United States Environmental Protection Agency

Office of Water EPA 821-R-16-009

www.epa.gov December 2016

Method 608.3: Organochlorine Pesticides and PCBs by GC/HSD

This document contains the text of Method 608.3 as published in 40 CFR part 136, Appendix A; but formatted as a more user friendly stand-alone document.

Please address questions or comments to:

CWA Methods Team
Engineering and Analytical Support Branch/EAD (4303T)
Office of Science and Technology
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460
www.epa.gov/cwa-methods/forms/contact-us-about-cwa-analytical-methods

METHOD 608.3 – ORGANOCHLORINE PESTICIDES AND PCBS BY GC/HSD

1. Scope and Application

1.1 This method is for determination of organochlorine pesticides and polychlorinated biphenyls (PCBs) in industrial discharges and other environmental samples by gas chromatography (GC) combined with a halogen-specific detector (HSD; e.g., electron capture, electrolytic conductivity), as provided under 40 CFR 136.1. This revision is based on a previous protocol (Reference 1), on the revision promulgated October 26, 1984 (49 FR 43234), on an inter-laboratory method validation study (Reference 2), and on EPA Method 1656 (Reference 16). The analytes that may be qualitatively and quantitatively determined using this method and their CAS Registry numbers are listed in Table 1.

1.2 This method may be extended to determine the analytes listed in Table 2. However, extraction or gas chromatography challenges for some of these analytes may make quantitative determination difficult.

1.3 When this method is used to analyze unfamiliar samples for an analyte listed in Table 1 or Table 2, analyte identification must be supported by at least one additional qualitative technique. This method gives analytical conditions for a second GC column that can be used to confirm and quantify measurements.

Additionally, Method 625.1 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative confirmation of results for the analytes listed in Tables 1 and 2 using the extract produced by this method, and Method 1699 (Reference 18) provides high resolution GC/MS conditions for qualitative confirmation of results using the original sample. When such methods are used to confirm the identifications of the target analytes, the quantitative results should be derived from the procedure with the calibration range and sensitivity that are most appropriate for the intended application.

1.4 The large number of analytes in Tables 1 and 2 makes testing difficult if all analytes are determined simultaneously. Therefore, it is necessary to determine and perform quality control (QC) tests for the "analytes of interest" only. The analytes of interest are those required to be determined by a regulatory/control authority or in a permit, or by a client. If a list of analytes is not specified, the analytes in Table 1 must be determined, at a minimum, and QC testing must be performed for these analytes. The analytes in Table 1 and some of the analytes in Table 2 have been identified as Toxic Pollutants (40 CFR 401.15), expanded to a list of Priority Pollutants (40 CFR part 423, appendix A).

1.5 In this revision to Method 608, Chlordane has been listed as the alpha- and gamma- isomers in Table 1. Reporting may be by the individual isomers, or as the sum of the concentrations of these isomers, as requested or required by a regulatory/control authority or in a permit. Technical Chlordane is listed in Table 2 and may be used in cases where historical reporting has only been the Technical Chlordane. Toxaphene and the PCBs have been moved from Table 1 to Table 2 (Additional Analytes) to distinguish these analytes from the analytes required in quality control tests (Table 1). QC acceptance criteria for Toxaphene and the PCBs have been retained in Table 4 and may continue to be applied if desired, or if these analytes are requested or required by a regulatory/control authority or in a permit. Method 1668C (Reference 17) may be useful for determination of PCBs as individual chlorinated biphenyl congeners, and Method 1699 (Reference 18) may be useful for determination of the pesticides listed in this method. However, at the time of

writing of this revision, Methods 1668C and 1699 had not been approved for use at 40 CFR part 136.

1.6 Method detection limits (MDLs; Reference 3) for the analytes in Tables 1 and some of the analytes in Table 2 are listed in those tables. These MDLs were determined in reagent water (Reference 3). Advances in analytical technology, particularly the use of capillary (open-tubular) columns, allowed laboratories to routinely achieve MDLs for the analytes in this method that are 2 - 10 times lower than those in the version promulgated in 1984 (49 FR 43234). The MDL for an analyte in a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.6.1 EPA has promulgated this method at 40 CFR part 136 for use in wastewater compliance monitoring under the National Pollutant Discharge Elimination System (NPDES). The data reporting practices described in Section 15.6 are focused on such monitoring needs and may not be relevant to other uses of the method.

1.6.2 This method includes "reporting limits" based on EPA's "minimum level" (ML) concept (see the glossary in Section 23). Tables 1 and 2 contain MDL values and ML values for many of the analytes.

1.7 The separatory funnel and continuous liquid-liquid sample extraction and concentration steps in this method are essentially the same as those steps in Methods 606, 609, 611, and 612. Thus, a single sample may be extracted to measure the analytes included in the scope of each of these methods. Samples may also be extracted using a disk-based solid-phase extraction (SPE) procedure developed by the 3M Corporation and approved by EPA as an Alternate Test Procedure (ATP) for wastewater analyses in 1995 (Reference 20).

1.8 This method is performance-based. It may be modified to improve performance (e.g., to overcome interferences or improve the accuracy of results) provided all performance requirements are met.

1.8.1 Examples of allowed method modifications are described at 40 CFR 136.6. Other examples of allowed modifications specific to this method are described in Section 8.1.2.

1.8.2 Any modification beyond those expressly permitted at 40 CFR 136.6 or in Section 8.1.2 of this method shall be considered a major modification subject to application and approval of an alternate test procedure under 40 CFR 136.4 and 136.5.

1.8.3 For regulatory compliance, any modification must be demonstrated to produce results equivalent or superior to results produced by this method when applied to relevant wastewaters (Section 8.1.2).

1.9 This method is restricted to use by or under the supervision of analysts experienced in the use of GC/HSD. The laboratory must demonstrate the ability to generate acceptable results with this method using the procedure in Section 8.2.

1.10 Terms and units of measure used in this method are given in the glossary at the end of the method.

2. Summary of Method

2.1 A measured volume of sample, the amount required to meet an MDL or reporting limit (nominally 1-L), is extracted with methylene chloride using a separatory funnel, a continuous liquid/liquid extractor, or disk-based solid-phase extraction equipment. The extract is dried and concentrated for cleanup, if required. After cleanup, or if cleanup is not required, the extract is exchanged into an appropriate solvent and concentrated to the volume necessary to meet the required compliance or detection limit, and analyzed by GC/HSD.

2.2 Qualitative identification of an analyte in the extract is performed using the retention times on dissimilar GC columns. Quantitative analysis is performed using the peak areas or peak heights for the analyte on the dissimilar columns with either the external or internal standard technique.

2.3 Florisil®, alumina, a C18 solid-phase cleanup, and an elemental sulfur cleanup procedure are provided to aid in elimination of interferences that may be encountered. Other cleanup procedures may be used if demonstrated to be effective for the analytes in a wastewater matrix.

3. Contamination and Interferences

3.1 Solvents, reagents, glassware, and other sample processing lab ware may yield artifacts, elevated baselines, or matrix interferences causing misinterpretation of chromatograms. All materials used in the analysis must be demonstrated free from contamination and interferences by running blanks initially and with each extraction batch (samples started through the extraction process in a given 24-hour period, to a maximum of 20 samples - see Glossary for detailed definition), as described in Section 8.5. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Where possible, labware is cleaned by extraction or solvent rinse, or baking in a kiln or oven.

3.2 Glassware must be scrupulously cleaned (Reference 4). Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. Solvent rinsing should be followed by detergent washing with hot water, and rinses with tap water and reagent water. The glassware should then be drained dry, and heated at 400 °C for 15-30 minutes. Some thermally stable materials, such as PCBs, may require higher temperatures and longer baking times for removal. Solvent rinses with pesticide quality acetone, hexane, or other solvents may be substituted for heating. Do not heat volumetric labware above 90 °C. After drying and cooling, store inverted or capped with solvent-rinsed or baked aluminum foil in a clean environment to prevent accumulation of dust or other contaminants.

3.3 Interferences by phthalate esters can pose a major problem in pesticide analysis when using the electron capture detector. The phthalate esters generally appear in the chromatogram as large late eluting peaks, especially in the 15 and 50% fractions from Florisil®. Common flexible plastics contain varying amounts of phthalates that may be extracted or leached from such materials during laboratory operations. Cross contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalates can best be minimized by avoiding use of non-fluoropolymer plastics in the laboratory. Exhaustive cleanup of reagents and glassware may be required to eliminate background phthalate contamination (References 5 and 6). Interferences from phthalate esters can be avoided by using a microcoulometric or electrolytic conductivity detector.

3.4 Matrix interferences may be caused by contaminants co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and

diversity of the industrial complex or municipality being sampled. Interferences extracted from samples high in total organic carbon (TOC) may result in elevated baselines, or by enhancing or suppressing a signal at or near the retention time of an analyte of interest. Analyses of the matrix spike and matrix spike duplicate (Section 8.3) may be useful in identifying matrix interferences, and the cleanup procedures in Section 11 may aid in eliminating these interferences. EPA has provided guidance that may aid in overcoming matrix interferences (Reference 7); however, unique samples may require additional cleanup approaches to achieve the MDLs listed in Tables 1 and 2.

4. Safety

4.1 Hazards associated with each reagent used in this method have not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. 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 safety data sheets (SDSs, OSHA, 29 CFR 1910.12009(g)) should also be made available to all personnel involved in sample handling and chemical analysis. Additional references to laboratory safety are available and have been identified (References 8 and 9) for the information of the analyst.

4.2 The following analytes covered by this method have been tentatively classified as known or suspected human or mammalian carcinogens: 4,4'-DDT, 4,4'-DDD, the BHCs, and the PCBs. Primary standards of these toxic analytes should be prepared in a chemical fume hood, and a NIOSH/MESA approved toxic gas respirator should be worn when high concentrations are handled.

4.3 This method allows the use of hydrogen as a carrier gas in place of helium (Section 5.8.2). The laboratory should take the necessary precautions in dealing with hydrogen, and should limit hydrogen flow at the source to prevent buildup of an explosive mixture of hydrogen in air.

5. Apparatus and Materials

Note: *Brand names and suppliers are for illustration purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here. Demonstrating that the equipment and supplies used in the laboratory achieve the required performance is the responsibility of the laboratory. Suppliers for equipment and materials in this method may be found through an on-line search. Please do not contact EPA for supplier information.*

5.1 Sampling equipment, for discrete or composite sampling

5.1.1 Grab sample bottle – Amber glass bottle large enough to contain the necessary sample volume (nominally 1 L), fitted with a fluoropolymer-lined screw cap. Foil may be substituted for fluoropolymer if the sample is not corrosive. If amber bottles are not available, protect samples from light. Unless pre-cleaned, the bottle and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) – The sampler must use a glass or fluoropolymer container and tubing for sample collection. If the sampler uses a peristaltic pump, a minimum length

of compressible silicone rubber tubing may be used. Before use, rinse the compressible tubing thoroughly with methanol, followed by repeated rinsing with reagent water to minimize the potential for sample contamination. An integrating flow meter is required to collect flow proportional composites. The sample container must be kept refrigerated at ≤6 °C and protected from light during compositing.

5.2. Lab ware

5.2.1 Extraction

5.2.1.1 pH measurement

5.2.1.1.1 pH meter, with combination glass electrode

5.2.1.1.2 pH paper, wide range (Hydrion Papers, or equivalent)

5.2.1.2 Separatory funnel – Size appropriate to hold the sample and extraction solvent volumes, equipped with fluoropolymer stopcock.

5.2.1.3 Continuous liquid-liquid extractor – Equipped with fluoropolymer or glass connecting joints and stopcocks requiring no lubrication. (Hershberg-Wolf Extractor, Ace Glass Company, Vineland, NJ, or equivalent.)

5.2.1.3.1 Round-bottom flask, 500-mL, with heating mantle

5.2.1.3.2 Condenser, Graham, to fit extractor

5.2.1.4 Solid-phase extractor – 90-mm filter apparatus (Figure 2) or multi-position manifold

Note: *The approved ATP for solid-phase extraction is limited to disk-based extraction media and associated peripheral equipment.*

5.2.1.4.1 Vacuum system – Capable of achieving 0.1 bar (25 inch) Hg (house vacuum, vacuum pump, or water aspirator), equipped with shutoff valve and vacuum gauge

5.2.1.4.2 Vacuum trap – Made from 500-mL sidearm flask fitted with single-hole rubber stopper and glass tubing

5.2.2 Filtration

5.2.2.1 Glass powder funnel, 125- to 250-mL

5.2.2.2 Filter paper for above, Whatman 41, or equivalent

5.2.2.3 Prefiltering aids – 90-mm 1-µm glass fiber filter or Empore® Filter Aid 400

5.2.3 Drying column

5.2.3.1 Chromatographic column – Approximately 400 mm long x 15 mm ID, with fluoropolymer stopcock and coarse frit filter disc (Kontes or equivalent).

5.2.3.2 Glass wool – Pyrex, extracted with methylene chloride or baked at 450 °C for 1 hour minimum

5.2.4 Column for Florisil® or alumina cleanup – Approximately 300 mm long x 10 mm ID, with fluoropolymer stopcock. (This column is not required if cartridges containing Florisil® are used.)

5.2.5 Concentration/evaporation

Note: *Use of a solvent recovery system with the K-D or other solvent evaporation apparatus is strongly recommended.*

5.2.5.1 Kuderna-Danish concentrator

5.2.5.1.1 Concentrator tube, Kuderna-Danish – 10-mL, graduated (Kontes or equivalent). Calibration must be checked at the volumes employed for extract volume measurement. A ground-glass stopper is used to prevent evaporation of extracts.

5.2.5.1.2 Evaporative flask, Kuderna-Danish – 500-mL (Kontes or equivalent). Attach to concentrator tube with connectors.

5.2.5.1.3 Snyder column, Kuderna/Danish – Three-ball macro (Kontes or equivalent)

5.2.5.1.4 Snyder column – Two-ball micro (Kontes or equivalent)

5.2.5.1.5 Water bath – Heated, with concentric ring cover, capable of temperature control (± 2 °C), installed in a hood using appropriate engineering controls to limit exposure to solvent vapors.

5.2.5.2 Nitrogen evaporation device – Equipped with heated bath that can be maintained at an appropriate temperature for the solvent and analytes. (N-Evap, Organomation Associates, Inc., or equivalent)

5.2.5.3 Rotary evaporator – Buchi/Brinkman-American Scientific or equivalent, equipped with a variable temperature water bath, vacuum source with shutoff valve at the evaporator, and vacuum gauge.

5.2.5.3.1 A recirculating water pump and chiller are recommended, as use of tap water for cooling the evaporator wastes large volumes of water and can lead to inconsistent performance as water temperatures and pressures vary.

5.2.5.3.2 Round-bottom flask – 100-mL and 500-mL or larger, with ground-glass fitting compatible with the rotary evaporator

Note: *This equipment is used to prepare copper foil or copper powder for removing sulfur from sample extracts (see Section 6.7.4).*

5.2.5.4 Automated concentrator – Equipped with glassware sufficient to concentrate 3-400 mL extract to a final volume of 1-10 mL under controlled conditions of temperature and nitrogen flow (Turbovap, or equivalent). Follow manufacturer's directions and requirements.

5.2.5.5 Boiling chips – Glass, silicon carbide, or equivalent, approximately 10/40 mesh. Heat at 400 °C for 30 minutes, or solvent rinse or Soxhlet extract with methylene chloride.

5.2.6 Solid-phase extraction disks – 90-mm extraction disks containing 2 g of 8-µm octadecyl (C18) bonded silica uniformly enmeshed in a matrix of inert PTFE fibrils (3M Empore® or equivalent). The disks should not contain any organic compounds, either from the PTFE or the bonded silica, which will leach into the methylene chloride eluant. One liter of reagent water should pass through the disks in 2-5 minutes, using a vacuum of at least 25 inches of mercury.

Note: *Extraction disks from other manufacturers may be used in this procedure, provided that they use the same solid-phase materials (i.e., octadecyl bonded silica). Disks of other diameters also may be used, but may adversely affect the flow rate of the sample through the disk.*

5.3 Vials

5.3.1 Extract storage – 10- to 15-mL, amber glass, with fluoropolymer-lined screw cap

5.3.2 GC autosampler – 1- to 5-mL, amber glass, with fluoropolymer-lined screw- or crimp-cap, to fit GC autosampler

5.4 Balances

5.4.1 Analytical – Capable of accurately weighing 0.1 mg

5.4.2 Top loading – Capable of weighing 10 mg

5.5 Sample cleanup

5.5.1 Oven – For baking and storage of adsorbents, capable of maintaining a constant temperature (± 5 °C) in the range of 105-250 °C.

