve are prepared by taking into account the difference in molecular weight between 2,4-D methyl ester and 2,4-D. The "1000 μg/mL" solution of 2,4-D methyl ester prepared in Step 10.6 actually has a concentration of 1064 μg/mL. This concentration is equivalent to 1000 μg/mL of 2,4-D. The 1064 μg/mL solution is used to prepare the 2,4-D methyl ester calibration standards with acid equivalent concentrations of 0.02 ppm, 0.04 ppm, 0.10 ppm, etc. and, therefore, any 2,4-D methyl ester peaks detected can be converted to 2,4-D concentrations directly.

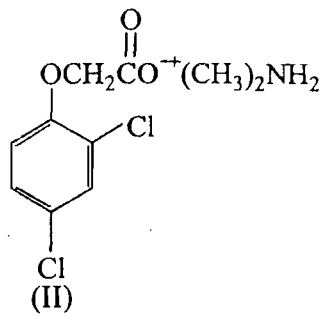
A. Scope

This method is applicable for the quantitative determination of 2,4-D 2-EHE, 2,4-D DMAS as its 2,4-D acid equivalent, 2,4-D, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA in soil sediment ranging in concentration from 0.01 to 0.10 µg/g (**Note O.1**).

B. Structures



(I)



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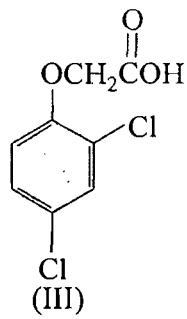

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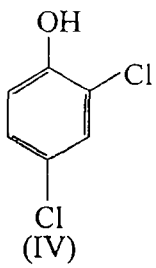

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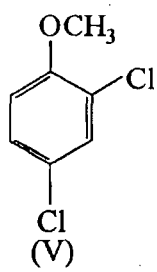
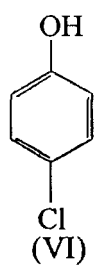
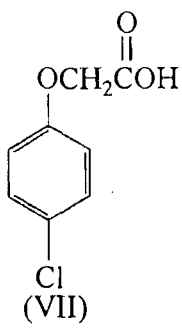


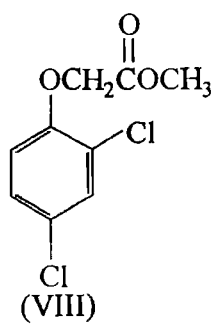
(V)



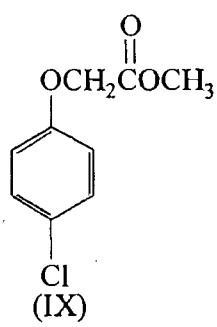
(VI)



(VII)



(VIII)



(IX)

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

C. Principle

A sample of soil sediment is vortexed and sonicated in 5% acetic acid in methanol, 50:50, 5 % acetic acid in methanol:5 % acetic acid in deionized water, and 5 % acetic acid in deionized water. The extracts are decanted, filtered after each extraction, combined, and brought to a known volume. An aliquot of extract is combined with hexane, a sodium sulfite solution, sodium chloride, and a sodium hydroxide solution. The 2,4-D 2-EHE and 2,4-DCA are partitioned into hexane and held for later work-up **(FRACTION A)**. The sodium hydroxide solution contains 2,4-D, 2,4-DCP, 4-CP, and 4-CPA.

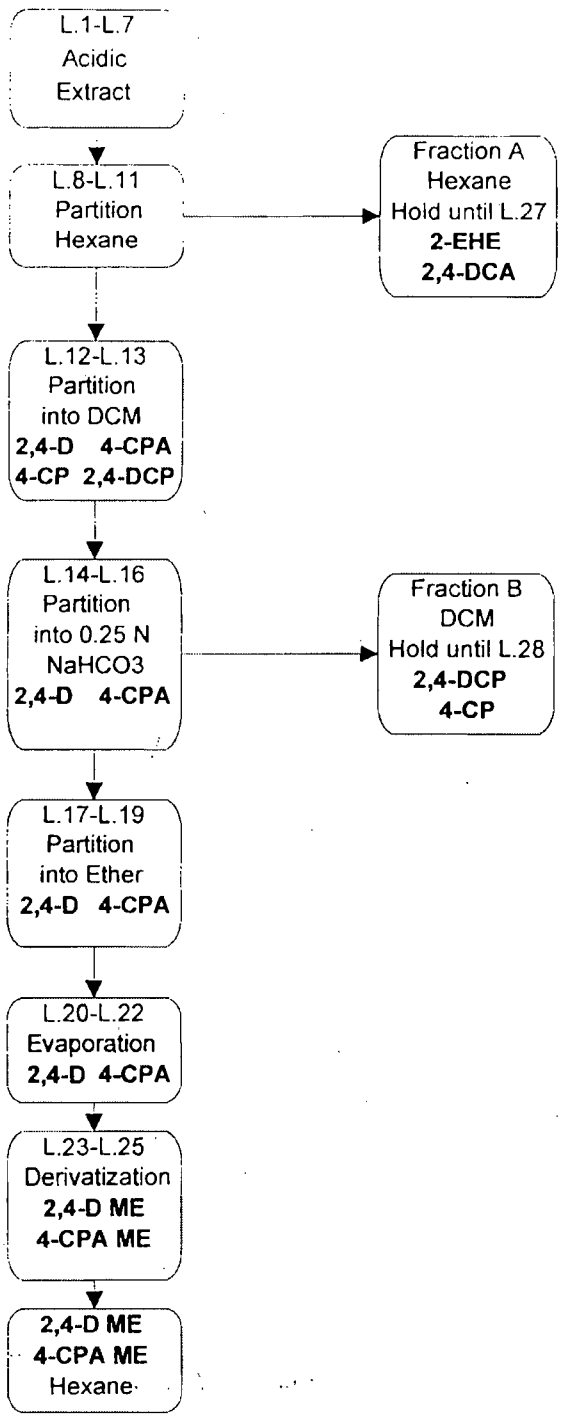
The sodium hydroxide solution is acidified, saturated with sodium chloride, and 2,4-D, 2,4-DCP, 4-CP, and 4-CPA are partitioned to dichloromethane (DCM). The 2,4-D and 4-CPA are back-partitioned to a sodium bicarbonate solution. The DCM containing 2,4-DCP and 4-CP, is designated **FRACTION B,** and held for further work-up.

