METHOD 508A

SCREENING FOR POLYCHLORINATED BIPHENYLS BY PERCHLORINATION AND GAS CHROMATOGRAPHY

Revision 1.0

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METHOD 508A

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1.0 SCOPE AND APPLICATION

1.1 This procedure may be used for screening finished drinking water, raw source water, or drinking water in any treatment stage for polychlorinated biphenyls (PCBs). This procedure is applicable to samples containing PCBs as single congeners or as complex mixtures such as weathered, intact, or mixtures of commercial Aroclors. The procedure is incapable of identifying the parent PCBs because the original PCBs are chemically converted to a common product, decachlorobiphenyl (DCB). The procedure has only been evaluated using Aroclors and 2-chlorobiphenyl as a source of PCBs.

1.2 This procedure is primarily designed to function as a pass/fail test for DCB at 0.5 µg/L. However, it will accurately measure DCB from the method detection limit (MDL) to 5.0 µg/L. It is prone to false positive interferences and can result in a calculated weight of PCBs significantly greater than that of PCB originally present in the sample. If DCB is detected at 0.5 µg/L or above, then an approved method for the analysis of PCBs should be used to accurately identify the source and measure the concentration of the PCBs.

1.3 This procedure can be used to help confirm the presence of PCBs for other methods using electron capture or halogen specific detectors whenever chromatographic patterns are not representative of those described in the method.

2.0 SUMMARY OF PROCEDURE

2.1 A 1 L water sample is placed into a separatory funnel and extracted with methylene chloride or one of several optional solvents. The extract is dried, concentrated, and the solvent is exchanged to chloroform. The PCBs are then reacted with antimony pentachloride ($SbCl_5$) (in the presence of an iron catalyst and heat) to form DCB. The DCB is extracted with hexane from the reaction mixture; after the extract is purified, an aliquot is injected into a gas chromatograph (GC) equipped with an electron capture detector (ECD) for separation and measurement. The GC is calibrated using DCB as the standard.

3.0 DEFINITIONS

3.1 Calibration Standard (CAL) -- A solution of DCB used to calibrate the ECD.

3.2 Congener Number -- Throughout this procedure, individual PCBs are described with the number assigned by Ballschmiter and Zell[1]. (This number is also used to describe PCB congeners in catalogs produced by Ultra Scientific, Hope, RI.)

3.3 Laboratory Duplicates (LD1 and LD2) -- Two sample aliquots taken in the analytical laboratory are analyzed with identical procedures. Analysis of laboratory duplicates indicates precision associated with laboratory procedures, but not with sample collection, preservation or storage procedures.

3.4 Laboratory Performance Check Solution (LPC) -- A solution of method analytes used to evaluate the analytical system performance with respect to a defined set of criteria.

3.5 Laboratory Reagent Blank (LRB) -- An aliquot of reagent water that is treated as a sample. It is exposed to all glassware and apparatus, and all method solvents and reagents are used. The extract is concentrated to the final volume used for samples and is analyzed the same as a sample extract.

3.6 Laboratory Fortified Sample Matrix (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.7 Quality Control (QC) Sample -- A sample containing known concentrations of analytes that is analyzed by a laboratory to demonstrate that it can obtain acceptable identifications and measurements with procedures to be used to analyze environmental samples containing the same or similar analytes. Analyte concentrations are known by the analyst. Preparation of the QC check sample by a laboratory other than the laboratory performing the analysis is highly desirable.

4.0 INTERFERENCES

4.1 Interferences may be caused by contaminants in solvents reagents, glassware, and other sample processing equipment. Laboratory reagent blanks (LRBs) are analyzed routinely to demonstrate that these materials are free of interferences under the analytical conditions used for samples.

4.2 To minimize interferences, glassware (including sample bottles) should be meticulously cleaned. As soon as possible after use, rinse glassware with the last solvent used. Then wash with detergent in hot water and rinse with tap water followed by distilled water. Drain dry and heat in a muffle furnace at 450°C for a few hours. After cooling, store glassware inverted or covered with aluminum foil. Before using, rinse each piece with an appropriate solvent. Volumetric glassware should not be heated in a muffle furnace.

4.3 In addition to PCBs, several compounds and classes of compounds will form DCB with varying yields when extracted and perchlorinated according to this procedure. Based upon a literature search[2] such compounds include biphenyl, polyhalogenated biphenyls, hydrogenated biphenyls, and polyhalogenated

terphenyls. If such compounds are present in the extract, false positive or positively biased data will be generated.

