Method 1657

The Determination of Organo-Phosphorus Pesticides in Municipal and Industrial Wastewater

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1. SCOPE AND APPLICATION

1.1 This method is designed to meet the survey requirements of the Environmental Protection Agency (EPA). It is used to determine the (1) organo-phosphorus pesticides associated with the Clean Water Act, the Resource Conservation and Recovery Act, and the Comprehensive Environmental Response, Compensation and Liability Act; and (2) other compounds amenable to extraction and analysis by automated, wide-bore capillary column gas chromatography (GC) with a flame photometric detector.

1.2 The compounds listed in Table 1 may be determined in waters, soils, sediments, and sludges by this method. The method is a consolidation of several EPA methods. For waters, the sample extraction and concentration steps are essentially the same as in these methods. However, the extraction and concentration steps have been extended to other sample matrices. The method may be applicable to other phosphorus containing pesticides. The quality assurance/quality control requirements in this method give the steps necessary to determine this applicability. Not all compounds listed in Table 1 have corresponding calibration data in Table 3 and acceptance criteria in Table 4. Calibration data for such analytes may be found in other EPA methods (References 1 and 2).

1.3 This method is applicable to a large number of compounds. Calibrating the GC systems for all compounds is time-consuming. If only a single compound or small number of compounds is to be tested for, it is necessary to calibrate the GC systems and meet the performance specifications in this method for these compounds only. In addition, the GC conditions can be optimized for these compounds provided that all performance specifications in this method are met.

1.4 When this method is applied to analysis of unfamiliar samples, compound identity must be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Gas chromatography/mass spectrometry (GC/MS) can be used to confirm compounds in extracts produced by this method when analyte levels are sufficient.

1.5 The detection limits of this method are usually dependent on the level of interferences rather than instrumental limitations. The limits in Table 2 typify the minimum quantities that can be detected with no interferences present.

1.6 This method is for use by or under the supervision of analysts experienced in the use of a gas chromatograph and in the interpretation of gas chromatographic data. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 8.2.

2. SUMMARY OF METHOD

2.1 Extraction.

2.1.1 The percent solids content of a sample is determined.

2.1.2 Aqueous samples containing less than or equal to 1% solids.

2.1.2.1 Samples containing water-insoluble compounds: A 1-L sample is extracted with methylene chloride using continuous extraction techniques.

2.1.2.2 Samples containing highly water-soluble compounds such as methamidophos: Salt is added to a 1-L sample and the sample is extracted with an azeotropic mixture of chloroform:acetone using continuous extraction techniques.

2.1.3 Samples containing greater than 1% solids.

2.1.3.1 Non-sludge samples: If the solids content is 1 to 30%, the sample is diluted to 1% solids with reagent water, homogenized ultrasonically, and extracted as an aqueous sample. If the solids content is greater than 30%, the sample is extracted with methylene chloride:acetone using ultrasonic techniques.

2.1.3.2 Municipal sludge samples and other intractable sample types: If the solids content is less than 30%, the sample is diluted to 1% solids and extracted as an aqueous sample. If the solids content is greater than 30%, the sample is extracted with acetonitrile and then methylene chloride using ultrasonic techniques. The extract is back-extracted with 2% (w/v) sodium sulfate in reagent water to remove water-soluble interferences and residual acetonitrile.

2.2 Concentration and cleanup: Each extract is dried over sodium sulfate, concentrated using a Kuderna-Danish evaporator, cleaned up (if necessary) using gel permeation chromatography (GPC) and/or solid-phase extraction, and concentrated to 1 mL.

2.3 Gas chromatography: A fixed volume of the extract is injected into the gas chromatograph (GC). The compounds are separated on a wide-bore, fused-silica capillary column and detected using a flame photometric detector.

2.4 Identification of a pollutant (qualitative analysis) is performed by comparing the GC retention times of the compound on two dissimilar columns with the respective retention times of an authentic standard. Compound identity is confirmed when the retention times agree within their respective windows.

2.5 Quantitative analysis is performed by using an authentic standard to produce a calibration factor or calibration curve, and using the calibration data to determine the concentration of a pollutant in the extract. The concentration in the sample is calculated using the sample weight or volume and the extract volume.

2.6 Quality is assured through reproducible calibration and testing of the extraction and GC systems.

3. CONTAMINATION AND INTERFERENCES

3.1 Solvents, reagents, glassware, and other sample-processing hardware may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms. All materials used in the analysis shall be demonstrated to be free from interferences under the conditions of analysis by running method blanks as described in Section 8.5.

3.2 Glassware and, where possible, reagents are cleaned by rinsing with solvent and baking at 450°C for a minimum of 1 hour in a muffle furnace or kiln. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment and thorough rinsing with acetone and pesticide-quality hexane may be required.

3.3 Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

3.4 Interferences coextracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled. The cleanup procedures given in this method can be used to overcome many of these interferences, but unique samples may require additional cleanup to achieve the minimum levels given in Table 2.

4. SAFETY

4.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 3 through 5.

4.2 Unknown samples may contain high concentrations of volatile toxic compounds. Sample containers should be opened in a hood and handled with gloves that will prevent exposure. The oven used for sample drying to determine percent moisture should be located in a hood so that vapors from samples do not create a health hazard in the laboratory.

5. APPARATUS AND MATERIALS

NOTE: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance meeting the requirements of this method is the responsibility of the laboratory.

5.1 Sampling equipment for discrete or composite sampling.

5.1.1 Sample bottles and caps.

5.1.1.1 Liquid samples (waters, sludges and similar materials that contain less than 5% solids): Sample bottle, amber glass, 1-L or 1-quart, with screw-cap.

5.1.1.2 Solid samples (soils, sediments, sludges, filter cake, compost, and similar materials that contain greater than 5% solids): Sample bottle, wide-mouth, amber glass, 500-mL minimum.

5.1.1.3 If amber bottles are not available, samples shall be protected from light.

5.1.1.4 Bottle caps: Threaded to fit sample bottles. Caps shall be lined with PTFE.

5.1.1.5 Cleaning.

5.1.1.5.1 Bottles are detergent-water washed, then rinsed with solvent or baked at 450°C for a minimum of 1 hour before use.

5.1.1.5.2 Liners are detergent water washed, then rinsed with reagent water and solvent, and baked at approximately 200°C for a minimum of 1 hour prior to use.

5.1.2 Compositing equipment: Automatic or manual compositing system incorporating glass containers cleaned per bottle cleaning procedure above. Sample containers are kept at 0 to 4°C during sampling. Glass or PTFE tubing only shall be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing shall be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

5.2 Equipment for determining percent moisture.

5.2.1 Oven, capable of maintaining a temperature of 110°C (±5°C).

5.2.2 Desiccator.

5.2.3 Crucibles, porcelain.

5.2.4 Weighing pans, aluminum.

5.3 Extraction equipment.

5.3.1 Equipment for ultrasonic extraction.

5.3.1.1 Sonic disrupter: 375-watt with pulsing capability and ½" or ¾" disrupter horn (Ultrasonics, Inc, Model 375C, or equivalent).

5.3.1.2 Sonabox (or equivalent): For use with disrupter.

5.3.2 Equipment for liquid-liquid extraction.

5.3.2.1 Continuous liquid-liquid extractor: PTFE or glass connecting joints and stopcocks without lubrication, 1.5- to 2-L capacity (Hershberg-Wolf Extractor, Cal-Glass, Costa Mesa, California, 1000- to 2000-mL continuous extractor, or equivalent).

5.3.2.2 Round-bottom flask: 500-mL, with heating mantle.

5.3.2.3 Condenser: Graham, to fit extractor.

5.3.2.4 pH meter: With combination glass electrode.

5.3.2.5 pH paper: Wide-range (Hydrion Papers, or equivalent).

5.3.3 Separatory funnels: 250-, 500-, 1000-, and 2000-mL, with PTFE stopcocks.

5.3.4 Filtration apparatus.

5.3.4.1 Glass powder funnels: 125- to 250-mL.

5.3.4.2 Filter paper for above (Whatman 41, or equivalent).

5.3.5 Beakers.

5.3.5.1 1.5- to 2-L, calibrated to 1 L.

5.3.5.2 400- to 500-mL.

5.3.6 Spatulas: Stainless steel or PTFE.

5.3.7 Drying column: 400 mm long x 15 to 20 mm ID Pyrex chromatographic column equipped with coarse glass frit or glass wool plug.

5.3.7.1 Pyrex glass wool: Extracted with solvent or baked at 450°C for a minimum of 1 hour.

5.4 Evaporation/concentration apparatus.

5.4.1 Kuderna-Danish (K-D) apparatus.

5.4.1.1 Evaporation flask: 500-mL (Kontes K-570001-0500, or equivalent), attached to concentrator tube with springs (Kontes K-662750-0012).

5.4.1.2 Concentrator tube: 10-mL, graduated (Kontes K-570050-1025, or equivalent) with calibration verified. Ground-glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

5.4.1.3 Snyder column: Three-ball macro (Kontes K-503000-0232, or equivalent).

5.4.1.4 Snyder column: Two-ball micro (Kontes K-469002-0219, or equivalent).

5.4.1.5 Boiling chips.

5.4.1.5.1 Glass or silicon carbide: Approximately 10/40 mesh, extracted with methylene chloride and baked at 450°C for a minimum of 1 hour.