The sodium bicarbonate solution is acidified, saturated with sodium chloride, and 2,4-D and 4-CPA are partitioned to ether. The ether is evaporated to incipient dryness and the two analytes are derivatized with BF_3/methanol to form 2,4-D ME and 4-CPA ME, respectively. The reactants are swamped with water and held for combination with other analytes for final quantitation.

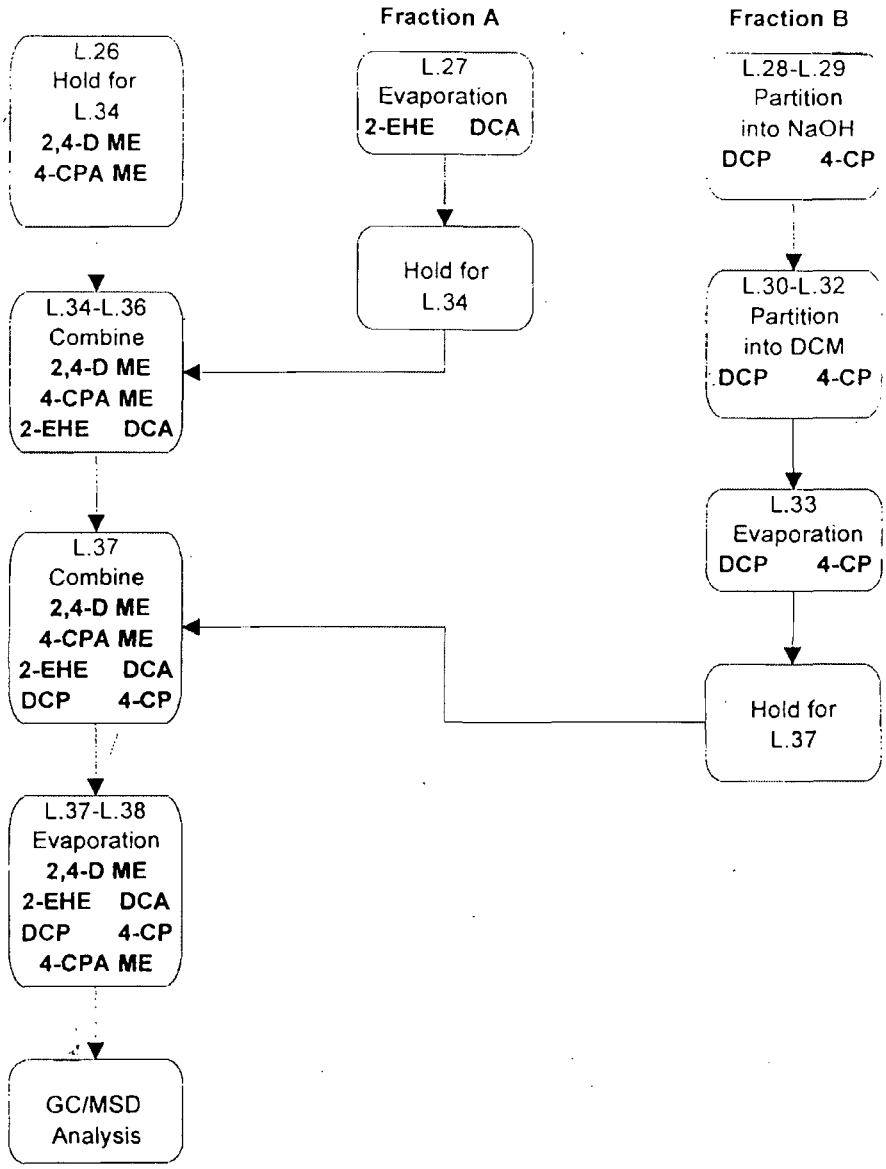
The hexane from **FRACTION A** is concentrated and combined with the analytes from derivatization of 2,4-D and 4-CPA. The 2,4-D ME and 4-CPA ME are partitioned into hexane, concentrated, and held for combination with analytes for final quantitation.