5.5.2 Muffle furnace – Capable of cleaning glassware or baking sodium sulfate in the range of 400-450 °C.

5.5.3 Vacuum system and cartridges for solid-phase cleanup (see Section 11.2)

5.5.3.1 Vacuum system – Capable of achieving 0.1 bar (25 in.) Hg (house vacuum, vacuum pump, or water aspirator), equipped with shutoff valve and vacuum gauge

5.5.3.2 VacElute Manifold (Analytichem International, or equivalent)

5.5.3.3 Vacuum trap – Made from 500-mL sidearm flask fitted with single-hole rubber stopper and glass tubing

5.5.3.4 Rack for holding 50-mL volumetric flasks in the manifold

5.5.3.5 Cartridge – Mega Bond Elute, Non-polar, C18 Octadecyl, 10 g/60 mL (Analytichem International or equivalent), used for solid-phase cleanup of sample extracts (see Section 11.2)

5.5.4 Sulfur removal tube – 40- to 50-mL bottle, test tube, or Erlenmeyer flask with fluoropolymer-lined screw cap

5.6 Centrifuge apparatus

5.6.1 Centrifuge – Capable of rotating 500-mL centrifuge bottles or 15-mL centrifuge tubes at 5,000 rpm minimum

5.6.2 Centrifuge bottle – 500-mL, with screw cap, to fit centrifuge

5.6.3 Centrifuge tube – 15-mL, with screw cap, to fit centrifuge

5.7 Miscellaneous lab ware – Graduated cylinders, pipettes, beakers, volumetric flasks, vials, syringes, and other lab ware necessary to support the operations in this method

5.8 Gas chromatograph – Dual-column with simultaneous split/splitless, temperature programmable split/splitless (PTV), or on-column injection; temperature program with isothermal holds, and all required accessories including syringes, analytical columns, gases, and detectors. An autosampler is highly recommended because it injects volumes more reproducibly than manual injection techniques. Alternatively, two separate single-column gas chromatographic systems may be employed.

5.8.1 Example columns and operating conditions

5.8.1.1 DB-608 (or equivalent), 30-m long x 0.53-mm ID fused-silica capillary, 0.83-µm film thickness.

5.8.1.2 DB-1701 (or equivalent), 30-m long x 0.53-mm ID fused-silica capillary, 1.0-µm film thickness.

5.8.1.3 Suggested operating conditions used to meet the retention times shown in Table 3 are:
Carrier gas flow rate: approximately 7 mL/min
Initial temperature: 150 °C for 0.5 minute,
Temperature program: 150-270 °C at 5 °C/min, and
Final temperature: 270 °C, until trans-Permethrin elutes

Note: *Other columns, internal diameters, film thicknesses, and operating conditions may be used, provided that the performance requirements in this method are met. However, the column pair chosen must have dissimilar phases/chemical properties in order to separate the compounds of interest in different retention time order. Columns that only differ in the length, ID, or film thickness, but use the same stationary phase do not qualify as "dissimilar."*

5.8.2 Carrier gas – Helium or hydrogen. Data in the tables in this method were obtained using helium carrier gas. If hydrogen is used, analytical conditions may need to be adjusted for

optimum performance, and calibration and all QC tests must be performed with hydrogen carrier gas. See Section 4.3 for precautions regarding the use of hydrogen as a carrier gas.

5.8.3 Detector – Halogen-specific detector (electron capture detector [ECD], electrolytic conductivity detector [ELCD], or equivalent). The ECD has proven effective in the analysis of wastewaters for the analytes listed in Tables 1 and 2, and was used to develop the method performance data in Section 17 and Tables 4 and 5.

5.8.4 Data system – A computer system must be interfaced to the GC that allows continuous acquisition and storage of data from the detectors throughout the chromatographic program. The computer must have software that allows searching GC data for specific analytes, and for plotting responses versus time. Software must also be available that allows integrating peak areas or peak heights in selected retention time windows and calculating concentrations of the analytes.

6. Reagents and Standards

6.1 pH adjustment

6.1.1 Sodium hydroxide solutions

6.1.1.1 Concentrated (10 M) – Dissolve 40 g of NaOH (ACS) in reagent water and dilute to 100 mL.

6.1.1.2 Dilute (1 M) – Dissolve 40 g NaOH in 1 L of reagent water.

6.1.2 Sulfuric acid (1+1) – Slowly add 50 mL of H_2SO_4 (ACS, sp. gr. 1.84) to 50 mL of reagent water.

6.1.3 Hydrochloric acid – Reagent grade, 6 N

6.2 Sodium thiosulfate – (ACS) granular.

6.3 Sodium sulfate – Sodium sulfate, reagent grade, granular anhydrous (Baker or equivalent), rinsed with methylene chloride, baked in a shallow tray at 450 °C for 1 hour minimum, cooled in a desiccator, and stored in a pre-cleaned glass bottle with screw cap which prevents moisture from entering. If, after heating, the sodium sulfate develops a noticeable grayish cast (due to the presence of carbon in the crystal matrix), that batch of reagent is not suitable for use and should be discarded. Extraction with methylene chloride (as opposed to simple rinsing) and baking at a lower temperature may produce sodium sulfate suitable for use.

6.4 Reagent water – Reagent water is defined as water in which the analytes of interest and interfering compounds are not observed at the MDLs of the analytes in this method.

6.5 Solvents – Methylene chloride, acetone, methanol, hexane, acetonitrile, and isooctane, high purity pesticide quality, or equivalent, demonstrated to be free of the analytes and interferences (Section 3). Purification of solvents by distillation in all-glass systems may be required.

Note: *The standards and final sample extracts must be prepared in the same final solvent.*

6.6 Ethyl ether – Nanograde, redistilled in glass if necessary

Ethyl ether must be shown to be free of peroxides before use, as indicated by EM Laboratories Quant test strips (available from Scientific Products Co. and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips. After removal of peroxides, add 20 mL of ethyl alcohol preservative to each liter of ether.

6.7 Materials for sample cleanup

6.7.1 Florisil® – PR grade (60/100 mesh), activated at 650 - 700 °C, stored in the dark in a glass container with fluoropolymer-lined screw cap. Activate each batch immediately prior to use for 16 hours minimum at 130 °C in a foil-covered glass container and allow to cool. Alternatively, 500 mg cartridges (J.T. Baker, or equivalent) may be used.

6.7.1.1 Cartridge certification – Each cartridge lot must be certified to ensure recovery of the analytes of interest and removal of 2,4,6-trichlorophenol. To make the test mixture, add the trichlorophenol solution (Section 6.7.1.3) to the same standard used to prepare the Quality Control Check Sample (Section 6.8.3). Transfer the mixture to the column and dry the column. Pre-elute with three 10-mL portions of elution solvent, drying the column between elutions. Elute the cartridge with 10 mL each of methanol and water, as in Section 11.2.3.3.

6.7.1.2 Concentrate the eluant to per Section 10.3.3, exchange to isooctane or hexane per Section 10.3.3, and inject 1.0 µL of the concentrated eluant into the GC using the procedure in Section 12. The recovery of all analytes (including the unresolved GC peaks) shall be within the ranges for calibration verification (Section 13.6 and Table 4), the recovery of trichlorophenol shall be less than 5%, and no peaks interfering with the target analytes shall be detected. Otherwise the Florisil® cartridge is not performing properly and the cartridge lot shall be rejected.

6.7.1.3 Florisil® cartridge calibration solution – 2,4,6-Trichlorophenol, 0.1 µg/mL in acetone.

6.7.2 SPE elution solvent – Methylene chloride:acetonitrile:hexane (50:3:47).

6.7.3 Alumina, neutral, Brockman Activity I, 80-200 mesh (Fisher Scientific certified, or equivalent). Heat in a glass bottle for 16 hours at 400 to 450 °C. Seal and cool to room temperature. Add 7% (w/w) reagent water and mix for 10 to 12 hours. Keep bottle tightly sealed.

6.7.4 Sulfur removal

6.7.4.1 Copper foil or powder – Fisher, Alfa Aesar, or equivalent. Cut copper foil into approximately 1-cm squares. Copper must be activated before it may be used, as described below.

6.7.4.1.1 Place the quantity of copper needed for sulfur removal (Section 11.5.1.3) in a ground-glass-stoppered Erlenmeyer flask or bottle. Cover the foil or powder with methanol.

6.7.4.1.2 Add HCl dropwise (0.5 - 1.0 mL) while swirling, until the copper brightens.

6.7.4.1.3 Pour off the methanol/HCl and rinse 3 times with reagent water to remove all traces of acid, then 3 times with acetone, then 3 times with hexane.

6.7.4.1.4 For copper foil, cover with hexane after the final rinse. Store in a stoppered flask under nitrogen until used. For the powder, dry on a rotary evaporator. Store in a stoppered flask under nitrogen until used. Inspect the copper foil or powder before each use. It must have a bright, non-oxidized appearance to be effective. Copper foil or powder that has oxidized may be reactivated using the procedure described above.

6.7.4.2 Tetrabutylammonium sulfite (TBA sulfite) – Prepare as described below.

6.7.4.2.1 Tetrabutylammonium hydrogen sulfate, $[CH_3(CH_2)_3]_4NHSO_4$

6.7.4.2.2 Sodium sulfite, Na_2SO_3

6.7.4.2.3 Dissolve approximately 3 g tetrabutylammonium hydrogen sulfate in 100 mL of reagent water in an amber bottle with fluoropolymer-lined screw cap. Extract with three 20-mL portions of hexane and discard the hexane extracts.

6.7.4.2.4 Add 25 g sodium sulfite to produce a saturated solution. Store at room temperature. Replace after 1 month.

6.7.5 Sodium chloride – Reagent grade, prepare at 5% (w/v) solution in reagent water.

6.8 Stock standard solutions – Stock standard solutions may be prepared from pure materials, or purchased as certified solutions. Traceability must be to the National Institute of Standards and Technology (NIST) or other national or international standard, when available. Stock solution concentrations alternative to those below may be used. Because of the toxicity of some of the compounds, primary dilutions should be prepared in a hood, and a NIOSH/MESA approved toxic gas respirator should be worn when high concentrations of neat materials are handled. The following procedure may be used to prepare standards from neat materials.

6.8.1 Accurately weigh about 0.0100 g of pure material in a 10-mL volumetric flask. Dilute to volume in pesticide quality hexane, isooctane, or other suitable solvent. Larger volumes may be used at the convenience of the laboratory. When compound purity is assayed to be 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.8.1.1 Unless stated otherwise in this method, store non-aqueous standards in fluoropolymer-lined screw-cap, or heat-sealed, glass containers, in the dark at -20 to -10 °C. Store aqueous standards; e.g., the aqueous LCS (Section 8.4), in the dark at ≤ 6 °C, but do not freeze.

6.8.1.2 Standards prepared by the laboratory may be stored for up to one year, except when comparison with QC check standards indicates that a standard has degraded or become more concentrated due to evaporation, or unless the laboratory has data on file to prove stability for a longer period. Commercially prepared standards

may be stored until the expiration date provided by the vendor, except when comparison with QC check standards indicates that a standard has degraded or become more concentrated due to evaporation, or unless the laboratory has data from the vendor on file to prove stability for a longer period.

6.8.2 Calibration solutions – It is necessary to prepare calibration solutions for the analytes of interest (Section 1.4) only using an appropriate solvent (isooctane or hexane may be used). Whatever solvent is used, both the calibration standards and the final sample extracts must use the same solvent. Other analytes may be included as desired.

6.8.2.1 Prepare calibration standards for the single-component analytes of interest and surrogates at a minimum of three concentration levels (five are suggested) by adding appropriate volumes of one or more stock standards to volumetric flasks. One of the calibration standards should be at a concentration at or below the ML specified in Table 1, or 2, or as specified by a regulatory/control authority or in a permit. The ML value may be rounded to a whole number that is more convenient for preparing the standard, but must not exceed the ML value listed in Tables 1 or 2 for those analytes which list ML values. Alternatively, the laboratory may establish an ML for each analyte based on the concentration of the lowest calibration standard in a series of standards produced by the laboratory or obtained from a commercial vendor, again, provided that the ML does not exceed the ML in Table 1 and 2, and provided that the resulting calibration meets the acceptance criteria in Section 7.5.2 based on the RSD, RSE, or R^2.

The other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the GC system. A minimum of six concentration levels is required for a second order, non-linear (e.g., quadratic; $ax^2 + bx + c = 0$) calibration (Section 7.5.2 or 7.6.2). Calibrations higher than second order are not allowed. A separate standard near the MDL may be analyzed as a check on sensitivity, but should not be included in the linearity assessment. The solvent for the standards must match the final solvent for the sample extracts (e.g., isooctane or hexane).

Note: *The option for non-linear calibration may be necessary to address specific instrumental techniques. However, it is not EPA's intent to allow non-linear calibration to be used to compensate for detector saturation or to avoid proper instrument maintenance.*

Given the number of analytes included in this method, it is highly likely that some will coelute on one or both of the GC columns used for the analysis. Divide the analytes into two or more groups and prepare separate calibration standards for each group, at multiple concentrations (e.g., a five-point calibration will require ten solutions to cover two groups of analytes). Table 7 provides information on dividing the target analytes into separate calibration mixtures that should minimize or eliminate co-elutions. This table is provided solely as guidance, based on the GC columns suggested in this method. If an analyte listed in Table 7 is not an analyte of interest in a given laboratory setting, then it need not be included in a calibration mixture.

Note: *Many commercially available standards are divided into separate mixtures to address this issue.*

If co-elutions occur in analysis if a sample, a co-elution on one column is acceptable so long as effective separation of the co-eluting compounds can be achieved on the second column.

6.8.2.2 Multi-component analytes (e.g., PCBs as Aroclors, and Toxaphene)

6.8.2.2.1 A standard containing a mixture of Aroclor 1016 and Aroclor 1260 will include many of the peaks represented in the other Aroclor mixtures. As a result, a multi-point initial calibration employing a mixture of Aroclors 1016 and 1260 at three to five concentrations should be sufficient to demonstrate the linearity of the detector response without the necessity of performing multi-point initial calibrations for each of the seven Aroclors. In addition, such a mixture can be used as a standard to demonstrate that a sample does not contain peaks that represent any one of the Aroclors. This standard can also be used to determine the concentrations of either Aroclor 1016 or Aroclor 1260, should they be present in a sample.

Therefore, prepare a minimum of three calibration standards containing equal concentrations of both Aroclor 1016 and Aroclor 1260 by dilution of the stock standard with isooctane or hexane. The concentrations should correspond to the expected range of concentrations found in real samples and should bracket the linear range of the detector.

6.8.2.2.2 Single standards of each of the other five Aroclors are required to aid the analyst in pattern recognition. Assuming that the Aroclor 1016/1260 standards described in Section 6.8.2.2.1 have been used to demonstrate the linearity of the detector, these single standards of the remaining five Aroclors also may be used to determine the calibration factor for each Aroclor. Prepare a standard for each of the other Aroclors. The concentrations should generally correspond to the mid-point of the linear range of the detector, but lower concentrations may be employed at the discretion of the analyst based on project requirements.

6.8.2.2.3 For Toxaphene, prepare a minimum of three calibration standards containing Toxaphene by dilution of the stock standard with isooctane or hexane. The concentrations should correspond to the expected range of concentrations found in real samples and should bracket the linear range of the detector.

6.8.3 Quality Control (QC) Check Sample Concentrate – Prepare one or more mid-level standard mixtures (concentrates) in acetone (or other water miscible solvent). The concentrate is used as the spiking solution with which to prepare the Demonstration of Capabilities (DOC) samples, the Laboratory Control Sample (LCS), and Matrix Spike (MS) and Matrix Spike Duplicate (MSD) samples described in Section 8. If prepared by the laboratory (as opposed the purchasing it from a commercial supplier), the concentrate must be prepared independently from the standards used for calibration, but may be prepared from the same source as the second-source standard used for calibration verification (Section 7.7). Regardless of the source, the concentrate must be in a water-miscible solvent, as noted above.

The concentrate is used to prepare the DOC and LCS (Sections 8.2.1 and 8.4) and MS/MSD samples (Section 8.3). Depending on the analytes of interest for a given sample (see Section 1.4), multiple solutions and multiple LCS or MS/MSD samples may be required to account for co-eluting analytes. However, a co-elution on one column is acceptable so long as effective separation of the co-eluting compounds can be achieved on the second column. In addition, the concentrations of the MS/MSD samples should reflect any relevant compliance limits for the analytes of interest, as described in Section 8.3.1. If a custom spiking solution is required for a specific discharge (Section 8.3.1), prepare it separately from the DOC and LCS solution.

Note: *Some commercially available standards are divided into separate mixtures to address the co-elution issue.*

6.8.4 Calibration Verification Standards – In order to verify the results of the initial calibration standards, prepare one or more mid-level standard mixtures in isooctane or hexane, using standards obtained from a second source (different manufacturer or different certified lot from the calibration standards). These standards will be analyzed to verify the accuracy of the calibration (Sections 7.7 and 13.6.2). As with the QC sample concentrate in Section 6.8.3, multiple solutions may be required to address co-elutions among all of the analytes.

6.8.5 Internal standard solution – If the internal standard calibration technique is to be used, prepare pentachloronitrobenzene (PCNB) at a concentration of 10 µg/mL in ethyl acetate. Alternative and multiple internal standards; e.g., tetrachloro-m-xylene, 4,4'-dibromo-biphenyl, and/or decachlorobiphenyl may be used provided that the laboratory performs all QC tests and meets all QC acceptance criteria with the alternative or additional internal standard(s) as an integral part of this method.

6.8.6 Surrogate solution – Prepare a solution containing one or more surrogates at a concentration of 2 µg/mL in acetone. Potential surrogates include: dibutyl chlorendate (DBC), tetrachloro-m-xylene (TCMX), 4,4'-dibromobiphenyl, or decachlorobiphenyl. Alternative surrogates and concentrations may be used, provided the laboratory performs all QC tests and meets all QC acceptance criteria with the alternative surrogate(s) as an integral part of this method. If the internal standard calibration technique is used, do not use the internal standard as a surrogate.

6.8.7 DDT and endrin decomposition (breakdown) solution – Prepare a solution containing endrin at a concentration of 50 ng/mL and 4,4'-DDT at a concentration of 100 ng/mL, in isooctane or hexane. A 1-µL injection of this standard will contain 50 picograms (pg) of endrin and 100 pg of DDT. The concentration of the solution may be adjusted by the laboratory to accommodate other injection volumes such that the same masses of the two analytes are introduced into the instrument.