5.4.1.5.2 PTFE (optional): Extracted with methylene chloride.

5.4.2 Water bath: Heated, with concentric ring cover, capable of temperature control (±2°C), installed in a fume hood.

5.4.3 Nitrogen-evaporation device: Equipped with heated bath that can be maintained at 35 to 40°C (N-Evap, Organomation Associates, Inc., or equivalent).

5.4.4 Sample vials: Amber glass, 1- to 5-mL with PTFE-lined screw- or crimp-cap, to fit GC autosampler.

5.5 Balances.

5.5.1 Analytical: Capable of weighing 0.1 mg.

5.5.2 Top loading: Capable of weighing 10 mg.

5.6 Apparatus for sample cleanup.

5.6.1 Automated gel permeation chromatograph (Analytical Biochemical Labs, Inc, Columbia, MO, Model GPC Autoprep 1002, or equivalent).

5.6.1.1 Column: 600 to 700 mm long x 25 mm ID, packed with 70 g of SX-3 Bio-beads (Bio-Rad Laboratories, Richmond, CA, or equivalent).

5.6.1.2 Syringe: 10-mL, with Luer fitting.

5.6.1.3 Syringe-filter holder: Stainless steel. Glass fiber or PTFE filters (Gelman Acrodisc-CR, 1 to 5 µ, or equivalent).

5.6.1.4 UV detectors: 254 nm, preparative or semi-prep flow cell (Isco, Inc., Type 6; Schmadzu, 5 mm path length; Beckman-Altex 152W, 8 µL micro-prep flow cell, 2 mm path; Pharmacia UV-1, 3 mm flow cell; LDC Milton-Roy UV-3, monitor #1203; or equivalent).

5.6.2 Vacuum system and cartridges for solid-phase extraction (SPE).

5.6.2.1 Vacuum system: Capable of achieving 0.1 bar (house vacuum, vacuum pump, or water aspirator), with vacuum gauge.

5.6.2.2 VacElute Manifold (Analytichem International, or equivalent).

5.6.2.3 Vacuum trap: Made from 500-mL sidearm flask fitted with single-hole rubber stopper and glass tubing.

5.6.2.4 Rack: For holding 50-mL volumetric flasks in the manifold.

5.6.2.5 Column: Mega Bond Elut, Non-polar, C18 Octadecyl, 10 g/60 mL (Analytichem International Cat. No. 607H060, or equivalent).

5.7 Centrifuge apparatus.

5.7.1 Centrifuge: Capable of rotating 500-mL centrifuge bottles or 15-mL centrifuge tubes at 5,000 rpm minimum.

5.7.2 Centrifuge bottles: 500-mL, with screw-caps, to fit centrifuge.

5.7.3 Centrifuge tubes: 12- to 15-mL, with screw-caps, to fit centrifuge.

5.7.4 Funnel: Buchner, 15 cm.

5.7.4.1 Flask: Filter, for use with Buchner funnel.

5.7.4.2 Filter paper: 15 cm (Whatman #41, or equivalent).

5.8 Miscellaneous glassware.

5.8.1 Pipettes: Glass, volumetric, 1-, 5-, and 10-mL.

5.8.2 Syringes: Glass, with Luerlok tip, 0.1-, 1- and 5-mL. Needles for syringes, 2", 22-gauge.

5.8.3 Volumetric flasks: 10-, 25-, and 50-mL.

5.8.4 Scintillation vials: Glass, 20- to 50-mL, with PTFE-lined screw-caps.

5.9 Gas chromatograph: Shall have splitless or on-column simultaneous automated injection into separate capillary columns with a flame photometric detector at the end of each column, temperature program with isothermal holds, data system capable of recording simultaneous signals from the two detectors, and shall meet all of the performance specifications in Section 14.

5.9.1 GC columns: Bonded-phase fused-silica capillary.

5.9.1.1 Primary: 30 m (±3 m) long x 0.5 mm (±0.05 mm) ID DB-1, or equivalent.

5.9.1.2 Confirmatory: DB-1701, or equivalent, with same dimensions as primary column.

5.9.2 Data system: Shall collect and record GC data, store GC runs on magnetic disk or tape, process GC data, compute peak areas, store calibration data including retention times and calibration factors, identify GC peaks through retention times, compute concentrations, and generate reports.

5.9.2.1 Data acquisition: GC data shall be collected continuously throughout the analysis and stored on a mass storage device.

5.9.2.2 Calibration factors and calibration curves: The data system shall be used to record and maintain lists of calibration factors and multi-point calibration curves (Section 7). Computations of relative standard deviation (coefficient of variation) are used for testing calibration linearity. Statistics on initial (Section 8.2) and ongoing (Section 13.6) performance shall be computed and maintained.

5.9.2.3 Data processing: The data system shall be used to search, locate, identify, and quantify the compounds of interest in each GC analysis. Software routines shall be employed to compute and record retention times and peak areas. Displays of chromatograms and library comparisons are required to verify results.

5.9.2.4 Flame photometric detector: Capable of detecting 11 pg of malathion under the analysis conditions given in Table 2.

6. REAGENTS AND STANDARDS

6.1 Sample preservation: Sodium thiosulfate (ACS), granular.

6.2 pH adjustment.

6.2.1 Sodium hydroxide: Reagent grade.

6.2.1.1 Concentrated solution (10N): Dissolve 40 g NaOH in 100-mL reagent water.

6.2.1.2 Dilute solution (0.1M): Dissolve 4 g NaOH in 1 L of reagent water.

6.2.2 Sulfuric acid (1+1): Reagent grade, 6N in reagent water. Slowly add 50 mL H_2SO_4 (specific gravity 1.84) to 50 mL reagent water.

6.2.3 Potassium hydroxide: 37% (w/v); dissolve 37 g KOH in 100 mL reagent water.

6.3 Solution drying and back-extraction.

6.3.1 Sodium sulfate: Reagent grade, granular anhydrous (Baker 3375, or equivalent), rinsed with methylene chloride (20 mL/g), baked at 450°C for a minimum of 1 hour, cooled in a desiccator, and stored in a pre-cleaned glass bottle with screw-cap which prevents moisture from entering.

6.3.2 Sodium sulfate solution: 2% (w/v) in reagent water, pH adjusted to 8.5 to 9.0 with KOH or H_2SO_4.

6.4 Solvents: Methylene chloride, hexane, acetone, acetonitrile, isooctane, and methanol; pesticide-quality; lot-certified to be free of interferences.

6.5 GPC calibration solution: Solution containing 300 mg/mL corn oil, 15 mg/mL bis(2-ethylhexyl)phthalate, 1.4 mg/mL pentachlorophenol, 0.1 mg/mL perylene, and 0.5 mg/mL sulfur.

6.6 Sample cleanup.

6.6.1 Solid-phase extraction.

6.6.1.1 SPE cartridge calibration solution: 2,4,6-trichlorophenol, 0.1 µg/mL in acetone.

6.6.1.2 SPE elution solvent: methylene chloride:acetonitrile:hexane (50:3:47).

6.7 Reagent water: Water in which the compounds of interest and interfering compounds are not detected by this method.

6.8 High-solids reference matrix: Playground sand or similar material in which the compounds of interest and interfering compounds are not detected by this method. May be prepared by extraction with methylene chloride and/or baking at 450°C for 4 hours minimum.

6.9 Standard solutions: Purchased as solutions or mixtures with certification to their purity, concentration, and authenticity, or prepared from materials of known purity and composition. If compound purity is 96% or greater, the weight may be used without correction to compute the concentration of the standard. When not being used, standards are stored in the dark at -20 to -10°C in screw-capped vials with PTFE-lined lids. A mark is placed on the vial at the level of the solution so that solvent evaporation loss can be detected. The vials are brought to room temperature prior to use. Any precipitate is redissolved and solvent is added if solvent loss has occurred.

6.10 Preparation of stock solutions: Prepare in isooctane per the steps below. Observe the safety precautions in Section 4.

6.10.1 Dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 10 mg of malathion in a 10-mL ground-glass stoppered volumetric flask and fill to the mark with isooctane. After the malathion is completely dissolved, transfer the solution to a 15-mL vial with PTFE-lined cap.

6.10.2 Stock solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards.

6.10.3 Stock standard solutions shall be replaced after 6 months, or sooner if comparison with quality control check standards indicates a change in concentration.

6.11 Secondary mixtures: Using stock solutions (Section 6.10), prepare mixtures at the levels shown in Table 3 for calibration and calibration verification (Sections 7.3 and 13.5), for initial and ongoing precision and recovery (Sections 8.2 and 13.6), and for spiking into the sample matrix (Section 8.4).

6.12 Surrogate spiking solution: Prepare tributyl phosphate and triphenyl phosphate each at a concentration of 2 µg/mL in acetone.

6.13 Stability of solutions: All standard solutions (Sections 6.9 to 6.12) shall be analyzed within 48 hours of preparation and on a monthly basis thereafter for signs of degradation. Standards will remain acceptable if the peak area remains within ±15% of the area obtained in the initial analysis of the standard.

7. SETUP AND CALIBRATION

7.1 Configure the GC system as given in Section 5.9 and establish the operating conditions in Table 2.

7.2 Attainment of method detection limit (MDL): Determine that each column/detector system meets the MDL's in Table 2.

7.3 Calibration.

7.3.1 Injection of calibration solutions.

7.3.1.1 Compounds with calibration data in Table 3: The compounds in each calibration group in Table 3 were chosen so that each compound would be separated from the others by approximately 1 minute on the primary column. The concentrations were chosen to bracket the working range of the FPD. However, because the response of some models of FPD are greater than others, it may be necessary to inject a larger volume of calibration solution for these detectors.

