

METHOD 1656

**ORGANO-HALIDE PESTICIDES IN WASTEWATER, SOIL,
SLUDGE, SEDIMENT, AND TISSUE BY GC/HSD**

Revision A

September 2000

Acknowledgments

This method was developed under the direction of William A. Telliard of the Engineering and Analysis Division (EAD) within the U.S. Environmental Protection Agency's (EPA's) Office of Science and Technology (OST) by DynCorp Environmental under EPA Contract 68-C-98-139 with assistance from Interface, Inc.

Disclaimer

This Method has been reviewed and approved for publication by the Analytical Methods Staff within EPA's Engineering and Analysis Division. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Introduction

Method 1656 was developed by the U.S. Environmental Protection Agency's (EPA's) Office of Science and Technology for measurement of organo-halide (O-C) pesticides in wastewater, soil, sludge, and sediment. EPA Method 1656 has been expanded through revision A to include measurement of O-C pesticides in fish to support EPA's National Fish Survey.

Further Information

Questions concerning this Method or its application should be addressed to:

W.A. Telliard, Director
Analytical Methods Staff (4303)
Office of Science and Technology
U.S. Environmental Protection Agency
Ariel Rios Building
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460
Phone: 202/260-7134
Fax: 202/260-7185

Requests for copies of this Method should be addressed to:

EPA Sample Control Center
c/o DynCorp Environmental
6101 Stevenson Avenue
Alexandria, VA 22314
Phone: 703-461-2100
Fax: 703-461-8056
E-mail: SCC@DYNCORP.COM

Note: This method is performance based. The laboratory is permitted to omit any step or modify any procedure provided that all performance requirements in this method are met. The laboratory may not omit any quality control analyses. The terms "shall," "must," and "may not" define procedures required for producing reliable results. The terms "should" and "may" indicate optional steps that may be modified or omitted if the laboratory can demonstrate that the modified method produces results equivalent or superior to results produced by this method.

Method 1656, Revision A

Organo-Halide Pesticides in Wastewater, Soil, Sludge, Sediment, and Tissue by GC/HSD

1.0 Scope and Application

1.1 Method 1656, Revision A (Method 1656A; the "Method") is for determination of organo-halide pesticides, polychlorinated biphenyls (PCBs), and other analytes amenable to extraction and analysis by wide-bore capillary column gas chromatography (GC) with a halogen-specific detector (HSD). The Method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.

1.2 The analytes listed in Table 1 may be determined in wastewater, soil, sludge, sediment, and tissue by this Method. The Method is a consolidation of EPA Methods 608, 608.1, 608.2, and 617. The sample extraction and concentration steps given in these methods have been extended to other sample matrices in this Method.

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

1.3 This Method may be applicable to analytes other than those listed in Table 1. The quality control requirements in this Method give the steps necessary to determine this applicability. Not all analytes listed in Table 1 have corresponding calibration data in Table 3 and quality control (QC) acceptance criteria in Table 4. Calibration data for additional analytes may be found in other EPA methods (References 1 - 3).

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

1.5 The method detection limits (MDLs) and minimum levels of quantitation (MLs) for the analytes in this Method are usually dependent on the level of interferences rather than instrumental limitations. The MDLs (40 CFR 136, Appendix B) and estimated MDLs in Table 2 typify the minimum quantities that can be detected when no interferences are present. MLs have been established as the lowest calibration points in Table 3.

1.6 This Method is for use by or under the supervision of analysts experienced in the use of a gas chromatography and in the interpretation of gas chromatographic data. Each laboratory that uses this Method must demonstrate the ability to generate acceptable results using the procedure in Section 9.2.

2.0 Summary of Method

2.1 Extraction—The extraction technique depends on the nature and solids content of the sample

2.1.1 Samples containing less than 1% solids are extracted with methylene chloride using continuous liquid-liquid extraction techniques.

2.1.2 Samples containing greater than 1% solids are extracted using techniques dependent on the nature of the matrix.

2.1.2.1 Non-sludge samples—If the solids content is 1 to 30%, the sample is diluted to 1% solids with reagent water, homogenized ultrasonically, and extracted with methylene chloride using continuous liquid-liquid extraction techniques. If the solids content is greater than 30%, the sample is extracted ultrasonically with methylene chloride:acetone.

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

2.1.3 Fish and other tissue samples are homogenized and dried with sodium sulfate, then extracted using methylene chloride:hexane (1:1) in a Soxhlet extractor.

2.2 Concentration—The extract is dried over sodium sulfate and concentrated using a Kuderna-Danish evaporator.

2.3 Cleanup—Cleanup procedures include gel permeation chromatography (GPC), Florisil and alumina column chromatography, solid-phase cartridge, and sulfur removal. After cleanup, the extract is concentrated to 1.0 mL (0.5 mL if GPC has been used).

2.4 Gas chromatography—A 1- μ L aliquot of the extract is injected into the gas chromatography (GC). The analytes are separated on a wide-bore, fused-silica capillary column. The analytes are detected by an electron capture, microcoulometric, or electrolytic conductivity detector. For analyte confirmation, a 1- μ L aliquot of the extract is injected into a column containing a dissimilar liquid phase.

-
- 2.5** Identification of an analyte (qualitative analysis) is performed by comparing the GC retention times of the analyte on two dissimilar columns with the respective retention times of an authentic standard. Analyte identity is confirmed when the retention times agree within their respective windows.
- 2.6** Quantitative analysis is performed using an authentic standard to produce a calibration factor or calibration curve, and using the calibration data to determine the concentration of a pollutant in the extract. The concentration in the sample is calculated using the sample weight or volume and the extract volume.
- 2.7** Quality is assured through reproducible calibration and testing of the extraction and GC systems.

3.0 Definitions

Definitions are given in the glossary at the end of this Method.

4.0 Contamination and Interferences

- 4.1** Solvents, reagents, glassware, and other sample-processing hardware may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms. All materials used in the analysis shall be demonstrated to be free from interferences under the conditions of analysis by running method blanks as described in Section 9.5.
- 4.2** Glassware and, where possible, reagents are cleaned by solvent rinse and baking at 450 °C for a minimum of 1 hour in a muffle furnace or kiln. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment, and thorough rinsing with acetone and pesticide-quality hexane may be required.
- 4.3** Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.
- 4.4** Interference by phthalate esters can pose a major problem in pesticide analysis when using the electron capture detector (ECD). Phthalates usually appear in the chromatogram as large, late-eluting peaks. Phthalates may be leached from common flexible plastic tubing and other plastic materials during the extraction and cleanup processes. Cross contamination of clean glassware routinely occurs when plastics are handled during extraction, especially when solvent-wetted surfaces are handled. Interferences from phthalates can best be minimized by avoiding the use of plastics in the laboratory, or by using a microcoulometric or electrolytic conductivity detector.
- 4.5** Interferences coextracted from samples will vary considerably from source to source. The cleanup procedures given in this Method can be used to overcome many of these interferences, but unique samples may require additional cleanups to achieve the MDLs in Table 2.
- 4.6** Cleanup of tissue—The natural lipid content of tissue can interfere in the analysis of tissue samples. The lipid contents of different species and portions of tissue can vary widely. Lipids are soluble to varying degrees in various organic solvents and may be present in sufficient quantity to overwhelm

the column chromatographic purification procedures used for sample extracts. Lipids are removed by the GPC procedure in Section 13.2.

5.0 Safety

- 5.1** The toxicity or carcinogenicity of each analyte or reagent used in this Method has not been precisely determined; however, each analyte or reagent should be treated as a potential health hazard. Exposure to these substances should be reduced to the lowest possible level.
- 5.2** This Method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a current awareness file of OSHA regulations for the safe handling of the chemicals specified in this Method. OSHA rules require that a reference file of material safety data sheets (MSDSs) must be made available to all personnel involved in these analyses (29 CFR 1917.28, appendix E). It is also suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this Method and that the results of this monitoring be made available to the analyst. Personnel hygiene monitoring should be performed using OSHA or NIOSH approved personal hygiene monitoring methods. Additional information on laboratory safety can be found in References 4-6.
- 5.3** The following analytes covered by this Method have been tentatively classified as known or suspected human or mammalian carcinogens: 4,4'-DDD, 4,4'-DDT, the BHCs and the PCBs. Primary standards of these analytes shall be prepared in a hood, and a NIOSH/MESA-approved toxic gas respirator should be worn when high concentrations are handled.
- 5.4** Mercury vapor is highly toxic. If mercury is used for sulfur removal, all operations involving mercury shall be performed in a hood.
- 5.5** Unknown samples may contain high concentrations of volatile toxic substances. Sample containers should be opened in a hood and handled with gloves that will prevent exposure. The oven used for sample drying to determine percent moisture should be located in a hood so that vapors from samples do not create a health hazard in the laboratory.

6.0 Apparatus and Materials

Note: *The mention of trade names or commercial products in this Method is for illustrative purposes only and does not constitute endorsement or recommendation for use by the Environmental Protection Agency. Equivalent performance may be achievable using apparatus, materials, or cleaning procedures other than those suggested here. The laboratory is responsible for demonstrating equivalent performance.*

- 6.1** Sampling equipment for discrete or composite sampling.
- 6.1.1** Sample bottles and caps.

-
- 6.1.1.1** Liquid samples (waters, sludges, and similar materials that contain less than 5% solids)—Sample bottle, amber glass, 1.1-L or 1-quart, with screw-cap.
 - 6.1.1.2** Solid samples (soils, sediments, tissues, sludges, filter cake, compost, and similar materials that contain >5% solids)—Sample bottle, wide mouth, amber glass, volume as required for the amount of samples to be tested.
 - 6.1.1.3** If amber bottles are not available, samples shall be protected from light.
 - 6.1.1.4** Bottle caps—Threaded to fit sample bottles. Caps shall be lined with fluoropolymer.
 - 6.1.1.5** Cleaning.
 - 6.1.1.5.1** Bottles are detergent-water washed, then rinsed with solvent or baked at >450 °C for a minimum of 1 hour before use.
 - 6.1.1.5.2** Liners are detergent-water washed, then rinsed with reagent water and solvent, and baked at approximately 200 °C for a minimum of 1 hour prior to use.
 - 6.1.2** Compositing equipment—Automatic or manual compositing system incorporating glass containers cleaned per bottle-cleaning procedure above. Sample containers are kept at 0 to 4 °C during sampling. Glass or fluoropolymer tubing only shall be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing shall be thoroughly rinsed with methanol, followed by repeated rinses with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.
 - 6.2** Equipment for glassware cleaning—Laboratory sink with overhead fume hood.
 - 6.3** Equipment for sample preparation
 - 6.3.1** Laboratory fume hood of sufficient size to contain the sample preparation equipment listed below.
 - 6.3.2** Glove box (optional).
 - 6.3.3** Tissue homogenizer—VirTis Model 45 Macro homogenizer (American Scientific Products H-3515, or equivalent) with stainless steel Macro-shaft and Turbo-shear blade.
 - 6.3.4** Meat grinder—Hobart, or equivalent, with 3-5 mm holes in inner plate.
 - 6.3.5** Equipment for determining percent moisture.
 - 6.3.5.1** Oven—Capable of maintaining a temperature of 110 ± 5 °C.

6.3.5.2 Desiccator.

6.3.5.3 Crucibles, porcelain.

6.3.5.4 Weighing pans, aluminum.

6.3.6 Balances.

6.3.6.1 Analytical—Capable of weighing 0.1 mg.

6.3.6.2 Top loading—Capable of weighing 10 mg.

6.4 Extraction equipment.

6.4.1 Equipment for liquid-liquid extraction.

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

6.4.1.2 Round-bottom flask, 500-mL, with heating mantle.

6.4.1.3 Condenser, Graham, to fit extractor.

6.4.1.4 pH meter, with combination glass electrode.

6.4.1.5 pH paper, wide range (Hydriion Papers, or equivalent).

6.4.2 Equipment for ultrasonic extraction.

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

6.4.2.2 Sonobox (or equivalent), for use with disrupter.

6.4.3 Equipment for Soxhlet extraction.

6.4.3.1 Soxhlet—50-mm ID, 200-mL capacity with 500-mL flask (Cal-Glass LG-6900, or equivalent, except substitute 500-mL round-bottom flask for 300-mL flat-bottom flask).