The 2,4-DCP and 4-CP in DCM **(FRACTION B)** are back-partitioned to sodium hydroxide. The sodium hydroxide is acidified and the analytes are partitioned to DCM. The DCM is concentrated, combined with the hexane fraction containing 2,4-D 2-EHE, 2,4-D ME, 2,4-DCA, and 4-CPA ME, and concentrated to a known volume.

An aliquot is injected on the gas chromatograph for quantitation by mass selective detection (MSD). A flow chart of the method, with the relevant step numbers from Section L. indicated in the blocks, is presented below:


L.1-L.7
Acidic
Extract
L.8-L.11
Partition
Hexane
Fraction A
Hexane
Hold until L.27
2-EHE
2,4-DCA
L.12-L.13
Partition
into DCM
2,4-D 4-CPA
4-CP 2,4-DCP
L.14-L.16
Partition
into 0.25 N
NaHCO3
2,4-D 4-CPA
Fraction B
DCM
Hold until L.28
2,4-DCP
4-CP
L.17-L.19
Partition
into Ether
2,4-D 4-CPA
L.20-L.22
Evaporation
2,4-D 4-CPA
L.23-L.25
Derivatization
2,4-D ME
4-CPA ME
2,4-D ME
4-CPA ME
Hexane

(Continued)


Fraction A
Fraction B
L.26
Hold for
L.34
2,4-D ME
4-CPA ME
L.27
Evaporation
2-EHE DCA
L.28-L.29
Partition
into NaOH
DCP 4-CP
Hold for
L.34
L.30-L.32
Partition
into DCM
DCP 4-CP
L.34-L.36
Combine
2,4-D ME
4-CPA ME
2-EHE DCA
L.33
Evaporation
DCP 4-CP
L.37
Combine
2,4-D ME
4-CPA ME
2-EHE DCA
DCP 4-CP
Hold for
L.37
L.37-L.38
Evaporation
2,4-D ME
2-EHE DCA
DCP 4-CP
4-CPA ME
GC/MSD
Analysis

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 suppliers.

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 or 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.2**)

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 TPK4D, 0 to 4000 g, O'haus Corporation, P.O. Box 900, 29 Hanover Road, Florham Park, NJ 07932.

E.3 Bath, ultrasonic, Model B2200R-4, Branson Ultrasonics Corporation, Eagle Road, Danbury, CT 06810-1951.

E.4 Centrifuge, Model K, International Equipment Company, 300 2nd Avenue, Needham Heights, MA 02194.

E.5 Crimper, catalog number 8710-0979, Hewlett-Packard Company, 2850 Centerville Road, Wilmington, DE 19808.

E.6 Evaporator, QMAS Model 100, Quality Management and Analytical Services, Inc., Hwy 32N, Walhalla, ND 58282.

E.7 Evaporator, Rotavapor®, Rotary Vacuum Evaporators, Buchi, catalog number 270-623, Curtin Matheson Scientific, Inc., 7677 Equitable Drive, Eden Prairie, MN 55344.

E.8 Gas chromatograph, Model 5890 Series II, equipped with a Mass Selective Detector, Model 5972, Hewlett-Packard Company, 2025 West Larpenteur Avenue, St. Paul, MN 55113.

E.9 Pipetters, to deliver between 200- to 1000-µL, Oxford, catalog number 273-132, Curtin Matheson Scientific, Inc.

E.10 Shaker, reciprocating, capable of achieving 180 excursions per minute (epm), Model 6000, Eberbach Corporation, 505 S. Maple Road, P.O. Box 1024, Ann Arbor, MI 48103.

E.11 Shaker, vortex, Model G-560, Scientific Industries, Inc. Bohemia, NY 11716.

E.12 Water bath, Equatherm, Model 273-811, Curtin Matheson Scientific, Inc.

F. Glassware (**Note O.2**)

F.1 Bottles, 950-mL, glass with PTFE-lined caps, catalog number 294-311, Curtin Matheson Scientific, Inc.

F.2 Centrifuge bottles, 200-mL, glass, Pyrex, catalog number 054-817, Curtin Matheson Scientific, Inc.

F.3 Conical tubes, 15-mL, graduated, Kimax, catalog number 253-822, Curtin Matheson Scientific, Inc.

F.4 Conical tubes, 50-mL, graduated, Pyrex, catalog number 054-171, Curtin Matheson Scientific, Inc.