7. Calibration

7.1 Establish gas chromatographic operating conditions equivalent to those in Section 5.8.1 and Footnote 2 to Table 3. Alternative temperature program and flow rate conditions may be used. The system may be calibrated using the external standard technique (Section 7.5) or the internal standard technique (Section 7.6). It is necessary to calibrate the system for the analytes of interest (Section 1.4) only.

7.2 Separately inject the mid-level calibration standard for each calibration mixture. Store the retention time on each GC column.

7.3 Injection of calibration solutions – Inject a constant volume in the range of 0.5 to 2.0 μL of each calibration solution into the GC column/detector pairs. An alternative volume (see Section 12.3) may be used provided all requirements in this method are met. Beginning with the lowest level mixture and proceeding to the highest level mixture may limit the risk of carryover from one standard to the next, but other sequences may be used. An instrument blank should be analyzed after the highest standard to demonstrate that there is no carry-over within the system for this calibration range.

7.4 For each analyte, compute, record, and store, as a function of the concentration injected, the retention time and peak area on each column/detector system. If multi-component analytes are to be analyzed, store the retention time and peak area for the three to five exclusive (unique large) peaks for each PCB or technical chlordane. Use four to six peaks for toxaphene.

7.5 External standard calibration

7.5.1 From the calibration data (Section 7.4), calculate the calibration factor (CF) for each analyte at each concentration according to the following equation:

$$CF = \frac{A_s}{C_s}$$

where:
C_s = Concentration of the analyte in the standard (ng/mL)
A_s = Peak height or area

For multi-component analytes, choose a series of characteristic peaks for each analyte (3 to 5 for each Aroclor, 4 to 6 for toxaphene) and calculate individual calibration factors for each peak. Alternatively, for toxaphene, sum the areas of all of the peaks in the standard chromatogram and use the summed area to determine the calibration factor. (If this alternative is used, the same approach must be used to quantitate the analyte in the samples.)

7.5.2 Calculate the mean (average) and relative standard deviation (RSD) of the calibration factors. If the RSD is less than 20%, linearity through the origin can be assumed and the average CF can be used for calculations. Alternatively, the results can be used to fit a linear or quadratic regression of response, As, vs. concentration Cs. If used, the regression must be weighted inversely proportional to concentration. The coefficient of determination (R^2) of the weighted regression must be greater than 0.920. Alternatively, the relative standard error (Reference 10) may be used as an acceptance criterion. As with the RSD, the RSE must be less than 20%. If an RSE less than 20% cannot be achieved for a quadratic regression, system performance is unacceptable and the system must be adjusted and re-calibrated.

Note: *Regression calculations are not included in this method because the calculations are cumbersome and because many GC/ECD data systems allow selection of weighted regression for calibration and calculation of analyte concentrations.*

7.6 Internal standard calibration

7.6.1 From the calibration data (Section 7.4), calculate the response factor (RF) for each analyte at each concentration according to the following equation:

$$RF = \frac{(A_s \times C_{is})}{(A_{is} \times C_s)}$$

where:

A_s = Response for the analyte to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard (ng/mL)
C_s = Concentration of the analyte to be measured (ng/mL).

7.6.2 Calculate the mean (average) and relative standard deviation (RSD) of the response factors. If the RSD is less than 15%, linearity through the origin can be assumed and the average RF can be used for calculations. Alternatively, the results can be used to prepare a calibration curve of response ratios, A_s/A_{is}, vs. concentration ratios, C_s/C_{is}, for the analyte. A minimum of six concentration levels is required for a non-linear (e.g., quadratic) regression. If used, the regression must be weighted inversely proportional to concentration, and the coefficient of determination of the weighted regression must be greater than 0.920. Alternatively, the relative standard error (Reference 10) may be used as an acceptance criterion. As with the RSD, the RSE must be less than 15%. If an RSE less than 15% cannot be achieved for a quadratic regression, system performance is unacceptable and the system must be adjusted and re-calibrated.

7.7 The working calibration curve, CF, or RF must be verified immediately after calibration and at the beginning and end of each 24-hour shift by the analysis of a mid-level calibration standard. The calibration verification standard(s) must be obtained from a second manufacturer or a manufacturer's batch prepared independently from the batch used for calibration (Section 6.8.4). Requirements for calibration verification are given in Section 13.6 and Table 4. Alternatively, calibration verification may be performed after a set number of injections (e.g., every 20 injections), to include injection of extracts of field samples, QC samples, instrument blanks, etc. (i.e., it is based on the number of injections performed, not sample extracts). The time for the injections may not exceed 24 hours.

Note: *The 24-hour shift begins after analysis of the combined QC standard (calibration verification) and ends 24 hours later. The ending calibration verification standard is run immediately after the last sample run during the 24-hour shift, so the beginning and ending calibration verifications are outside of the 24-hour shift. If calibration verification is based on the number of injections instead of time, then the ending verification standard for one group of injections may be used as the beginning verification for the next group of injections.*

7.8 Florisil® calibration – The column cleanup procedure in Section 11.3 utilizes Florisil® column chromatography. Florisil® from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil® which is used, use of the lauric acid value (Reference 11) is suggested. The referenced procedure determines the adsorption from a hexane solution of lauric acid (mg) per g of Florisil®. The amount of Florisil® to be used for each column is calculated by dividing 110 by this ratio and multiplying by 20 g. If cartridges containing Florisil® are used, then this step is not necessary.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality assurance program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and ongoing analysis of spiked samples and blanks to evaluate and document data quality. The laboratory must maintain records to document the quality of data generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet performance requirements of this method. A quality control check standard (LCS, Section 8.4) must be prepared and analyzed with each batch of samples to confirm that the measurements were performed in an in-control mode of operation. A laboratory may develop its own performance criteria (as QC acceptance criteria), provided such criteria are as or more restrictive than the criteria in this method.

8.1.1 The laboratory must make an initial demonstration of the capability (IDC) to generate acceptable precision and recovery with this method. This demonstration is detailed in Section 8.2. On a continuing basis, the laboratory must repeat demonstration of capability (DOC) at least annually.

8.1.2 In recognition of advances that are occurring in analytical technology, and to overcome matrix interferences, the laboratory is permitted certain options (Section 1.8 and 40 CFR 136.6(b) [Reference 12]) to improve separations or lower the costs of measurements. These options may include alternative extraction (e.g., other solid-phase extraction materials and formats), concentration, and cleanup procedures, and changes in GC columns (Reference 12). Alternative determinative techniques, such as the substitution of spectroscopic or immunoassay techniques, and changes that degrade method performance, are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or greater than the specificity of the techniques in this method for the analytes of interest. The laboratory is also encouraged to participate in performance evaluation studies (see Section 8.8).

8.1.2.1 Each time a modification listed above is made to this method, the laboratory is required to repeat the procedure in Section 8.2. If the detection limit of the method will be affected by the change, the laboratory is required to demonstrate that the MDLs (40 CFR part 136, appendix B) are lower than one-third the regulatory compliance limit or as low as the MDLs in this method, whichever are greater. If calibration will be affected by the change, the instrument must be recalibrated per Section 7. Once the modification is demonstrated to produce results equivalent or superior to results produced by this method as written, that modification may be used routinely thereafter, so long as the other requirements in this method are met (e.g., matrix spike/matrix spike duplicate recovery and relative percent difference).

8.1.2.1.1 If an allowed method modification, is to be applied to a specific discharge, the laboratory must prepare and analyze matrix spike/matrix spike duplicate (MS/MSD) samples (Section 8.3) and LCS samples (Section 8.4). The laboratory must include surrogates (Section 8.7) in each of the samples. The MS/MSD and LCS samples must be fortified with the analytes of interest (Section 1.4). If the modification is for nationwide use, MS/MSD samples must be prepared from a minimum of nine different discharges (See Section 8.1.2.1.2), and all QC acceptance criteria in this method must be met. This evaluation only needs to be performed once other than for the

routine QC required by this method (for example it could be performed by the vendor of an alternative material) but any laboratory using that specific material must have the results of the study available. This includes a full data package with the raw data that will allow an independent reviewer to verify each determination and calculation performed by the laboratory (see Section 8.1.2.2.5, items a-q).

8.1.2.1.2 Sample matrices on which MS/MSD tests must be performed for nationwide use of an allowed modification:

(a) Effluent from a publicly owned treatment works (POTW)
(b) ASTM D5905 Standard Specification for Substitute Wastewater
(c) Sewage sludge, if sewage sludge will be in the permit
(d) ASTM D1141 Standard Specification for Substitute Ocean Water, if ocean water will be in the permit
(e) Untreated and treated wastewaters up to a total of nine matrix types (see www.epa.gov/eg/industrial-effluent-guidelines for a list of industrial categories with existing effluent guidelines).

At least one of the above wastewater matrix types must have at least one of the following characteristics:

(i) Total suspended solids greater than 40 mg/L
(ii) Total dissolved solids greater than 100 mg/L
(iii) Oil and grease greater than 20 mg/L
(iv) NaCl greater than 120 mg/L
(v) CaCO3 greater than 140 mg/L

The interim acceptance criteria for MS, MSD recoveries that do not have recovery limits in Table 4 or developed in Section 8.3.3, and for surrogates that do not have recovery limits developed in Section 8.6, must be no wider than 60 -140 %, and the relative percent difference (RPD) of the concentrations in the MS and MSD that do not have RPD limits in Table 4 or developed in Section 8.3.3, must be less than 30%. Alternatively, the laboratory may use the laboratory's in-house limits if they are tighter.

(f) A proficiency testing (PT) sample from a recognized provider, in addition to tests of the nine matrices (Section 8.1.2.1.1).

8.1.2.2 The laboratory must maintain records of modifications made to this method. These records include the following, at a minimum:

8.1.2.2.1 The names, titles, and business street addresses, telephone numbers, and e-mail addresses of the analyst(s) that performed the analyses and modification, and of the quality control officer that witnessed and will verify the analyses and modifications.

8.1.2.2.2 A list of analytes, by name and CAS Registry number.

8.1.2.2.3 A narrative stating reason(s) for the modifications.

8.1.2.2.4 Results from all quality control (QC) tests comparing the modified method to this method, including:

a) Calibration (Section 7).
b) Calibration verification (Section 13.6).
c) Initial demonstration of capability (Section 8.2).
d) Analysis of blanks (Section 8.5).
e) Matrix spike/matrix spike duplicate analysis (Section 8.3).
f) Laboratory control sample analysis (Section 8.4).

8.1.2.2.5 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include:

a) Sample numbers and other identifiers.
b) Extraction dates.
c) Analysis dates and times.
d) Analysis sequence/run chronology.
e) Sample weight or volume (Section 10).
f) Extract volume prior to each cleanup step (Sections 10 and 11).
g) Extract volume after each cleanup step (Section 11).
h) Final extract volume prior to injection (Sections 10 and 12).
i) Injection volume (Sections 12.3 and 13.2).
j) Sample or extract dilution (Section 15.4).
k) Instrument and operating conditions.
l) Column (dimensions, material, etc.).
m) Operating conditions (temperatures, flow rates, etc.).
n) Detector (type, operating conditions, etc.).
o) Chromatograms and other recordings of raw data.
p) Quantitation reports, data system outputs, and other data to link the raw data to the results reported.
q) A written Standard Operating Procedure (SOP)

8.1.2.2.6 Each individual laboratory wishing to use a given modification must perform the start-up tests in Section 8.1.2 (e.g., DOC, MDL), with the modification as an integral part of this method prior to applying the modification to specific discharges. Results of the DOC must meet the QC acceptance criteria in Table 5 for the analytes of interest (Section 1.4), and the MDLs must be equal to or lower than the MDLs in Tables 1 and 2 for the analytes of interest.

8.1.3 Before analyzing samples, the laboratory must analyze a blank to demonstrate that interferences from the analytical system, lab ware, and reagents, are under control. Each time a batch of samples is extracted or reagents are changed, a blank must be extracted and analyzed as a safeguard against laboratory contamination. Requirements for the blank are given in Section 8.5.

8.1.4 The laboratory must, on an ongoing basis, spike and analyze samples to monitor and evaluate method and laboratory performance on the sample matrix. The procedure for spiking and analysis is given in Section 8.3.

8.1.5 The laboratory must, on an ongoing basis, demonstrate through analysis of a quality control check sample (laboratory control sample, LCS; on-going precision and recovery sample,

OPR) that the measurement system is in control. This procedure is described in Section 8.4.

8.1.6 The laboratory should maintain performance records to document the quality of data that is generated. This procedure is given in Section 8.7.

8.1.7 The large number of analytes tested in performance tests in this method present a substantial probability that one or more will fail acceptance criteria when all analytes are tested simultaneously, and a re-test (reanalysis) is allowed if this situation should occur. If, however, continued re-testing results in further repeated failures, the laboratory should document the failures and either avoid reporting results for the analytes that failed or report the problem and failures with the data. A QC failure does not relieve a discharger or permittee of reporting timely results.

8.2 Demonstration of capability (DOC) – To establish the ability to generate acceptable recovery and precision, the laboratory must perform the DOC in Sections 8.2.1 through 8.2.6 for the analytes of interest initially and in an on-going manner at least annually. The laboratory must also establish MDLs for the analytes of interest using the MDL procedure at 40 CFR part 136, appendix B. The laboratory's MDLs must be equal to or lower than those listed in Tables 1 or 2, or lower than one-third the regulatory compliance limit, whichever is greater. For MDLs not listed in Tables 1 or 2, the laboratory must determine the MDLs using the MDL procedure at 40 CFR part 136, appendix B under the same conditions used to determine the MDLs for the analytes listed in Tables 1 and 2. When analyzing the PCBs as Aroclors, it is only necessary to establish an MDL for one of the multi-component analytes (e.g., PCB 1254), or the mixture of Aroclors 1016 and 1260 may be used to establish MDLs for all of the Aroclors. Similarly, MDLs for other multi-component analytes (e.g., Chlordanes) may be determined using only one of the major components. All procedures used in the analysis, including cleanup procedures, must be included in the DOC.

8.2.1 For the DOC, a QC check sample concentrate containing each analyte of interest (Section 1.4) is prepared in a water-miscible solvent using the solution in Section 6.8.3.

Note: *QC check sample concentrates are no longer available from EPA.*

8.2.2 Using a pipet or syringe, prepare four QC check samples by adding an appropriate volume of the concentrate and of the surrogate(s) to each of four 1-L aliquots of reagent water. Swirl or stir to mix.

8.2.3 Extract and analyze the well-mixed QC check samples according to the method beginning in Section 10.

8.2.4 Calculate the average percent recovery ($\overline{X}$) and the standard deviation (s) of the percent recovery for each analyte using the four results.

8.2.5 For each analyte, compare s and $\overline{X}$ with the corresponding acceptance criteria for precision and recovery in Table 4. For analytes in Table 2 that are not listed in Table 4, QC acceptance criteria must be developed by the laboratory. EPA has provided guidance for development of QC acceptance criteria (References 12 and 13). If s and $\overline{X}$ for all analytes of interest meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples can begin. If any individual s exceeds the precision limit or any individual $\overline{X}$ falls outside the range for recovery, system performance is unacceptable for that analyte.

Note: *The large number of analytes in Tables 1 and 2 present a substantial probability that one or more will fail at least one of the acceptance criteria when many or all analytes are determined simultaneously.*

8.2.6 When one or more of the analytes tested fail at least one of the acceptance criteria, repeat the test for only the analytes that failed. If results for these analytes pass, system performance is acceptable and analysis of samples and blanks may proceed. If one or more of the analytes again fail, system performance is unacceptable for the analytes that failed the acceptance criteria. Correct the problem and repeat the test (Section 8.2). See Section 8.1.7 for disposition of repeated failures.

Note: *To maintain the validity of the test and re-test, system maintenance and/or adjustment is not permitted between this pair of tests.*

8.3 Matrix spike and matrix spike duplicate (MS/MSD) – The purpose of the MS/MSD requirement is to provide data that demonstrate the effectiveness of the method as applied to the samples in question by a given laboratory, and both the data user (discharger, permittee, regulated entity, regulatory/control authority, customer, other) and the laboratory share responsibility for provision of such data. The data user should identify the sample and the analytes of interest (Section 1.4) to be spiked and provide sufficient sample volume to perform MS/MSD analyses. The laboratory must, on an ongoing basis, spike at least 5% of the samples in duplicate from each discharge being monitored to assess accuracy (recovery and precision). If direction cannot be obtained from the data user, the laboratory must spike at least one sample in duplicate per extraction batch of up to 20 samples with the analytes in Table 1. Spiked sample results should be reported only to the data user whose sample was spiked, or as requested or required by a regulatory/control authority, or in a permit.

8.3.1. If, as in compliance monitoring, the concentration of a specific analyte will be checked against a regulatory concentration limit, the concentration of the spike should be at that limit; otherwise, the concentration of the spike should be one to five times higher than the background concentration determined in Section 8.3.2, at or near the midpoint of the calibration range, or at the concentration in the LCS (Section 8.4) whichever concentration would be larger. When no information is available, the mid-point of the calibration may be used.

8.3.2 Analyze one sample aliquot to determine the background concentration (B) of the each analyte of interest. If necessary to meet the requirement in Section 8.3.1, prepare a new check sample concentrate (Section 8.2.1) appropriate for the background concentration. Spike and analyze two additional sample aliquots of the same volume as the original sample, and determine the concentrations after spiking (A_1 and A_2) of each analyte. Calculate the percent recoveries (P_1 and P_2) as:

$$P_x = \frac{A_x - B}{T} \times 100$$

where T is the known true value of the spike.