6.4.3.2 Thimble—43 × 123 to fit Soxhlet (Cal-Glass LG-6901-122, or equivalent).

6.4.3.3 Moisture trap—Dean Stark or Barret with fluoropolymer stopcock, to fit Soxhlet.

- 6.4.3.4** Heating mantle—Hemispherical, to fit 500-mL round-bottom flask (Cal-Glass LG-8801-112, or equivalent).
- 6.4.3.5** Variable transformer—Powerstat (or equivalent), 110-volt, 10-amp.
- 6.4.4** Separatory funnels—250-, 500-, 1000-, and 2000-mL, with fluoropolymer stopcocks.
- 6.4.5** Filtration apparatus.
 - 6.4.5.1** Glass powder funnels—125- to 250- mL.
 - 6.4.5.2** Filter paper for above (Whitman 41, or equivalent).
- 6.4.6** Beakers.
 - 6.4.6.1** 1.5- to 2-L, calibrated to 1 L.
 - 6.4.6.2** 400- to 500-mL.
- 6.4.7** Spatulas—Stainless steel or fluoropolymer.
- 6.4.8** Drying column—400 mm long x 15 to 20 mm ID Pyrex chromatographic column equipped with coarse glass frit or glass wool plug.
 - 6.4.8.1** Pyrex glass wool—Extracted with solvent or baked at 450 °C for a minimum of 1 hour.
- 6.4.9** Evaporation/concentration apparatus.
 - 6.4.9.1** Kuderna-Danish (K-D) apparatus.
 - 6.4.9.1.1** Evaporation flask—500-mL (Kontes K-570001-0500, or equivalent), attached to concentrator tube with springs (Kontes K-662750-0012).
 - 6.4.9.1.2** Concentrator tube—10-mL, graduated (Kontes K-570050-1025, or equivalent) with calibration verified. Ground-glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.
 - 6.4.9.1.3** Snyder column—Three-ball macro (Kontes K-503000-0232, or equivalent).
 - 6.4.9.1.4** Snyder column—Two-ball micro (Kontes K-469002-0219, or equivalent).
 - 6.4.9.1.5** Boiling chips.

- 6.4.9.1.5.1** Glass or silicon carbide—Approximately 10/40 mesh, extracted with methylene chloride and baked at 450 °C for a minimum of 1 hour.
 - 6.4.9.1.5.2** Fluoropolymer (optional)—Extracted with methylene chloride.
 - 6.4.9.2** Water bath—Heated, with concentric ring cover, capable of temperature control (± 5 °C), installed in a fume hood.
 - 6.4.9.3** Nitrogen evaporation device—Equipped with heated bath that can be maintained at 35-60 °C (N-Evap, Organomation Associates, Inc., or equivalent).
 - 6.4.9.4** Sample vials—Amber glass, 1- to 5-mL with fluoropolymer-lined screw- or crimp-cap, to fit GC autosampler.
- 6.5** Apparatus for sample cleanup.
 - 6.5.1** Gel permeation chromatography.
 - 6.5.1.1** Automated gel permeation chromatograph (O.I. Analytical Inc., Columbia, MO, Model AP 1000, or equivalent).
 - 6.5.1.2** Column—600 to 700 mm long \times 25 mm ID.
 - 6.5.1.3** Syringe, 10-mL, with Luer fitting.
 - 6.5.1.4** Syringe-filter holder, stainless steel, and glass fiber or fluoropolymer filters (Gelman Acrodisc-CR, 1 to 5 μm , or equivalent).
 - 6.5.1.5** UV detector—254-nm, preparative or semi-prep flow cell: (Isco, Inc., Type 6; Schmadzu, 5 mm path length; Beckman-Altex 152W, 8 μL micro-prep flow cell, 2 mm path; Pharmacia UV-1, 3 mm flow cell; LDC Milton-Roy UV-3, monitor #1203; or equivalent).
 - 6.5.2** Oven—For baking and storage of adsorbents, capable of maintaining a constant temperature (± 5 °C) in the range of 105-250 °C.
 - 6.5.3** Vacuum system and cartridges for solid-phase extraction (SPE).
 - 6.5.3.1** Vacuum system—Capable of achieving 0.1 bar (house vacuum, vacuum pump, or water aspirator), with vacuum gauge.
 - 6.5.3.2** VacElute Manifold (Analytichem International, or equivalent).
 - 6.5.3.3** Vacuum trap—Made from 500-mL sidearm flask fitted with single-hole rubber stopper and glass tubing.

-
- 6.5.3.4** Rack for holding 50-mL volumetric flasks in the manifold.
 - 6.5.3.5** Column—Mega Bond Elut, Non-polar, C18 Octadecyl, 10 g/60 mL (Analytichem International Cat. No. 607H060, or equivalent).
 - 6.5.4** Chromatographic column—400 mm long x 22 mm ID, with fluoropolymer stopcock and coarse frit (Kontes K-42054, or equivalent).
 - 6.5.5** Sulfur removal tubes—40- to 50-mL bottle or test tube with fluoropolymer-lined screw-cap.
 - 6.6** Centrifuge apparatus
 - 6.6.1** Centrifuge—Capable of rotating 500-mL centrifuge bottles or 15-mL centrifuge tubes at 5,000 rpm minimum.
 - 6.6.2** Centrifuge bottles—500-mL, with screw-caps, to fit the centrifuge.
 - 6.6.3** Centrifuge tubes—12- to 15-mL, with screw-caps, to fit the centrifuge.
 - 6.6.4** Funnel, Buchner, 15 cm.
 - 6.6.4.1** Flask, filter, for use with Buchner funnel.
 - 6.6.4.2** Filter paper, 15 cm (Whatman #41, or equivalent).
 - 6.7** Miscellaneous glassware.
 - 6.7.1** Pipettes, glass - volumetric, 1.00-, 5.00-, and 10.0-mL.
 - 6.7.2** Syringes, glass - with Luerlok tip, 0.1-, 1.0-, and 5.0-mL. Needles for syringes, 2", 22-gauge.
 - 6.7.3** Volumetric flasks - 10.0-, 25.0-, and 50.0-mL.
 - 6.7.4** Scintillation vials - glass, 20- to 50-mL, with fluoropolymer-lined screw-caps.
 - 6.7.5** Glass funnel—125-250 mL.
 - 6.7.6** Glass-fiber filter paper—Whatman GF/D (or equivalent), to fit glass funnel in Section 6.5.2.
 - 6.8** Gas chromatography —Shall have splitless or on-column simultaneous automated injection into separate capillary columns with a halide-specific detector at the end of each column, temperature program with isothermal holds, data system capable of recording simultaneous signals from the two detectors, and shall meet all of the performance specifications in Section 15.

6.8.1 GC columns—Bonded-phase, fused-silica capillary.

6.8.1.1 Primary—30 m (± 3 m) long \times 0.5 mm (± 0.05 mm) ID DB-608 (or equivalent).

6.8.1.2 Confirmatory—DB-1701, or equivalent, with same dimensions as primary column.

6.8.2 Data system—Shall collect and record GC data, store runs on magnetic media, process GC data, compute peak areas, store calibration data including retention times and calibration factors, identify GC peaks through retention times, compute concentrations, and generate reports.

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

6.8.2.2 Calibration factors and calibration curves—The data system shall be used to record and maintain lists of calibration factors, and multipoint calibration curves (Section 10). Computations of relative standard deviation (coefficient of variation) are used for testing calibration linearity. Statistics on initial (Section 9.2) and ongoing (Section 15.6) performance may be computed and maintained, if desired.

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

6.8.3 Halide-specific detector—Electron capture or electrolytic conductivity (Micoulometric, Hall, O.I., or equivalent), capable of detecting 8 pg of aldrin under the analysis conditions given in Table 2.

7.0 Reagents and Standards.

7.1 Sodium thiosulfate (ACS), granular.

7.2 pH adjustment.

7.2.1 Sodium hydroxide—Reagent grade

7.2.1.1 Concentrated solution (10 N)—Dissolve 40 g NaOH in 100 mL reagent water.

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

7.2.2 Sulfuric acid (1+1)—Reagent grade, 6 N in reagent water. Slowly add 50 mL concentrated H₂SO₄ (specific gravity 1.84) to 50 mL reagent water.

7.2.3 Potassium hydroxide—37 w/v percent. Dissolve 37 g KOH in 100 mL reagent water.

7.3 Sample and solution drying and back-extraction

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

7.3.2 Sodium sulfate, reagent grade, powdered, treated as in Section 7.3.1—Used for drying solids and tissue samples.

7.3.3 Sodium sulfate solution—2% (w/v) in reagent water, pH-adjusted to 8.5 to 9.0 with KOH or H₂SO₄.

7.3.4 Activated silica gel—100-200 mesh, Supelco 1-3651 (or equivalent), rinsed with methylene chloride, baked at 180°C for a minimum of 1 hour, cooled in a desiccator, and stored in a pre-cleaned glass bottle with screw-cap that will prevent moisture from entering.

7.3.5 Prepurified nitrogen

7.4 Solvents—Methylene chloride, hexane, ethyl ether, acetone, acetonitrile, isooctane, and methanol; pesticide-quality; lot-certified to be free of interferences.

7.4.1 Ethyl ether must be shown to be free of peroxides before it is used, as indicated by EM Laboratories Quant Test Strips (Scientific Products P1126-8, or equivalent).

7.4.2 Procedures recommended for removal of peroxides are provided with the test strips. After the cleanup, 20 mL of ethyl alcohol is added to each liter of ether as a preservative.

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

7.6 Sample cleanup.

7.6.1 Florisil—PR grade, 60/100 mesh, activated at 650 to 700 °C, stored in the dark in glass container with fluoropolymer-lined screw-cap. Activate at 130 °C for 16 hours minimum immediately prior to use. Standardize with lauric acid (see Section 10.5 and Reference 7). Alternatively, 500-mg cartridges (J.T. Baker, or equivalent) may be used.

7.6.2 Solid-phase extraction.

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

7.6.2.2 SPE elution solvent—Methylene chloride:acetonitrile:hexane (50:3:47).

- 7.6.3** Alumina, neutral, Brockman Activity I, 80-200 mesh (Fisher Scientific Certified, or equivalent). Heat 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 2 hours. Keep bottle tightly sealed.
- 7.6.4** Silicic acid, 100 mesh.
- 7.6.5** Sulfur removal—Copper powder (bright, non-oxidized), or TBA sodium sulfite. If mercury is used, observe the handling precautions in Section 5.
- 7.6.6** GPC column packing—Bio-beads, SX-3, (Bio-Rad Laboratories, Richmond, CA, or equivalent).
- 7.7** Reference matrices.
- 7.7.1** Reagent water—Water in which the analytes of interest and interfering substances are not detected by this Method.
- 7.7.2** High-solids reference matrix—Playground sand or similar material in which the analytes of interest and interfering substances are not detected by this Method. May be prepared by extraction with methylene chloride and/or baking at 450 °C for four hours minimum.
- 7.7.3** Tissue reference matrix—Corn or other vegetable oil. Fish oil may be used provided it is demonstrated to be free of the analytes of interest and interfering substances at the detection limit of this Method.
- 7.8** Standard solutions—Purchased as solutions or mixtures with certification to their purity, concentration, and authenticity, or prepared from materials of known purity and composition. If analyte purity is 96% or greater, the weight may be used without correction to compute the concentration of the standard. When not being used, standards are stored in the dark at -20 to -10 °C in screw-capped vials with fluoropolymer-lined lids. A mark is placed on the vial at the level of the solution so that solvent evaporation loss can be detected. The vials are brought to room temperature prior to use. Any precipitate is redissolved and solvent is added if solvent loss has occurred.
- 7.9** Preparation of stock solutions—Prepare in isooctane per the steps below. Observe the safety precautions in Section 5.
- 7.9.1** Dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 10 mg aldrin in a 10-mL ground-glass stoppered volumetric flask and fill to the mark with isooctane. After the aldrin is completely dissolved, transfer the solution to a 15-mL vial with fluoropolymer-lined cap.
- 7.9.2** Stock solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards.

- 7.9.3** Stock solutions shall be replaced after 6 months, or sooner if comparison with quality control check standards indicates a change in concentration.
- 7.10** Secondary mixtures—Using stock solutions (Section 7.9), prepare mixtures at the levels shown in Table 3 for calibration and calibration verification (Sections 10.3 and 15.5), for initial and ongoing precision and recovery (Sections 9.2.1 and 15.6), and for spiking into the sample matrix (Section 9.4).
- 7.11** Combined QC standard—Mix equal volumes of the high concentration calibration solutions (Table 3) to produce this standard. It is used for calibration (Section 10.4), calibration verification (Section 15.5), and for initial (Section 9.2.1) and ongoing (Section 15.6) demonstrations of method performance.
- 7.12** Surrogate spiking solution—Prepare tetrachloro-m-xylene or decachlorobiphenyl at a concentration of 2 µg/mL in acetone.
- 7.13** DDT and endrin decomposition solution—Prepare a solution containing endrin at a concentration of 1 µg/mL and DDT at a concentration of 2 µg/mL.
- 7.14** Stability of solutions—All standard solutions (Sections 7.9 through 7.13) shall be analyzed within 48 hours of preparation and on a monthly basis thereafter for signs of degradation. Standards will remain acceptable if the concentration remains within ±15% of the concentration obtained in the initial analysis of the standard.

8.0 Sample Preservation

- 8.1** Collect aqueous samples in glass containers following conventional sampling practices (Reference 8), except that the bottle shall not be prerinsed with sample before collection.
- 8.1.1** Aqueous samples that flow freely are collected in refrigerated bottles using automatic sampling equipment. Collect a volume as close to 1.00 L as possible, but in no case less than 0.9 or more than 1.1 L.
- 8.1.2** Solid samples are collected as grab samples using wide-mouth jars. Collect a minimum of 50 g.
- 8.1.3** Tissue samples may be collected in jars or wrapped in aluminum foil. Collect a minimum of 50 g of the tissue to be analyzed. For example, if only the fillet will be analyzed, a minimum amount of tissue necessary to yield 50 g of filet is required.
- 8.2** Storage temperature
- 8.2.1** Aqueous samples—Maintain at 0 - 4 °C from the time of collection until extraction.