F.5 Cylinder, graduated, glass, to deliver 100-mL, Pyrex, catalog number 312-404, Curtin Matheson Scientific, Inc.

F.6 Cylinder, graduated, glass, to deliver 500-mL, Pyrex, catalog number 074-591, Curtin Matheson Scientific, Inc.

F.7 Cylinder, mixing, graduated, glass, to contain 100-mL, with ground-glass stopper, Kimax, catalog number 101-469, Curtin Matheson Scientific, Inc.

F.8 Flasks, flat-bottom, boiling, 250-mL, with 24/40 joint, short neck, Pyrex, catalog number 095-943, Curtin Matheson Scientific, Inc.

F.9 Flasks, filtering, 500-mL, Pyrex, catalog number 103-010, Curtin Matheson Scientific, Inc.

F.10 Flasks, volumetric, glass, 100-mL, Kimax, with ground-glass stopper, catalog number 104-323, Curtin Matheson Scientific, Inc.

F.11 Flasks, volumetric, glass, 1000-mL, Kimax, with ground-glass stopper, catalog number 104-364, Curtin Matheson Scientific, Inc.

F.12 Funnels, Buchner, 104-mm dia., Coors, catalog number 109-900, Curtin Matheson Scientific, Inc.

F.13 Pipets, 10-mL, graduated in 1/10 mL increments, Pyrex, catalog number 250-814, Curtin Matheson Scientific, Inc.

F.14 Pipets, volumetric, to deliver 1.0 mL, Pyrex, catalog number 250-816, Curtin Matheson Scientific, Inc.

F.15 Pipets, volumetric, to deliver 4.0 mL, Pyrex, catalog number 250-819, Curtin Matheson Scientific, Inc.

F.16 Pipets, volumetric, to deliver 5.0 mL, Pyrex, catalog number 250-820, Curtin Matheson Scientific, Inc.

F.17 Pipets, volumetric, to deliver 10.0 mL, Pyrex, catalog number 250-821, Curtin Matheson Scientific, Inc.

F.18 Pipets, volumetric, to deliver 20.0 mL, Pyrex, catalog number 250-823, Curtin Matheson Scientific, Inc.

F.19 Pipets, volumetric, to deliver 50.0 mL, Pyrex, catalog number 190-363, Curtin Matheson Scientific, Inc.

F.20 Stoppers, number 13, ground-glass, Kimax, catalog number 219-121, to fit F.10, Curtin Matheson Scientific, Inc.

F.21 Stoppers, number 22, ground-glass, Kimax, catalog number 104-364, to fit F.7 and F.11, Curtin Matheson Scientific, Inc.

F.22 Syringe, 10-µL, Hamilton, glass, part number 9301-0725, Hewlett-Packard Company.

F.23 Test tubes, 20-mL, threaded, disposable, Kimble, catalog number 254-268, Curtin Matheson Scientific, Inc.

F.24 Vials, glass, 2-mL, part number 5181-3400, Hewlett-Packard Company.

G. Materials (**Note O.2**)

G.1 Air, compressed, catalog number UN1002, Genex, 700 2nd Avenue, Des Moines, IA 50302.

G.2 Caps, plastic, PTFE-lined, size 53-400, catalog number 237-623, to fit F.1 above, Curtin Matheson Scientific, Inc.

G.3 Caps, plastic, PTFE-lined, size 38-400, catalog number 237-619, to fit F.2 above, Curtin Matheson Scientific, Inc.

G.4 Caps, phenolic, PTFE-lined, size 15-415, catalog number 226-167, to fit F.3 and F.23 above, Curtin Matheson Scientific, Inc.

G.5 Caps, plastic, PTFE-lined, size 24-410, catalog number 256-216, to fit F.4 above, Curtin Matheson Scientific, Inc.

G.6 Column, HP-5MS, 0.25 mm X 30 meter, capillary, 0.25 μm film thickness, part number 19091S-433, Hewlett-Packard Company.

G.7 Filter paper, 9-cm, number 3, Whatman, catalog number 091-934, Curtin Matheson Scientific, Inc.

G.8 Gloves, clean, lint-free, part number 8650-0030, Hewlett-Packard Company.

G.9 Liner, injection, single-taper, deactivated, part number 5181-3316, Hewlett-Packard Company.

G.10 pH test strips, pH 0-14, ColorpHast, catalog number 393-209, Curtin Matheson Scientific, Inc.

G.11 Pipet tips, 100- to 1000-μL, Labcraft, catalog number 044-826, to fit E.9, Curtin Matheson Scientific, Inc.

G.12 Rubber bands, 1/4" x 3-1/2", catalog number 942-7-90064, Quill Corp., P.O. Box 94080, Palatine, IL 60094-4080.

G.13 Septum, 11-mm, low-bleed, part number 5181-1263, Hewlett-Packard Company.

G.14 Stopper, neoprene, number 7, catalog number 202-127,to fit F.9, Curtin Matheson Scientific, Inc.