7.3.1.2 Compounds without calibration data in Table 3: Prepare calibration standards at a minimum of three concentration levels. One of these concentrations should be near, but above, the MDL (Table 2) and the other concentrations should define the working range of the detectors.

7.3.1.3 Set the automatic injector to inject a constant volume in the range of 0.5 to 5.0 µL of each calibration solution into the GC column/detector pairs, beginning with the lowest level mixture and proceeding to the highest. For each compound, compute and store, as a function of the concentration injected, the retention time and peak area on each column/detector system (primary and confirmatory).

7.3.2 Retention time: The polar nature of some analytes causes the retention time to decrease as the quantity injected increases. To compensate this effect, the retention time for compound identification is correlated with the analyte level.

7.3.2.1 If the difference between the maximum and minimum retention times for any compound is less than 5 seconds over the calibration range, the retention time for that compound can be considered constant and an average retention time may be used for compound identification.

7.3.2.2 Retention-time calibration curve (retention time vs. amount): If the retention time for a compound in the lowest level standard is more than 5 seconds greater than the retention time for the compound in the highest level standard, a retention-time calibration curve shall be used for identification of that compound.

7.3.3 Calibration factor (ratio of area to amount injected).

7.3.3.1 Compute the coefficient of variation (relative standard deviation) of the calibration factor over the calibration range for each compound on each column/detector system.

7.3.3.2 Linearity: If the calibration factor for any compound is constant (C_v <20%) over the calibration range, an average calibration factor may be used for that compound; otherwise, the complete calibration curve (area vs. amount) for that compound shall be used.

7.**4** Combined QC standards: To preclude periodic analysis of all of the individual calibration groups of compounds (Table 3), the GC systems are calibrated with combined solutions as a final step. Not all of the compounds in these standards will be separated by the GC columns used in this method. Retention times and calibration factors are verified for the compounds that are resolved, and calibration factors are obtained for the unresolved peaks. These combined QC standards are prepared at the level the mid-range calibration standard (Table 3).

7.4.1 Analyze the combined QC standard on each column/detector pair.

7.4.1.1 For those compounds that exhibit a single, resolved GC peak, the retention time shall be within ±5 seconds of the retention time of the peak in the medium level calibration standard (Section 7.3.1), and the calibration factor using the primary column shall be within ±20% of the calibration factor in the medium level standard (Table 3).

7.4.1.2 For the peaks containing two or more compounds, compute and store the retention times at the peak maxima on both columns (primary and confirmatory), and also compute and store the calibration factors on both columns. These results will be used for calibration verification (Section 13.2 and 13.5) and for precision and recovery studies (Sections 8.2 and 13.6).

8. QUALITY CONTROL

8.1 Each laboratory that uses this method is required to operate a formal quality control program (Reference 6). The minimum requirements of this program consist of an initial demonstration of laboratory capability, an ongoing analysis of standards and blanks as tests of continued performance, and analysis of spiked samples to assess accuracy. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method. If the method is to be applied routinely to samples containing high solids with very little moisture (e.g., soils, compost), the high-solids reference matrix (Section 6.8) is substituted for the reagent water (Section 6.8) in all performance tests, and the high-solids method (Section 10) is used for these tests.

8.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 The analyst is permitted to modify this method to improve separations or lower the costs of measurements, provided all performance requirements are met. Each time a modification is made to the method or a cleanup procedure is added, the analyst is required to repeat the procedure in Section 8.2 to demonstrate method performance. If detection limits will be affected by the modification, the analyst is required to repeat the demonstration of detection limits (Section 7.2).

8.1.3 The laboratory shall spike all samples with at least one surrogate compound to monitor method performance. This test is described in Section 8.3. When results of these spikes indicate atypical method performance for samples, the samples are diluted to bring method performance within acceptable limits (Section 16).

8.1.4 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and the analysis of the combined QC standard (Section 7.4) that the analysis system is in control. These procedures are described in Sections 13.1, 13.5, and 13.6.

8.1.5 The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Section 8.4.

8.1.6 Analyses of blanks are required to demonstrate freedom from contamination. The procedures and criteria for analysis of a blank are described in Section 8.5.

8.1.7 Other analytes may be determined by this method. The procedure for establishing a preliminary quality control limit for a new analyte is given in Section 8.6.

8.2 Initial precision and recovery: To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations.

8.2.1 For analysis of samples containing low solids (aqueous samples), extract, concentrate, and analyze one set of four 1-L aliquots of reagent water spiked with the combined QC standard (Section 7.4) according to the procedure in Section 10. Alternatively, sets of four replicates of the individual calibration groups (Section 7.3) may be used. For samples containing high solids, sets of four 30-g aliquots of the high-solids reference matrix are used.

8.2.2 Using results of the set of four analyses, compute the average percent recovery (X) and the coefficient of variation (C_v) of percent recovery (s) for each compound.

8.2.3 For each compound, compare s and X with the corresponding limits for initial precision and accuracy in Table 4. For coeluting compounds, use the coeluted compound with the least restrictive specification (largest C_v and widest range). If s and X for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any individual s exceeds the precision limit or any individual X falls outside the range for recovery, system performance is unacceptable for that compound. In this case, correct the problem and repeat the test.

8.3 The laboratory shall spike all samples with at least one surrogate compound to assess method performance on the sample matrix.

8.3.1 Analyze each sample according to the method beginning in Section 10.

8.3.2 Compute the percent recovery (P) of the surrogate compounds.

8.3.3 The recovery of the surrogate compounds shall be within the limits of 40 to 120%. If the recovery of any surrogate falls outside of these limits, method performance is unacceptable for that sample, and the sample is complex. Water samples are diluted, and smaller amounts of soils, sludges, and sediments are reanalyzed per Section 16.

8.4 Method accuracy: The laboratory shall spike (matrix spike) at least 10% of the samples from a given site type (e.g., influent to treatment, treated effluent, produced water, river sediment). If only one sample from a given site type is analyzed, a separate aliquot of that sample shall be spiked.

8.4.1 The concentration of the matrix spike shall be determined as follows.

8.4.1.1 If, as in compliance monitoring, the concentration of a specific analyte in the sample is being checked against a regulatory concentration limit, the matrix spike shall be at that limit or at 1 to 5 times higher than the background concentration determined in Section 8.4.2, whichever

concentration is larger.

8.4.1.2 If the concentration of an analyte in the sample is not being checked against a limit specific to that analyte, the matrix spike shall be at the concentration of the combined QC standard (Section 7.4) or at 1 to 5 times higher than the background concentration, whichever concentration is larger.

8.4.1.3 If it is impractical to determine the background concentration before spiking (e.g., maximum holding times will be exceeded), the matrix spike concentration shall be the regulatory concentration limit, if any; otherwise, the larger of either 5 times the expected background concentration or at the concentration of the combined QC standard (Section 7.4).

8.4.2 Analyze one sample aliquot to determine the background concentration (B) of each analyte. If necessary, prepare a standard solution appropriate to produce a level in the sample 1 to 5 times the background concentration. Spike a second sample aliquot with the standard solution and analyze it to determine the concentration after spiking (A) of each analyte. Calculate the percent recovery (P) of each analyte:

Equation 1

$$P = \frac{100\ (A-B)}{T}$$

where

T = *True value of the spike*

8.4.3 Compare the percent recovery for each analyte with the corresponding QC acceptance criteria in Table 4. If any analyte fails the acceptance criteria for recovery, the sample is complex and must be diluted and reanalyzed per Section 16.

8.4.4 As part of the QC program for the laboratory, method accuracy for samples shall be assessed and records shall be maintained. After the analysis of five spiked samples of a given matrix type (water, soil, sludge, sediment) in which the analytes pass the tests in Section 8.4, compute the average percent recovery (P) and the standard deviation of the percent recovery (s_p) for each compound (or coeluting compound group). Express the accuracy assessment as a percent recovery interval from $P-2s_p$ to $P + 2s_p$ for each matrix. For example, if P = 90% and s_p = 10% for five analyses of compost, the accuracy interval is expressed as 70 to 110%. Update the accuracy assessment for each compound in each matrix on a regular basis (e.g., after each five to ten new accuracy measurements).

8.5 Blanks: Reagent water and high-solids reference matrix blanks are analyzed to demonstrate freedom from contamination.

8.5.1 Extract and concentrate a 1-L reagent water blank or a 30-g high-solids reference matrix blank with each sample batch (samples started through the extraction process on the same 8-hour shift, to a maximum of 20 samples). Analyze the blank
immediately after analysis of the combined QC standard (Section 13.6) to demonstrate freedom from contamination.

8.5.2 If any of the compounds of interest (Table 1) or any potentially interfering compound is found in an aqueous blank at greater than 0.05 µg/L, or in a high-solids reference matrix blank at greater than 1 µg/kg (assuming the same calibration factor as malathion for compounds not listed in Table 1), analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.

8.6 Other analytes may be determined by this method. To establish a quality control limit for an analyte, determine the precision and accuracy by analyzing four replicates of the analyte along with the combined QC standard per the procedure in Section 8.2. If the analyte coelutes with an analyte in the QC standard, prepare a new QC standard without the coeluting component(s). Compute the average percent recovery (A) and the standard deviation of percent recovery (s_n) for the analyte, and measure the recovery and standard deviation of recovery for the other analytes. The data for the new analyte is assumed to be valid if the precision and recovery specifications for the other analytes are met; otherwise, the analytical problem is corrected and the test is repeated. Establish a preliminary quality control limit of $A \pm 2s_n$ for the new analyte and add the limit to Table 4.