8.2.2 Solid samples—Maintain at 0 - 4 °C from time of collection until extraction, or freeze at <-10 °C.

8.2.3 Tissue samples—Freeze at <-10 °C upon collection unless the sample will be extracted within 12 hours, in which case maintain at 0 - 4 °C from the time of collection until extraction.

8.3 Aqueous sample preservation

8.3.1 If the sample will not be extracted within 72 hours of collection, adjust the sample to a pH of 5.0 to 9.0 using sodium hydroxide or sulfuric acid solution. Record the volume of acid or base used.

8.3.2 If residual chlorine is present in an aqueous sample, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine (Reference 9).

8.4 Holding times

8.4.1 Aqueous and unfrozen solid samples—Begin sample extraction within 7 days of collection

8.4.2 Frozen solid and tissue samples—If stored in the dark at <-10°C, solid, semi-solid, multi-phase, and tissue samples may be stored for up to one year prior to extraction.

8.4.3 Analyze all extracts within 40 days of extraction.

9.0 Quality Control

9.1 Each laboratory that uses this Method is required to operate a formal quality control (QC) program (Reference 10). The minimum requirements of this program consist of an initial demonstration of laboratory capability, an ongoing analysis of standards and blanks as tests of continued performance, and analysis of matrix spike samples to assess accuracy and precision. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the Method. For performance tests, a reference matrix is used that most closely simulates the matrix of interest. For aqueous, solid, and tissue samples, reference matrices of reagent water (Section 7.7.1), playground sand (Section 7.7.2), or corn oil (Section 7.7.3) are suggested to simulate waters, soils, and tissue, respectively.

9.1.1 The laboratory shall make an initial demonstration of the ability to generate acceptable precision and recovery with this Method. This ability is established as described in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, the laboratory is permitted certain options to improve results or lower the cost of measurements. These options include use of alternate extraction techniques such as solid-phase extraction and accelerated solvent extraction, alternate concentration techniques such as rotary evaporation, and alternate

cleanup techniques such as high-performance liquid chromatography. Changes in the principle of the determinative technique, such as the use of colorimetry, are not allowed.

- 9.1.2.1** Each time a modification is made to the Method, the laboratory is required to repeat the procedure in Section 9.2 to demonstrate method performance. If a detection limit will be affected by the modification, the laboratory is required to demonstrate that the MDL (40 CFR Part 136, Appendix B) is less than or equal to the MDL in this Method for the analyte(s) of interest. If the Method will be used for compliance monitoring, the MDL for the modified method must be less than or equal to the MDL of this Method or one-third the regulatory compliance limit, whichever is greater. If the change will affect calibration, the instrument must be recalibrated according to Section 10.
- 9.1.2.2** The laboratory is required to maintain records of modifications made to this Method. These records include the following, at a minimum:
- 9.1.2.2.1** The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and the quality control officer who witnessed and will verify the analyses and modification
 - 9.1.2.2.2** A narrative stating the reason(s) for the modification(s)
 - 9.1.2.2.3** Results from all quality control (QC) tests comparing the modified method to this Method, including the following:
 - (a) Calibration (Section 10)
 - (b) Initial precision and recovery (Section 9.2)
 - (c) Analysis of blanks (Section 9.5)
 - (d) Matrix spike analysis (Section 9.4)
 - (e) Ongoing precision and recovery (Section 15.6)
 - (f) Surrogate recovery (Section 9.3)
 - (g) Method detection limit (Section 9.1.2.1)
 - 9.1.2.2.4** Data that will allow an independent reviewer to validate each determination by tracking the instrument output to the final result. These data are to include the following:
 - (a) Sample numbers and other identifiers
 - (b) Processing dates
 - (c) Analysis dates
 - (d) Analysis sequence/run chronology
 - (e) Sample weight or volume
 - (f) Copies of logbooks, recorder charts, or other raw data output
 - (g) Calculations linking raw data to the results reported
- 9.1.3** The laboratory shall spike all samples with at least one surrogate compound to monitor method performance. This test is described in Section 9.3. When results of these spikes

indicate atypical method performance for samples, the samples are diluted to bring method performance within acceptable limits (Section 18).

- 9.1.4** Analyses of matrix spike (MS) samples are required to demonstrate the recovery and to monitor matrix interferences. Section 9.4 describes the procedure and QC criteria for spiking.
 - 9.1.5** The laboratory shall, on an ongoing basis, demonstrate through calibration verification and the analysis of the combined QC standard (Section 7.11) that the analysis system is in control. These procedures are described in Sections 15.1, 15.5, and 15.6.
 - 9.1.6** The laboratory should maintain records to define the quality of data that is generated. Development of accuracy statements is described in Section 9.4.4 and 15.6.4
 - 9.1.7** Analyses of blanks are required to demonstrate freedom from contamination. The procedures and criteria for analysis of a blank are described in Section 9.5.
 - 9.1.8** Other analytes may be determined by this Method. The procedure for establishing a preliminary quality control limit for a new analyte is given in Section 9.6.
 - 9.1.9** QC using this Method is controlled by an analytical batch. An analytical batch is a set of samples started through the extraction process during a given 12-hour shift, to a maximum of 20. A batch may be from 1 to as many as 20 samples. Each batch must be accompanied by a blank, OPR, and MS.
- 9.2** Initial demonstration of laboratory capability
- 9.2.1** Method detection limit—To establish the ability to detect the analytes of interest in this Method, the laboratory shall achieve MDLs that are less than or equal to the MDLs listed in Table 2 or one-third the regulatory compliance limit, whichever is greater. The MDLs shall be determined according to the procedure at 40 CFR 136, Appendix B using the apparatus, reagents, and standards that will be used in the practice of this Method. These MDLs shall be used for determination of laboratory capability only, and should be determined when a new operator begins work or whenever, in the judgment of the laboratory, a change in instrument hardware or operating conditions would dictate reevaluation of capability.
 - 9.2.2** Initial precision and recovery—To establish the ability to generate acceptable precision and recovery, the laboratory shall perform the following operations.
 - 9.2.2.1** For aqueous samples, extract, concentrate, and analyze one set of four 1-L aliquots of reagent water spiked with the combined QC standard (Section 7.11) according to the procedure in Section 11. Alternatively, sets of four replicates of the individual calibration groups (Table 3) may be used. For samples containing high solids, a set of four 30-g aliquots of the high-solids reference matrix (Section 7.7.2) are used. For tissue samples, a set of four 10-g aliquots of the tissue reference matrix (Section 7.7.3) are used.

- 9.2.2.2** Using results of the set of four analyses, compute the average percent recovery (\bar{X}) and the relative standard deviation (RSD) of percent recovery for each analyte.
- 9.2.2.3** For each analyte, compare RSD and \bar{X} with the corresponding limits for initial precision and recovery in Table 4. For coeluting analytes, use the coeluted compound with the least restrictive specification (largest RSD and widest range). If RSD and \bar{X} for all analytes meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any individual RSD exceeds the precision limit or any individual \bar{X} falls outside the range for accuracy, system performance is unacceptable for that analyte. In this case, correct the problem and repeat the test.
- 9.3** Surrogates - The laboratory shall spike all samples with at least one surrogate analyte to assess method performance on the sample matrix.
- 9.3.1** Analyze each sample according to the Method beginning in Section 11.
- 9.3.2** Compute the percent recovery (P) of the surrogate.
- 9.3.3** The surrogate recovery shall be 40 to 120%. If the recovery of the surrogate falls outside of these limits, method performance is unacceptable for that sample. Water samples are diluted, and smaller amounts of soils, sludges, sediments, and tissues are reanalyzed per Section 18.
- 9.4** Matrix spike—The laboratory shall spike (matrix spike) at least 5% of the samples from a given matrix type (e.g., influent to treatment, treated effluent, produced water, river sediment). If only one sample from a given matrix type is analyzed, a separate aliquot of that sample shall be spiked.
- 9.4.1** The concentration of the matrix spike shall be determined as follows:
- 9.4.1.1** If, as in compliance monitoring, the concentration of a specific analyte in the sample is being checked against a regulatory concentration limit, the MS shall be at that limit or at 1 to 5 times higher than the background concentration determined in Section 9.4.2, whichever concentration is greater.
- 9.4.1.2** If the concentration of an analyte in the sample is not being checked against a limit specific to the analyte, the MS shall be at the concentration of the combined QC standard (Section 7.11) or at 1 to 5 times higher than the background concentration, whichever concentration is greater.
- 9.4.1.3** If it is impractical to determine the background concentration before spiking (e.g., maximum holding times will be exceeded), the matrix spike concentration shall be the regulatory concentration limit, if any; otherwise, the larger of either 5 times the expected background concentration or at the concentration of the combined QC standard (Section 7.11).

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

Equation 1

$$P = [100(A-B)/T]$$

where

T = True value of the spike

- 9.4.3** The matrix spike recoveries in sample should be 25-150%. For the OPR, compare the percent recovery for each analyte with the corresponding QC acceptance criteria in Table 4.

9.4.3.1 If the results of both the MS and the OPR test fall outside the acceptance criteria, the analytical system is judged to be not in control, and the results may not be reported or used for permitting or regulatory compliance purposes. The laboratory must identify and correct the problem and reanalyze all samples in the sample batch.

9.4.3.2 If results for the MS fail the acceptance criteria, and recovery for the OPR standard for the analytical batch is within the acceptance criteria in Table 4, an interference is present and the results may not be reported or otherwise used for permitting or regulatory compliance purposes. If the interference can be attributed to sampling, the site or discharge should be resampled. If the interference can be attributed to a method deficiency, the laboratory must modify the Method, repeat the test required in Section 9.1.2, and repeat analysis of the sample and MS.

9.4.3.3 If the interference cannot be overcome after the Method has been modified, the sample is complex and must be diluted and reanalyzed per Section 18.

- 9.4.4** As part of the QC program for the laboratory, it is suggested but not required that method precision and recovery be assessed and records be maintained. After the analysis of five spiked samples of a given matrix type (water, soil, sludge, sediment, tissue) in which the analytes pass the tests in Section 9.4.3, compute the average percent recovery (P) and the relative standard deviation of the percent recovery (RSD_p) for each analyte (or coeluting analyte group). Express the accuracy assessment as a percent recovery interval from $P - 2RSD_p$ to $P + 2RSD_p$ for each matrix. For example, if $P=90\%$ and $RSD_p=10\%$ for five analyses of compost, the accuracy interval is expressed as 70 to 110%. Update the accuracy assessment for each analyte in each matrix on a regular basis (e.g., after each 5 to 10 new accuracy measurements).

- 9.5** Blanks—Reference matrix blanks are analyzed to demonstrate freedom from contamination.

- 9.5.1** Extract and concentrate a 1-L reagent water blank, a 30-g high-solids reference matrix blank, or a 10-g tissue matrix blank (whichever is appropriate) with each sample batch (Section 9.1.9). Analyze the blank immediately after analysis of the combined QC standard (Section 15.6) to demonstrate freedom from contamination.
- 9.5.2** If any of the analytes of interest (Table 1) or any potentially interfering substance is found in a blank at a concentration greater than the concentration of the low concentration calibration solution in Table 3 (assuming the same calibration factor as aldrin for analytes not listed in Table 1), analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.
- 9.6** Other analytes may be determined by this Method.
- 9.6.1** To establish a quality control limit for an analyte, determine the precision and accuracy by analyzing four replicates of the analyte along with the combined QC standard per the procedure in Section 9.2. If the analyte coelutes with an analyte in the QC standard, prepare a new QC standard without the coeluting component(s).
- 9.6.2** Compute the average percent recovery (A) and the RSD of percent recovery (RSD_n) for the new analyte, and measure the percent recovery and RSD of percent recovery for the other analytes. The data for the new analyte is assumed to be valid if the precision and recovery specifications for the other analytes are met; otherwise, the analytical problem is corrected and the test is repeated. Establish a preliminary QC limit for precision of $2RSD_n$ and a QC limit for recovery of $A \pm 2RSD_n$ for the new analyte and add the limit to Table 4.
- 9.7** The specifications contained in this Method can be met if the apparatus used is calibrated properly, then maintained in a calibrated state. The standards used for calibration (Sections 7.10 and 7.11), calibration verification (Section 15.5), and for initial (Section 9.2) and ongoing (Section 15.6) precision and recovery should be identical, so that the most precise results will be obtained. The GC instruments will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of the analytes given in this Method.
- 9.8** Depending on specific program requirements, field replicates and field spikes of the analytes of interest into samples may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

10.0 Setup and Calibration.

- 10.1** Configure the GC system as given in Section 6.8 and establish the operating conditions in Table 2.
- 10.2** Attainment of method detection limit (MDL) and DDT/Endrin decomposition requirements—Determine that each column/detector system meets the MDLs (Table 2), and the DDT and endrin decomposition test (Section 15.4).
- 10.3** Calibration.