G.15 Vial closures, 11-mm aluminum, PTFE-lined, part number 5181-1210, Hewlett-Packard Company.

G.16 Weighing paper, 6" x 6", Labcraft, catalog number 340-919, Curtin Matheson Scientific, Inc.

H. Chemicals (**Note O.2**)

H.1 Acetic acid, ACS-grade, E.M. Science, catalog number MAX0073-9, Curtin Matheson Scientific, Inc.

H.2 Ammonium hydroxide solution, 28 %, E.M. Science, catalog number MAX1303-3, Curtin Matheson Scientific, Inc.

H.3 Boron trifluoride/methanol (BF_3/methanol), 12 %, catalog number 26,412-1, Aldrich Chemical Company, 1001 West Saint Paul Avenue, Milwaukee, WI 53233.

H.4 Dichloromethane (DCM), Omnisolv, Pesticide Residue Quality, E.M. Science, catalog number MDX0831-6, Curtin Matheson Scientific, Inc.

H.5 Diethyl ether, anhydrous, E.M. Science, catalog number MEX0190-3, Curtin Matheson Scientific, Inc.

H.6 Helium, carrier gas, Ultra High Purity, Genex.

H.7 Hexane, OmniSolv, Pesticide Residue Quality, E. M. Science, catalog number MHX0298-1, Curtin Matheson Scientific, Inc.

H.8 Methanol, OmniSolv, Pesticide Residue Quality, E.M. Science, catalog number MMX0484-1, Curtin Matheson Scientific, Inc.

H.9 PFTBA 99.9 %, for tuning mass spectrometer, catalog number 8500-0656, Hewlett-Packard Company.

H.10 Phosphoric acid (H_3PO_4), 85 %, ACS, Chempure, catalog number 832-536, Curtin Matheson Scientific, Inc.

H.11 Sodium bicarbonate ($NaHCO_3$), powder, ACS, E.M. Science, catalog number MSX0325-5, Curtin Matheson Scientific, Inc.

H.12 Sodium chloride (NaCl), crystals, ACS, E.M. Science, catalog number MSX0420-5, Curtin Matheson Scientific, Inc.

H.13 Sodium hydroxide (NaOH), pellets, E.M. Science, catalog number MSX0600-1, Curtin Matheson Scientific, Inc.

H.14 Sodium sulfite (Na_2SO_3), powder, E.M. Science, catalog number MSX0785-1, Curtin Matheson Scientific, Inc.

H.15 Standards, analytical (**Note O.3**):

H.15.1 2,4-dichlorophenoxyacetic acid 2-ethylhexyl ester
H.15.2 2,4-dichlorophenoxyacetic acid dimethylamine salt
H.15.3 2,4-dichlorophenoxyacetic acid
H.15.4 2,4-dichlorophenol
H.15.5 2,4-dichloroanisole
H.15.6 4-chlorophenol
H.15.7 4-chlorophenoxyacetic acid
H.15.8 2,4-dichlorophenoxyacetic acid methyl ester
H.15.9 4-chlorophenoxyacetic acid methyl ester

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

I. Reagents (**Note O.2**)

I.1 5 % ammonium hydroxide in methanol: dilute 5 mL of 28 % ammonium hydroxide solution to 100 mL with methanol.

I.2 5 % acetic acid in methanol: mix 50 mL of 99 % acetic acid and 950 mL of methanol.

I.3 5 % acetic acid in water: mix 50 mL of 99 % acetic acid and 950 mL of deionized water.

I.4 50:50, 5 % acetic acid in methanol:5 % acetic acid in deionized water: mix 50 mL of 99 % acetic acid with 475 mL methanol and 475 mL deionized water.

I.5 0.5 % phosphoric acid in methanol: dilute 5.0 mL of 85 % phosphoric acid in a 1000-mL volumetric flask and bring to volume with methanol and mix.

I.6 0.25 N sodium bicarbonate: dissolve 21.0 g of sodium bicarbonate in deionized water and dilute to 1000-mL in a volumetric flask with water and mix. (**Note O.4**).

I.7 0.5 N sodium hydroxide: dissolve 20.0 g of sodium hydroxide pellets in deionized water, dilute to 1000-mL in a volumetric flask with water and mix.

I.8 1.0 N sodium hydroxide: dissolve 40.0 g of sodium hydroxide pellets in deionized water, dilute to 1000-mL in a volumetric flask with water and mix.

I.9 Saturated sodium sulfite: add 65 g sodium sulfite to a 950-mL bottle containing 500 mL of deionized water and mix on a reciprocating shaker for 20 min.

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 paper with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1000 µg/mL stock solution.

J.2 2,4-D DMAS analytical standard. Weigh out 0.1204 g 2,4-D DMAS 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 1204 µg/mL stock solution equivalent to 1000 µg/mL 2-4,D (**Note O.5**).

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 paper with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1000 µg/mL 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 paper with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1000 µg/mL stock solution.