Also calculate the relative percent difference (RPD) between the concentrations (A_1 and A_2):

$$RPD = \frac{|A_1 - A_2|}{\frac{A_1 + A_2}{2}} \times 100$$

8.3.3 Compare the percent recoveries (P_1 and P_2) and the RPD for each analyte in the MS/MSD aliquots with the corresponding QC acceptance criteria for recovery (P) and RPD in Table 4.

If any individual P falls outside the designated range for recovery in either aliquot, or the RPD limit is exceeded, the result for the analyte in the unspiked sample is suspect and may not be reported or used for permitting or regulatory compliance. See Section 8.1.7 for disposition of failures.

For analytes in Table 2 not listed in Table 4, QC acceptance criteria must be developed by the laboratory. EPA has provided guidance for development of QC acceptance criteria (References 12 and 13).

8.3.4 After analysis of a minimum of 20 MS/MSD samples for each target analyte and surrogate, and if the laboratory chooses to develop and apply optional in-house QC limits, the laboratory should calculate and apply the optional in-house QC limits for recovery and RPD of future MS/MSD samples (Section 8.3). The optional in-house QC limits for recovery are calculated as the mean observed recovery ± 3 standard deviations, and the upper QC limit for RPD is calculated as the mean RPD plus 3 standard deviations of the RPDs. The in-house QC limits must be updated at least every two years and re-established after any major change in the analytical instrumentation or process. At least 80% of the analytes tested in the MS/MSD must have in-house QC acceptance criteria that are tighter than those in Table 4 and the remaining analytes (those not included in the 80%) must meet the acceptance criteria in Table 4. If an in-house QC limit for the RPD is greater than the limit in Table 4, then the limit in Table 4 must be used. Similarly, if an in-house lower limit for recovery is below the lower limit in Table 4, then the lower limit in Table 4 must be used, and if an in-house upper limit for recovery is above the upper limit in Table 4, then the upper limit in Table 4 must be used. The laboratory must evaluate surrogate recovery data in each sample against its in-house surrogate recovery limits. The laboratory may use 60 -140% as interim acceptance criteria for surrogate recoveries until in-house limits are developed. Alternatively, surrogate recovery limits may be developed from laboratory control charts. In-house QC acceptance criteria must be updated at least every two years.

8.4 Laboratory control sample (LCS) – A QC check sample (laboratory control sample, LCS; on-going precision and recovery sample, OPR) containing each single-component analyte of interest (Section 1.4) must be extracted, concentrated, and analyzed with each extraction batch of up to 20 samples (Section 3.1) to demonstrate acceptable recovery of the analytes of interest from a clean sample matrix. If multi-peak analytes are required, extract and prepare at least one as an LCS for each batch. Alternatively, the laboratory may set up a program where multi-peak LCS is rotated with a single-peak LCS.

8.4.1 Prepare the LCS by adding QC check sample concentrate (Sections 6.8.3 and 8.2.1) to reagent water. Include all analytes of interest (Section 1.4) in the LCS. The volume of reagent water must be the same as the nominal volume used for the sample, the DOC (Section 8.2), the blank (Section 8.5), and the MS/MSD (Section 8.3). Also add a volume of the surrogate solution (Section 6.8.6).

8.4.2 Analyze the LCS prior to analysis of samples in the extraction batch (Section 3.1). Determine the concentration (A) of each analyte. Calculate the percent recovery as:

$$P_s = \frac{A}{T} \times 100$$

where T is the true value of the concentration in the LCS.

8.4.3 For each analyte, compare the percent recovery (P) with its corresponding QC acceptance criterion in Table 4. For analytes of interest in Table 2 not listed in Table 4, use the QC acceptance criteria developed for the MS/MSD (Section 8.3.3.2), or limits based on laboratory control charts. If the recoveries for all analytes of interest fall within the designated ranges, analysis of blanks and field samples may proceed. If any individual recovery falls outside the range, proceed according to Section 8.4.4.

Note: *The large number of analytes in Tables 1 and 2 present a substantial probability that one or more will fail the acceptance criteria when all analytes are tested simultaneously. Because a re-test is allowed in event of failure (Sections 8.1.7 and 8.4.4), it may be prudent to extract and analyze two LCSs together and evaluate results of the second analysis against the QC acceptance criteria only if an analyte fails the first test.*

8.4.4 Repeat the test only for those analytes that failed to meet the acceptance criteria (P). If these analytes now pass, system performance is acceptable and analysis of blanks and samples may proceed. Repeated failure, however, will confirm a general problem with the measurement system. If this occurs, repeat the test using a fresh LCS (Section 8.2.1) or an LCS prepared with a fresh QC check sample concentrate (Section 8.2.1), or perform and document system repair. Subsequent to analysis of the LCS prepared with a fresh sample concentrate, or to system repair, repeat the LCS test (Section 8.4). If failure of the LCS indicates a systemic problem with samples in the batch, re-extract and re-analyze the samples in the batch. See Section 8.1.7 for disposition of repeated failures.

8.4.5 After analysis of 20 LCS samples, and if the laboratory chooses to develop and apply optional in-house QC limits, the laboratory should calculate and apply the optional in-house QC limits for recovery of future LCS samples (Section 8.4). Limits for recovery in the LCS should be calculated as the mean recovery ±3 standard deviations. A minimum of 80% of the analytes tested for in the LCS must have QC acceptance criteria tighter than those in Table 4, and the remaining analytes (those not included in the 80%) must meet the acceptance criteria in Table 4. If an in-house lower limit for recovery is lower than the lower limit in Table 4, the lower limit in Table 4 must be used, and if an in-house upper limit for recovery is higher than the upper limit in Table 4, the upper limit in Table 4 must be used. Many of the analytes and surrogates do not contain acceptance criteria. The laboratory should use 60 -140% as interim acceptance criteria for recoveries of spiked analytes and surrogates that do not have recovery limits specified in Table 4, and at least 80% of the surrogates must meet the 60 - 140% interim criteria until in-house LCS and surrogate limits are developed. Alternatively, acceptance criteria for analytes that do not have recovery limits in Table 4 may be based on laboratory control charts. In-house QC acceptance criteria must be updated at least every two years.

8.5 Blank – Extract and analyze a blank with each extraction batch (Section 3.1) to demonstrate that the reagents and equipment used for preparation and analysis are free from contamination.

8.5.1 Prepare the blank from reagent water and spike it with the surrogates. The volume of reagent water must be the same as the volume used for samples, the DOC (Section 8.2), the LCS (Section 8.4), and the MS/MSD (Section 8.3). Extract, concentrate, and analyze the blank using the same procedures and reagents used for the samples, LCS, and MS/MSD in the batch. Analyze the blank immediately after analysis of the LCS (Section 8.4) and prior to analysis of the MS/MSD and samples to demonstrate freedom from contamination.

8.5.2 If any analyte of interest is found in the blank at a concentration greater than the MDL for the analyte, at a concentration greater than one-third the regulatory compliance limit, or at a concentration greater than one-tenth the concentration in a sample in the batch (Section 3.1), whichever is greatest, analysis of samples must be halted and samples in the batch must be re-extracted and the extracts reanalyzed. Samples in a batch must be associated with an uncontaminated blank before the results for those samples may be reported or used for permitting or regulatory compliance purposes. If re-testing of blanks results in repeated failures, the laboratory should document the failures and report the problem and failures with the data.

8.6 Surrogate recovery – The laboratory must spike all samples with the surrogate standard spiking solution (Section 6.8.6) per Section 10.2.2 or 10.4.2, analyze the samples, and calculate the percent recovery of each surrogate. QC acceptance criteria for surrogates must be developed by the laboratory (Section 8.4). If any recovery fails its criterion, attempt to find and correct the cause of the failure, and if sufficient volume is available, re-extract another aliquot of the affected sample; otherwise, see Section 8.1.7 for disposition of repeated failures.

8.7 As part of the QC program for the laboratory, it is suggested but not required that method accuracy for wastewater samples be assessed and records maintained. After analysis of five or more spiked wastewater samples as in Section 8.3, calculate the average percent recovery ($\overline{X}$) and the standard deviation of the percent recovery (sp). Express the accuracy assessment as a percent interval from $\overline{X}$-2sp to $\overline{X}$+2sp. For example, if $\overline{X}$ = 90% and sp = 10%, the accuracy interval is expressed as 70 - 110%. Update the accuracy assessment for each analyte on a regular basis to ensure process control (e.g., after each 5 - 10 new accuracy measurements). If desired, statements of accuracy for laboratory performance, independent of performance on samples, may be developed using LCSs.

8.8 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to assess the precision of environmental measurements. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with another dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Collect samples as grab samples in glass bottles, or in refrigerated bottles using automatic sampling equipment. Collect 1-L of ambient waters, effluents, and other aqueous samples. If high concentrations of the analytes of interest are expected (e.g., for untreated effluents or in-process waters), collect a smaller volume (e.g., 250 mL), but not less than 100 mL, in addition to the 1-L sample. Follow conventional sampling practices, except do not pre-rinse the bottle with sample before collection. Automatic sampling equipment must be as free as possible of polyvinyl chloride

or other tubing or other potential sources of contamination. If needed, collect additional sample(s) for the MS/MSD (Section 8.3).

9.2 Ice or refrigerate the sample at ≤ 6 °C from the time of collection until extraction, but do not freeze. If aldrin is to be determined and residual chlorine is present, add 80 mg/L of sodium thiosulfate but do not add excess. Any method suitable for field use may be employed to test for residual chlorine (Reference 14). If sodium thiosulfate interferes in the determination of the analytes, an alternative preservative (e.g., ascorbic acid or sodium sulfite) may be used.

9.3 Extract all samples within seven days of collection and completely analyze within 40 days of extraction (Reference 1). If the sample will not be extracted within 72 hours of collection, adjust the sample pH to a range of 5.0 - 9.0 with sodium hydroxide solution or sulfuric acid. Record the volume of acid or base used.

10. Sample Extraction

10.1 This section contains procedures for separatory funnel liquid-liquid extraction (SFLLE, Section 10.2), continuous liquid-liquid extraction (CLLE, Section 10.4), and disk-based solid-phase extraction (SPE, Section 10.5). SFLLE is faster, but may not be as effective as CLLE for extracting polar analytes. SFLLE is labor intensive and may result in formation of emulsions that are difficult to break. CLLE is less labor intensive, avoids emulsion formation, but requires more time (18-24 hours), more hood space, and may require more solvent. SPE can be faster, unless the particulate load in an aqueous sample is so high that it slows the filtration process. If an alternative extraction scheme to those detailed in this method is used, all QC tests must be performed and all QC acceptance criteria must be met with that extraction scheme as an integral part of this method.

10.2 Separatory funnel liquid-liquid extraction (SFLLE)

10.2.1 The SFLLE procedure below assumes a sample volume of 1 L. When a different sample volume is extracted, adjust the volume of methylene chloride accordingly.

10.2.2 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into the separatory funnel. Pipet the surrogate standard spiking solution (Section 6.8.6) into the separatory funnel. If the sample will be used for the LCS or MS or MSD, pipet the appropriate QC check sample concentrate (Section 8.3 or 8.4) into the separatory funnel. Mix well. If the sample arrives in a larger sample bottle, 1 L may be measured in a graduated cylinder, then added to the separatory funnel.

Note: *Instances in which the sample is collected in an oversized bottle should be reported by the laboratory to the data user. Of particular concern is that fact that this practice precludes rinsing the empty bottle with solvent as described below, which could leave hydrophobic pesticides on the wall of the bottle, and underestimate the actual sample concentrations.*

10.2.3 Add 60 mL of methylene chloride to the sample bottle, seal, and shake for 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If an emulsion forms and the emulsion interface between the layers is more than one-third the volume of the solvent layer, employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring,

filtration of the emulsion through glass wool, use of phase-separation paper, centrifugation, salting, freezing, or other physical methods. Collect the methylene chloride extract in a flask. If the emulsion cannot be broken (recovery of less than 80% of the methylene chloride, corrected for the water solubility of methylene chloride), transfer the sample, solvent, and emulsion into the extraction chamber of a continuous extractor and proceed as described in Section 10.4.

10.2.4 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the flask. Perform a third extraction in the same manner. Proceed to macro-concentration (Section 10.3.1).

10.2.5 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to an appropriately sized graduated cylinder. Record the sample volume to the nearest 5 mL. Sample volumes may also be determined by weighing the container before and after extraction or filling to the mark with water.

10.3 Concentration

10.3.1 Macro concentration

10.3.1.1 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D concentrator so long as the requirements of Section 8.2 are met.

10.3.1.2 Pour the extract through a solvent-rinsed drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the flask and column with 20-30 mL of methylene chloride to complete the quantitative transfer.

10.3.1.3 If no cleanup is to be performed on the sample, add 500 µL (0.5 mL) of isooctane to the extract to act as a keeper during concentration.

10.3.1.4 Add one or two clean boiling chips and attach a three-ball Snyder column to the K-D evaporative flask. Pre-wet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 - 65 °C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 - 20 minutes. At the proper rate of evaporation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL or other determined amount, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.

10.3.1.5 If the extract is to be cleaned up by sulfur removal or acid back extraction, 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 in methylene chloride and proceed to sulfur removal (Section 11.5) or acid back extraction (Section 11.6). If the extract is to cleaned up using one of the other cleanup procedures or is to be injected into the GC, proceed to Kuderna-Danish micro-

concentration (Section 10.3.2) or nitrogen evaporation and solvent exchange (Section 10.3.3).

10.3.2 Kuderna-Danish micro concentration – Add another one or two clean boiling chips to the concentrator tube and attach a two-ball micro-Snyder column. Pre-wet the Snyder column by adding about 0.5 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 – 65 °C) so that the concentrator tube is partially immersed in hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 5 - 10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches approximately 1 mL or other required amount, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with approximately 0.2 mL of methylene chloride, and proceed to Section 10.3.3 for nitrogen evaporation and solvent exchange.

10.3.3 Nitrogen evaporation and solvent exchange – Extracts to be subjected to solid-phase cleanup (SPE) are exchanged into 1.0 mL of the SPE elution solvent (Section 6.7.2.2). Extracts to be subjected to Florisil® or alumina cleanups are exchanged into hexane. Extracts that have been cleaned up and are ready for analysis are exchanged into isooctane or hexane, to match the solvent used for the calibration standards.

10.3.3.1 Transfer the vial containing the sample extract to the nitrogen evaporation (blowdown) device (Section 5.2.5.2). Lower the vial into a 50-55 °C water bath and begin concentrating. During the solvent evaporation process, do not allow the extract to become dry. Adjust the flow of nitrogen so that the surface of the solvent is just visibly disturbed. A large vortex in the solvent may cause analyte loss.

10.3.3.2 Solvent exchange

10.3.3.2.1 When the volume of the liquid is approximately 500 µL, add 2 to 3 mL of the desired solvent (SPE elution solvent for SPE cleanup, hexane for Florisil® or alumina, or isooctane for final injection into the GC) and continue concentrating to approximately 500 µL. Repeat the addition of solvent and concentrate once more.

10.3.3.3.2 Adjust the volume of an extract to be cleaned up by SPE, Florisil®, or alumina to 1.0 mL. Proceed to extract cleanup (Section 11).

10.3.3.3 Extracts that have been cleaned up and are ready for analysis – Adjust the final extract volume to be consistent with the volume extracted and the sensitivity desired. The goal is for a full-volume sample (e.g., 1-L) to have a final extract volume of 10 mL, but other volumes may be used.

10.3.4 Transfer the concentrated extract to a vial with fluoropolymer-lined cap. Seal the vial and label with the sample number. Store in the dark at room temperature until ready for GC analysis. If GC analysis will not be performed on the same day, store the vial in the dark at ≤ 6 C. Analyze the extract by GC per the procedure in Section 12.

10.4 Continuous liquid/liquid extraction (CLLE)

10.4.1 Use CLLE when experience with a sample from a given source indicates an emulsion problem, or when an emulsion is encountered using SFLLE. CLLE may be used for all samples, if desired.

10.4.2 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Transfer the sample to the continuous extractor and, using a pipet, add surrogate standard spiking solution. If the sample will be used for the LCS, MS, or MSD, pipet the appropriate check sample concentrate (Section 8.2.1 or 8.3.2) into the separatory funnel. Mix well. Add 60 mL of methylene chloride to the sample bottle, seal, and shake for 30 seconds to rinse the inner surface. Transfer the solvent to the extractor.

10.4.3 Repeat the sample bottle rinse with two additional 50-100 mL portions of methylene chloride and add the rinses to the extractor.

10.4.4 Add a suitable volume of methylene chloride to the distilling flask (generally 200 - 500 mL) and sufficient reagent water to ensure proper operation of the extractor, and extract the sample for 18 - 24 hours. A shorter or longer extraction time may be used if all QC acceptance criteria are met. Test and, if necessary, adjust the pH of the water to a range of 5.0 - 9.0 during the second or third hour of the extraction. After extraction, allow the apparatus to cool, then detach the distilling flask. Dry, concentrate, solvent exchange, and transfer the extract to a vial with fluoropolymer-lined cap, per Section 10.3.

10.4.5 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to an appropriately sized graduated cylinder. Record the sample volume to the nearest 5 mL. Sample volumes may also be determined by weighing the container before and after extraction or filling to the mark with water.