10.3.1 Injection of calibration solutions.

10.3.1.1 Analytes with calibration data in Table 3—The analytes in each calibration group in Table 3 were chosen so that each analyte would be separated from the others by approximately 1 minute on the primary column. The concentrations were chosen to bracket the working range of either the ECD or the ELCD. However, because the response of the ELCD is less for some analytes than that of the ECD, it may be necessary to inject a larger volume of calibration solution when the ELCD is used.

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

10.3.1.3 Set the automatic injector to inject a constant volume in the range of 0.5 to 5.0 μL of each calibration solution into the GC column/detector pairs, beginning with the lowest level mixture and proceeding to the highest. For each analyte, compute, record, and store, as a function of the concentration injected, the retention time and peak area on each column/detector system (primary and confirmatory). For the multi-component analytes (PCBs, toxaphene), store the retention time and peak area for the five largest peaks.

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

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

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

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

10.3.3.1 Compute the relative standard deviation of the calibration factor over the calibration range for each analyte on each column/detector system.

10.3.3.2 Linearity—If the calibration factor for any analyte is constant ($\text{RSD} < 20\%$) over the calibration range, an average calibration factor may be used for that analyte; otherwise, the complete calibration curve (area vs. amount) for that analyte shall be used.

10.4 Combined QC standard—To preclude periodic analysis of all of the individual calibration groups of analytes (Table 3), the GC systems are calibrated with the combined QC standard (Section 7.11) as a final step. Not all of the analytes in these standards will be separated by the GC columns used in this Method. Retention times and calibration factors are verified for the analytes that are resolved, and calibration factors are obtained for the unresolved peaks.

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

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

10.4.1.2 For the peaks containing two or more analytes, compute and store the retention times at the peak maxima on both columns (primary and confirmatory), and also compute and store the calibration factors on both columns. These results will be used for calibration verification (Section 15.5) and for precision and recovery studies (Sections 9.2 and 15.6).

10.5 Florisil calibration—The cleanup procedure in Section 13.4 utilizes Florisil column chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil that is used, the use of the lauric acid value (Reference 7) is suggested. The referenced procedure determines the adsorption of lauric acid (in milligrams per gram of Florisil) from hexane solution. The amount of Florisil to be used for each column is calculated by dividing 110 by this ratio and multiplying by 20 g.

11.0 Sample Preparation.

11.1 Determination of percent solids (for non-tissue samples expected or known to contain $>1\%$ solids).

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

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

11.1.3 Determine percent solids as follows:

Equation 2

$$\% \text{ solids} = 100 (\text{weight of dry sample}) / (\text{weight of wet sample})$$

11.2 Preparation of samples for extraction.

11.2.1 Samples containing 0-30% solids.

11.2.1.1 Samples containing less than 1% solids—Extract the sample directly using continuous liquid-liquid extraction techniques.

11.2.1.1.1 Allow the sample to come to room temperature. Dry the exterior of the sample container. Weigh the sample bottle. Pour the entire volume of sample (Section 8.1.1) into a clean 1.5 to 2.0-L beaker.

11.2.1.1.2 Spike 1.0 mL of the surrogate spiking solution (Section 7.12) into the sample in the beaker. Proceed to preparation of the QC aliquots for low solids samples (Section 11.2.1.3).

11.2.1.2 Samples containing 1 to 30% solids.

11.2.1.2.1 Mix sample thoroughly.

11.2.1.2.2 Using the percent solids found in Section 11.1.3, determine the weight of sample required to produce 1 L of solution containing 1% solids as follows:

Equation 3

$$\text{sample weight} = (1000 \text{ g})/(\% \text{ solids})$$

11.2.1.2.3 Place the weight determined in Section 11.2.1.2.2 in a clean 1.5- to 2.0-L beaker. Discard all sticks, rocks, leaves, and other foreign material prior to weighing.

11.2.1.2.4 Bring the sample volume 100 - 200 mL with reagent water.

11.2.1.2.5 Spike 1.0 mL of the surrogate spiking solution (Section 7.12) into each sample aliquot.

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

11.2.1.2.7 Place the $\frac{3}{4}$ " horn on the ultrasonic probe approximately $\frac{1}{2}$ " below the surface of each sample aliquot and pulse at 50% for 3 minutes at full power. If necessary, remove the probe from the solution and break any large pieces using the metal spatula or a stirring rod and repeat the sonication. Clean the probe with methylene chloride:acetone (1:1) between samples to preclude cross-contamination.

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

11.2.1.3 Preparation of QC aliquots for samples containing 0 - 30% solids

-
- 11.2.1.3.1** For each sample or sample batch (Section 9.1.9), place two 1.0-L (± 0.1 L) aliquots of reagent water in clean 1.5- to 2.0-L beakers.
 - 11.2.1.3.2** Blank—Spike 1.0 mL of the surrogate spiking solution (Section 7.12) into the aliquots.
 - 11.2.1.3.3** OPR—Spike the combined QC standard (Section 7.11) into one of the aliquots.
 - 11.2.1.3.4** MS—Prepare an aliquot at the concentrations specified in Section 9.4.
 - 11.2.1.3.5** Stir and equilibrate all sample and QC solutions for one to two hours. Extract the samples and QC aliquots per Section 12.1.
- 11.2.2** Samples containing >30% solids.
- 11.2.2.1** Mix the sample thoroughly.
 - 11.2.2.2** Weigh 30 g (± 0.3 g) into a clean 400- to 500-mL beaker. Discard all sticks, rocks, leaves, and other foreign material prior to weighing.
 - 11.2.2.3** Spike 1.0 mL of the surrogate spiking solution (Section 7.12) into the sample.
 - 11.2.2.4** Preparation of QC aliquots for samples containing high solids (>30%).
 - 11.2.2.4.1** For each sample or sample batch (Section 9.1.9), weigh two 30-g (± 3 g) aliquots of the high solids reference matrix (Section 7.7.2) into clean 400- to 500-mL beakers. One aliquot will serve as the blank, the other as the OPR.
 - 11.2.2.4.2** Spike 1.0 mL of the surrogate spiking solution (Section 7.12) into each aliquot.
 - 11.2.2.4.3** OPR—Spike the combined QC standard (Section 7.11) into one of the aliquots.
 - 11.2.2.4.4** MS—Prepare an aliquot at the concentration specified in Section 9.4.
 - 11.2.2.5** Extract the high-solids samples per Section 12.2.
- 11.2.3** Tissue samples
- 11.2.3.1** Prior to processing tissue samples, the laboratory must determine the exact tissue to be analyzed. Common requests for analysis of fish tissue include whole fish-skin on, whole fish-skin removed, edible fish fillets (filleted in the field or by the laboratory), specific organs, and other portions. Once the appropriate tissue has been determined, the sample must be homogenized.
 - 11.2.3.2** Homogenization

- 11.2.3.2.1** Samples are homogenized while still frozen, where practical. If the laboratory must dissect the whole fish to obtain the appropriate tissue for analysis, the unused tissues may be rapidly refrozen and stored in a clean glass jar for subsequent use.
- 11.2.3.2.2** Each analysis requires 10 g of tissue (wet weight). Therefore, homogenize at least 30 g of tissue to allow for the MS and for re-extraction of a second aliquot of the same homogenized sample, if re-analysis is required. When whole fish analysis is required, the entire fish is homogenized.
- 11.2.3.2.3** Homogenize the sample in a tissue homogenizer (Section 6.3.3) or grind in a meat grinder (Section 6.3.4). Cut tissue too large to feed into the grinder into smaller pieces. To assure homogeneity, grind three times.
- 11.2.3.2.4** Transfer approximately 10 g (wet weight) of homogenized tissue to a clean, tared, 400- to 500-mL beaker. Record the weight to 3 significant figures.
- 11.2.3.2.5** Spike 1.0 mL of the surrogate spiking solution (Section 7.12) into the sample.
- 11.2.3.2.6** Transfer the remaining homogenized tissue to a clean jar with a fluoropolymer-lined lid. Seal the jar and store the tissue at <-10 °C. Return any tissue that was not homogenized to its original container and store at <-10 °C.

11.2.3.3 Preparation of QC aliquots.

- 11.2.3.3.1** For each sample or sample batch (Section 9.1.9), weigh two 10-g (± 1 g) aliquots of the tissue reference matrix (Section 7.7.3) into clean 400- to 500-mL beakers. One aliquot will serve as the blank and the other as the OPR.
- 11.2.3.3.2** Spike 1.0 mL of the surrogate spiking solution (Section 7.12) into each aliquot.
- 11.2.3.3.4** OPR—Spike the combined QC standard (Section 7.11) into one of the aliquots.
- 11.2.3.3.5** MS—Prepare an aliquot at the concentrations specified in Section 9.4.

12.0 Extraction and Concentration.

12.1 Continuous extraction of samples containing 0-30% solids.

- 12.1.1** Place 100-150 mL of methylene chloride in each continuous extractor and 200 to 300 mL in each distilling flask.
- 12.1.2** Pour the sample(s), blank, OPR, and MS aliquots into their respective extractors.

-
- 12.1.2.1** For each sample, rinse the sample container then the beaker that contained the sample with 20 - 30 mL of methylene chloride and add to the respective extractor. Repeat the rinsing twice. Include all solids in the extraction process.
- 12.1.2.2** For samples containing <1% solids, dry and weigh the sample container and cap and determine the sample volume using the weight determined in Section 11.2.1.1.1, assuming a density of 1.00 g/mL.
- 12.1.2.3** For the blank, OPR, and MS, rinse the beakers that contained these aliquots with 20-30 mL of methylene chloride and add to the respective extractors. Repeat the rinsing twice.
- 12.1.3** Adjust the pH of the waters in the extractors to 5 - 9 with NaOH or H₂SO₄ while monitoring with a pH meter.

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

- 12.1.4** Begin the extraction by heating the flask until the methylene chloride is boiling. When properly adjusted, 1 to 2 drops of methylene chloride per second will fall from the condenser tip into the water. Test and adjust the pH of the waters during the first 1 to 2 hours and between the 3rd and 5th hours of extraction. Extract for 18-24 hours.
- 12.1.5** Remove the distilling flask, estimate and record the volume of extract (to the nearest 100 mL), and pour the contents through a prerinsed drying column containing 7 to 10 cm of granular anhydrous sodium sulfate. Rinse the distilling flask with 30 to 50 mL of methylene chloride and pour through the drying column. Collect the solution in a 500-mL K-D evaporator flask equipped with a 10-mL concentrator tube. Seal, label, and concentrate per Sections 12.4 to 12.5.
- 12.2** Ultrasonic extraction of high solids samples—Procedures are provided for extraction of non-municipal sludge (Section 12.2.1) and municipal sludge samples (Section 12.2.2).
- 12.2.1** Ultrasonic extraction of non-municipal sludge high-solids aliquots.
- 12.2.1.1** Add 60 to 70 g of powdered anhydrous sodium sulfate (Section 7.3.2) to the sample and QC aliquots. Mix each aliquot thoroughly. Some wet samples may require more than 70 g for complete removal of water. All water must be removed prior to addition of organic solvent so that the extraction process is efficient.
- 12.2.1.2** Add 100 ± 10 mL of acetone:methylene chloride (1:1) to each of the aliquots and mix thoroughly.
- 12.2.1.3** Place the ¾" horn on the ultrasonic probe approximately ½" below the surface of the solvent but above the solids layer and pulse at 50% for 3 minutes at full power. If

necessary, remove the probe from the solution and break any large pieces using a metal spatula or a stirring rod and repeat the sonication.

12.2.1.4 Decant the extract through a prerinsed drying column containing 7 to 10 cm of granular anhydrous sodium sulfate into a 500-mL K-D evaporator flask.

12.2.1.5 Repeat the extraction steps (Sections 12.2.1.2 to 12.2.1.4) twice more for each sample and QC aliquot. On the final extraction, swirl the sample or QC aliquot, pour into its respective drying column, and rinse with acetone:methylene chloride. If necessary, transfer the extract to a centrifuge tube and centrifuge for 10 minutes to settle fine particles. Record the approximate extract volume. Concentrate the extract per Sections 12.4 to 12.5.

12.2.2 Ultrasonic extraction of high-solids municipal sludge aliquots.

12.2.2.1 Add 100 ± 10 mL of acetonitrile to each of the aliquots and mix thoroughly.

12.2.2.2 Place the $\frac{3}{4}$ " horn on the ultrasonic probe approximately $\frac{1}{2}$ " below the surface of the solvent but above the solids layer and pulse at 50% for 3 minutes at full power. If necessary, remove the probe from the solution and break any large pieces using a metal spatula or a stirring rod and repeat the sonication.

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

12.2.2.4 Repeat the extraction and filtration steps (Sections 12.2.2.2 to 12.2.2.3) using a second 100 mL of acetonitrile.

12.2.2.5 Repeat the extraction step (Section 12.2.2.2) using 100 ± 10 mL of methylene chloride. On this final extraction, swirl the sample or QC aliquot, pour into its respective filter paper, and rinse with methylene chloride. Record the total extract volume.