J.5 2,4-DCA analytical standard. Weigh out 0.1000 g 2,4-DCA 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 µg/mL stock solution.

J.6 4-CP analytical standard. Weigh out 0.1000 g 4-CP 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 µg/mL stock solution.

J.7 4-CPA 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 µg/mL stock solution.

Take the 1000 µg/mL solutions from J.1 to J.7 and serially dilute in methanol as given in table J.8. Each standard can be prepared separately or in combination by combining 1 mL of each 1000 µg/mL solution from J.1, J.4 to J.7, and either J.2 or J.3 in a 100-mL volumetric flask and bringing it to volume with methanol (**Note O.6**).

J.8

Conc. of Initial Solution µg/mL	Aliquot of Initial Solution mL	Final Volume of Diluted Solution mL	Conc. of Final Solution µg/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 µg/mL	Aliquot of Initial Solution mL	Mass of Soil Sediment g	Fort. Conc. on Soil Sediment µg/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 paper with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1063 µg/mL stock solution equivalent to 1000 µg/mL 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 paper with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1075 µg/mL stock solution equivalent to 1000 µg/mL 4-CPA (**Note O.8**).

Dilute the 1000 µg/mL stock solution of J.1, J.4, J.5, J.6, J.10, and J.11 with hexane 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.

Each standard can be prepared separately or as a combination by combining 1 mL of each 10 µg/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 (**Note O.9**).

J.12

Conc. of Initial Solution µg/mL	Aliquot of Initial Solution mL	Final Volume of Diluted Solution mL	Conc. of Final Solution (a) µg/mL
1000	1.0	100	10.0
10.0	10.0	100	1.0
10.0	5.0	100	0.50 (b)
1.0	20.0	100	0.20 (b)
10.0	1.0	100	0.10 (b)
0.50	10.0	100	0.050 (b)(c)
0.50	5.0	100	0.025 (b)(d)

(a) Concentrations of J.10 and J.11 are based on 2,4-D and 4-CPA equivalence, respectively.
(b) These are the series of standards that make up the calibration curve.
(c) This standard is equivalent to the method LOQ of 0.01 µg/g.
(d) This standard is 1/2 the LOQ.

K. Instrument Operating Conditions

K.1 Inlet liner, septum and column 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 °C which is mid-range on the GC temperature program where most of the analytes elute. 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.

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
2,4-DCP	162	164	166
2,4-DCA	176	178	163
4-CP	128	130	100
4-CPA ME	200	202	141

K.4 Typical operating conditions for the analysis of 2,4-D 2-EHE, 2,4-D ME, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA ME 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 μm film thickness
Oven Temperature	Hold at 50°C for 1 min, then 50 to 100°C 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
Injection Liner	Silanized single taper
Injector Purge Delay	1.5 min
Septum Purge	50 mL/min (Helium)
Injection Volume	2 µL
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.12 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. Average confirmation ratio will be used to confirm the presence of each analyte in the soil sediment samples.

For 2,4-D ME:

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

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

e.g. $$\textit{Confirmation Ratio} = \frac{289}{426}$$

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

The presence of each analyte is confirmed when the confirmation ratio for the analyte in a sample is within ± 20 % of the average found for its respective 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-EHE, 2,4-D, 2,4-DCP, 2,4-DCA, 4-CP and 4-CPA from soil sediment

L.1 Place 10 g of soil sediment into a series of 50-mL conical 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.9.

Treat each sample as follows:

L.3 Add 20 mL of 5 % acetic acid in methanol, cap, and vortex for 30 seconds on high speed.

L.4 Suspend the samples to the depth of the contents in the container in an ultrasonic bath and sonicate for 20 minutes, then centrifuge at 2000 rpm for 10 min.

L.5 Decant the supernatant into a 104-mm Buchner funnel fitted with a 9-cm Whatman number 3 filter paper and vacuum filter into a 500-mL filtering flask. Transfer the filtrate to a 100-mL graduated mixing cylinder.

L.6 To the remaining soil sediment add back 20 mL of 50:50, 5 % acetic acid in methanol:5 % acetic acid in deionized water and repeat the vortexing, sonication, centrifugation, and filtering. Combine the filtrate in the graduated mixing cylinder from L.5.

L.7 Add 20 mL of 5 % acetic acid in water to the remaining sediment, cap, and repeat the extraction in the same manner as above. Combine the filtrate with the first two extractions and bring to 100 mL with deionized water and stopper. Mix well by inversion.

The solution in step L.7 contains 2,4-D 2-EHE, 2,4-D, 2,4-DCP, 2,4-DCA, 4-CP and 4-CPA.

L.8 Transfer a 50-mL aliquot of the methanolic extract into a 200-mL centrifuge bottle.

L.9 Add 30 mL of hexane and mix by hand for 30 seconds, then add 2 mL saturated sodium sulfite solution, 20 g of sodium chloride, and mix vigorously by hand for 15 seconds (**Note O.10**).