4.4 A splitless injection capillary column GC can be used but standards and samples should be contained in the same solvent, or results may be significantly biased.

4.5 PCBs are converted to DCB on a mole for mole basis. Converting DCB concentrations back to the original PCB concentration is beyond the scope of this method. For informational purposes and in order to demonstrate the degree of increased weight of PCBs generated by the procedure, Table 1 lists the conversion of 0.5 µg/L of DCB back to various sources of PCBs assuming 100% method recovery.

5.0 SAFETY

5.1 Chloroform and methylene chloride have been tentatively classified as known or suspected human or mammalian carcinogens. The toxicity or carcinogenicity of the remaining chemicals used in this method has not been precisely defined. Therefore, each should be treated as a potential health hazard, and exposure should be reduced to the lowest feasible level. Each laboratory is responsible for safely disposing materials and for maintaining awareness of OSHA regulations regarding safe handling of the chemicals used in this method. A reference file of material data handling sheets should be made available to all personnel involved in analyses. Additional information on laboratory safety is available[3-5].

5.2 Polychlorinated biphenyls have been classified as known or suspected human or mammalian carcinogens. Primary standards of these compounds should be prepared in an area specifically designed to handle carcinogens. It is recommended that primary dilutions be obtained from certified sources such as the EPA repository.

5.3 $SbCl_5$ is a corrosive reagent that reacts violently with water. This compound must be used with extreme caution. All operations involving the pure reagent must be performed in a hood because appreciable quantities of volatile, potentially harmful materials will be lost to the atmosphere.

5.4 The perchlorination reaction described in this procedure requires that the sample extract be heated to 205°C for about 30 minutes while hermetically sealed in a glass test tube. The solvents and volumes described in the procedure should be carefully reproduced; otherwise dangerous pressures may be generated during perchlorination. The following safety precautions are strongly recommended.

5.4.1 Use only the prescribed perchlorination glassware and visually check for flaws such as chips, strains, or scratches. Discard if any abnormalities are noted.

5.4.2 After cooling the perchlorinated product is still under slight pressure and should be carefully vented in a hood (Section 11.2.8.).

5.4.3 The $SbCl_5$ neutralization step involves an exothermic reaction and should be performed in a hood (Section 11.2.9.).

5.4.4 An explosion shield should be used during the perchlorination and neutralization procedures along with additional eye protection such as an 8 in. face shield. An oil bath heater should not be substituted for the block digester.

5.5 Storage, labelling and disposal of PCBs must conform to all applicable laws and regulations. See Reference 6 for USEPA requirements. Call the Toxics Substances Control Act hotline for further assistance (1-800-424-9065).

5.6 Methylene chloride is described in the procedure (Section 11.1.2) as the extraction solvent; however, hexane, hexane + 15% methylene chloride or hexane + 15% ethylether may be substituted to minimize laboratory personnel exposure to methylene chloride.

5.7 Chloroform is described in the procedure (Section 11.2.1) as the solvent for the perchlorination reaction. Other less toxic solvents including methylene chloride and hydrocarbons were evaluated but were found to be unsuitable. Prior to implementing this procedure, all laboratory personnel must be trained in safe handling practices for chloroform.

6.0 APPARATUS AND EQUIPMENT

6.1 Sampling Equipment

6.1.1 Water sample bottles -- Meticulously cleaned 1 L glass bottles fitted with Teflon-lined screw caps.

6.2 Glassware

6.2.1 Separatory funnel -- 2 L with Teflon stopcock.

6.2.2 Drying column -- Glass column approximately 400 mm long x 19 mm i.d. with coarse frit filter disc.

6.2.3 Concentrator tube -- 10 mL graduated Kuderna-Danish design with ground-glass stopper.

6.2.4 Evaporative flask -- 500 mL Kuderna-Danish design.

6.2.5 Snyder column -- Three-ball macro Kuderna-Danish design.

6.2.6 Snyder column -- Three-ball micro Kuderna-Danish design.

6.2.7 Vials -- 10-15 mL amber glass with Teflon-lined screw caps.

6.2.8 Screw cap culture test tubes -- 100 mm x 13 mm i.d. Pyrex with a Teflon-lined screw cap, Sargent-Welch #S-79533A or equivalent.