10.5 Solid-phase extraction of aqueous samples

The steps in this section address the extraction of aqueous field samples using disk-based solid-phase extraction (SPE) media, based on an ATP approved by EPA in 1995 (Reference 20). This application of SPE is distinct from that used in this method for the cleanup of sample extracts in Section 11.2. Analysts must be careful not to confuse the equipment, supplies, or the procedural steps from these two different uses of SPE.

Note: *Changes to the extraction conditions described below may be made by the laboratory under the allowance for method flexibility described in Section 8.1, provided that the performance requirements in Section 8.2 are met. However, changes in SPE materials, formats, and solvents must meet the requirements in Section 8.1.2 and its subsections.*

10.5.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. If the sample contains particulates, let stand to settle out the particulates before extraction.

10.5.2 Extract the sample as follows:

10.5.2.1 Place a 90-mm standard filter apparatus on a vacuum filtration flask or manifold and attach to a vacuum source. The vacuum gauge must read at least 25 in. of mercury when all valves are closed. Position a 90-mm C18 extraction disk onto the filter screen. Wet the entire disk with methanol. To aid in filtering samples

with particulates, a 1-µm glass fiber filter or Empore® Filter Aid 400 can be placed on the top of the disk and wetted with methanol. Install the reservoir and clamp. Resume vacuum to dry the disk. Interrupt the vacuum. Wash the disk and reservoir with 20 mL of methylene chloride. Resume the vacuum briefly to pull methylene chloride through the disk. Interrupt the vacuum and allow the disk to soak for about a minute. Resume vacuum and completely dry the disk.

10.5.2.2 Condition the disk with 20 mL of methanol. Apply vacuum until nearly all the solvent has passed through the disk, interrupting it while solvent remains on the disk. Allow the disk to soak for about a minute. Resume vacuum to pull most of the methanol through, but interrupting it to leave a layer of methanol on the surface of the disk. Do not allow disk to dry.

For uniform flow and good recovery, it is critical the disk not be allowed to dry from now until the end of the extraction. Discard waste solvent. Rinse the disk with 20 mL of deionized water. Resume vacuum to pull most of the water through, but interrupt it to leave a layer of water on the surface of the disk. Do not allow the disk to dry. If disk does dry, recondition with methanol as above.

10.5.2.3 Add the water sample to the reservoir and immediately apply the vacuum. If particulates have settled in the sample, gently decant the clear layer into the apparatus until most of the sample has been processed. Then pour the remainder including the particulates into the reservoir. Empty the sample bottle completely. When the filtration is complete, dry the disk for three minutes. Turn off the vacuum.

10.5.3 Discard sample filtrate. Insert tube to collect the eluant. The tube should fit around the drip tip of the base. Reassemble the apparatus. Add 5.0 mL of acetone to the center of the disk, allowing it to spread evenly over the disk. Turn the vacuum on and quickly off when the filter surface nears dryness but still remains wet. Allow to soak for 15 seconds. Add 20 mL of methylene chloride to the sample bottle, seal and shake to rinse the inside of the bottle. Transfer the methylene chloride from the bottle to the filter. Resume the vacuum slowly so as to avoid splashing.

Interrupt the vacuum when the filter surface nears dryness but still remains wet. Allow disk to soak in solvent for 20 seconds. Rinse the reservoir glass and disk with 10 mL of methylene chloride. Resume vacuum slowly. Interrupt vacuum when disk is covered with solvent. Allow to soak for 20 seconds. Resume vacuum to dry the disk. Remove the sample tube.

10.5.4 Dry, concentrate, solvent exchange, and transfer the extract to a vial with fluoropolymer-lined cap, per Section 10.3.

10.5.5 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to an appropriately sized graduated cylinder. Record the sample volume to the nearest 5 mL. Sample volumes may also be determined by weighing the container before and after extraction or filling to the mark with water.

11. Extract Cleanup

11.1 Cleanup may not be necessary for relatively clean samples (e.g., treated effluents, groundwater, drinking water). If particular circumstances require the use of a cleanup procedure, the laboratory may use any or all of the procedures below or any other appropriate procedure (e.g., gel permeation chromatography). However, the laboratory must first repeat the tests in Sections 8.2, 8.3, and 8.4 to demonstrate that the requirements of those sections can be met using the cleanup procedure(s) as an integral part of this method. This is particularly important when the target analytes for the analysis include any of the single component pesticides in Table 2, because some cleanups have not been optimized for all of those analytes.

11.1.1 The solid-phase cartridge (Section 11.2) removes polar organic compounds such as phenols.

11.1.2 The Florisil® column (Section 11.3) allows for selected fractionation of the organochlorine analytes and will also eliminate polar interferences.

11.1.3 Alumina column cleanup (Section 11.4) also removes polar materials.

11.1.4 Elemental sulfur, which interferes with the electron capture gas chromatography of some of the pesticides, may be removed using activated copper, or TBA sulfite. Sulfur removal (Section 11.5) is required when sulfur is known or suspected to be present. Some chlorinated pesticides which also contain sulfur may be removed by this cleanup.

11.1.5 Acid back extraction (Section 11.6) may be useful for cleanup of PCBs and other compounds not adversely affected by sulfuric acid.

11.2 Solid-phase extraction (SPE) as a cleanup

In order to use the C18 SPE cartridge in Section 5.5.3.5 as a cleanup procedure, the sample extract must be exchanged from methylene chloride to methylene chloride:acetonitrile:hexane (50:3:47). Follow the solvent exchange steps in Section 10.3.3.2 prior to attempting solid-phase cleanup.

Note: *This application of SPE is distinct from that used in this method for the extraction of aqueous samples in Section 10.5. Analysts must be careful not to confuse the equipment, supplies, or procedural steps from these two different uses of SPE.*

11.2.1 Setup

11.2.1.1 Attach the VacElute Manifold (Section 5.5.3.2) to a water aspirator or vacuum pump with the trap and gauge installed between the manifold and vacuum source.

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

11.2.2 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 eluting solvent. Follow this pre-elution with 1 mL methylene chloride and three 10-mL portions of the elution solvent (Section 6.7.2.2) using vacuum for 5 minutes after each eluting solvent. Tap the cartridge lightly while under vacuum to dry between solvent rinses. The three portions of elution solvent may be collected and used as a cartridge blank, if desired.

Finally, elute the cartridge with 10 mL each of methanol and water, using the vacuum for 30 seconds after each eluant.

11.2.3 Extract cleanup

11.2.3.1 After cartridge washing (Section 11.2.2), release the vacuum and place the rack containing the 50-mL volumetric flasks (Section 5.5.3.4) in the vacuum manifold. Re-establish the vacuum at 5 to 10 psi.

11.2.3.2 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.

11.2.3.3 Elute each cartridge into its volumetric flask sequentially with three 10-mL portions of the methylene chloride:acetonitrile:hexane (50:3:47) elution solvent (Section 6.7.2.2), using vacuum for five minutes after each portion. Collect the eluants in the 50-mL volumetric flasks.

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

11.2.3.5 Concentrate the eluted extracts per Section 10.3.

11.3 Florisil®

In order to use Florisil® cleanup, the sample extract must be exchanged from methylene chloride to hexane. Follow the solvent exchange steps in Section 10.3.3.2 prior to attempting Florisil® cleanup.

Note: *Alternative formats for this cleanup may be used by the laboratory, including cartridges containing Florisil®. If an alternative format is used, consult the manufacturer's instructions and develop a formal documented procedure to replace the steps in Section 11.3 of this method and demonstrate that the alternative meets the relevant quality control requirements of this method.*

11.3.1 If the chromatographic column does not contain a frit at the bottom, place a small plug of pre-cleaned glass wool in the column (Section 5.2.4) to retain the Florisil®. Place the mass of Florisil® (nominally 20 g) predetermined by calibration (Section 7.8 and Table 6) in a chromatographic column. Tap the column to settle the Florisil® and add 1 to 2 cm of granular anhydrous sodium sulfate to the top.

11.3.2 Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil®. Just prior to exposure of the sodium sulfate layer to the air, stop the elution of the hexane by closing the stopcock on the chromatographic column. Discard the eluant.

11.3.3 Transfer the concentrated extract (Section 10.3.3) onto the column. Complete the transfer with two 1-mL hexane rinses, drawing the extract and rinses down to the level of the sodium sulfate.

11.3.4 Place a clean 500-mL K-D flask and concentrator tube under the column. Elute Fraction 1 with 200 mL of 6% (v/v) ethyl ether in hexane at a rate of approximately 5 mL/min. Remove the K-D flask and set it aside for later concentration. Elute Fraction 2 with 200 mL of 15% (v/v) ethyl ether in hexane into a second K-D flask. Elute Fraction 3 with 200

mL of 50% (v/v) ethyl ether in hexane into a third K-D flask. The elution patterns for the pesticides and PCBs are shown in Table 6.

11.3.5 Concentrate the fractions as in Section 10.3, except use hexane to prewet the column and set the water bath at about 85 °C. When the apparatus is cool, remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with hexane. Adjust the volume of Fraction 1 to approximately 10 mL for sulfur removal (Section 11.5), if required; otherwise, adjust the volume of the fractions to 10 mL, 1.0 mL, or other volume needed for the sensitivity desired. Analyze the concentrated extract by gas chromatography (Section 12).

11.4 Alumina

The sample extract must be exchanged from methylene chloride to hexane. Follow the solvent exchange steps in Section 10.3.3.2 prior to attempting alumina cleanup.

11.4.1 If the chromatographic column does not contain a frit at the bottom, place a small plug of pre-cleaned glass wool in the chromatographic column (Section 5.2.4) to retain the alumina. Add 10 g of alumina (Section 6.7.3) on top of the plug. Tap the column to settle the alumina. Place 1 - 2 g of anhydrous sodium sulfate on top of the alumina.

11.4.2 Close the stopcock and fill the column to just above the sodium sulfate with hexane. Add 25 mL of hexane. Open the stopcock and adjust the flow rate of hexane to approximately 2 mL/min. Do not allow the column to go dry throughout the elutions.

11.4.3 When the level of the hexane is at the top of the column, quantitatively transfer the extract to the column. When the level of the extract is at the top of the column, slowly add 25 mL of hexane and elute the column to the level of the sodium sulfate. Discard the hexane.

11.4.4 Place a K-D flask (Section 5.2.5.1.2) under the column and elute the pesticides with approximately 150 mL of hexane:ethyl ether (80:20 v/v). It may be necessary to adjust the volume of elution solvent for slightly different alumina activities.

11.4.5 Concentrate the extract per Section 10.3.

11.5 Sulfur removal – Elemental sulfur will usually elute in Fraction 1 of the Florisil® column cleanup. If Florisil® cleanup is not used, or to remove sulfur from any of the Florisil® fractions, use one of the sulfur removal procedures below. These procedures may be applied to extracts in hexane, ethyl ether, or methylene chloride.

Note: *Separate procedures using copper or TBA sulfite are provided in this section for sulfur removal. They may be used separately or in combination, if desired.*

11.5.1 Removal with copper (Reference 15)

Note: *Some of the analytes in Table 2 are not amenable to sulfur removal with copper (e.g., atrazine and diazinon). Therefore, before using copper to remove sulfur from an extract that will be analyzed for any of the non-PCB analytes in Table 2, the laboratory must demonstrate that the analytes can be extracted from an aqueous sample matrix that contains sulfur and recovered from an extract treated with copper. Acceptable performance can be demonstrated through the preparation and analysis of a matrix spike sample that meets the QC requirements for recovery.*

11.5.1.1 Quantitatively transfer the extract to a 40- to 50-mL flask or bottle. If there is evidence of water in the K-D or round-bottom flask after the transfer, rinse the flask with small portions of hexane:acetone (40:60) and add to the flask or bottle. Mark and set aside the concentration flask for future use.

11.5.1.2 Add 10 - 20 g of granular anhydrous sodium sulfate to the flask. Swirl to dry the extract.

11.5.1.3 Add activated copper (Section 6.7.4.1.4) and allow to stand for 30 - 60 minutes, swirling occasionally. If the copper does not remain bright, add more and swirl occasionally for another 30 - 60 minutes.

11.5.1.4 After drying and sulfur removal, quantitatively transfer the extract to a nitrogen-evaporation vial or tube and proceed to Section 10.3.3 for nitrogen evaporation and solvent exchange, taking care to leave the sodium sulfate and copper foil in the flask.

11.5.2 Removal with TBA sulfite

11.5.2.1 Using small volumes of hexane, quantitatively transfer the extract to a 40- to 50-mL centrifuge tube with fluoropolymer-lined screw cap.

11.5.2.2 Add 1 - 2 mL of TBA sulfite reagent (Section 6.7.4.2.4), 2 - 3 mL of 2-propanol, and approximately 0.7 g of sodium sulfite (Section 6.7.4.2.2) crystals to the tube. Cap and shake for 1 - 2 minutes. If the sample is colorless or if the initial color is unchanged, and if clear crystals (precipitated sodium sulfite) are observed, sufficient sodium sulfite is present. If the precipitated sodium sulfite disappears, add more crystalline sodium sulfite in approximately 0.5-g portions until a solid residue remains after repeated shaking.

11.5.2.3 Add 5 - 10 mL of reagent water and shake for 1 - 2 minutes. Centrifuge to settle the solids.

11.5.2.4 Quantitatively transfer the hexane (top) layer through a small funnel containing a few grams of granular anhydrous sodium sulfate to a nitrogen-evaporation vial or tube and proceed to Section 10.3.3 for micro-concentration and solvent exchange.

11.6 Acid back extraction (Section 6.1.2)

11.6.1 Quantitatively transfer the extract (Section 10.3.1.5) to a 250-mL separatory funnel.

11.6.2 Partition the extract against 50 mL of sulfuric acid solution (Section 6.1.2). Discard the aqueous layer. Repeat the acid washing until no color is visible in the aqueous layer, to a maximum of four washings.

11.6.3 Partition the extract against 50 mL of sodium chloride solution (Section 6.7.5). Discard the aqueous layer.

11.6.4 Proceed to Section 10.3.3 for micro-concentration and solvent exchange.

12. Gas Chromatography

12.1 Establish the same operating conditions used in Section 7.1 for instrument calibration.

12.2 If the internal standard calibration procedure is used, add the internal standard solution (Section 6.9.3) to the extract as close as possible to the time of injection to minimize the possibility of loss by evaporation, adsorption, or reaction. For example, add 1 µL of 10 µg/mL internal standard solution into the extract, assuming no dilutions. Mix thoroughly.

12.3 Simultaneously inject an appropriate volume of the sample extract or standard solution onto both columns, using split, splitless, solvent purge, large-volume, or on-column injection. Alternatively, if using a single-column GC configuration, inject an appropriate volume of the sample extract or standard solution onto each GC column independently. If the sample is injected manually, the solvent-flush technique should be used. The injection volume depends upon the technique used and the sensitivity needed to meet MDLs or reporting limits for regulatory compliance. Injection volumes must be the same for all extracts. Record the volume injected to the nearest 0.05 µL.

12.4 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.

12.5 Perform all qualitative and quantitative measurements as described in Sections 14 and 15. When standards and extracts are not being used for analyses, store them refrigerated at <6 °C, protected from light, in screw-cap vials equipped with un-pierced fluoropolymer-lined septa.

13. System and Laboratory Performance

13.1 At the beginning of each shift during which standards or extracts are analyzed, GC system performance and calibration must be verified for all analytes and surrogates on both column/detector systems. Adjustment and/or recalibration (per Section 7) are performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks and other QC samples, and standards be analyzed.

13.2 Inject an aliquot of the calibration verification standard (Section 6.8.4) on both columns. Inject an aliquot of each of the multi-component standards.

13.3 Retention times – The absolute retention times of the peak maxima shall be within ±2 seconds of the retention times in the calibration verification (Section 7.8).

13.4 GC resolution – Resolution is acceptable if the valley height between two peaks (as measured from the baseline) is less than 40% of the shorter of the two peaks.

13.4.1 DB-608 column – DDT and endrin aldehyde

13.4.2 DB-1701 column – alpha and gamma chlordane

Note: *If using other GC columns or stationary phases, these resolution criteria apply to these four target analytes and any other closely eluting analytes on those other GC columns.*

13.5 Decomposition of DDT and endrin – If DDT, endrin, or their breakdown products are to be determined, this test must be performed prior to calibration verification (Section 13.6). DDT decomposes to DDE and DDD. Endrin decomposes to endrin aldehyde and endrin ketone.

13.5.1 Inject 1 µL of the DDT and endrin decomposition solution (Section 6.8.7). As noted in Section 6.8.7, other injection volumes may be used as long as the concentrations of DDT and endrin in the solution are adjusted to introduce the masses of the two analytes into the instrument that are listed in Section 6.8.7.

13.5.2 Measure the areas of the peaks for DDT, DDE, DDD, endrin, endrin aldehyde, and endrin ketone in the chromatogram and calculate the percent breakdown as shown in the equations below:

$$\%\ \text{breakdown of DDT} = \frac{\text{sum of degradation peak areas (DDD + DDE)}}{\text{sum of all peak areas (DDT + DDE + DDD)}}\ \text{x } 100$$

$$\%\ \text{breakdown of Endrin} = \frac{\text{sum of degradation peak areas (Endrin aldehyde + Endrin ketone)}}{\text{sum of all peak areas (Endrin + Endrin aldehyde + Endrin ketone)}}\ \text{x } 100$$

13.5.3 Both the % breakdown of DDT and of endrin must be less than 20%, otherwise the system is not performing acceptably for DDT and endrin. In this case, repair the GC column system that failed and repeat the performance tests (Sections 13.2 to 13.6) until the specification is met.