8.7 The specifications contained in this method can be met if the apparatus used is calibrated properly, then maintained in a calibrated state. The standards used for calibration (Section 7), calibration verification (Section 13.5), and for initial (Section 8.2) and ongoing (Section 13.6) precision and recovery should be identical, so that the most precise results will be obtained. The GC instruments will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of the analytes given in this method.

8.8 Depending on specific program requirements, field replicates and field spikes of the analytes of interest into samples may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

9.1 Collect samples in glass containers following conventional sampling practices (Reference 7), except that the bottle shall not be prerinsed with sample before collection. Aqueous samples which flow freely are collected in refrigerated bottles using automatic sampling equipment. Solid samples are collected as grab samples using wide-mouth jars.

9.2 Maintain samples at 0 to 4°C from the time of collection until extraction. If the samples will not be extracted within 72 hours of collection, adjust the sample to a pH of 5.0 to 9.0 using sodium hydroxide or sulfuric acid solution. Record the volume of acid or base used. If residual chlorine is present in aqueous samples, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine (Reference 8).

9.3 Begin sample extraction within 7 days of collection, and analyze all extracts within 40 days of extraction.

10. SAMPLE EXTRACTION AND CONCENTRATION

Samples containing 1% solids or less are extracted directly using continuous liquid-liquid extraction techniques (Section 10.2.1). Samples containing 1 to 30% solids are diluted to the 1% level with reagent water (Section 10.2.2) and extracted using continuous liquid-liquid extraction techniques. Samples containing greater than 30% solids are extracted using ultrasonic techniques (Section 10.2.5). For highly water soluble compounds such as methamidophos, samples are salted and extracted using a chloroform:acetone azeotrope (Section 10.2.6). Figure 1 outlines the extraction and concentration steps.

10.1 Determination of percent solids.

10.1.1 Weigh 5 to 10 g of sample into a tared beaker. Record the weight to three significant figures.

10.1.2 Dry overnight (12 hours minimum) at 110°C (±5°C), and cool in a desiccator.

10.1.3 Determine percent solids as follows:

Equation 2

$$\%\ solids = \frac{weight\ of\ dry\ sample}{weight\ of\ wet\ sample} \times 100$$

10.2 Preparation of samples for extraction.

10.2.1 Aqueous samples containing 1% solids or less: Extract the sample directly using continuous liquid-liquid extraction techniques.

10.2.1.1 Measure 1 L (±0.01 L) of sample into a clean 1.5- to 2-L beaker.

10.2.1.2 Spike 0.5 mL of the surrogate spiking solution (Section 6.12) into the sample aliquot. Proceed to preparation of the QC aliquots for low-solids samples (Section 10.2.3).

10.2.2 Samples containing 1 to 30% solids.

10.2.2.1 Mix sample thoroughly.

10.2.2.2 Using the percent solids found in Section 10.1.3, determine the weight of sample required to produce 1-L of solution containing 1% solids as follows:

Equation 3

$$sample\ weight = \frac{1000\ g}{\%\ solids}$$

10.2.2.3 Place the weight determined in Section 10.2.2.2 in a clean 1.5- to 2.0-L beaker. Discard all sticks, rocks, leaves, and other foreign material prior to weighing.

10.2.2.4 Bring the volume of the sample aliquot(s) to 100- to 200-mL with reagent water.

10.2.2.5 Spike 0.5 mL of the appropriate surrogate spiking solution (Section 6.12) into each sample aliquot.

10.2.2.6 Using a clean metal spatula, break any solid portions of the sample into small pieces.

10.2.2.7 Place the ¾" horn on the ultrasonic probe approximately ½" below the surface of each sample aliquot and pulse at 50% for 3 minutes at full power. If necessary, remove the probe from the solution and break any large pieces using the metal spatula or a stirring rod and repeat the sonication. Clean the probe with methylene chloride:acetone (1:1) between samples to preclude cross-contamination.

10.2.2.8 Bring the sample volume to 1.0 L (±0.1 L) with reagent water.

10.2.3 Preparation of QC aliquots for samples containing low solids (less than 30%).

10.2.3.1 For each sample or sample batch (to a maximum of 20) to be extracted at the same time, place two 1.0-L (±0.01 L) aliquots of reagent water in clean 1.5- to 2.0-L beakers.

10.2.3.2 Blank: Spike 0.5 mL of the pesticide surrogate spiking solution (Section 6.12) into one reagent water aliquot.

10.2.3.3 Spike the combined QC standard (Section 7.4) into a reagent water aliquot.

10.2.3.4 If a matrix spike is required, prepare an aliquot at the concentrations specified in Section 8.4.

10.2.4 Stir and equilibrate all sample and QC solutions for 1 to 2 hours. Extract the samples and QC aliquots per Section 10.3.

10.2.5 Samples containing 30% solids or greater.

10.2.5.1 Mix the sample thoroughly.

10.2.5.2 Weigh 30 g (±0.3 g) into a clean 400- to 500-mL beaker. Discard all sticks, rocks, leaves, and other foreign material prior to weighing.

10.2.5.3 Spike 0.5 mL of the surrogate spiking solution (Section 6.12) into the sample aliquot.

10.2.5.4 QC aliquots: For each sample or sample batch (to a maximum of 20) to be extracted at the same time, place two 30-g (±0.3 g) aliquots of the high-solids reference matrix in clean 400- to 500-mL beakers.

10.2.5.5 Blank: Spike 0.5 mL of the surrogate spiking solution (Section 6.12) into an aliquot of the high-solids reference matrix.

10.2.5.6 Spike the combined QC standard (Section 7.4) into a high-solids reference matrix aliquot. Extract the high-solids samples per Section 10.4.

10.2.6 Samples containing methamidophos and other highly water-soluble compounds: Prepare samples containing less than 30% solids per Sections 10.2.6.1 to 10.2.6.5; prepare samples containing greater than 30% solids per Section 10.2.5.

10.2.6.1 Interferences: If interferences are expected, aqueous samples can be pre-extracted with methylene chloride to remove these interferences. This extract can be used for determination of insoluble or slightly soluble compounds and the surrogates. Methamidophos is only sightly soluble in methylene chloride and will not be in this extract unless carried by polar species in the sample matrix. If compounds other than methamidophos are not to be determined, the methylene chloride extract can be discarded.

10.2.6.2 Determine the percent solids and prepare a 1-L sample aliquot and the QC aliquots per Sections 10.2.2 and 10.2.4 or 10.2.3 and 10.2.4, except do not spike the surrogate into the sample aliquot if the methylene chloride extract will be discarded (Section 10.2.6.1).

10.2.6.3 Extract the aliquots per Section 10.3 using methylene chloride to remove interferences.

10.2.6.4 After extraction, remove the water and methylene chloride from the extractor. Decant the aqueous portion into a beaker and combine the remaining methylene chloride with the extract in the distilling flask. If the methylene chloride extract is to be used for determination of other analytes and the surrogate, proceed to Section 10.5 with that extract.

10.2.6.5 Saturate the water sample with sodium chloride. Approximately 350 g will be required.

10.2.6.6 If the methylene chloride extract was discarded, spike the surrogates into the sample aliquot.

10.2.6.7 Extract the sample per Section 10.3 except use a chloroform:acetone azeotrope (2:1 v/v or 4:1 w/w) for the extraction.

NOTE: As a result of the increased density of the water caused by saturation with salt, the sample may sink to where the water enters the siphon tube of the continuous extractor. To prevent this from occurring, use a smaller volume of water (e.g., 800 mL) in the extractor. Correct for this adjustment in the calculation of the concentration of the pollutants in the extract (Section 15).

10.3 Continuous extraction of low-solids (aqueous) samples: Place 100 to 150 mL methylene chloride in each continuous extractor and 200 to 300 mL in each distilling flask.

10.3.1 Pour the sample(s), blank, and standard aliquots into the extractors. Rinse the glass containers with 50 to 100 mL methylene chloride and add to the respective extractors. Include all solids in the extraction process.

10.3.2 Extraction: Adjust the pH of the waters in the extractors to 5 to 9 with NaOH or H_2SO_4 while monitoring with a pH meter.

NOTE: Caution: Some samples require acidification in a hood because of the potential for generating hydrogen sulfide.

10.3.3 Begin the extraction by heating the flask until the methylene chloride is boiling. When properly adjusted, one to two drops of methylene chloride per second will fall from the condenser tip into the water. Test and adjust the pH of the waters during the first 1 to 2 hours of extraction. Extract for 18 to 24 hours.

10.3.4 Remove the distilling flask, estimate and record the volume of extract (to the nearest 100 mL), and pour the contents through a prerinsed drying column containing 7 to 10 cm of anhydrous sodium sulfate. Rinse the distilling flask with 30 to 50 mL of methylene chloride and pour through the drying column. For extracts to be cleaned up using GPC, collect the solution in a 500-mL K-D evaporator flask equipped with a 10-mL concentrator tube. Seal, label, and concentrate per Sections 10.5 to 10.6.

10.4 Ultrasonic extraction of high-solids samples: Procedures are provided for extraction of non-municipal sludge (Section 10.4.1) and municipal sludge samples (Section 10.4.2).

10.4.1 Ultrasonic extraction of non-municipal sludge high-solids aliquots.

10.4.1.1 Add 60 to 70 g of powdered sodium sulfate to the sample and QC aliquots. Mix each aliquot thoroughly. Some wet sludge samples may require more than 70 g for complete removal of water. All water must be removed prior to addition of organic solvent so that the extraction process is efficient.