12.2.2.6 Back-extract each extract three times sequentially with 500 mL of the aqueous sodium sulfate solution (Section 7.3.3), returning the bottom (organic) layer to the separatory funnel the first two times while discarding the top (aqueous) layer. On the final back extraction, filter each extract through a prerinsed drying column containing 7 - 10 cm granular anhydrous sodium sulfate (Section 7.3.2) into a 500-mL K-D evaporator flask. Record the approximate final extract volume. Concentrate the extract per Sections 12.4 and 12.5.

12.3 Tissue extraction and determination of lipid content.

12.3.1 Pre-extraction of the apparatus.

12.3.1.1 Charge a clean extraction thimble (Section 6.4.3.2) with 5.0 g of silica (Section 7.3.4).

- 12.3.1.2** Place the thimble in a clean extractor. Place 30 to 40 mL of methylene chloride:hexane (1:1) mixture in the receiver and 200 to 250 mL of methylene chloride:hexane (1:1) mixture in the flask.
- 12.3.1.3** Pre-extract by heating the flask until the methylene chloride:hexane (1:1) mixture is boiling. When properly adjusted, one to two drops of methylene chloride:hexane (1:1) mixture will fall per second from the condenser tip into the receiver. Extract the apparatus for a minimum of three hours.
- 12.3.1.4** After pre-extraction, cool and disassemble the apparatus. Rinse the thimble with methylene chloride:hexane (1:1) mixture and allow to air dry.
- 12.3.2** Sample extraction.
- 12.3.2.1** Add 30 to 40 g of powdered anhydrous sodium sulfate (Section 7.3.2) to each of the sample and QC aliquots (Sections 11.2.3.2 and 11.2.3.3) and mix thoroughly. Cover the beakers with aluminum foil and allow to equilibrate for 12-24 hours. Remix prior to extraction to prevent clumping.
- 12.3.2.2** Reassemble the pre-extracted Soxhlet apparatus and add a fresh charge of methylene chloride:hexane to the reflux flask.
- 12.3.2.3** Transfer the sample/sodium sulfate mixture (Section 12.3.2.1) to the Soxhlet thimble, and install the thimble in the Soxhlet apparatus.
- 12.3.2.4** Rinse the beaker with several portions of solvent mixture and add to the thimble. Fill the thimble/receiver with solvent. Extract for 18 to 24 hours.
- 12.3.2.5** After extraction, cool and disassemble the apparatus. Filter each extract through Whatman #41 paper into a 500-mL K-D evaporator flask equipped with a 10-mL concentrator tube. Rinse the extraction apparatus with 30 to 50 mL of methylene chloride and add to the K-D flask.
- 12.3.3** Percent lipid determination—The lipid content is determined by extraction of tissue with the same solvent system (methylene chloride:hexane) that was used in EPA's National Dioxin Study so that lipid contents are consistent with that study.
- 12.3.3.1** Concentrate the tissue extract to near dryness using the macro concentration procedure in Section 12.4.
- 12.3.3.2** Complete the removal of the solvent using the nitrogen blowdown procedure (Section 12.5) and a water bath temperature of approximately 60 °C. Weigh the receiver, record the weight, and return the receiver to the blowdown apparatus, concentrating the residue until a constant weight is obtained. Record the weight of the receiver, boiling chips, and residue.

- 12.3.3.3** Redissolve the residue in methylene chloride and quantitatively transfer to a vial for GPC cleanup (Section 13.2), retaining the boiling chips in the receiver.
- 12.3.3.4** Allow the receiver and chips to dry and weigh the receiver (including the boiling chips).
- 12.3.3.5** Calculate the lipid content of the sample, blank, and OPR to the nearest three significant figures as follows:
-

Equation 4

$$\text{Percent lipid} = \frac{\text{Weight of residue (g)}}{\text{Weight of tissue (g)}} \times 100$$

12.4 Macro concentration.

- 12.4.1** Concentrate the extracts in separate 500-mL K-D flasks equipped with 10-mL concentrator tubes. Add one to two clean boiling chips to the flask and attach a three-ball macro Snyder column. Prewet the column by adding approximately 1 mL of methylene chloride through the top. Place the K-D apparatus in a hot water bath so that the entire lower rounded surface of the flask is bathed with steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.
- 12.4.2** When the liquid has reached an apparent volume of 1 mL, remove the K-D apparatus from the bath and allow the solvent to drain and cool for at least 10 minutes.
- 12.4.3** If the extract is to be cleaned up using GPC, remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation. Adjust the final volume to 10 mL and proceed to GPC cleanup in Section 13.2. If the extract is to be cleaned up by SPE or injected into the GC, proceed per Section 12.5.

12.5 Hexane exchange

- 12.5.1** Remove the Snyder column, add approximately 50 mL of hexane and a clean boiling chip, and reattach the Snyder column. Concentrate the extract as in Section 12.4 except use hexane to prewet the column. The elapsed time of the concentration should be 5 to 10 minutes.
- 12.5.2** Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane. Proceed to micro concentration and solvent exchange

(Section 12.6). After the clean up, adjust the final volume of extract with hexane to 10 mL, 5.0 mL if the cleaned up were done by GPC.

- 12.6** Micro-concentration and solvent exchange—Extracts to be cleaned up using SPE are exchanged into 1.0 mL of SPE elution solvent (Section 7.6.2.2). Extracts to be cleaned up using Florisil or alumina are exchanged into 1.0 mL of hexane. Extracts that have been cleaned up and are ready for GC analysis are exchanged into hexane.
- 12.6.1** Transfer the vial containing the sample extract to the nitrogen blowdown device (Section 6.4.9.3). 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.
- 12.6.2** Lower the vial into a 45 °C water bath and continue concentrating.
- 12.6.2.1** If the extract is to be concentrated to dryness for weight determination (Section 12.3.3.2), blow dry at 60 °C until a constant weight is obtained.
- 12.6.2.2** If the extract is to be concentrated for injection into the GC or the solvent is to be exchanged for extract cleanup, proceed as follows:
- 12.6.3** When the volume of the liquid is approximately 200 µL, add 2 to 3 mL of the desired solvent (acetone for alumina or hexane for the other cleanups) and continue concentrating to approximately 100 µL. Repeat the addition of solvent and concentrate once more.
- 12.6.4** Adjust the volume of an extract to be cleaned up by SPE, Florisil, alumina, or sulfur removal to 1.0 mL. Proceed to extract cleanup (Section 13).
- 12.6.5** For an extract that was cleaned up and is ready for GC analysis, adjust the volume as follows:
- 12.6.5.1** Aqueous samples containing <1% solids—Adjust the final extract volume to be consistent with the volume extracted.
- 12.6.5.1.1** For extracts that have been cleaned up using GPC, adjust the final extract volume to 1/200 of the volume extracted. For example if the volume extracted is 950 mL, adjust the final extract volume to 4.8 mL.
- 12.6.5.1.2** For extracts that have not been cleaned up using GPC, adjust the final extract volume to 1/100 of the volume extracted. For example if the volume extracted is 1000 mL, adjust the final extract volume to 10 mL.

Note: *The difference in volume fractions (1/200 or 1/100) accounts for the 50% loss in the GPC cleanup. Also, by preserving the ratio between the volume extracted and the final extract volume, the concentrations, detection limits, and minimum levels of quantitation do not need to be adjusted for differences in the volume extracted.*

12.6.5.2 For aqueous samples containing >1% solids, soil, sludge, and tissue samples, adjust the final extract volume to 5 mL if the extract was cleaned up by GPC; otherwise, adjust the volume to 10 mL (the difference accounts for the 50% loss in the GPC cleanup).

12.6.6 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 <-10 °C. Analyze the extract by GC per the procedure in Section 14.

13.0 Extract Cleanup

13.1 Except for tissues, cleanup procedures may not be necessary. 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. However, the laboratory shall first repeat the tests in Section 9.2 to demonstrate that the requirements of Section 9.2 can be met using the cleanup procedure(s) as an integral part of this Method. Figure 1 outlines the cleanup steps.

13.1.1 Gel permeation chromatography (Section 13.2) removes many high molecular weight interferences that cause GC column performance to degrade. It is used for all soil, sediment, and tissue extracts and may be used for water extracts that are expected to contain high molecular weight organic substances (e.g., polymeric materials, humic acids).

13.1.2 The solid-phase extraction cartridge (Section 13.3) removes polar organic compounds such as phenols.

13.1.3 The Florisil column (Section 13.4) allows for selected fractionation of the organo-chlorine compounds and will also eliminate polar interferences.

13.1.4 Alumina column cleanup (Section 13.5) also removes polar materials.

13.1.5 Elemental sulfur, which interferes with the electron capture gas chromatography of some of the pesticides, is removed using GPC, mercury, or activated copper. Sulfur removal (Section 13.6) is required when sulfur is known or suspected to be present.

13.2 Gel permeation chromatography (GPC).

13.2.1 Column packing.

13.2.1.1 Place 70 to 75 g of SX-3 Bio-beads (Section 7.6.6) in a 400- to 500-mL beaker.

- 13.2.1.2** Cover the beads with methylene chloride and allow to swell overnight (12 hours minimum).
- 13.2.1.3** Transfer the swelled beads to the column and pump solvent through the column, from bottom to top, at 4.5 to 5.5 mL/min prior to connecting the column to the detector.
- 13.2.1.4** After purging the column with solvent for one to two hours, adjust the column head pressure to 7 to 10 psig, and purge for 4 to 5 hours to remove air. Maintain a head pressure of 7 to 10 psig. Connect the column to the detector.
- 13.2.2** Column calibration.
- 13.2.2.1** Load 5 mL of the calibration solution (Section 7.5) into the sample loop.
- 13.2.2.2** Inject the calibration solution and record the signal from the detector. The elution pattern will be corn oil, bis (2-ethylhexyl) phthalate, pentachlorophenol, perylene, and sulfur.
- 13.2.2.3** Set the "dump time" to allow greater than 85% removal of the corn oil and greater than 85% collection of the phthalate.
- 13.2.2.4** Set the "collect time" to the peak minimum between perylene and sulfur.
- 13.2.2.5** Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the pentachlorophenol is greater than 85%. If calibration is not verified, the system shall be recalibrated using the calibration solution, and the sample batch (those samples affected by the calibration) shall be re-extracted and cleaned up using a calibrated GPC system.
- 13.2.3** Extract cleanup—GPC requires that the column not be overloaded. The column specified in this Method is designed to handle a maximum of 0.5 g of high molecular weight material in a 5-mL extract. If the extract is known or expected to contain more than 0.5 g, the extract is split into fractions for GPC and the fractions are combined after elution from the column. The solids content of the extract may be obtained gravimetrically by evaporating the solvent from a 50- μ L aliquot.
- 13.2.3.1** Filter the extract or load through the filter holder to remove particulates. Load the extract onto the column.
- 13.2.3.2** Elute the extract using the calibration data determined in Section 13.2.2. Collect the eluate in a clean 400- to 500-mL beaker.
- 13.2.3.3** Rinse the sample loading tube thoroughly with methylene chloride between extracts to prepare for the next sample.

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

13.2.3.5 Concentrate the extract and exchange into hexane per Sections 12.4 and 12.5.

13.3 Solid-phase extraction (SPE).

13.3.1 Setup.

13.3.1.1 Attach the Vac-elute Manifold (Section 6.5.4.2) to a water aspirator or vacuum pump with the trap and gauge installed between the manifold and vacuum source.

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

13.3.2 Cartridge washing—Pre-elute each cartridge prior to use sequentially with 10-mL portions each of hexane, methanol, and water using vacuum for 30 seconds after each eluant. Follow this pre-elution with 1 mL methylene chloride and three 10-mL portions of the elution solvent (Section 7.6.2.2) using vacuum for 5 minutes after each eluant. Tap the cartridge lightly while under vacuum to dry between eluants. The three portions of elution solvent may be collected and used as a blank if desired. Finally, elute the cartridge with 10 mL each of methanol and water, using the vacuum for 30 seconds after each eluant.

13.3.3 Cartridge certification—Each cartridge lot must be certified to ensure recovery of the analytes of interest and removal of 2,4,6-trichlorophenol.

13.3.3.1 To make the test mixture, add the trichlorophenol solution (Section 7.6.2.1) to the combined QC standard (Section 7.11). Elute the mixture using the procedure in Section 13.3.4.

13.3.3.2 Concentrate the eluant to 1.0 mL and inject 1.0 μ L of the concentrated eluant into the GC using the procedure in Section 14. The recovery of all analytes (including the unresolved GC peaks) shall be within the ranges for recovery specified in Table 4, and the peak for trichlorophenol shall not be detectable; otherwise the SPE cartridge is not performing properly and the cartridge lot shall be rejected.

13.3.4 Extract cleanup.

13.3.4.1 After cartridge washing (Section 13.3.2), release the vacuum and place the rack containing the 50-mL volumetric flasks (Section 6.5.3.4) in the vacuum manifold. Re-establish the vacuum at 5 to 10 psi.