Note: *DDT and endrin decomposition are usually caused by accumulations of particulates in the injector and in the front end of the column. Cleaning and silanizing the injection port liner, and breaking off a short section of the front end of the column will usually eliminate the decomposition problem. Either of these corrective actions may affect retention times, GC resolution, and calibration linearity.*

13.6 Calibration verification

13.6.1 Compute the percent recovery of each analyte and of the coeluting analytes, based on the initial calibration data (Section 7.5 or 7.6).

13.6.2 For each analyte or for coeluting analytes, compare the concentration with the limits for calibration verification in Table 4. For coeluting analytes, use the coeluting analyte with the least restrictive specification (the widest range). For analytes in Table 2 not listed in Table 4, QC acceptance criteria must be developed by the laboratory. EPA has provided guidance for development of QC acceptance criteria (References 13 and 14). If the recoveries for all analytes meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may continue. If, however, any recovery falls outside the calibration verification range, system performance is unacceptable for that analyte. If this occurs, repair the system and repeat the test (Section 13.6), or prepare a fresh calibration standard and repeat the test, or recalibrate (Section 7). See Section 8.1.7 for information on repeated test failures.

13.7 Laboratory control sample

13.7.1 Analyze the extract of the LCS (Section 6.8.3) extracted with each sample batch (Section 8.4). See Section 8.4 for criteria acceptance of the LCS.

13.7.2 It is suggested, but not required, that the laboratory update statements of data quality. Add results that pass the specifications in Section 13.7.3 to initial (Section 8.7) 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, sr. Express the accuracy as a recovery interval from R - 2sr to R + 2sr. For example, if R = 95% and sr = 5%, the accuracy is 85 to 105%.

13.8 Internal standard response – If internal standard calibration is used, verify that detector sensitivity has not changed by comparing the response (area or height) of each internal standard in the sample, blank, LCS, MS, and MSD to the response in calibration verification (Section 6.8.3). The peak area or height of the internal standard should be within 50% to 200% (1/2 to 2x) of its respective peak area or height in the verification standard. If the area or height is not within this range, compute the concentration of the analytes using the external standard method (Section 7.5). If the analytes are affected, re-prepare and reanalyze the sample, blank, LCS, MS, or MSD, and repeat the pertinent test.

14. Qualitative Identification

14.1 Identification is accomplished by comparison of data from analysis of a sample, blank, or other QC sample with data from calibration verification (Section 7.7.1 or 13.5), and with data stored in the retention-time and calibration libraries (Section 7.7). The retention time window is determined as described in Section 14.2. Identification is confirmed when retention time agrees on both GC columns, as described below. Alternatively, GC/MS identification may be used to provide another means of identification.

14.2 Establishing retention time windows

14.2.1 Using the data from the multi-point initial calibration (Section 7.4), determine the retention time in decimal minutes (not minutes:seconds) of each peak representing a single-component target analyte on each column/detector system. For the multi-component analytes, use the retention times of the five largest peaks in the chromatograms on each column/detector system.

14.2.2 Calculate the standard deviation of the retention times for each single-component analyte on each column/detector system and for the three to five exclusive (unique large) peaks for each multi-component analyte.

14.2.3 Define the width of the retention time window as three times that standard deviation. Establish the center of the retention time window for each analyte by using the absolute retention time for each analyte from the calibration verification standard at the beginning of the analytical shift. For samples run during the same shift as an initial calibration, use the retention time of the mid-point standard of the initial calibration. If the calculated RT window is less than 0.02 minutes, then use 0.02 minutes as the window.

Note: *Procedures for establishing retention time windows from other sources may be employed provided that they are clearly documented and provide acceptable performance. Such performance may be evaluated using the results for the spiked QC samples described in this method, such as laboratory control samples and matrix spike samples.*

14.2.4 The retention time windows must be recentered when a new GC column is installed or if a GC column has been shortened during maintenance to a degree that the retention times of analytes in the calibration verification standard have shifted close to the lower limits of the established retention time windows.

14.2.5 RT windows should be checked periodically by examining the peaks in spiked samples such as the LCS or MS/MSD to confirm that peaks for known analytes are properly identified.

14.2.6 If the retention time of an analyte in the calibration (Section 7.4) varies by more than 5 seconds across the calibration range as a function of the concentration of the standard, using the standard deviation of the retention times (Section 14.2.3) to set the width of the retention time window may not adequately serve to identify the analyte in question under routine conditions. In such cases, data from additional analyses of standards may be required to adequately model the chromatographic behavior of the analyte.

14.3 Identifying the analyte in a sample

14.3.1 In order to identify a single-component analyte from analysis of a sample, blank, or other QC sample, the peak representing the analyte must fall within its respective retention time windows on both column/detector systems (as defined in Section 14.2). That identification is further supported by the comparison of the numerical results on both columns, as described in Section 15.7.

14.3.2 In order to identify a multi-component analyte, pattern matching (fingerprinting) may be used, or the three to five exclusive (unique and largest) peaks for that analyte must fall within their respective retention time windows on both column/detector systems (as defined in Section 14.2). That identification is further supported by the comparison of the numerical results on both columns, as described in Section 15.7. Alternatively, GC/MS identification may be used. Differentiation among some of the Aroclors may require evaluation of more than five peaks to ensure correct identification.

14.4 GC/MS confirmation

When the concentration of an analyte is sufficient and the presence or identity is suspect, its presence should be confirmed by GC/MS. In order to match the sensitivity of the GC/ECD, confirmation would need to be by GC/MS-SIM, or the estimated concentration would need to be 100 times higher than the GC/ECD calibration range. The extract may be concentrated by an additional amount to allow a further attempt at GC/MS confirmation.

14.5 Additional information that may aid the laboratory in the identification of an analyte

The occurrence of peaks eluting near the retention time of an analyte of interest increases the probability of a false positive for the analyte. If the concentration is insufficient for confirmation by GC/MS, the laboratory may use the cleanup procedures in this method (Section 11) on a new sample aliquot to attempt to remove the interferent. After attempts at cleanup are exhausted, the following steps may be helpful to assure that the substance that appears in the RT windows on both columns is the analyte of interest.

14.5.1 Determine the consistency of the RT data for the analyte on each column. For example, if the RT is very stable (i.e., varies by no more than a few seconds) for the calibration, calibration verification, blank, LCS, and MS/MSD, the RT for the analyte of interest in the

sample should be within this variation regardless of the window established in Section 14.2. If the analyte is not within this variation on both columns, it is likely not present.

14.5.2 The possibility exists that the RT for the analyte in a sample could shift if extraneous materials are present. This possibility may be able to be confirmed or refuted by the behavior of the surrogates in the sample. If multiple surrogates are used that span the length of the chromatographic run, the RTs for the surrogates on both columns are consistent with their RTs in calibration, calibration verification, blank, LCS, and MS/MSD, it is unlikely that the RT for the analyte of interest has shifted.

14.5.3 If the RT for the analyte is shifted slightly later on one column and earlier on the other, and the surrogates have not shifted, it is highly unlikely that the analyte is present, because shifts nearly always occur in the same direction on both columns.

15. Quantitative Determination

15.1 External standard quantitation – Calculate the concentration of the analyte in the extract using the calibration curve or average calibration factor determined in calibration (Section 7.5.2) and the following equation:

$$C_{ex} = \frac{A_s}{CF}$$

where:
C_{ex} = Concentration of the analyte in the extract (ng/mL)
A_s = Peak height or area for the analyte in the standard or sample
CF = Calibration factor, as defined in Section 7.5.1

15.2 Internal standard quantitation – Calculate the concentration of the analyte in the extract using the calibration curve or average response factor determined in calibration (Section 7.6.2) and the following equation:

$$C_{ex} = \frac{A_s \times C_{is}}{A_{is} \times RF}$$

where:
C_{ex} = Concentration of the analyte in the extract (ng/mL)
A_s = Peak height or area for the analyte in the standard or sample
C_{is} = Concentration of the internal standard (ng/mL)
A_{is} = Area of the internal standard
RF = Response factor, as defined in Section 7.6.1

15.3 Calculate the concentration of the analyte in the sample using the concentration in the extract, the extract volume, the sample volume, and the dilution factor, per the following equation:

$$C_s = \frac{C_{ex} \times V_{ex} \times DF}{V_s \times 1000}$$

where:
C_s = Concentration of the analyte in the sample (µg/L)
V_{ex} = Final extract volume (mL)
C_{ex} = Concentration in the extract (ng/mL)

V_s = Volume of sample (L)
DF = Dilution factor

and the factor of 1,000 in the denominator converts the final units from ng/L to µg/L

15.4 If the concentration of any target analyte exceeds the calibration range, either extract and analyze a smaller sample volume, or dilute and analyze the diluted extract.

15.5 Quantitation of multi-component analytes

15.5.1 PCBs as Aroclors

Quantify an Aroclor by comparing the sample chromatogram to that of the most similar Aroclor standard as indicated in Section 14.3.2. Compare the responses of 3 to 5 major peaks in the calibration standard for that Aroclor with the peaks observed in the sample extract. The amount of Aroclor is calculated using the individual calibration factor for each of the 3 to 5 characteristic peaks chosen in Section 7.5.1. Determine the concentration of each of the characteristic peaks, using the average calibration factor calculated for that peak in Section 7.5.2, and then those 3 to 5 concentrations are averaged to determine the concentration of that Aroclor.

15.5.2 Other multi-component analytes

Quantify any other multi-component analytes (technical chlordane or toxaphene) using the same peaks used to develop the average calibration factors in Section 7.5.2. Determine the concentration of each of the characteristic peaks, and then the concentrations represented by those characteristic peaks are averaged to determine the concentration of the analyte. Alternatively, for toxaphene, the analyst may determine the calibration factor in Section 7.5.2 by summing the areas of all of the peaks for the analyte and using the summed of the peak areas in the sample chromatogram to determine the concentration. However, the approach used for toxaphene must be the same for the calibration and the sample analyses.

15.6 Reporting of results

As noted in Section 1.6.1, EPA has promulgated this method at 40 CFR part 136 for use in wastewater compliance monitoring under the National Pollutant Discharge Elimination System (NPDES). The data reporting practices described here are focused on such monitoring needs and may not be relevant to other uses of the method.

15.6.1 Report results for wastewater samples in µg/L without correction for recovery. (Other units may be used if required by in a permit.) Report all QC data with the sample results.

15.6.2 Reporting level

Unless specified otherwise by a regulatory authority or in a discharge permit, results for analytes that meet the identification criteria are reported down to the concentration of the ML established by the laboratory through calibration of the instrument (see Section 7.5 or 7.6 and the glossary for the derivation of the ML). EPA considers the terms “reporting limit,” “quantitation limit,” and “minimum level” to be synonymous.

15.6.2.1 Report the lower result from the two columns (see Section 15.7 below) for each analyte in each sample or QC standard at or above the ML to 3 significant

figures. Report a result for each analyte in each sample or QC standard below the ML as "<ML," where "ML" is the concentration of the analyte at the ML (e.g., if the ML is 10 µg/L, then report the result as <10 µg/L), or as required by the regulatory authority or permit. Report a result for each analyte in a blank at or above the MDL to 2 significant figures. Report a result for each analyte found in a blank below the MDL as "<MDL," where MDL is the concentration of the analyte at the MDL, or as required by the regulatory/control authority or permit.

15.6.2.2 In addition to reporting results for samples and blank(s) separately, the concentration of each analyte in a blank or field blank associated with that sample may be subtracted from the result for that sample, but only if requested or required by a regulatory authority or in a permit. In this case, both the sample result and the blank results must be reported together.

15.6.2.3 Report the result for an analyte in a sample or extract that has been diluted at the least dilute level at which the peak area is within the calibration range (i.e., above the ML for the analyte) and the MS/MSD recovery and RPD are within their respective QC acceptance criteria (Table 4). This may require reporting results for some analytes from different analyses.

Results for each analyte in MS/MSD samples should be reported from the same GC column as used to report the results for that analyte in the unspiked sample. If the MS/MSD recoveries and RPDs calculated in this manner do not meet the acceptance criteria in Table 4, the analyst may use the results from the other GC column to determine if the MS/MSD results meet the acceptance criteria. If such a situation occurs, the results for the sample should be recalculated using the same GC column data as used for the MS/MSD samples, and reported with appropriate annotations that alert the data user of the issue.

15.6.2.4 Results from tests performed with an analytical system that is not in control (i.e., that does not meet acceptance criteria for all of QC tests in this method) must not be reported or otherwise used for permitting or regulatory compliance purposes, but do not relieve a discharger or permittee of reporting timely results. See Section 8.1.7 for dispositions of failures. If the holding time would be exceeded for a re-analysis of the sample, the regulatory/control authority should be consulted for disposition.

15.6.3 Analyze the sample by GC/MS or on a third column when analytes have co-eluted or interfere with determination on both columns.

Note: *Dichlone and kepone do not elute from the DB-1701 column and must be confirmed on a DB-5 column, or by GC/MS.*

15.7 Quantitative information that may aid in the confirmation of the presence of an analyte

15.7.1 As noted in Section 14.3, the relative agreement between the numerical results from the two GC columns may be used to support the identification of the target analyte by providing evidence that co-eluting interferences are not present at the retention time of the target analyte. Calculate the percent difference (%D) between the results for the analyte from both columns, as follows:

$$\%D = \frac{\text{Higher result} - \text{Lower result}}{\text{Higher result}} \text{ x } 100$$

In general, if the %D of the two results is less than 50% (e.g., a factor of 2), then the pesticide is present. This %D is generous and allows for the pesticide that has the largest measurement error.

Note: *Laboratories may employ metrics less than 50% for this comparison, including those specified in other analytical methods for these pesticides (e.g., CLP or SW-846).*

15.7.2 If the amounts do not agree, and the RT data indicate the presence of the analyte (per Section 14), it is likely that a positive interference is present on the column that yielded the higher result. That interferent may be represented by a separate peak on the other column that does not coincide with the retention time of any of the target analytes. If the interfering peak is evident on the other column, report the result from that column and advise the data user that the interference resulted in a %D value greater than 50%.

If an interferent is not identifiable on the second column, then the results must be reported as "not detected" at the lower concentration. In this event, the pesticide is not confirmed and the reporting limit is elevated. See Section 8.1.7 for disposition of problem results.

Note: *The resulting elevation of the reporting limit may not meet the requirements for compliance monitoring and the use of additional cleanup procedures may be required.*

16. Analysis of Complex Samples

16.1 Some samples may contain high levels (greater than 1 µg/L) of the analytes of interest, interfering analytes, and/or polymeric materials. Some samples may not concentrate to 1.0 mL (Section 10.3.3.3.2); others may overload the GC column and/or detector.

16.2 When an interference is known or suspected to be present, the laboratory should attempt to clean up the sample extract using the SPE cartridge (Section 11.2), by Florisil® (Section 11.3), Alumina (Section 11.4), sulfur removal (Section 11.5), or another clean up procedure appropriate to the analytes of interest. If these techniques do not remove the interference, the extract is diluted by a known factor and reanalyzed (Section 12). Dilution until the extract is lightly colored is preferable. Typical dilution factors are 2, 5, and 10.

16.3 Recovery of surrogate(s) – In most samples, surrogate recoveries will be similar to those from reagent water. If surrogate recovery is outside the limits developed in Section 8.6, re-extract and reanalyze the sample if there is sufficient sample and if it is within the 7-day extraction holding time. If surrogate recovery is still outside this range, extract and analyze one-tenth the volume of sample to overcome any matrix interference problems. If a sample is highly colored or suspected to be high in concentration, a 1-L sample aliquot and a 100-mL sample aliquot could be extracted simultaneously and still meet the holding time criteria, while providing information about a complex matrix.

16.4 Recovery of the matrix spike and matrix spike duplicate (MS/MSD) – In most samples, MS/MSD recoveries will be similar to those from reagent water. If either the MS or MSD recovery is outside the range specified in Section 8.3.3, one-tenth the volume of sample is spiked and analyzed. If the

matrix spike recovery is still outside the range, the result for the unspiked sample may not be reported or used for permitting or regulatory compliance purposes. See Section 8.1.7 for dispositions of failures. Poor matrix spike recovery does not relieve a discharger or permittee of reporting timely results.

17. Method Performance

17.1 This method was tested for linearity of spike recovery from reagent water and has been demonstrated to be applicable over the concentration range from 4x MDL to 1000x MDL with the following exceptions: Chlordane recovery at 4x MDL was low (60%); Toxaphene recovery was demonstrated linear over the range of 10x MDL to 1000x MDL (Reference 3).

17.2 The 1984 version of this method was tested by 20 laboratories using reagent water, drinking water, surface water, and three industrial wastewaters spiked at six concentrations (Reference 2). Concentrations used in the study ranged from 0.5 to 30 μg/L for single-component pesticides and from 8.5 to 400 μg/L for multi-component analytes. These data are for a subset of analytes described in the current version of the method.

17.3 During the development of Method 1656, a similar EPA procedure for the organochlorine pesticides, single-operator precision, overall precision, and method accuracy were found to be directly related to the concentration of the analyte and essentially independent of the sample matrix. Linear equations to describe these relationships are presented in Table 5.

18. Pollution Prevention

18.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operations. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, the laboratory should use pollution prevention techniques to address waste generation. When wastes cannot be reduced at the source, the Agency recommends recycling as the next best option.

18.2 The analytes in this method are used in extremely small amounts and pose little threat to the environment when managed properly. Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards. This method utilizes significant quantities of methylene chloride. Laboratories are encouraged to recover and recycle this and other solvents during extract concentration.