10.4.1.2 Add 100 mL (±10 mL) of acetone:methylene chloride (1:1) to each of the aliquots and mix thoroughly.

10.4.1.3 Place the ¾" horn on the ultrasonic probe approximately ½" below the surface of the solvent but above the solids layer and pulse at 50% for 3 minutes at full power. If necessary, remove the probe from the solution and break any large pieces using a metal spatula or a stirring rod and repeat the sonication.

10.4.1.4 Decant the pesticide extracts through a prerinsed drying column containing 7 to 10 cm anhydrous sodium sulfate into 500- to 1000-mL graduated cylinders.

10.4.1.5 Repeat the extraction steps (Sections 10.4.1.2 to 10.4.1.4) twice more for each sample and QC aliquot. On the final extraction, swirl the sample or QC aliquot, pour into its respective drying column, and rinse with acetone:methylene chloride. Record the total extract volume. If necessary, transfer the extract to a centrifuge tube and centrifuge for 10 minutes to settle fine particles.

10.4.2 Ultrasonic extraction of high solids municipal sludge aliquots.

10.4.2.1 Add 100 mL (±10 mL) of acetonitrile to each of the aliquots and mix thoroughly.

10.4.2.2 Place the ¾" horn on the ultrasonic probe approximately ½" below the surface of the solvent but above the solids layer and pulse at 50% for 3 minutes at full power. If necessary, remove the probe from the solution and break any large pieces using a metal spatula or a stirring rod and repeat the sonication.

10.4.2.3 Decant the extract through filter paper into a 1000- to 2000-mL separatory funnel.

10.4.2.4 Repeat the extraction and filtration steps (Sections 10.4.2.1 to 10.4.2.3) using a second 100 mL (±10 mL) of acetonitrile.

10.4.2.5 Repeat the extraction step (Sections 10.4.2.1 and 10.4.2.2) using 100 mL (±10 mL) of methylene chloride. On this final extraction,

swirl the sample or QC aliquot, pour into its respective filter paper, and rinse with methylene chloride. Record the total extract volume.

10.4.2.6 For each extract, prepare 1.5 to 2 L of reagent water containing 2% sodium sulfate. Adjust the pH of the water to 6.0 to 9.0 with NaOH or H_2SO_4.

10.4.2.7 Back-extract each extract three times sequentially with 500 mL of the aqueous sodium sulfate solution, returning the bottom (organic) layer to the separatory funnel the first two times while discarding the top (aqueous) layer. On the final back-extraction, filter each pesticide extract through a prerinsed drying column containing 7 to 10 cm anhydrous sodium sulfate into a 500- to 1000-mL graduated cylinder. Record the final extract volume.

10.4.3 For extracts to be cleaned up using GPC, filter these extracts through Whatman #41 paper into a 500-mL K-D evaporator flask equipped with a 10-mL concentrator tube. Rinse the graduated cylinder or centrifuge tube with 30 to 50 mL of methylene chloride and pour through filter to complete the transfer. Seal and label the K-D flask. Concentrate these fractions per Sections 10.5 through 10.8.

10.5 Macro concentration.

10.5.1 Concentrate the extracts in separate 500-mL K-D flasks equipped with 10-mL concentrator tubes. Add one to two clean boiling chips to the flask and attach a three-ball macro Snyder column. Prewet the column by adding approximately 1 mL of methylene chloride through the top. Place the K-D apparatus in a hot water bath so that the entire lower rounded surface of the flask is bathed with steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

10.5.2 When the liquid has reached an apparent volume of 1 mL, remove the K-D apparatus from the bath and allow the solvent to drain and cool for at least 10 minutes.

10.5.3 If the extract is to be cleaned up using GPC, remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation. Adjust the final volume to 10 mL and proceed to GPC cleanup in Section 11.

10.6 Hexane exchange: Extracts that have been cleaned up are exchanged into hexane.

10.6.1 Remove the Snyder column, add approximately 50 mL of hexane and a clean boiling chip, and reattach the Snyder column. Concentrate the extract as in Section 10.5 except use hexane to prewet the column. The elapsed time of the concentration should be 5 to 10 minutes.

10.6.2 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane. Adjust the final volume of extracts that have not been cleaned up by GPC to 10 mL and those that have been cleaned up by GPC to 5 mL (the difference accounts for the 50% loss in the GPC cleanup).

11. CLEANUP AND SEPARATION

11.1 Cleanup procedures may not be necessary for relatively clean samples (treated effluents, ground water, drinking water). If particular circumstances require the use of a cleanup procedure, the analyst may use any or all of the procedures below or any other appropriate procedure. However, the analyst shall first repeat the tests in Section 8.2 to demonstrate that the requirements of Section 8.2 can be met using the cleanup procedure(s) as an integral part of the method. Figure 1 outlines the cleanup steps.

11.1.1 Gel permeation chromatography (Section 11.2) removes many high molecular weight interferents that cause GC column performance to degrade. It is used for all soil and sediment extracts and may be used for water extracts that are expected to contain high molecular weight organic compounds (e.g., polymeric materials, humic acids).

11.1.2 The solid-phase extraction cartridge (Section 11.3) removes polar organic compounds such as phenols. It is used for cleanup of organo-chlorine and organo-phosphate extracts.

11.2 Gel permeation chromatography (GPC).

11.2.1 Column packing.

11.2.1.1 Place 70 to 75 g of SX-3 Bio-beads in a 400- to 500-mL beaker.

11.2.1.2 Cover the beads with methylene chloride and allow to swell overnight (12 hours minimum).

11.2.1.3 Transfer the swelled beads to the column and pump solvent through the column, from bottom to top, at 4.5 to 5.5 mL/min prior to connecting the column to the detector.

11.2.1.4 After purging the column with solvent for 1 to 2 hours, adjust the column head pressure to 7 to 10 psig, and purge for 4 to 5 hours to remove air. Maintain a head pressure of 7 to 10 psig. Connect the column to the detector.

11.2.2 Column calibration.

11.2.2.1 Load 5 mL of the calibration solution (Section 6.5) into the sample loop.

11.2.2.2 Inject the calibration solution and record the signal from the detector. The elution pattern will be corn oil, bis(2-ethylhexyl) phthalate, pentachlorophenol, perylene, and sulfur.

11.2.2.3 Set the "dump time" to allow >85% removal of the corn oil and >85% collection of the phthalate.

11.2.2.4 Set the "collect time" to the peak minimum between perylene and sulfur.

11.2.2.5 Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the pentachlorophenol is greater than 85%. If calibration is not verified, the system shall be recalibrated using the calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated GPC system.

11.2.3 Extract cleanup: GPC requires that the column not be overloaded. The column specified in this method is designed to handle a maximum of 0.5 g of high molecular weight material in a 5-mL extract. If the extract is known or expected to contain more than 0.5 g, the extract is split into fractions for GPC and the fractions are combined after elution from the column. The solids content of the extract may be obtained gravimetrically by evaporating the solvent from a 50-µL aliquot.

11.2.3.1 Filter the extract or load through the filter holder to remove particulates. Load the 5.0 mL extract onto the column.

11.2.3.2 Elute the extract using the calibration data determined in Section 11.2.2. Collect the eluate in a clean 400- to 500-mL beaker.

11.2.3.3 Rinse the sample loading tube thoroughly with methylene chloride between extracts to prepare for the next sample.

11.2.3.4 If a particularly dirty extract is encountered, a 5.0-mL methylene chloride blank shall be run through the system to check for carry-over.

11.2.3.5 Concentrate the extract and exchange into hexane per Sections 10.5 and 10.6. Adjust the final volume to 5.0 mL.

11.3 Solid-phase extraction (SPE).

11.3.1 Setup.

11.3.1.1 Attach the Vac-elute manifold to a water aspirator or vacuum pump with the trap and gauge installed between the manifold and vacuum source.

11.3.1.2 Place the SPE cartridges in the manifold, turn on the vacuum source, and adjust the vacuum to 5 to 10 psia.

11.3.2 Cartridge washing: Pre-elute each cartridge prior to use sequentially with 10-mL portions each of hexane, methanol, and water using vacuum for 30 seconds after each eluant. Follow this pre-elution with 1 mL methylene chloride and three 10-mL portions of the elution solvent (Section 6.6.2.2) using vacuum for 5 minutes after each eluant. Tap the cartridge lightly while under vacuum to dry between eluants. The three portions of elution solvent may be collected and used as a blank if desired. Finally, elute the cartridge with 10 mL each of methanol and water, using the vacuum for 30 seconds after each eluant.

11.3.3 Cartridge certification: Each cartridge lot must be certified to ensure recovery of the compounds of interest and removal of 2,4,6-trichlorophenol.

11.3.3.1 To make the test mixture, add the trichlorophenol solution (Section 6.6.2.1) to the combined calibration standard (Section 7.4). Elute the mixture using the procedure in Section 11.3.4.

11.3.3.2 Concentrate the eluant to 1.0 mL and inject 1.0 µL of the concentrated eluant into the GC using the procedure in Section 13. The recovery of all analytes (including the unresolved GC peaks) shall be within the ranges for recovery specified in Table 4, and the peak for trichlorophenol shall not be detectable; otherwise the SPE cartridge is not performing properly and the cartridge lot shall be rejected.

11.3.4 Extract cleanup.

11.3.4.1 After cartridge washing (Section 11.3.2), release the vacuum and place the rack containing the 50 mL volumetric flasks (Section 5.6.2.4) in the vacuum manifold. Reestablish the vacuum at 5 to 10 psia.