L.10 Add 80 mL of 0.5 N sodium hydroxide. (**Note O.11**) Cap and shake on a reciprocating shaker at 180 epm for 5 minutes (**Note O.12**). Centrifuge at 1000 rpm for 2 minutes, then using a 50-mL pipet, transfer the hexane layer to a 250-mL flat-bottom boiling flask.

L.11 Add a second 30 mL of hexane to the sodium hydroxide and repeat the shaking and centrifugation, combining the hexane with the first partition solution in the 250-mL flat-bottom boiling flask.

The hexane now contains the 2,4-D 2-EHE and 2,4-DCA and is designated **FRACTION A**. Hold for further cleanup later. The sodium hydroxide contains the 2,4-D, 2,4-DCP, 4-CP, and 4-CPA.

L.12. To the sodium hydroxide add 10 mL of concentrated phosphoric acid, and 60 mL of dichloromethane. Cap and shake for 5 minutes on a reciprocating shaker at 180 epm, then centrifuge the sample at 1000 rpm for 2 minutes.

L.13. Using a 50-mL pipet, transfer the DCM layer to a 200-mL centrifuge tube, add back an additional 60 mL of DCM, and repeat the partitioning, combining all of the DCM in the 200-mL centrifuge tube.

The DCM contains the 2,4-D, 2,4-DCP, 4-CP, and 2,4-CPA.

L.14 Add 60 mL of 0.25 N sodium bicarbonate to the DCM, shake for 5 minutes on a reciprocating shaker at 180 epm **(Note O.12)**, then centrifuge for 2 minutes at 1000 rpm.

L.15 Using a 50-mL pipet, transfer the sodium bicarbonate layer to a 200-mL centrifuge tube.

L.16 The DCM contains the 2,4-DCP and 4-CP and is designated **FRACTION B**. Save the DCM for further cleanup later.

The sodium bicarbonate contains the 2,4-D and 4-CPA.

L.17 To the sodium bicarbonate, add: 20 g of sodium chloride, 10 mL of concentrated phosphoric acid, (drop-wise), **(Note O.13)** and 30 mL of diethyl ether.

L.18 Cap and shake the sample on a reciprocating shaker at 180 epm for 5 minutes **(Note O.12)**, centrifuge at 1000 rpm for 2 minutes, then transfer the ether layer to a 250-mL flat-bottom boiling flask.

L.19 Repeat the ether partition with an additional 30 mL combining with the first ether partition in the 250-mL flat-bottom boiling flask.

L.20 Concentrate the ether to approximately 5 mL using a rotovap set at 40°C and transfer to a 20-mL test tube using two 2-mL ether washes.

L.21 Add 1 mL of 5 % ammonium hydroxide in methanol and evaporate the ether to incipient dryness at 40°C under 200 mL/min air flow (**Note O.14**).

L.22 Add 0.5 mL of methanol to azeotrope off the water carried over from the ether partition.

L.23 Add 0.2 mL of 0.5 % phosphoric acid in methanol to the sample tube, 1 mL of 12 % boron trifluoride/methanol, cap tightly, and mix by hand for 15 seconds.

L.24 Incubate the sample tube in a water bath at 70°C for 30 minutes, check the caps occasionally during the incubation for tightness (**Note O.15**).

L.25 Remove from the water bath, cool, add 5 mL of deionized water, and hold for later work-up.

L.26 This solution contains the 2,4-D methyl ester and 4-CPA methyl ester and will be combined with the other analytes for final quantitation.

L.27 The hexane from step L.11, **FRACTION A**, containing the 2,4-D 2-EHE and the 2,4-DCA is concentrated to approximately 10 mL using a rotovap at 40°C, transferred to a 20-mL test tube using two 2-mL hexane rinses and concentrated to 5 mL at 40°C under 200 mL/min air flow (**Note O.14**).

(This solution will be combined later in the method with the other analytes for quantitation.)

L.28 To the DCM from step L.16 containing the 2,4-DCP and 4-CP, add 50 mL of 1 N sodium hydroxide, cap, and shake for 5 minutes on a reciprocating shaker at 180 epm (**Note O.12**).

L.29 Centrifuge the sample at 1000 rpm for 2 minutes, then transfer the sodium hydroxide to a 200-mL centrifuge tube using a 50-mL pipet. Discard the DCM.

L.30 To the sodium hydroxide add 20 g of sodium chloride, 10 mL of concentrated phosphoric acid, and 30 mL of DCM and cap (**Note O.13**).

L.31 Shake the sample on a reciprocating shaker at 180 epm for 5 minutes (**Note O.12**). The DCM and aqueous layers will separate quickly on standing.

L.32 Transfer the DCM layer to a 250-mL flat-bottom boiling flask. Repeat the partitioning with an additional 30 mL of DCM, shake on a reciprocating shaker for 5 minutes at 180 epm, and combine the two DCM fractions in a 250-mL flask.