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

13.3.4.3 Elute each cartridge into its volumetric flask sequentially with three 10 mL portions of the elution solvent (Section 7.6.2.2), using vacuum for five minutes after each portion. Collect the eluants in the 50-mL volumetric flasks.

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

13.3.4.5 Concentrate the eluted extracts per Sections 12.4 and 12.5.

13.4 Florisil column.

13.4.1 Place a weight of Florisil (nominally 20 g) predetermined by calibration (Section 10.5) 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.

13.4.2 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 eluate.

13.4.3 Transfer the concentrated extract (Section 12.5) onto the column. Complete the transfer with two 1-mL hexane rinses.

13.4.4 Place a clean 500-mL K-D flask and concentrator tube under the column. Drain the column into the flask until the sodium sulfate layer is nearly exposed. 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. 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.

13.4.5 Concentrate the extracts per Sections 12.4 and 12.5.

13.5 Alumina column.

13.5.1 Add 3 g of activity III neutral alumina to a 10-mL chromatographic column. Tap the column to settle the alumina.

13.5.2 Transfer the extract to the top of the column and collect the eluate in a clean 10 mL concentrator tube. Rinse the extract container with 1-2 mL portions of hexane (to a total volume of 9 mL) and add to the alumina column. Do not allow the column to go dry.

13.5.3 Concentrate the extract per Sections 12.4 and 12.5.

13.6 Sulfur removal—Elemental sulfur will usually elute entirely in Fraction 1 of the Florisil column cleanup.

- 13.6.1** Transfer the concentrated extract into a clean concentrator tube or fluoropolymer-sealed vial. Add 1 to 2 drops of mercury or 100 mg of activated copper powder and seal (Reference 11). If TBA sulfite is used, add 1 mL of the TBA sulfite reagent and 2 mL of isopropanol.
- 13.6.2** Agitate the contents of the vial for 1 to 2 hours on a reciprocal shaker. If the mercury or copper appears shiny, or if precipitated sodium sulfite crystals from the TBA sulfite reagent are present, and if the color remains unchanged, all sulfur has been removed; if not, repeat the addition and shaking.
- 13.6.2.1** If mercury or copper is used, centrifuge and filter the extract to remove all residual mercury or copper. Dispose of the mercury waste properly. Bring the final volume to 1.0 mL and analyze by gas chromatography per the procedure in Section 14.
- 13.6.2.2** If TBA sulfite is used, add 5 mL of reagent water and shake for 1 to 2 minutes. Centrifuge and filter the extract to remove all precipitate. Transfer the hexane (top) layer to a K-D concentrator tube (Section 6.4.9.1.2) and concentrate per Section 12.5.

14.0 Gas Chromatography.

Note: *Table 2 shows the retention times and MDLs that can be achieved. Examples of the separations achieved by the primary and confirmatory columns are shown in Figure 2.*

- 14.1** Calibrate the system as described in Section 10.
- 14.2** Set the auto-sampler to inject the same volume that was chosen for calibration (Section 10.3.1.3) for all standards and extracts of blanks and samples.
- 14.3** Set the data system or GC control to start the temperature program upon sample injection, and begin data collection after the solvent peak elutes. Set the data system to stop data collection after the last analyte is expected to elute and to return the column to the initial temperature.

15.0 System and Laboratory Performance.

- 15.1** At the beginning of each 12-hour shift during which analyses are performed, GC system performance and calibration are verified for all pollutants and surrogates on both column/detector systems. For these tests, analysis of the combined QC standard (Section 7.11) shall be used to verify all performance criteria. Adjustment and/or recalibration (per Section 10) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, and precision and recovery standards be analyzed.
- 15.2** Retention times—The absolute retention times of the peak maxima shall be within ± 10 seconds of the retention times in the initial calibration (Section 10.4.1).

15.3 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.

15.3.1 Primary column (DB-608)—DDT and endrin aldehyde.

15.3.2 Confirmatory column (DB-1701)—Alpha and gamma chlordane.

15.4 Decomposition of DDT and endrin.

15.4.1 Analyze 1 μ L of the DDT and endrin decomposition solution (Section 7.13).

15.4.2 Measure the total area of all peaks in the chromatogram.

15.4.3 The area of peaks other than the sum of the areas of the DDT and endrin peaks shall be less than 20% the sum of the areas of these two peaks. If the area is greater than this sum, the system is not performing acceptably for DDT and endrin. In this case, the GC column system that failed shall be repaired and the performance tests (Sections 15.1 to 15.4) shall be repeated 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.*

15.5 Calibration verification—Calibration is verified for the combined QC standard only.

15.5.1 Inject the combined QC standard (Section 7.11).

15.5.2 Compute the percent recovery of each analyte or coeluting analytes, based on the calibration data (Section 10.4).

15.5.3 For each analyte or coeluted analytes, compare this calibration verification recovery with the corresponding limits for ongoing recovery in Table 4. For coeluting analytes, use the coeluted analyte with the least restrictive specification (the widest range). If the recoveries for all analytes meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any recovery falls outside the calibration verification range, system performance is unacceptable for that analyte. In this case, correct the problem and repeat the test, or recalibrate (Section 10). If verification requirements are met, the calibration is assumed to be valid for the multicomponent analytes (PCBs and toxaphene).

15.6 Ongoing precision and recovery.

15.6.1 Analyze the extract of the combined QC standard (Section 7.11) extracted with each sample batch (Sections 11.2.1.3.3, 11.2.2.4.3, and 11.2.3.3.3).

- 15.6.2** Compute the percent recovery of each analyte and for coeluting analytes.
- 15.6.3** For each analyte or coeluted analytes, compare the percent recovery with the limits for ongoing recovery in Table 4. For coeluted analytes, use the coeluted analyte with the least restrictive specification (widest range). If all analytes pass, the extraction, concentration, and cleanup processes are in control and analysis of blanks and samples may proceed. If, however, any of the analytes fail, these processes are not in control. In this event, correct the problem, re-extract the sample batch, and repeat the ongoing precision and recovery test.
- 15.6.4** It is suggested but not required that the laboratory update statements of laboratory data quality. Add results that pass the specifications in Section 15.6.3 to initial and previous ongoing data. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory data quality for each analyte by calculating the average percent recovery (R) and the standard deviation of percent recovery s_r . Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if $R = 95\%$ and $s_r = 5\%$, the accuracy is 85 to 105%.

16.0 Qualitative Determination.

- 16.1** Qualitative determination is accomplished by comparison of data from analysis of a sample or blank with data from analysis of the shift standard (Section 15.1), and with data stored in the retention-time and calibration libraries (Section 10.4.1.2). The pesticide window is defined by the CAL data but may be modified by slight changes that will be reflected in calibration verification (VER), blank (BLK), ongoing precision and recovery (OPR), and matrix spike (MS) data. Identification is confirmed when retention time and amounts agree per the criteria below.
- 16.2** For each analyte on each column/detector system, establish a retention-time window of 3 RSD on either side of the average retention time in the calibration data (Section 10.4.1.2). For analytes that have a retention-time curve (Section 10.3.2.2), establish this window as the minimum -10 seconds and maximum +10 seconds. For the multi-component analytes, use the retention times of the five largest peaks in the chromatogram from the calibration data (Section 10.4.1.2).
- 16.2.1** Analytes not requiring a retention-time calibration curve—If a peak representing an analyte from the analysis of a sample or blank is within its respective window (as defined in Section 16.2), on both column/detector systems, it is considered identified. Identity is further confirmed when the computed amounts (Section 17) on both column/detector systems agree within a factor of 2.
- 16.2.2** Analytes requiring a retention-time calibration curve—If a peak representing an analyte from the analysis of a sample or blank is within ± 10 seconds of the retention times for the computed amounts (Section 17), as determined by the retention-time calibration curve (Section 10.3.2.2), it is considered identified. Identity is further confirmed when the computed amounts (Section 17) on both column/detector systems agree within a factor of 2.

- 16.2.3** When the concentration of an analyte is sufficient, it should be further identified by gas chromatography/mass spectrometry (GC/MS).
- 16.2.4** 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 must use all of the cleanup procedures in this Method (Section 18.2) to attempt to remove the interferent. After all 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.
- 16.2.4.1** 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 VER, BLK, OPR, and MS, the RT for the analyte of interest in the sample should be within this variation regardless of the window established in Section 16.2. If the analyte is not within this variation on both columns, it is likely not present.
- 16.2.4.2** The possibility exists that the RT for the analyte in a sample could shift if extraneous materials are present. This possibility can be confirmed or refuted by the behavior of the surrogates in the sample. If the RTs for the surrogates on both columns are consistent with their RTs in the VER, BLK, OPR and MS data, the RT for the analyte of interest cannot shift.
- 16.2.4.3** 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 pesticide is present because shifts nearly always occur in the same direction on both columns.

17.0 Quantitative Determination.

- 17.1** Using the GC data system, compute the concentration of the analyte detected in the extract (in $\mu\text{g/mL}$) using the calibration factor or calibration curve (Section 10.3.3.2).
- 17.2** Liquid samples
- 17.2.1** Compute the concentration in the sample using the following equation:

Equation 5

$$C_s = 10 C_{ex}/(V_s)$$

where

- C_s = Concentration in the sample, in $\mu\text{g/L}$
 - 10 = Final extract total volume, in mL
 - C_{ex} = Concentration in the extract, in $\mu\text{g/mL}$
 - V_s = Sample extracted, in L
-

17.2.2 Samples containing <1% solids - If the final extract volume was adjusted to be consistent with the sample volume extracted (Section 12.6.5.1), adjust the concentrations of the surrogate and matrix spike compounds for the sample volume (Section 12.6.5.1). For example, if the sample volume was 950 mL, adjust the concentration of the surrogates and matrix spike compounds by multiplying the concentration by 1000/950.

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

Equation 6

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

where

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

17.4 Tissue samples.

17.4.1 Total sample—calculate the concentration in the total tissue sample using the following equation:

Equation 7

$$C_s = (V_e C_{ex})/W_s$$

where

- C_s = Concentration in the sample, in $\mu\text{g/g}$
 V_e = Extract volume, in mL
 C_{ex} = Concentration in the extract, in $\mu\text{g/mL}$
 W_s = Sample weight, in g

17.4.2 Concentration in lipid fraction—Calculate the concentration in the lipid fraction using the following equation:

Equation 8

$$C_l = 100C_s/\%lipid$$

where

- C_l = concentration in lipid fraction, in $\mu\text{g/g}$
 C_s = total concentration (from Equation 7)
 $\%lipid$ = the percent lipid content

- 17.5** If the concentration of any analyte exceeds the calibration range of the system, the extract is diluted by a factor of 10, and a 1- μL aliquot of the diluted extract is analyzed.
- 17.6** Two or more PCBs in a given sample are quantitated and reported as total PCB.
- 17.7** Report results for all pollutants found in all standards, blanks, and samples to three significant figures. Results for samples that have been diluted are reported at the least dilute level at which the concentration is in the calibration range.
- 17.8** The laboratory must report the lower value from the two columns. The laboratory must analyze the sample by GC/MS or on a third column when analytes have co-eluted or interfere with determination on one of the two columns used in the analysis.

Note: *Dichlone and kepone do not elute from the DB-1701 column and must be confirmed on DB-5.*

- 17.9** Quantitative information that may aid in the confirmation of the presence of an analyte
- 17.9.1** Sections 16.2.1 and 16.2.2 state that if the amounts on both column/detector systems agree within a factor of 2, the pesticide is present. This factor of 2 is generous and allows for the pesticide that has the largest measurement error. A better indication of the actual measurement error can be gained from the IPR and VER data. Using these data, establish

a measurement window ± 3 standard deviations (or ± 3 RSD) around the mean on one column. If the concentration for the pesticide determined on the other column/detector system agrees within this window, and the RT data agree (from Section 16), the pesticide is definitely confirmed.

- 17.9.2** If the amounts do not agree, and the RT data indicate the presence of the analyte (per Section 16), it is likely that an interference is present on the column that yielded the highest result. Look for a separate peak for this interference on the other column. It should be near the peak for the analyte of interest, and the area of the peak, when added to the area of the peak for the analyte of interest, should provide an estimate of what the concentration would be if the peaks coincided. This concentration should roughly agree with the concentration determined for the analyte on the column on which the suspected interference occurred. In this event, the pesticide is confirmed and lowest concentration should be reported.

18.0 Analysis of Complex Samples.

- 18.1** Some samples may contain high levels (greater than 1000 ng/L) of the analytes of interest, interfering analytes, and/or polymeric materials. Some samples may not concentrate to 1.0 mL (Section 12.5); others may overload the GC column and/or detector.
- 18.2** The laboratory shall attempt to clean up all samples using GPC (Section 13.2), the SPE cartridge (Section 13.3), by Florisil (Section 13.4) or Alumina (Section 13.5), and sulfur removal (Section 13.6). If these techniques do not remove the interfering analytes, the extract is diluted by a factor of 10 and reanalyzed (Section 17.5).
- 18.3** Recovery of surrogate—In most samples, surrogate recoveries will be similar to those from the reference matrix. If the surrogate recovery is outside the range specified in Section 9.3.3, the sample is re-extracted and re-analyzed. If the surrogate recovery is still outside this range, 1/10 the amount of sample is analyzed to overcome any matrix interference problems.
- 18.4** Recovery of the matrix spike—In most samples, the matrix spike recoveries will be similar to those from the reference matrix. If the matrix spike recovery is outside the range specified in Section 9.4.3, 1/10 the amount of sample is respiked and reanalyzed. 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. Poor matrix spike recovery does not relieve a discharger or permittee of reporting timely results.