11.3.4.2 Using a pipette or a 1-mL syringe, transfer 1.0 mL of extract to the SPE cartridge. Apply vacuum for five minutes to dry the cartridge. Tap gently to aid in drying.

11.3.4.3 Elute each cartridge into its volumetric flask sequentially with three 10-mL portions of the elution solvent (Section 6.6.2.2), using vacuum for five minutes after each portion. Collect the eluants in the 50-mL volumetric flasks.

11.3.4.4 Release the vacuum and remove the 50-mL volumetric flasks.

11.3.4.5 Concentrate the eluted extracts to approximately 0.5 mL using the nitrogen blow-down apparatus. Adjust the final volume to 1.0 mL (per Section 10.6) and proceed to Section 13 for GC analysis.

12. GAS CHROMATOGRAPHY

Table 2 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are the retention times and estimated detection limits that can be achieved under these conditions. Examples of the separations achieved by the primary and confirmatory columns are shown in Figure 2.

12.1 Calibrate the system as described in Section 7.

12.2 Set the autosampler to inject the same volume that was chosen for calibration (Section 7.3.1.3) for all standards and extracts of blanks and samples.

12.3 Set the data system or GC control to start the temperature program upon sample injection, and begin data collection after the solvent peak elutes. Set the data system to stop data collection after the last analyte is expected to elute and to return the column to the initial temperature.

13. SYSTEM AND LABORATORY PERFORMANCE

13.1 At the beginning of each 8-hour shift during which analyses are performed, GC system performance and calibration are verified for all pollutants and surrogates on both column/detector systems. For these tests, analysis of the combined QC standard (Section 7.4) shall be used to verify all performance criteria. Adjustment and/or recalibration (per Section 7) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, and precision and recovery standards be analyzed.

13.2 Retention times: The absolute retention times of the peak maxima shall be within ±10 seconds of the retention times in the initial calibration (Section 7.4.1).

13.3 GC resolution: Resolution is acceptable if the valley height between two peaks (as measured from the baseline) is less than 10% of the taller of the two peaks.

13.3.1 Primary column (DB-1): Malathion and ethyl parathion.

13.3.2 Confirmatory column (DB-1701): Terbufos and diazinon.

13.4 Calibration verification: Calibration is verified for the combined QC standard only.

13.4.1 Inject the combined QC standard (Section 7.4).

13.4.2 Compute the percent recovery of each compound or coeluting compounds, based on the calibration data (Section 7.4).

13.4.3 For each compound or coeluted compounds, compare this calibration verification recovery with the corresponding limits for ongoing accuracy in Table 4. For coeluting compounds, use the coeluted compound with the least restrictive specification (the widest range). If the recoveries for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and

samples may begin. If, however, any recovery falls outside the calibration verification range, system performance is unacceptable for that compound. In this case, correct the problem and repeat the test, or recalibrate (Section 7).

13.5 Ongoing precision and recovery.

13.5.1 Analyze the extract of the precision and recovery standard extracted with each sample batch (Sections 10.2.3.3 and 10.2.5.7).

13.5.2 Compute the percent recovery of each analyte and coeluting compounds.

13.5.3 For each compound or coeluted compounds, compare the percent recovery with the limits for ongoing recovery in Table 4. For coeluted compounds, use the coeluted compound with the least restrictive specification (widest range). If all analytes pass, the extraction, concentration, and cleanup processes are in control and analysis of blanks and samples may proceed. If, however, any of the analytes fail, these processes are not in control. In this event, correct the problem, re-extract the sample lot, and repeat the ongoing precision and recovery test.

13.5.4 Add results which pass the specifications in Section 13.6.3 to initial and previous ongoing data. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory data quality for each analyte by calculating the average percent recovery (R) and the standard deviation of percent recovery s_r. Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if R = 95% and s_r = 5%, the accuracy is 85 to 105%.

14. QUALITATIVE DETERMINATION

14.1 Qualitative determination is accomplished by comparison of data from analysis of a sample or blank with data from analysis of the shift standard (Section 14.2), and with data stored in the retention-time and calibration libraries (Sections 7.3.2 and 7.3.3.2). Identification is confirmed when retention time and amounts agree per the criteria below.

14.2 For each compound on each column/detector system, establish a retention-time window ±20 seconds on either side of the retention time in the calibration data (Section 7.3.2). For compounds that have a retention-time curve (Section 7.3.2.2), establish this window as the minimum -20 seconds and maximum +20 seconds.

14.2.1 Compounds not requiring a retention-time calibration curve: If a peak from the analysis of a sample or blank is within a window (as defined in Section 14.2) on the primary column/detector system, it is considered tentatively identified. A tentatively identified compound is confirmed when (1) the retention time for the compound on the confirmatory column/detector system is within the retention-time window on that system, and (2) the computed amounts (Section 16) on each system (primary and confirmatory) agree within a factor of 3.

14.2.2 Compounds requiring a retention-time calibration curve: If a peak from the analysis of a sample or blank is within a window (as defined in Section 14.2) on

the primary column/detector system, it is considered tentatively identified. A tentatively identified compound is confirmed when (1) the retention times on both systems (primary and confirmatory) are within ±30 seconds of the retention times for the computed amounts (Section 15), as determined by the retention-time calibration curve (Section 7.3.2.2), and (2) the computed amounts (Section 15) on each system (primary and confirmatory) agree within a factor of 3.

15. QUANTITATIVE DETERMINATION

15.1 Using the GC data system, compute the concentration of the analyte detected in the extract (in micrograms per milliliter) using the calibration factor or calibration curve (Section 7.3.3.2).

15.2 Liquid samples: Compute the concentration in the sample using the following equation:

Equation 4

$$C_s = 10\ \frac{(C_{ex})}{(V_s)}$$

where

C_s = *Concentration in the sample, in µg/L*
10 = *Final extract total volume, in mL*
C_{ex} = *Concentration in the extract, in µg/mL*
V_s = *Sample extracted, in L*

15.3 Solid samples: Compute the concentration in the solid phase of the sample using the following equation:

Equation 5

$$C_s = 10\ \frac{(C_{ex})}{1000\ (W_s)\ (solids)}$$

where

C_s = *Concentration in the sample, in µg/kg*
10 = *Final extract total volume, in mL*
C_{ex} = *Concentration in the extract, in µg/mL*
1000 = *Conversion factor, g to kg*
W_s = *Sample weight, in g*
solids = *Percent solids in Section 10.1.3 divided by 100*

15.4 If the concentration of any analyte exceeds the calibration range of the system, the extract is diluted by a factor of 10, and a 1-µL aliquot of the diluted extract is analyzed.

15.5 Report results for all pollutants found in all standards, blanks, and samples to three significant figures. Results for samples that have been diluted are reported at the least dilute level at which the concentration is in the calibration range.

16. ANALYSIS OF COMPLEX SAMPLES

16.1 Some samples may contain high levels (>1000 ng/L) of the compounds of interest, interfering compounds, and/or polymeric materials. Some samples may not concentrate to 10 mL (Section 10.6); others may overload the GC column and/or detector.

16.2 The analyst shall attempt to clean up all samples using GPC (Section 11.2), and the SPE cartridge (Section 11.3). If these techniques do not remove the interfering compounds, the extract is diluted by a factor of 10 and reanalyzed (Section 16.4).

16.3 Recovery of surrogates: In most samples, surrogate recoveries will be similar to those from reagent water or from the high solids reference matrix. If the surrogate recovery is outside the range specified in Section 8.3, the sample shall be re-extracted and reanalyzed. If the surrogate recovery is still outside this range, the sample is diluted by a factor of 10 and reanalyzed (Section 15.4).

16.4 Recovery of matrix spikes: In most samples, matrix spike recoveries will be similar to those from reagent water or from the high solids reference matrix. If the matrix spike recovery is outside the range specified in Table 4, the sample shall be diluted by a factor of 10, respiked, and reanalyzed. If the matrix spike recovery is still outside the range, the method may not apply to the sample being analyzed and the result may not be reported for regulatory compliance purposes.

17. METHOD PERFORMANCE

17.1 Development of this method is detailed in References 9 and 10.

References

1. "Guideline Establishing Test Procedures for the Analysis of Pollutants under the Clean Water Act; Final Rule and Interim Final Rule and Proposed Rule," 40 *CFR* Part 136.

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5. "Safety in Academic Chemistry Laboratories," American Chemical Society Committee on Chemical Safety: 1979.

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10. "Method Development and Validation, EPA Method 1618," Pesticide Center, Department of Environmental Health, Colorado State University: November 1988, January 1989, and March 1992.