18.3 For information about pollution prevention that may be applied to laboratories and research institutions, consult “Less is Better: Laboratory Chemical Management for Waste Reduction” (Reference 19), available from the American Chemical Society’s Department of Governmental Relations and Science Policy, 1155 16th Street NW, Washington DC 20036, 202-872-4477.

19. Waste Management

19.1 The laboratory is responsible for complying with all Federal, State, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance is also required with any sewage discharge permits

and regulations. An overview of requirements can be found in Environmental Management Guide for Small Laboratories (EPA 233-B-98-001).

19.2 Samples at pH <2, or pH >12, are hazardous and must be handled and disposed of as hazardous waste, or neutralized and disposed of in accordance with all federal, state, and local regulations. It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions. The laboratory using this method has the responsibility to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance is also required with any sewage discharge permits and regulations. For further information on waste management, see "The Waste Management Manual for Laboratory Personnel," also available from the American Chemical Society at the address in Section 18.3.

19.3 Many analytes in this method decompose above 500 ºC. Low-level waste such as absorbent paper, tissues, animal remains, and plastic gloves may be burned in an appropriate incinerator. Gross quantities of neat or highly concentrated solutions of toxic or hazardous chemicals should be packaged securely and disposed of through commercial or governmental channels that are capable of handling toxic wastes.

19.4 For further information on waste management, consult The Waste Management Manual for Laboratory Personnel and Less is Better-Laboratory Chemical Management for Waste Reduction, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036, 202-872-4477.

20. References

1. "Determination of Pesticides and PCBs in Industrial and Municipal Wastewaters," EPA 600/4-82-023, National Technical Information Service, PB82-214222, Springfield, Virginia 22161, April 1982.

2. "EPA Method Study 18 Method 608-Organochlorine Pesticides and PCBs," EPA 600/4-84-061, National Technical Information Service, PB84-211358, Springfield, Virginia 22161, June 1984.

3. "Method Detection Limit and Analytical Curve Studies, EPA Methods 606, 607, and 608," Special letter report for EPA Contract 68-03-2606, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, June 1980.

4. ASTM Annual Book of Standards, Part 31, D3694-78. "Standard Practice for Preparation of Sample Containers and for Preservation of Organic Constituents," American Society for Testing and Materials, Philadelphia.

5. Giam, C.S., Chan, H.S., and Nef, G.S. "Sensitive Method for Determination of Phthalate Ester Plasticizers in Open-Ocean Biota Samples," Analytical Chemistry, 47: 2225 (1975).

6. Giam, C.S. and Chan, H.S. "Control of Blanks in the Analysis of Phthalates in Air and Ocean Biota Samples," U.S. National Bureau of Standards, Special Publication 442, pp. 701-708, 1976.

7. Solutions to Analytical Chemistry Problems with Clean Water Act Methods, EPA 821-R-07-002, March 2007.

8. "Carcinogens-Working With Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.

9. "Occupational Exposure to Hazardous Chemicals in Laboratories," (29 CFR Part 1910, Subpart 1450), Occupational Safety and Health Administration, OSHA.

10. 40 CFR 136.6(b)(4)(j).

11. Mills, P.A. "Variation of Florisil Activity: Simple Method for Measuring Absorbent Capacity and Its Use in Standardizing Florisil Columns," Journal of the Association of Official Analytical Chemists, 51: 29, (1968).

12. 40 CFR 136.6(b)(2)(i).

13. Protocol for EPA Approval of New Methods for Organic and Inorganic Analytes in Wastewater and Drinking Water (EPA-821-B-98-003) March 1999.

14. Methods 4500 Cl F and 4500 Cl G, Standard Methods for the Examination of Water and Wastewater, published jointly by the American Public Health Association, American Water Works Association, and Water Environment Federation, 1015 Fifteenth St., Washington, DC 20005, 20th Edition, 2000.

15. "Manual of Analytical Methods for the Analysis of Pesticides in Human and Environmental Samples," EPA-600/8-80-038, U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, North Carolina.

16. USEPA, 2000, Method 1656 Organo-Halide Pesticides In Wastewater, Soil, Sludge, Sediment, and Tissue by GC/HSD, EPA-821-R-00-017, September 2000.

17. USEPA, 2010, Method 1668C Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS, EPA-820-R-10-005, April 2010.

18. USEPA, 2007, Method 1699: Pesticides in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS, EPA-821-R-08-001, December 2007.

19. "Less is Better," American Chemical Society on-line publication (2002), www.acs.org/content/dam/acsorg/about/governance/committees/chemicalsafety/publications/less-is-better.pdf

20. EPA Method 608 ATP 3M0222, An alternative test procedure for the measurement of organochlorine pesticides and polychlorinated biphenyls in waste water. Federal Register, Vol. 60, No. 148 August 2, 1995.

21. Tables

TABLE 1 – PESTICIDES [1]			
Analyte	**CAS Number**	**MDL[2] (ng/L)**	**ML[3] (ng/L)**
Aldrin	309-00-2	4	12
alpha-BHC	319-84-6	3	9
beta-BHC	319-85-7	6	18
delta-BHC	319-86-8	9	27
gamma-BHC (Lindane)	58-89-9	4	12
alpha-Chlordane [4]	5103-71-9	14	42
gamma-Chlordane [4]	5103-74-2	14	42
4,4'-DDD	72-54-8	11	33
4,4'-DDE	72-55-9	4	12
4,4'-DDT	50-29-3	12	36
Dieldrin	60-57-1	2	6
Endosulfan I	959-98-8	14	42
Endosulfan II	33213-65-9	4	12
Endosulfan sulfate	1031-07-8	66	198
Endrin	72-20-8	6	18
Endrin aldehyde	7421-93-4	23	70
Heptachlor	76-44-8	3	9
Heptachlor epoxide	1024-57-3	83	249

[1] All analytes in this table are Priority Pollutants (40 CFR part 423, appendix A)
[2] 40 CFR part 136, appendix B, June 30, 1986.
[3] ML = Minimum Level – see Glossary for definition and derivation, calculated as 3 times the MDL.
[4] MDL based on the MDL for Chlordane

TABLE 2 – ADDITIONAL ANALYTES			
Analyte	**CAS Number**	**MDL[3] (ng/L)**	**ML[4] (ng/L**
Acephate	30560-19-1		
Alachlor	15972-60-8		
Atrazine	1912-24-9		
Benfluralin (Benefin)	1861-40-1		
Bromacil	314-40-9		
Bromoxynil octanoate	1689-99-2		
Butachlor	23184-66-9		
Captafol	2425-06-1		
Captan	133-06-2		
Carbophenothion (Trithion)	786-19-6		
Chlorobenzilate	510-15-6		
Chloroneb (Terraneb)	2675-77-6		
Chloropropylate (Acaralate)	5836-10-2		
Chlorothalonil	1897-45-6		
Cyanazine	21725-46-2		
DCPA (Dacthal)	1861-32-1		
2,4'-DDD	53-19-0		
2,4'-DDE	3424-82-6		
2,4'-DDT	789-02-6		
Diallate (Avadex)	2303-16-4		
1,2-Dibromo-3-chloropropane (DBCP)	96-12-8		
Dichlone	117-80-6		
Dichloran	99-30-9		
Dicofol	115-32-2		
Endrin ketone	53494-70-5		
Ethalfluralin (Sonalan)	55283-68-6		
Etridiazole	2593-15-9		
Fenarimol (Rubigan)	60168-88-9		
Hexachlorobenzene [1]	118-74-1		
Hexachlorocyclopentadiene [1]	77-47-4		
Isodrin	465-73-6		
Isopropalin (Paarlan)	33820-53-0		
Kepone	143-50-0		
Methoxychlor	72-43-5		
Metolachlor	51218-45-2		
Metribuzin	21087-64-9		
Mirex	2385-85-5		
Nitrofen (TOK)	1836-75-5		
cis-Nonachlor	5103-73-1		
trans-Nonachlor	39765-80-5		
Norfluorazon	27314-13-2		
Octachlorostyrene	29082-74-4		
Oxychlordane	27304-13-8		
PCNB (Pentachloronitrobenzene)	82-68-8		
Pendamethalin (Prowl)	40487-42-1		
cis-Permethrin	61949-76-6		
trans-Permethrin	61949-77-7		
Perthane (Ethylan)	72-56-0		

TABLE 2 – ADDITIONAL ANALYTES			
Analyte	**CAS Number**	**MDL[3] (ng/L)**	**ML[4] (ng/L**
Propachlor	1918-16-7		
Propanil	709-98-8		
Propazine	139-40-2		
Quintozene	82-68-8		
Simazine	122-34-9		
Strobane	8001-50-1		
Technazene	117-18-0		
Technical Chlordane[2]			
Terbacil	5902-51-2		
Terbuthylazine	5915-41-3		
Toxaphene [1]	8001-35-2	240	720
Trifluralin	1582-09-8		
PCB-1016 [1]	12674-11-2		
PCB-1221 [1]	11104-28-2		
PCB-1232 [1]	11141-16-5		
PCB-1242 [1]	53469-21-9	65	95
PCB-1248 [1]	12672-29-6		
PCB-1254 [1]	11097-69-1		
PCB-1260 [1]	11096-82-5		
PCB-1268	11100-14-4		

[1] Priority Pollutants (40 CFR part 423, appendix A)

[2] Technical Chlordane may be used in cases where historical reporting has only been for this form of Chlordane.

[3] 40 CFR part 136, appendix B, June 30, 1986.

[4] ML = Minimum Level – see Glossary for definition and derivation, calculated as 3 times the MDL.

TABLE 3 – EXAMPLE RETENTION TIMES[1]		
	Retention time (min)[2]	
Analyte	**DB-608**	**DB-1701**
Acephate	5.03	–[3]
Trifluralin	5.16	6.79
Ethalfluralin	5.28	6.49
Benfluralin	5.53	6.87
Diallate-A	7.15	6.23
Diallate-B	7.42	6.77
alpha-BHC	8.14	7.44
PCNB	9.03	7.58
Simazine	9.06	9.29
Atrazine	9.12	9.12
Terbuthylazine	9.17	9.46
gamma-BHC (Lindane)	9.52	9.91
beta-BHC	9.86	11.90
Heptachlor	10.66	10.55
Chlorothalonil	10.66	10.96
Dichlone	10.80	–[4]
Terbacil	11.11	12.63
delta-BHC	11.20	12.98
Alachlor	11.57	11.06
Propanil	11.60	14.10
Aldrin	11.84	11.46
DCPA	12.18	12.09
Metribuzin	12.80	11.68
Triadimefon	12.99	13.57
Isopropalin	13.06	13.37
Isodrin	13.47	11.12
Heptachlor epoxide	13.97	12.56
Pendamethalin	14.21	13.46
Bromacil	14.39	–[3]
alpha-Chlordane	14.63	14.20
Butachlor	15.03	15.69
gamma-Chlordane	15.24	14.36
Endosulfan I	15.25	13.87
4,4’-DDE	16.34	14.84
Dieldrin	16.41	15.25
Captan	16.83	15.43
Chlorobenzilate	17.58	17.28
Endrin	17.80	15.86
Nitrofen (TOK)	17.86	17.47
Kepone	17.92	–[3, 5]
4,4’-DDD	18.43	17.77
Endosulfan II	18.45	18.57

TABLE 3 – EXAMPLE RETENTION TIMES[1]		
	Retention time (min)[2]	
Analyte	**DB-608**	**DB-1701**
Bromoxynil octanoate	18.85	18.57
4,4'-DDT	19.48	18.32
Carbophenothion	19.65	18.21
Endrin aldehyde	19.72	19.18
Endosulfan sulfate	20.21	20.37
Captafol	22.51	21.22
Norfluorazon	20.68	22.01
Mirex	22.75	19.79
Methoxychlor	22.80	20.68
Endrin ketone	23.00	21.79
Fenarimol	24.53	23.79
cis-Permethrin	25.00	23.59
trans-Permethrin	25.62	23.92
PCB-1016		
PCB-1221		
PCB-1232		
PCB-1242		
PCB-1248		
PCB-1254		
PCB-1260 (5 peaks)	15.44	14.64
	15.73	15.36
	16.94	16.53
	17.28	18.70
	19.17	19.92
Toxaphene (5 peaks)	16.60	16.60
	17.37	17.52
	18.11	17.92
	19.46	18.73
	19.69	19.00

[1] Data from EPA Method 1656 (Reference 16)

[2] Columns: 30-m long x 0.53-mm ID fused-silica capillary; DB-608, 0.83 μm; and DB-1701, 1.0 μm.
Conditions suggested to meet retention times shown: 150 °C for 0.5 minute, 150-270 °C at 5 °C/min, and 270 °C until *trans*-Permethrin elutes.
Carrier gas flow rates approximately 7 mL/min.

[3] Does not elute from DB-1701 column at level tested.

[4] Not recovered from water at the levels tested.

[5] Dichlone and Kepone do not elute from the DB-1701 column and should be confirmed on DB-5.

TABLE 4 – QC ACCEPTANCE CRITERIA						
Analyte	**Calibration verification (%)**	**Test concen-tration (µg/L)**	**Limit for s (% SD)**	**Range for $\overline{X}$ (%)**	**Range for P (%)**	**Maximum MS/MSD RPD (%)**
Aldrin	75 - 125	2.0	25	54 - 130	42 - 140	35
alpha-BHC	69 - 125	2.0	28	49 - 130	37 - 140	36
beta-BHC	75 - 125	2.0	38	39 - 130	17 - 147	44
delta-BHC	75 - 125	2.0	43	51 - 130	19 - 140	52
gamma-BHC	75 - 125	2.0	29	43 - 130	32 - 140	39
alpha-Chlordane	73 - 125	50.0	24	55 - 130	45 - 140	35
gamma-Chlordane	75 - 125	50.0	24	55 - 130	45 - 140	35
4,4'-DDD	75 - 125	10.0	32	48 - 130	31 - 141	39
4,4'-DDE	75 - 125	2.0	30	54 - 130	30 - 145	35
4,4'-DDT	75 - 125	10.0	39	46 - 137	25 - 160	42
Dieldrin	48 - 125	2.0	42	58 - 130	36 - 146	49
Endosulfan I	75 - 125	2.0	25	57 - 141	45 - 153	28
Endosulfan II	75 - 125	10.0	63	22 - 171	D - 202	53
Endosulfan sulfate	70 - 125	10.0	32	38 - 132	26 - 144	38
Endrin	5 - 125	10.0	42	51 - 130	30 - 147	48
Heptachlor	75 - 125	2.0	28	43 - 130	34 - 140	43
Heptachlor epoxide	75 - 125	2.0	22	57 - 132	37 - 142	26
Toxaphene	68 - 134	50.0	30	56 - 130	41 - 140	41
PCB-1016	75 - 125	50.0	24	61 - 103	50 - 140	36
PCB-1221	75 - 125	50.0	50	44 - 150	15 - 178	48
PCB-1232	75 - 125	50.0	32	28 - 197	10 - 215	25
PCB-1242	75 - 125	50.0	26	50 - 139	39 - 150	29
PCB-1248	75 - 125	50.0	32	58 - 140	38 - 158	35
PCB-1254	75 - 125	50.0	34	44 - 130	29 - 140	45
PCB-1260	75 - 125	50.0	28	37 - 130	8 - 140	38

S = Standard deviation of four recovery measurements for the DOC (Section 8.2.4).
$\overline{X}$ = Average of four recovery measurements for the DOC (Section 8.2.4)
P = Recovery for the LCS (Section 8.4.3)

Note: *These criteria were developed from data in Table 5 (Reference 2). Where necessary, limits for recovery have been broadened to assure applicability to concentrations below those in Table 5.*

TABLE 5 – PRECISION AND RECOVERY AS FUNCTIONS OF CONCENTRATION			
Analyte	**Recovery, X' (µg/L)**	**Single analyst precision, s_r' (µg/L)**	**Overall precision, S' (µg/L)**
Aldrin	0.81C + 0.04	0.16($\overline{X}$) - 0.04	0.20($\overline{X}$) - 0.01
alpha-BHC	0.84C + 0.03	0.13($\overline{X}$) + 0.04	0.23($\overline{X}$) - 0.00
beta-BHC	0.81C + 0.07	0.22($\overline{X}$) - 0.02	0.33($\overline{X}$) - 0.05
delta-BHC	0.81C + 0.07	0.18($\overline{X}$) + 0.09	0.25($\overline{X}$) + 0.03
gamma-BHC (Lindane)	0.82C - 0.05	0.12($\overline{X}$) + 0.06	0.22($\overline{X}$) + 0.04
Chlordane	0.82C - 0.04	0.13 ($\overline{X}$) + 0.13	0.18($\overline{X}$) + 0.18
4,4'-DDD	0.84C + 0.30	0.20($\overline{X}$) - 0.18	0.27($\overline{X}$) - 0.14
4,4'-DDE	0.85C + 0.14	0.13($\overline{X}$) + 0.06	0.28($\overline{X}$) - 0.09
4,4'-DDT	0.93C - 0.13	0.17($\overline{X}$) + 0.39	0.31($\overline{X}$) - 0.21
Dieldrin	0.90C + 0.02	0.12($\overline{X}$) + 0.19	0.16($\overline{X}$) + 0.16
Endosulfan I	0.97C + 0.04	0.10($\overline{X}$) + 0.07	0.18($\overline{X}$) + 0.08
Endosulfan II	0.93C + 0.34	0.41($\overline{X}$) - 0.65	0.47($\overline{X}$) - 0.20
Endosulfan sulfate	0.89C - 0.37	0.13($\overline{X}$) + 0.33	0.24($\overline{X}$) + 0.35
Endrin	0.89C - 0.04	0.20($\overline{X}$) + 0.25	0.24($\overline{X}$) + 0.25
Heptachlor	0.69C + 0.04	0.06($\overline{X}$) + 0.13	0.16($\overline{X}$) + 0.08
Heptachlor epoxide	0.89C + 0.10	0.18($\overline{X}$) - 0.11	0.25($\overline{X}$) - 0.08
Toxaphene	0.80C + 1.74	0.09($\overline{X}$) + 3.20	0.20($\overline{X}$) + 0.22
PCB-1016	0.81C + 0.50	0.13($\overline{X}$) + 0.15	0.15($\overline{X}$) + 0.45
PCB-1221	0.96C + 0.65	0.29($\overline{X}$) - 0.76	0.35($\overline{X}$) - 0.62
PCB-1232	0.91C + 10.8	0.21($\overline{X}$) - 1.93	0.31($\overline{X}$) + 3.50
PCB-1242	0.93C + 0.70	0.11($\overline{X}$) + 1.40	0.21($\overline{X}$) + 1.52
PCB-1248	0.97C + 1.06	0.17($\overline{X}$) + 0.41	0.25($\overline{X}$) - 0.37
PCB-1254	0.76C + 2.07	0.15($\overline{X}$) + 1.66	0.17($\overline{X}$) + 3.62
PCB-1260	0.66C + 3.76	0.22($\overline{X}$) - 2.37	0.39($\overline{X}$) - 4.86

X' = Expected recovery for one or more measurements of a sample containing a concentration of C, in µg/L.