19.0 Method Performance

Development of this Method is detailed in References 12 and 13.

20.0 Pollution Prevention.

- 20.1** 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, laboratory personnel should use pollution prevention techniques to address waste generation. When wastes cannot be reduced feasibly at the source, the Agency recommends recycling as the next best option. The solvents used in this Method should be reused as practicable by purifying by distillation. The only other chemicals used in this Method are the neat materials used in preparing standards. These standards 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.
- 20.2** For information about pollution prevention that may be applied to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Governmental Relations and Science Policy, 1155 16th Street NW, Washington DC 20036, 202/872-4477.

21.0 Waste Management.

- 21.1** The laboratory is responsible for complying with all Federal, State, and local regulations governing waste management, particularly hazardous waste identification rules and land disposal restrictions, and for protecting the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required. An overview of requirements can be found in *Environmental Management Guide for Small Laboratories* (EPA 233-B-98-001).
- 21.2** Acids, bases, and samples at pH <2 must be neutralized before being disposed of, or must be handled as hazardous waste.
- 21.3** For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better: Laboratory Chemical Management for Waste Reduction*, both available from the American Chemical Society's Department of Governmental Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

22.0 References

- 22.1** "Guidelines Establishing Test Procedures for the Analysis of Pollutants under the Clean Water Act; Final Rule and Interim Final Rule and Proposed Rule," *Federal Register*, October 26, 1984 (49 FR 43234).
- 22.2** "Methods for the Determination of Organic Compounds in Drinking Water," U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio: EPA-600/4-88/039, December 1988.

- 22.3** "Methods for the Determination of Nonconventional Pesticides in Municipal and Industrial Wastewater," Volume 1 (EPA-821-R-93-010-A) and Volume 2 (EPA-821-R-93-010-B), August 1993.
- 22.4** "Carcinogens-Working with Carcinogens." Department of Health, Education, and Welfare; Public Health Service; Center for Disease Control; National Institute for Occupational Health and Safety: Publication 77-206, August 1977.
- 22.5** "OSHA Safety and Health Standards, General Industry" (29 CFR 1910). Occupational Safety and Health Administration: January 1976.
- 22.6** "Safety in Academic Chemistry Laboratories," American Chemical Society Committee on Chemical Safety: 1979.
- 22.7** Mills, P. A. "Variation of Florisil Activity: Simple Method for Measuring Adsorbent Capacity and Its Use in Standardizing Florisil Columns," Journal of the Association of Official Analytical Chemists, 51, 29: 1968.
- 22.8** "Standard Practice for Sampling Water" (ASTM Annual Book of Standards), American Society for Testing and Materials, Philadelphia, Pennsylvania: 76, 1980.
- 22.9** "Methods 330.4 and 330.5 for Total Residual Chlorine," U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH: EPA-600/4-70-020, March 1979.
- 22.10** "Handbook of Quality Control in Wastewater Laboratories," U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH: EPA-600/4-79-019, March 1979.
- 22.11** Goerlitz, D.F. and Law, L.M. "Bulletin for Environmental Contamination and Toxicology": 6, 9, 1971.
- 22.12** "Consolidated GC Method for the Determination of ITD/RCRA Pesticides using Selective GC Detectors," S-CUBED, A Division of Maxwell Laboratories, Inc., La Jolla, CA: Ref. 32145-01, Document R70, September 1986.
- 22.13** "Method Development and Validation, EPA Method 1618, Cleanup Procedures," Pesticide Center, Department of Environmental Health, Colorado State University: November 1988 and January 1989.

23.0 Tables

Table 1.

Organo-Halide Pesticides Determined by Large-Bore, Fused-Silica Capillary Column Gas Chromatography with Halide-Specific Detector

<i>EPA EGD</i>	<i>Analyte</i>	<i>CAS Registry</i>
	Acephate	30560-19-1
	Alachlor	15972-60-8
089	Aldrin	309-00-2
	Atrazine	1912-24-9
	Benfluralin (Benefin)	1861-40-1
102	α -BHC	319-84-6
103	β -BHC	319-85-7
104	γ -BHC (Lindane)	58-89-9
105	δ -BHC	319-86-8
	Bromacil	314-40-9
	Bromoxynil octanoate	1689-99-2
	Butachlor	23184-66-9
434	Captafol	2425-06-1
433	Captan	133-06-2
441	Carbophenothion (Trithion)	786-19-6
	α -Chlordane (cis-Chlordane)	5103-71-9
091	γ -Chlordane (trans-Chlordane)	5566-34-7
431	Chlorobenzilate	510-15-6
	Chloroneb (Terraneb)	2675-77-6
	Chloropropylate (Acaralate)	5836-10-2
	Chlorothalonil	1897-45-6
	DBCP (Dibromochloropropane)	96-12-8
	DCPA (Dacthal)	1861-32-1
094	4,4'-DDD (TDE)	72-54-8
093	4,4'-DDE	72-55-9
092	4,4'-DDT	50-29-3
432	Diallate (Avadex)	2303-16-4
478	Dichlone	117-80-6
	Dicofol	115-32-2
090	Dieldrin	60-57-1
095	Endosulfan I	959-98-8
096	Endosulfan II	33213-65-9
097	Endosulfan sulfate	1031-07-8
098	Endrin	72-20-8

Table 1.
Organo-Halide Pesticides Determined by Large-Bore, Fused-Silica Capillary Column Gas Chromatography
with Halide-Specific Detector

<i>EPA EGD</i>	<i>Analyte</i>	<i>CAS Registry</i>
099	Endrin aldehyde	7421-93-4
435	Endrin ketone	53494-70-5
	Ethalfuralin (Sonalan)	55283-68-6
	Etridiazole (Terrazole [®])	2593-15-9
	Fenarimol (Rubigan)	60168-88-9
100	Heptachlor	76-44-8
101	Heptachlor epoxide	1024-57-3
437	Isodrin	465-73-6
	Isopropalin (Paarlan)	33820-53-0
439	Kepone	143-50-0
430	Methoxychlor	72-43-5
	Metribuzin	21087-64-9
438	Mirex	2385-85-5
436	Nitrofen (TOK)	1836-75-5
	Norfluorazon	27314-13-2
112	PCB-1016	12674-11-2
108	PCB-1221	11104-28-2
109	PCB-1232	11141-16-5
106	PCB-1242	53469-21-9
110	PCB-1248	12672-29-6
107	PCB-1254	11097-69-1
111	PCB-1260	11096-82-5
440	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
	Propachlor	1918-16-7
	Propanil	709-98-8
	Propazine	139-40-2
	Simazine	122-34-9
	Strobane	8001-50-1
	Terbacil	5902-51-2
	Terbuthylazine	5915-41-3
113	Toxaphene	8001-35-2
	Triadimefon (Bayleton)	43121-43-3

Table 1.

Organo-Halide Pesticides Determined by Large-Bore, Fused-Silica Capillary Column Gas Chromatography with Halide-Specific Detector

<i>EPA EGD</i>	<i>Analyte</i>	<i>CAS Registry</i>
442	Trifluralin	1582-09-8

Table 2.

Gas Chromatography of Organo-Halide Pesticides

<i>EPA EGD</i>	<i>Analyte</i>	<i>Retention Time (min)¹</i>		<i>Method Detection Limit²</i>	
		<i>DB-608</i>	<i>DB-1701</i>	<i>(ng/L)</i>	
	Acephate	5.03	-- ³	2000	est (ECD)
442	Trifluralin	5.16	6.79	50	est
	Ethalfuralin	5.28	6.49	5	est (ECD)
	Benfluralin	5.53	6.87	20	est (ECD)
432	Diallate-A	7.15	6.23	45	
	Diallate-B	7.42	6.77	32	
102	α -BHC	8.14	7.44	6	
440	PCNB	9.03	7.58	6	
	Simazine	9.06	9.29	400	est (ECD)
	Atrazine	9.12	9.12	500	est (ECD)
	Terbutylazine	9.17	9.46	300	est (ECD)
104	γ -BHC (Lindane)	9.52	9.91	11	
103	β -BHC	9.86	11.90	7	
100	Heptachlor	10.66	10.55	5	
	Chlorothalonil	10.66	10.96	15	est (ECD)
478	Dichlone	10.80	-- ³	-- ⁴	
	Terbacil	11.11	12.63	200	est (ECD)
105	δ -BHC	11.20	12.98	5	
	Alachlor	11.57	11.06	20	est (ECD)
	Propanil	11.60	14.10		
089	Aldrin	11.84	11.46	8	
	DCPA	12.18	12.09	3	est (ECD)
	Metribuzin	12.80	11.68	5	est (ECD)
	Triadimefon	12.99	13.57	50	est (ECD)
	Isopropalin	13.06	13.37	20	est (ECD)
437	Isodrin	13.47	11.12	13	
101	Heptachlor epoxide	13.97	12.56	12	
	Pendamethalin	14.21	13.46	30	
	Bromacil	14.39	-- ³	70	est (ECD)
	γ -Chlordane	14.63	14.20	9	
	Butachlor	15.03	15.69	30	est (ECD)
091	α -Chlordane	15.24	14.36	8	

Table 2.
Gas Chromatography of Organo-Halide Pesticides

EPA EGD	Analyte	Retention Time (min) ¹		Method Detection Limit ² (ng/L)	
		DB-608	DB-1701		
095	Endosulfan I	15.25	13.87	11	
093	4,4'-DDE	16.34	14.84	10	
090	Dieldrin	16.41	15.25	6	
433	Captan	16.83	15.43	100	est (ECD)
431	Chlorobenzilate	17.58	17.28	25	
098	Endrin	17.80	15.86	4	
436	Nitrofen (TOK)	17.86	17.47	13	
439	Kepone	17.92	24.03	100	est (ECD)
094	4,4'-DDD	18.43	17.77	5	
096	Endosulfan II	18.45	18.57	8	
	Bromoxynil octanoate	18.85	18.57	30	est (ECD)
092	4,4'-DDT	19.48	18.32	12	
441	Carbophenothion	19.65	18.21	50	
099	Endrin aldehyde	19.72	19.18	11	
097	Endosulfan sulfate	20.21	20.37	7	
434	Captafol	22.51	21.22	100	est (ECD)
	Norfluorazon	20.68	22.01	50	est (ECD)
438	Mirex	22.75	19.79	4	
430	Methoxychlor	22.80	20.68	30	
435	Endrin ketone	23.00	21.79	8	
	Fenarimol	24.53	23.79	20	est (ECD)
	cis-Permethrin	25.00	23.59	200	est (ECD)
	trans-Permethrin	25.62	23.92	200	est (ECD)
106	PCB-1242			150	est
109	PCB-1232			150	est
112	PCB-1016			150	est
108	PCB-1221			150	est
110	PCB-1248			150	est
107	PCB-1254			150	est
111	PCB-1260	15.44	14.64	140	
		15.73	15.36		
		16.94	16.53		
		17.28	18.70		
		19.17	19.92		
113	Toxaphene	16.60	16.60	910	
		17.37	17.52		
		18.11	17.92		
		19.46	18.73		
		19.69	19.00		

Notes:

1. Columns: 30 m long × 0.53 mm ID; DB-608: 0.83 μ; DB-1701: 1.0 μ. Conditions suggested to meet retention times shown: 150°C for 0.5 minutes, 150-270° at 5°C/min, 270°C until trans-permethrin elutes. Carrier gas flow rates approximately 7 mL/min.
2. 40 *CFR* Part 136, Appendix B (49 *FR* 43234). MDLs were obtained by a single laboratory with an electrolytic conductivity detector, except as noted. MDL's for soils (in ng/kg) are estimated to be 30-100 times this level.
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 3.

Concentrations of Calibration Solutions for Electron Capture Detector and Suggested Calibration Groups

<i>EPA EGD</i>	<i>Analyte</i> ¹	<i>Concentration (ng/mL)</i>		
		<i>Low</i>	<i>Medium</i>	<i>High</i>
<i>Calibration group 1</i>				
	Acephate	2000	10000	40000
	Alachlor	20	100	400
	Atrazine	1000	5000	20000
103	β-BHC	10	50	200
	Bromoxynil octanoate	50	250	1000
434	Captafol	200	1000	4000
432	Diallate	200	1000	4000
097	Endosulfan sulfate	10	50	200
098	Endrin	20	100	400
437	Isodrin	10	50	200
	Pendimethalin (Prowl)	50	250	1000
	trans-Permethrin	200	1000	4000
<i>Calibration group 2</i>				
102	α-BHC	5.0	25	100
	DCPA	5.0	25	100
093	4,4'-DDE	10	50	200
092	4,4'-DDT	10	50	200
478	Dichlone	20	100	400
	Ethalfuralin	10	50	200
	Fenarimol	20	100	400
430	Methoxychlor	20	100	400
	Metribuzin	10	50	200
<i>Calibration group 3</i>				
105	γ-BHC (Lindane)	5	25	100
091	γ-Chlordane	5	25	100
435	Endrin ketone	10	50	200
101	Heptachlor epoxide	5	25	100
	Isopropalin	20	100	400
436	Nitrofen (TOK)	20	100	400
440	PCNB	5	25	100
	cis-Permethrin	200	1000	4000
442	Trifluralin	10	50	200
<i>Calibration group 4</i>				
	Benfluralin	20	100	400
431	Chlorobenzilate	50	500	5000
090	Dieldrin	5	20	100
095	Endosulfan I	10	50	200

Table 3.