Table 1. Organo-Phosphorus Pesticides Determined by Large-Bore, Fused-Silica Capillary Column Gas Chromatography with Flame Photometric Detector

EPA EGD	***Compound***	***CAS Registry***
	Acephate	30560-19-1
468	Azinphos ethyl	2642-71-9
453	Azinphos methyl	86-50-0
461	Chlorfevinphos	470-90-6
469	Chlorpyrifos	2921-88-2
443	Coumaphos	56-72-4
479	Crotoxyphos	7700-17-6
	DEF	78-48-8
471	Demeton	8065-48-3
460	Diazinon	333-41-5
	Dichlofenthion	97-17-6
450	Dichlorvos	62-73-7
455	Dicrotophos	141-66-2
449	Dimethoate	60-51-5
452	Dioxathion	78-34-2
458	Disulfoton	298-04-4
467	EPN	2104-64-5
463	Ethion	563-12-2
	Ethoprop	13194-48-4
446	Famphur	52-85-7
454	Fensulfothion	115-90-2
447	Fenthion	55-38-9
464	Hexamethylphosphoramide	680-31-9
474	Leptophos	21609-90-5
475	Malathion	121-75-5
	Merphos *	150-50-5
	Methamidophos	10265-92-6
	Methyl chlorpyrifos	5598-13-0
456	Methyl parathion	298-00-0
	Methyl trithion	953-17-3
444	Mevinphos	7786-34-7
470	Monocrotophos	6923-22-4
459	Naled	300-76-5
448	Parathion (ethyl)	56-38-2
457	Phorate	298-02-2
465	Phosmet	732-11-6
473	Phosphamidon	13171-21-6
	Ronnel	299-84-3
477	Sulfotepp	3689-24-5
	Sulprofos (Bolstar)	35400-43-2
476	TEPP	107-49-3
472	Terbufos	13071-79-9
466	Tetrachlorvinphos	22248-79-9
	Tokuthion	34643-46-4
445	Trichlorfon	52-68-6

Table 1. Organo-Phosphorus Pesticides Determined by Large-Bore, Fused-Silica Capillary Column Gas Chromatography with Flame Photometric Detector

EPA EGD	***Compound***	***CAS Registry***
	Trichloronate	327-98-0
451	Tricresylphosphate	78-30-8
462	Trimethylphosphate	512-56-1

* This analyte will convert to DEF in the very hot GC injection port.

Table 2. Gas Chromatography of Organo-Phosphorus Pesticides

		Retention Time [1] (min)		
EPA EGD	***Compound***	***DB-1***	***DB-1701***	***MDL [2] (ng/L)***
450	Dichlorvos	6.56	9.22	4
444	Mevinphos	11.85	16.20	74
	Acephate	12.60	17.40	500
445	Trichlorofon	12.69	18.85	150[3]
	Methamidophos	15.10	19.20	100
471	Demeton-A	17.70	20.57	19
	Ethoprop	18.49	21.43	7
459	Naled	18.92	23.00	18
455	Dicrotophos	19.33	26.30	81
470	Monocrotophos	19.62	29.24	85
477	Sulfotepp	20.04	23.68	6
457	Phorate	20.12	23.08	10
449	Dimethoate	20.59	29.29	27
	Demeton-B	21.40	25.52	21
452	Dioxathion	22.24	26.70	121
472	Terbufos	22.97	24.55	26
473	Phosphamidon-E	23.70	29.89	28
458	Disulfoton	23.89	27.01	32
460	Diazinon	24.03	26.10	38
	Tributyl phosphate (surr)	24.50	17.20	-
	Phosphamidon-Z	25.88	32.62	116
456	Methyl parathion	25.98	32.12	18
	Dichlorofenthion	26.11	28.66	6
	Methyl chlorpyrifos	26.29	29.53	13
	Ronnel	27.33	30.09	11
475	Malathion	28.87	33.49	11
447	Fenthion	29.14	32.16	22
448	Parathion	29.29	34.61	10
469	Chlorpyrifos	29.48	32.15	4
	Trichloronate	30.44	32.12	14
461	Chlorfevinphos	32.05	36.08	2
479	Crotoxyphos	32.65	37.58	81
	Tokuthion	33.30	37.17	2
466	Tetrachlorvinphos	33.40	37.85	12
	DEF	34.05	37.50	50
	Merphos-B	35.16	37.37	18
454	Fensulfothion	36.58	43.86	104
	Methyl trithion	36.62	40.52	10

Table 2. Gas Chromatography of Organo-Phosphorus Pesticides

EPA EGD	*Compound*	*Retention Time [1] (min)*		*MDL [2] (ng/L)*
		DB-1	*DB-1701*	
463	Ethion	37.61	41.67	13
	Sulprofos	38.10	41.74	6
446	Famphur	38.24	46.37	27
465	Phosmet	41.24	48.22	14
467	EPN	41.94	47.52	9
453	Azinphos methyl	43.33	50.26	9
474	Leptophos	44.32	47.36	14
468	Azinphos ethyl	45.55	51.88	22
	Triphenyl phosphate (surr)	47.68	40.43	-
443	Coumaphos	48.02	56.44	24

Notes:

1. Columns: 30 m long × 0.53 mm ID; DB-1: 1.5 µ; DB-1701: 1.0 µ. Conditions suggested to meet retention times shown: 110°C for 0.5 min, 110 to 250° at 3°C/min, 250°C until coumaphos elutes. Carrier gas flow rate approximately 7 mL/min.
2. 40 *CFR* Part 136, Appendix B (49 *FR* 43234).
3. Estimated. Detection limits for soils (in ng/kg) are estimated to be 30 to 100 times this level.

Table 3. Concentrations of Calibration Solutions

EPA EGD	*Compound*	*Concentration (µg/mL)*		
		Low	*Medium*	*High*
Calibration Group 1				
453	Azinphos methyl	0.1	0.5	2.0
450	Dichlorvos	0.5	2.5	10.0
458	Disulfoton	0.2	1.0	4.0
447	Fenthion	0.2	1.0	4.0
	Merphos-A	0.2	1.0	4.0
	Merphos-B	0.2	1.0	4.0
	Methyl trithion	0.5	2.5	10.0
	Ronnel	0.2	1.0	4.0
	Sulprofos	0.2	1.0	4.0
Calibration Group 2				
461	Chlorfevinphos	0.2	1.0	4.0

Table 3. Concentrations of Calibration Solutions

		Concentration (µg/mL)		
EPA EGD	***Compound***	***Low***	***Medium***	***High***
469	Chlorpyrifos	0.2	1.0	4.0
471	Demeton-A	0.2	1.0	4.0
	Demeton-B	0.2	1.0	4.0
	Dichlofenthion	0.2	1.0	4.0
449	Dimethoate	0.1	0.5	2.0
446	Famphur	0.5	2.5	10.0
474	Leptophos	0.2	1.0	4.0
456	Methyl parathion	0.2	1.0	4.0
445	Trichlorofon	0.5	2.5	10.0
451	Tricresylphosphate	1.0	5	20.0
Calibration Group 3				
468	Azinphos ethyl	0.2	1.0	4.0
479	Crotoxyphos	0.5	2.5	10.0
	DEF	0.2	1.0	4.0
454	Fensulfothion	0.5	2.5	10.0
	Methyl chlorpyrifos	0.2	1.0	4.0
444	Mevinphos	0.5	2.5	10.0
459	Naled	0.5	2.5	10.0
448	Parathion	0.2	1.0	4.0
465	Phosmet	0.5	2.5	10.0
473	Phosphamidon-E	0.5	2.5	10.0
	Phosphamidon-Z	0.5	2.5	10.0
477	Sulfotepp	0.2	1.0	4.0
472	Terbufos	0.2	1.0	4.0
Calibration Group 4				
443	Coumaphos	0.5	2.5	10.0
460	Diazinon	0.2	1.0	4.0
467	EPN	0.2	1.0	4.0
463	Ethion	0.2	1.0	4.0
	Ethoprop	0.2	1.0	4.0
475	Malathion	0.2	1.0	4.0
457	Phorate	0.2	1.0	4.0
466	Tetrachlorvinphos	0.2	1.0	4.0
	Trichloronate	0.2	1.0	4.0

For compounds listed in Table 2 that are not listed in this table, determine appropiate ranges for calibration standards.

Table 4. Acceptance Criteria for Performance Tests for Organo-Phosphorus Compounds

			Acceptance Criteria			
			Initial Precision and Accuracy (%)			
EGD No.	***Compound***	***Spike Level (ng/L)***	***s***	***x***	***Calibration Verification (%)***	***Recovery/ Ongoing Accuracy, R (%)***
	Acephate	50000	25	32-122	68-132	28-126
468	Azinphos ethyl	10	10	71-117	77-127	59-129
453	Azinphos methyl	5	10	52-112	83-119	37-127
461	Chlorfevinphos	10	11	56-132	83-114	37-151
469	Chlorpyrifos	10	10	61-112	80-119	48-125
443	Coumaphos	25	10	78-104	82-120	72-110
479	Crotoxyphos	25	46	28-116	68-136	6-138
	DEF	10	31	45-107	68-132	42-110
471	Demeton	10	23	33-101	64-123	16-118
460	Diazinon	10	10	70-110	86-114	60-120
	Dichlofenthion	10	10	75-115	80-110	65-125
450	Dichlorvos	25	18	52-106	77-103	39-119
455	Dicrotophos		not recovered		78-122	
449	Dimethoate	5	89	27-100	73-127	22-100
452	Dioxathion	--	22	59-101	79-121	49-111
458	Disulfoton	10	30	46-98	70-118	33-111
467	EPN	10	13	74-124	81-108	62-136
463	Ethion	10	11	72-134	70-118	47-149
	Ethoprop	10	14	79-103	84-108	73-109
446	Famphur	25	12	81-101	81-113	76-106
454	Fensulfothion	25	65	13-115	42-139	0-141
447	Fenthion	10	13	69-101	73-137	61-109
464	Hexamethylphos-phoramide		not recovered		70-130	
474	Leptophos	10	10	85-105	85-112	80-110
475	Malathion	10	10	75-109	82-108	66-118
	Merphos-B	10	10	68-102	72-118	59-111
	Methamidophos [1]	10000	33	66-132	70-128	63-135
	Methyl chlorpyrifos	10	10	88-108	81-114	83-113
456	Methyl parathion	10	15	72-112	89-114	61-123
	Methyl trithion	25	20	21-137	78-122	0-166
444	Mevinphos	25	23	24-100	73-135	7-107
470	Monocrotophos		not recovered		19-206	
459	Naled	25	10	0-148	77-114	0-176
448	Parathion	10	10	71-111	79-110	61-121