L.33 Concentrate the DCM to 5 mL using a rotovap set at 25°C.

Quantitation of 2,4-D 2-EHE, 2,4-D ME, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA ME.

L.34 Add the solution from L.27 to the solution from L.26 using two 1-mL hexane rinses.

L.35 Cap and shake for 5 min on a vortex shaker at high speed (**Note O.16**).

L.36 Transfer the hexane layer to a 15-mL graduated conical tube and concentrate to 1 mL at 40°C under 200 mL/min air flow (**Note O.14**).

L.37 Add the solution from L.33 to the solution from L.36 using two 1-mL DCM rinses and concentrate to 1 mL at 30°C under 200 mL/min air flow (**Note O.14**).

L.38 Adjust the final volume to 1 mL with hexane. Mix and place a portion of the hexane in a 2-mL injection vial. Cap and crimp the vial.

L.39 Inject a 2-µL aliquot on the gas chromatograph for quantitation using a mass selective detector.

L.40 Determine the concentration of the sample in µg/mL from the standard curve.

L.41 Calculate the µg/g by multiplying µg/mL found in the final solution times 0.20 times any additional dilution factor:

µg/g = µg/mL x 0.2 x any additional dilution factor

$$\textit{Percent Recovery} = \frac{\textit{total µg/g found (fortified sample) - total µg/g found (control)}}{\textit{total µg added}} \times 100$$

L.42 Average recoveries over the concentration range of 0.01 µg/g to 0.10 µg/g for each analyte are given below. Individual values are provided in Table I to Table VI.

Analyte	2,4-D 2-EHE	2,4-D ME	2,4-DCP	2,4-DCA	4-CP	4-CPA ME
% Recovery	102	102	93	92	85	87
Std Error	7	17	14	10	15	12
n	12	12	12	12	12	12

M. Determination of 2,4-D 2-EHE, 2,4-D, 2,4-DCP, 2,4-DCA, 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.39.

M.2 Determine the concentration of the sample in µg/mL from the standard curve.

M.3 Calculate the µg/g by multiplying µg/mL found in the final solution times 0.20 times any additional dilution factor:

µg/g = µg/mL x 0.2 x any additional dilution factor

M.4 A typical standard curve for each analyte is shown in Figures 7 through 12.

M.5 Typical chromatograms of standard, and control and recovery samples are shown in Figures 13 through 15.

N. Miscellaneous

N.1 A suggested analytical set is as follows (**Note O.17**):

0.025 µg/mL standard (1/2 LOQ)
one reagent blank
one control
one recovery at the LOQ
one recovery at the LOQ
0.05 µg/mL standard (LOQ)
field sample
field sample
field sample
field sample
0.10 µg/mL standard
field sample
field sample
field sample
field sample
0.20 µg/mL standard
0.50 µg/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.

O. Notes

O.1 When 2,4-D DMAS is shaken for 20 minutes in the presence of acetic acid in methanol or acetic acid in deionized water it is quantitatively dissociated to 2,4-D.

O.2 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.3 Obtain from Sampling Coordinator, Formulations, DowElanco, P.O. Box 63689, Indianapolis, Indiana 46268-1053.

O.4 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. Check with pH paper.

O.5 The molecular weight of 2,4-D DMAS is 266.01. The molecular weight of 2,4-D is 220.98. The ratio of 2,4-D DMAS to 2,4-D is 1.204. When 0.1204 g of 2,4-D DMAS is weighed out it is equivalent to 0.1000 g of 2,4-D. Weighing out the standard in this manner saves having to make a molecular weight correction for every analytical sample.

O.6 Stock solutions from J.2 and J.3 contribute 2,4-D, so only one should be added to the solution.

O.7 The molecular weight of 2,4-D ME is 234.99. The molecular weight of 2,4-D is 220.98. 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 0.1000 g of 2,4-D. Weighing out the standard 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 200.54. The molecular weight of 4-CPA is 186.53. 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 0.1000 g of 4-CPA. Weighing out the standard in this manner saves having to make a molecular weight correction for every analytical sample.

O.9 The initial dilution of the methanolic solution should be no greater than 1 mL methanol diluted to 100 mL with hexane to overcome any solvent immiscibility problems.

O.10 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.8.

O.11 Ensure that the pH of the aqueous layer is greater than 12 by adding more sodium hydroxide if necessary.

O.12 The samples are shaken horizontally by clamping them to the platform.

O.13 Add the acid to the water carefully.

O.14 The evaporation apparatus must be set up in the same fashion each time with conditions carefully controlled. The flow rate must be measured before the evaporation of each set and readjusted to 200 mL/min ± 10 mL/min.

O.15 Immerse the tube into the water to the same depth as the liquid in the vial.

O.16 The samples are shaken horizontally by attaching the vials to the platform with rubber bands.

O.17 A standard should be injected at the beginning and end of each sample run and at least every four samples throughout the run.