Concentrations of Calibration Solutions for Electron Capture Detector and Suggested Calibration Groups

<i>EPA EGD</i>	<i>Analyte</i> ¹	<i>Concentration (ng/mL)</i>		
		<i>Low</i>	<i>Medium</i>	<i>High</i>
438	Mirex	20	100	400
	Terbacil	200	1000	4000
	Terbutylazine	500	2500	10000
	Triadimefon	100	500	2000
<i>Calibration group 5</i>				
433	α -Chlordane	10	50	200
	Captan	100	500	2000
	Chlorothalonil	20	100	400
094	4,4'-DDD	20	100	400
	Norfluorazon	100	500	2000
	Simazine	800	4000	20000
<i>Calibration group 6</i>				
089	Aldrin	20	100	400
104	δ -BHC	5	25	100
	Bromacil	100	500	2000
	Butachlor	50	250	1000
096	Endosulfan II	10	50	200
100	Heptachlor	10	50	200
439	Kepone	100	500	2000
<i>Calibration group 7</i>				
	Carbophenothion	80	400	1600
	Chloroneb	300	1500	6000
	Chloropropylate	200	1000	4000
	DBCP	25	125	500
	Dicofol	300	1500	6000
	Endrin aldehyde	80	400	1600
	Etridiazole	80	400	1600
	Perthane	1000	5000	20000
	Propachlor	500	2500	10000
	Propanil	200	1000	4000
	Propazine	1000	5000	20000

¹ For analytes listed in Table 2 that are not listed in this table, determine appropriate ranges for calibration standards.

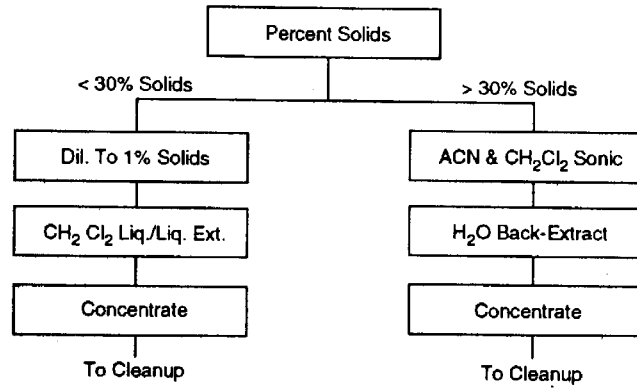
Table 4.
Acceptance Criteria for Performance Tests

EGD No.	Analyte	Acceptance Criteria				
		Spike Level in Extract (ng/mL)	Initial Precision and Recovery		Calibration Verification* (%)	Ongoing Recovery R (%)
			Maximum RSD (%)			
	Acephate	100000	94	0–195	6–194	0–209
	Alachlor	1000	40	26–140	70–130	23–150
089	Aldrin	1000	40	60–140	70–130	50–150
	Atrazine	50000	40	35–140	70–130	31–150
	Benfluralin	1000	40	45–140	70–130	42–150
102	α -BHC	250	40	57–140	69–130	38–154
103	β -BHC	500	40	60–140	70–130	50–150
105	δ -BHC	250	40	60–140	70–130	45–150
104	γ -BHC (Lindane)	250	40	60–140	70–130	50–150
	Bromacil	5000	84	0–263	16–184	0–275
	Bromoxynil octanoate	2500	40	31–140	70–130	27–150
	Butachlor	2500	40	21–140	68–132	17–150
434	Captafol	10000	76	0–221	24–176	0–232
433	Captan	5000	40	28–144	49–114	24–150
441	Carbophenothion	1000	40	60–141	70–130	43–161
091	Chlordane- α	500	40	60–140	70–130	50–150
	Chlordane- γ	250	40	32–140	70–130	4–169
431	Chlorobenzilate	5000	40	58–140	54–130	43–150
	Chlorothalonil	1000	40	37–140	70–130	34–150
	DCPA	250	40	57–140	70–130	50–150
094	4,4'-DDD	1000	40	60–140	70–130	50–150
093	4,4'-DDE	500	40	60–140	70–130	50–150
092	4,4'-DDT	500	40	60–140	70–130	50–150
432	Diallate	10000	40	44–140	70–130	24–150
478	Dichlone	1000	40	45–140	70–130	42–150
090	Dieldrin	200	40	60–140	48–130	48–158
095	Endosulfan I	500	40	41–140	70–130	18–156
096	Endosulfan II	500	40	60–142	70–130	62–158
097	Endosulfan sulfate	500	40	50–140	70–130	31–150
098	Endrin	1000	40	17–149	5–130	0–182

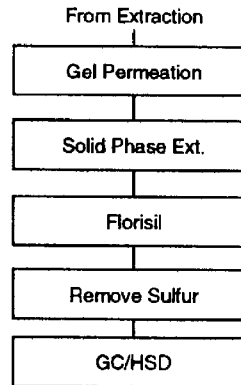
Table 4.
Acceptance Criteria for Performance Tests

<i>EGD No.</i>	<i>Analyte</i>	<i>Spike Level in Extract (ng/mL)</i>	<i>Acceptance Criteria</i>			
			<i>Initial Precision and Recovery</i>		<i>Calibration Verification* (%)</i>	<i>Ongoing Recovery R (%)</i>
			<i>Maximum RSD (%)</i>			
099	Endrin aldehyde	1000	40	0–149	70–130	0–190
435	Endrin ketone	500	40	36–140	68–135	14–150
	Ethalfuralin	500	40	46–140	70–130	42–150
	Fenarimol	1000	40	46–140	70–130	42–150
100	Heptachlor	500	40	60–140	70–130	50–150
101	Heptachlor epoxide	250	40	60–140	70–130	49–150
437	Isodrin	500	40	60–140	70–130	45–150
	Isopropalin	1000	40	47–140	70–130	50–150
439	Kepone	5000	46	31–197	47–134	25–203
430	Methoxychlor	1000	40	50–140	47–130	28–158
	Metribuzin	500	40	54–140	70–130	50–155
438	Mirex	1000	40	25–155	70–130	0–188
436	Nitrofen (TOK)	1000	40	15–140	59–142	0–170
	Norfluorazon	5000	40	60–143	70–130	50–150
112	PCB-1016					
108	PCB-1221					
109	PCB-1232					
106	PCB-1242					
110	PCB-1248					
107	PCB-1254					
111	PCB-1260	1000	40	60–140	70–130	50–150
440	PCNB	250	40	49–140	70–130	29–150
	Pendimethalin	2500	40	32–140	70–130	28–150
	cis-Permethrin	10000	40	45–153	70–130	41–157
	trans-Permethrin	10000	40	59–140	70–130	50–150
	Simazine	40000	40	16–140	70–130	13–150
	Terbacil	10000	82	0–217	18–182	0–228
	Terbutylazine	25000	40	32–140	70–130	29–150
113	Toxaphene	5000	40	60–140	68–134	50–150
	Triadimefon	5000	54	32–140	70–130	0–150
442	Trifluralin	500	40	32–148	47–134	3–177

*Verified at the level of the median standard in Table 3.



Extraction and Concentration Steps



Cleanup and Analysis Steps

A52-002-84

Figure 1. Extraction, Cleanup, Derivatization, and Analysis

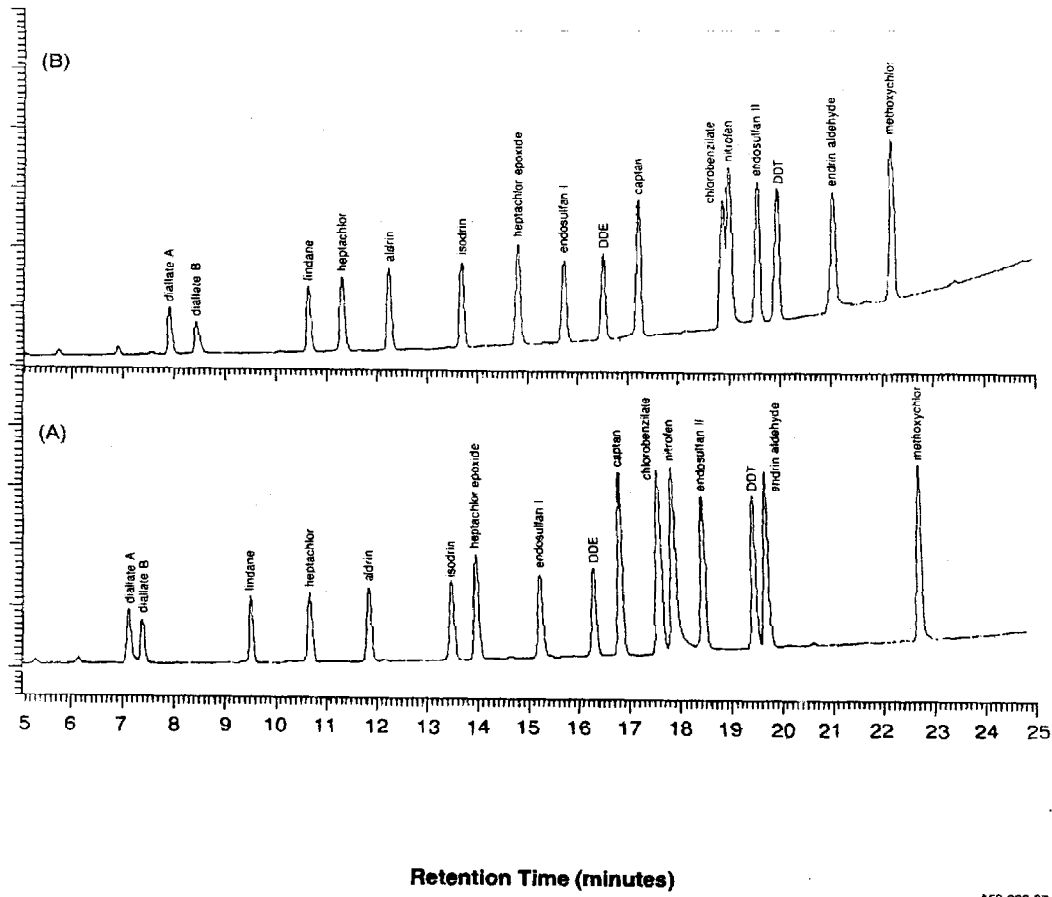


Figure 2. Gas Chromatogram of Selected Organo-Chlorine Compounds

24.0 Glossary

The definitions and purposes below are specific to this Method, but have been conformed to common usage as much as possible.

- 24.1 Ambient Water**—Waters in the natural environment (e.g., rivers, lakes, streams, and other receiving waters), as opposed to effluent discharges.
- 24.2 Analyte**—A substance listed in Table 1 of this Method.
- 24.3 Analytical Batch**—Up to 20 samples started through the extraction process during the same 12-hour shift. Each analytical batch must include an OPR, blank, and MS.
- 24.4 Matrix Spike (MS)**—Aliquots of an environmental sample to which known quantities of the analyte(s) of interest is added in the laboratory. The MS is analyzed exactly like a sample. Its purpose is to quantify the bias and precision 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 corrected for these background concentrations.
- 24.5 May**—This action, activity, or procedural step is allowed but not required.
- 24.6 May not**—This action, activity, or procedural step is prohibited.
- 24.7 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 method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.
- 24.8 Minimum Level (ML)**—The lowest level at which the entire analytical system must give a recognizable signal and acceptable calibration point for the analyte. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed. The ML is calculated by multiplying the MDL by 3.18 and rounding the result to the number nearest to $(1, 2, \text{ or } 5) \times 10^n$, where n is an integer.
- 24.9 Must**—This action, activity, or procedural step is required.
- 24.10 Quality Control Sample (QCS)**—A sample containing analytes at known concentrations. The QCS is obtained from a source external to the laboratory, or is prepared from a source of standards different from the source of calibration standards. It is used as an independent check of instrument calibration.
- 24.11 Reagent Water**—Water demonstrated to be free of analytes at the MDLs of this Method. It may be prepared by passage of distilled water through activated carbon. Reagent water is used to wash bottles, as trip and field blanks, and in the preparation of standards and reagents.

24.12 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 authority.

24.13 Shall—This action, activity, or procedure is required.

24.14 Should—This action, activity, or procedure is suggested, but not required.

24.15 Stock Solution—A solution containing an analyte that is prepared from a reference material traceable to EPA, NIST, or a source that will attest to the purity and authenticity of the reference material.