Table 4. Acceptance Criteria for Performance Tests for Organo-Phosphorus Compounds

			Acceptance Criteria			
			Initial Precision and Accuracy (%)			
EGD No.	***Compound***	***Spike Level (ng/L)***	***s***	***x***	***Calibration Verification (%)***	***Recovery/ Ongoing Accuracy, R (%)***
457	Phorate	10	19	54-100	70-118	43-109
465	Phosmet	25	39	44-119	61-159	25-138
473	Phosphamidon-Z	25	45	0-100	81-102	0-100
	Ronnel	10	10	79-111	78-113	71-119
477	Sulfotepp	10	10	70-120	75-115	58-132
	Sulprofos	10	10	75-100	81-118	70-100
476	TEPP		not recovered		70-130	
472	Terbufos	10	23	60-110	82-111	47-123
466	Tetrachlorvinphos	10	11	48-110	73-119	32-126
	Tokuthion	100	17	73-105	70-130	65-113
445	Trichlorofon [2]	25	42	43-195	58-142	37-201
	Trichloronate	10	10	82-102	80-113	77-107
451	Tricresylphosphate	50	10	81-101	70-130	74-114
462	Trimethylphosphate		not recovered		70-130	

Notes:

1. With salt and azeotropic extraction
2. With salt

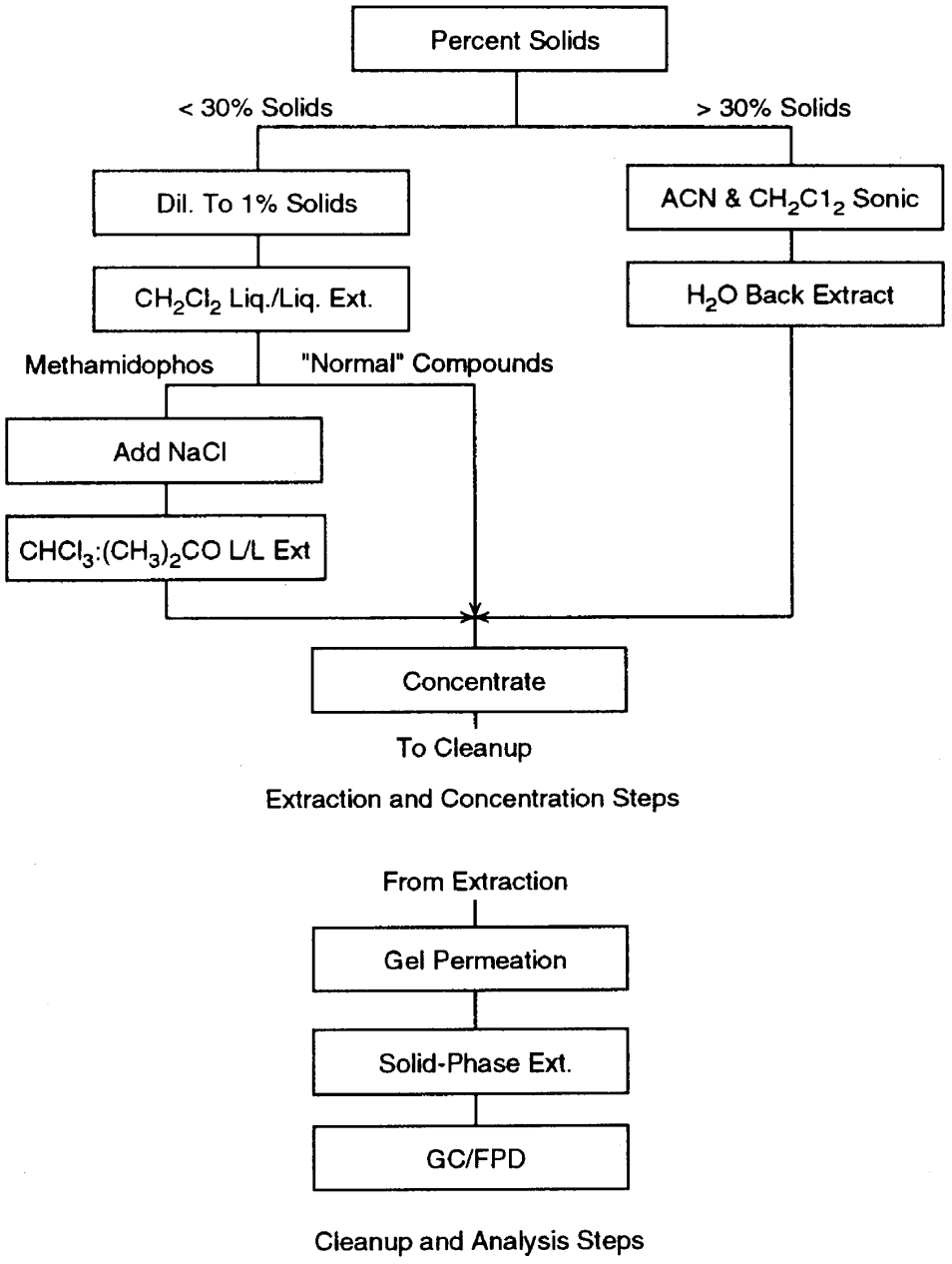

Figure 1. Extraction, Cleanup, Derivatization, and Analysis

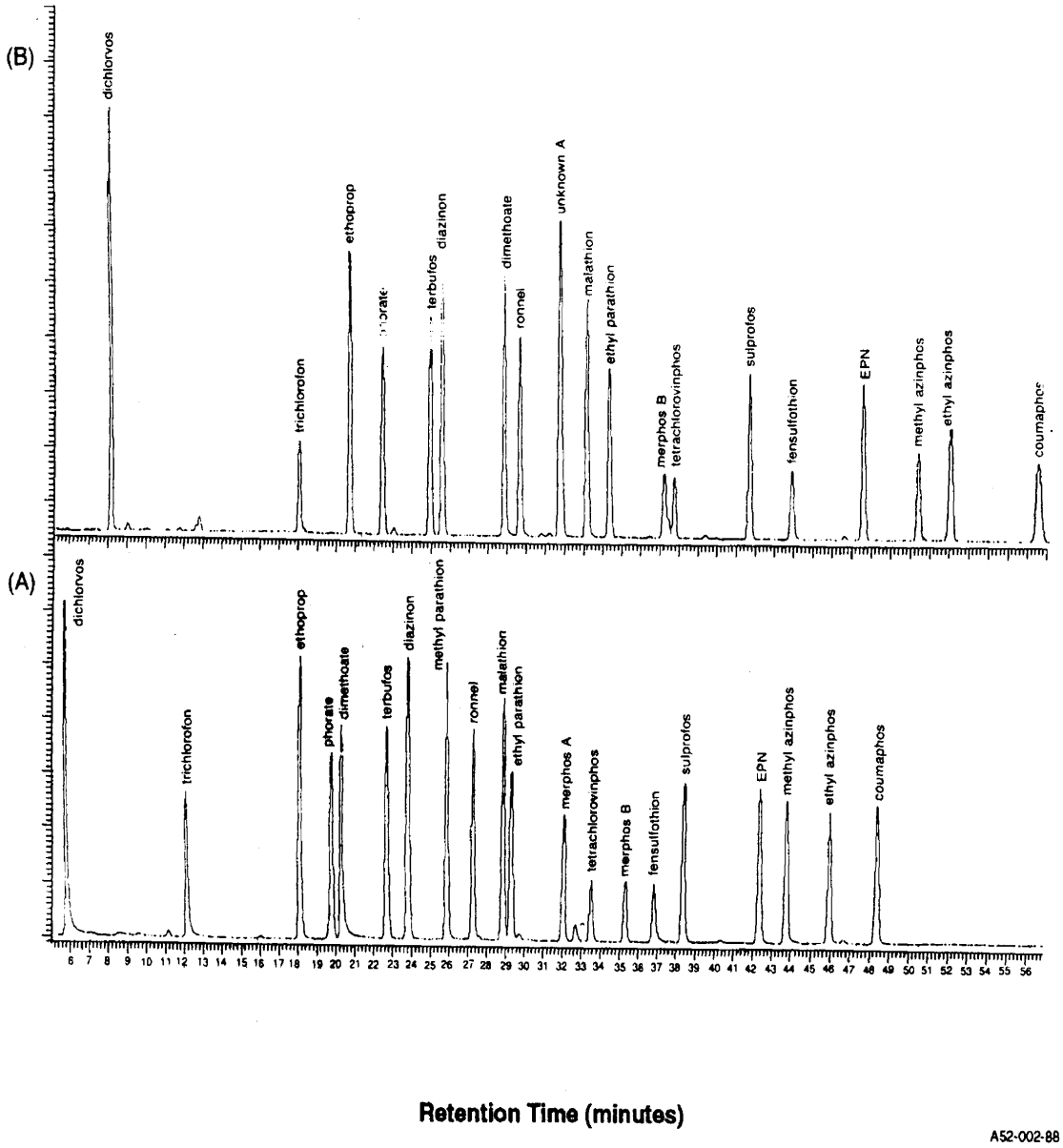

Figure 2. Gas Chromatogram of Selected Organo-Phosphorus Compounds