TABLE 6 – DISTRIBUTION OF CHLORINATED PESTICIDES AND PCBS INTO FLORISIL® COLUMN FRACTIONS			
	Percent Recovery by Fraction[1]		
Analyte	1	2	3
Aldrin	100		
alpha-BHC	100		
beta-BHC	97		
delta-BHC	98		
gamma-BHC (Lindane)	100		
Chlordane	100		
4,4'-DDD	99		
4,4'-DDE		98	
4,4'-DDT	100		
Dieldrin	0	100	
Endosulfan I	37	64	
Endosulfan II	0	7	91
Endosulfan sulfate	0	0	106
Endrin	4	96	
Endrin aldehyde	0	68	26
Heptachlor	100		
Heptachlor epoxide	100		
Toxaphene	96		
PCB-1016	97		
PCB-1221	97		
PCB-1232	95	4	
PCB-1242	97		
PCB-1248	103		
PCB-1254	90		
PCB-1260			

[1] Eluant composition:
Fraction 1 - 6% ethyl ether in hexane
Fraction 2 - 15% ethyl ether in hexane
Fraction 3 - 50% ethyl ether in hexane

TABLE 7 – SUGGESTED CALIBRATION GROUPS[1]	
Analyte	
Calibration Group 1	***Calibration Group 4***
Acephate	Benfluralin
Alachlor	Chlorobenzilate
Atrazine	Dieldrin
beta-BHC	Endosulfan I
Bromoxynil octanoate	Mirex
Captafol	Terbacil
Diallate	Terbuthylazine
Endosulfan sulfate	Triadimefon
Endrin	***Calibration Group 5***
Isodrin	*alpha*-Chlordane
Pendimethalin (Prowl)	Captan
trans-Permethrin	Chlorothalonil
Calibration Group 2	4,4'-DDD
alpha-BHC	Norfluorazon
DCPA	Simazine
4,4'-DDE	***Calibration Group 6***
4,4'-DDT	Aldrin
Dichlone	*delta*-BHC
Ethalfluralin	Bromacil
Fenarimol	Butachlor
Methoxychlor	Endosulfan II
Metribuzin	Heptachlor
Calibration Group 3	Kepone
gamma-BHC (Lindane)	***Calibration Group 7***
gamma-Chlordane	Carbophenothion
Endrin ketone	Chloroneb
Heptachlor epoxide	Chloropropylate
Isopropalin	DBCP
Nitrofen (TOK)	Dicofol
PCNB	Endrin aldehyde
cis-Permethrin	Etridiazole
Trifluralin	Perthane
	Propachlor
	Propanil
	Propazine

[1] The analytes may be organized in other calibration groups, provided that there are no coelution problems and that all QC requirements are met.

22. Figures

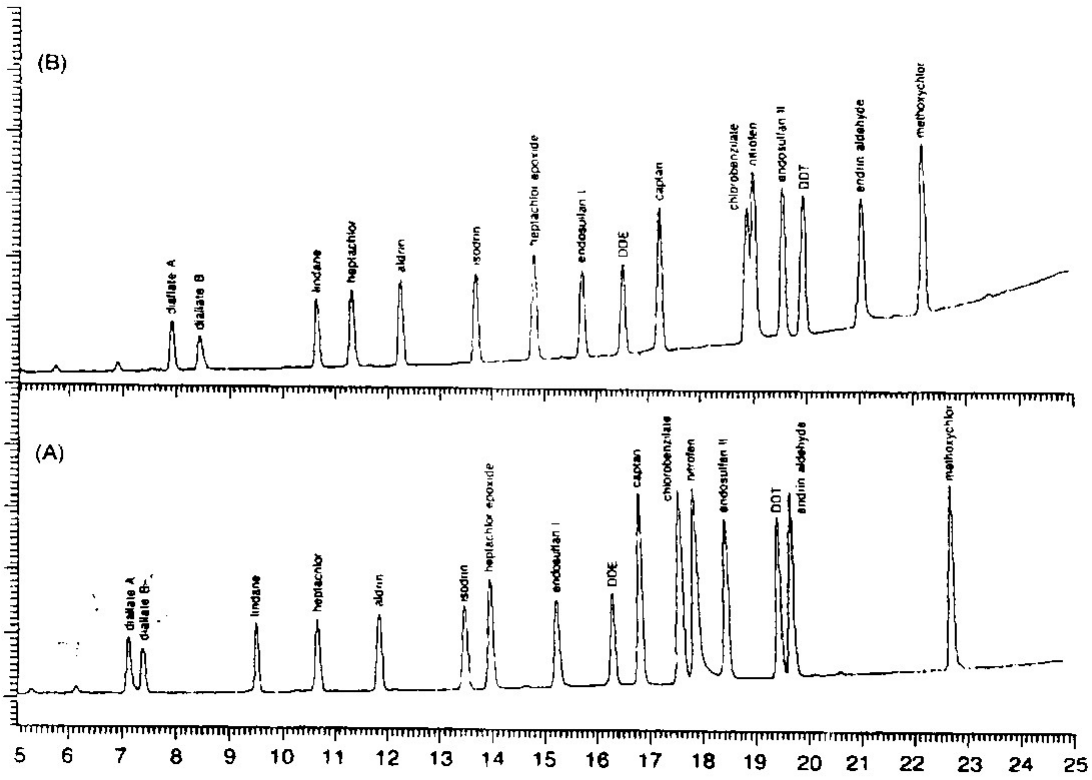

Figure 1 Example Chromatogram of Selected Organochlorine Pesticides

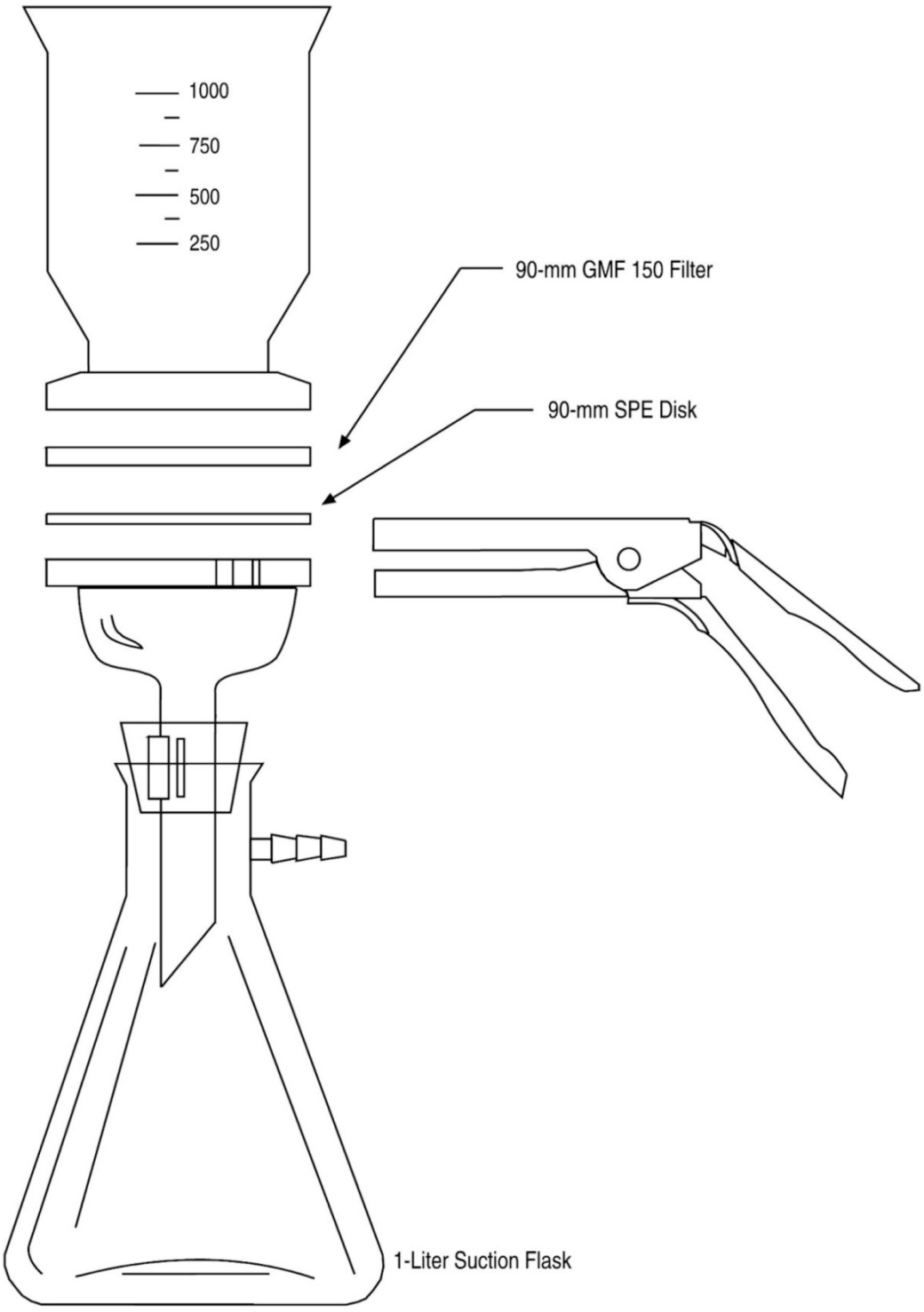

Figure 2 Disk-based solid-phase extraction apparatus

23. Glossary

These definitions and purposes are specific to this method but have been conformed to common usage to the extent possible.

23.1 Units of weight and measure and their abbreviations

23.1.1 Symbols

ºC	degrees Celsius
µg	microgram
µL	microliter
<	less than
≤	less than or equal to
>	greater than
%	percent

23.1.2 Abbreviations (in alphabetical order)

cm	centimeter
g	gram
hr	hour
ID	inside diameter
in.	inch
L	liter
M	molar solution - one mole or gram molecular weight of solute in one liter of solution
mg	milligram
min	minute
mL	milliliter
mm	millimeter
N	Normality - one equivalent of solute in one liter of solution
ng	nanogram
psia	pounds-per-square inch absolute
psig	pounds-per-square inch gauge
v/v	volume per unit volume
w/v	weight per unit volume

23.2 Definitions and acronyms (in alphabetical order)

Analyte – A compound or mixture of compounds (e.g., PCBs) tested for by this method. The analytes are listed in Tables 1 and 2.

Analytical batch – The set of samples analyzed on a given instrument during a 24-hour period that begins and ends with calibration verification (Sections 7.8 and 13). See also "Extraction batch."

Blank (method blank; laboratory blank) – An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with samples. The blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.
Calibration factor (CF) – See Section 7.5.1.

Calibration standard – A solution prepared from stock solutions and/or a secondary standards and containing the analytes of interest, surrogates, and internal standards. This standard is used to model the response of the GC instrument against analyte concentration.

Calibration verification – The process of confirming that the response of the analytical system remains within specified limits of the calibration.

Calibration verification standard – The standard (Section 6.8.4) used to verify calibration (Sections 7.8 and 13.6).

Extraction Batch – A set of up to 20 field samples (not including QC samples) started through the extraction process in a given 24-hour shift. Each extraction batch of 20 or fewer samples must be accompanied by a blank (Section 8.5), a laboratory control sample (LCS, Section 8.4), a matrix spike and duplicate (MS/MSD; Section 8.3), resulting in a minimum of five samples (1 field sample, 1 blank, 1 LCS, 1 MS, and 1 MSD) and a maximum of 24 samples (20 field samples, 1 blank, 1 LCS, 1 MS, and 1 MSD) for the batch. If greater than 20 samples are to be extracted in a 24-hour shift, the samples must be separated into extraction batches of 20 or fewer samples.

Field Duplicates – Two samples collected at the same time and place under identical conditions, and treated identically throughout field and laboratory procedures. Results of analyses the field duplicates provide an estimate of the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

Field blank – An aliquot of reagent water or other reference matrix that is placed in a sample container in the field, and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the field blank is to determine if the field or sample transporting procedures and environments have contaminated the sample. See also "Blank."

GC – Gas chromatograph or gas chromatography

Gel-permeation chromatography (GPC) – A form of liquid chromatography in which the analytes are separated based on exclusion from the solid phase by size.

Internal standard – A compound added to an extract or standard solution in a known amount and used as a reference for quantitation of the analytes of interest and surrogates. Also see Internal standard quantitation.

Internal standard quantitation – A means of determining the concentration of an analyte of interest (Tables 1 and 2) by reference to a compound not expected to be found in a sample.

IDC – Initial Demonstration of Capability (Section 8.2); four aliquots of a reference matrix spiked with the analytes of interest and analyzed to establish the ability of the laboratory to generate acceptable precision and recovery. An IDC is performed prior to the first time this method is used and any time the method or instrumentation is modified.

Laboratory Control Sample (LCS; laboratory fortified blank; Section 8.4) – An aliquot of reagent water spiked with known quantities of the analytes of interest and surrogates. The LCS is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

Laboratory Fortified Sample Matrix – See Matrix spike

Laboratory reagent blank – See blank

Matrix spike (MS) and matrix spike duplicate (MSD) (laboratory fortified sample matrix and duplicate) – Two aliquots of an environmental sample to which known quantities of the analytes of interest and surrogates are added in the laboratory. The MS/MSD are prepared and analyzed exactly like a field sample. Their purpose is to quantify any additional bias and imprecision caused by the sample matrix. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS/MSD corrected for background concentrations.

May – This action, activity, or procedural step is neither required nor prohibited.

May not – This action, activity, or procedural step is prohibited.

Method detection limit (MDL) – A detection limit determined by the procedure at 40 CFR part 136, appendix B. The MDLs determined by EPA are listed in Tables 1 and 2. As noted in Section 1.6, use the MDLs in Tables 1 and 2 in conjunction with current MDL data from the laboratory actually analyzing samples to assess the sensitivity of this procedure relative to project objectives and regulatory requirements (where applicable)

Minimum level (ML) – The term "minimum level" refers to either the sample concentration equivalent to the lowest calibration point in a method or a multiple of the method detection limit (MDL), whichever is higher. Minimum levels may be obtained in several ways: They may be published in a method; they may be based on the lowest acceptable calibration point used by a laboratory; or they may be calculated by multiplying the MDL in a method, or the MDL determined by a laboratory, by a factor of 3. For the purposes of NPDES compliance monitoring, EPA considers the following terms to be synonymous: "quantitation limit," "reporting limit," and "minimum level."

MS – Mass spectrometer or mass spectrometry

Must – This action, activity, or procedural step is required.

Preparation blank – See blank

Reagent water – Water demonstrated to be free from the analytes of interest and potentially interfering substances at the MDLs for the analytes in this method.

Regulatory compliance limit – A limit on the concentration or amount of a pollutant or contaminant specified in a nationwide standard, in a permit, or otherwise established by a regulatory/control authority.

Relative standard deviation (RSD) – The standard deviation times 100 divided by the mean. Also termed "coefficient of variation."

RF – Response factor. See Section 7.6.2

RPD – Relative percent difference

RSD – See relative standard deviation

Safety Data Sheet (SDS) – Written information on a chemical's toxicity, health hazards, physical properties, fire, and reactivity, including storage, spill, and handling precautions that meet the requirements of OSHA, 29 CFR 1910.1200(g) and appendix D to §1910.1200. United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), third revised edition, United Nations, 2009.

Should – This action, activity, or procedural step is suggested but not required.

SPE – Solid-phase extraction; a sample extraction or extract cleanup technique in which an analyte is selectively removed from a sample or extract by passage over or through a material capable of reversibly adsorbing the analyte.

Stock solution – A solution containing an analyte that is prepared using a reference material traceable to EPA, the National Institute of Science and Technology (NIST), or a source that will attest to the purity and authenticity of the reference material.

Surrogate – A compound unlikely to be found in a sample, which is spiked into the sample in a known amount before extraction, and which is quantified with the same procedures used to quantify other sample components. The purpose of the surrogate is to monitor method performance with each sample.