6.2.9 Disposable pasteur pipettes -- 9 in. heavy wall.

6.2.10 Screw cap test tube -- 15 mL with Teflon-lined screw cap.

6.3 GC System -- Packed column or capillary column.

6.3.1 Isothermal packed column GC equipped with an on-column injector and a linearized ECD capable of generating a linear response for DCB from at least 0.005-1.0 ng injected.

6.3.2 Programmable capillary column GC equipped with an on-column or splitless injector and a linearized ECD capable of generating a linear response for DCB from at least 0.005-1.0 ng injected. The column oven temperature programmer should have multi-ramp capabilities from at least 60-300°C. For most precise data, an autoinjector should be used.

6.4 GC Columns

6.4.1 Packed column -- A 2 mm i.d. x 3 m, glass column packed with 3% OV-1 on 80-100 mesh Supelcoport or equivalent.

6.4.2 Capillary column -- A 30 m x 0.32 mm i.d. fused silica capillary coated with a bonded 0.25 µm film of cross linked phenyl methyl silicone such as Durabond-5 (DB-5).

6.5 Miscellaneous Equipment

6.5.1 Volumetric flask -- 5 mL, 10 mL, and 100 mL with ground glass stoppers.

6.5.2 Microsyringes -- Various standard sizes.

6.5.3 Boiling chips -- Approximately 10/40 mesh. Heat at 400°C for 30 minutes or extract with methylene chloride in a Soxhlet apparatus.

6.5.4 Water bath -- Heated, with concentric ring cover, capable of temperature control with ±2°C.

6.5.5 Analytical balance -- Capable of accurately weighing to 0.0001 g.

6.5.6 One liter graduated cylinder.

6.5.7 Block digestor -- 1.4 cm i.d. x 5 cm deep holes. Operated at 205°C ±5°C.

NOTE: A Technicon Model BD-40 block digestor with specially fabricated aluminum insert bushings was used to conduct the procedure

development research. Block digestors with holes of other dimensions may adversely influence recoveries.

7.0 REAGENTS AND CONSUMABLE MATERIALS

7.1 Solvents -- High purity, distilled in glass toluene, hexane, methylene chloride, chloroform and methyl alcohol.

7.2 Sodium Sulfate -- ACS granular, anhydrous. Purify by heating at 400°C for four hours in a shallow dish. Store in a glass bottle with a Teflon-lined screw cap.

7.3 $SbCl_5$ >98%.

7.4 Iron Powder -- 99.1%.

7.5 PCB Solutions

7.5.1 Prepare a stock solution of Aroclor 1260 at 5.00 µg/µL in methyl alcohol or obtain a similar mixture from a certified source.

7.5.2 Prepare a stock solution of DCB at 1.00 µg/µL in toluene or obtain a similar mixture from a certified source.

7.5.3 PCB fortification solution -- Dilute an aliquot of the Aroclor 1260 stock solution in methyl alcohol to produce about 10 mL of a solution containing 50.0 ng/µL. Store in a 50-90% filled glass bottle with a Teflon-lined screw cap.

7.5.4 Calibration standards -- Five calibration solutions containing DCB from 0.01-1.0 ng/µL in hexane are required to calibrate the detector response. Prepare standards at 0.010, 0.080, 0.10, 0.25 and 1.0 ng/µL in hexane (see Section 4.4) from the stock solution of DCB. Store in 50-90% filled glass bottles with Teflon-lined screw caps. Monitor for solvent loss due to evaporation.

7.5.5 Extract matrix evaluation solution -- Dilute an aliquot of the DCB stock solution to produce about 10 mL of a solution containing 50.0 ng/µL in hexane. Store in a 50-90% filled glass bottle with a Teflon-lined screw cap.

7.6 Hydrochloric Acid Solution 1+1 -- Dilute one part concentrated hydrochloric acid with one part distilled water.

7.7 0.1 N Sodium Bicarbonate ($NaHCO_3$) Solution -- Dilute 0.84 g of ACS grade $NaHCO_3$ to 100 mL with reagent water.

7.8 Reagent Water -- Water in which DCB is found to be less than 0.1 µg/L as analyzed by this procedure. Distilled water met this criterion.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Sample Collection

8.1.1 Collect duplicate samples in clean 1 L glass containers and seal with a Teflon-lined screw cap. Fill the bottles to about 90-95% full.

8.1.2 Because PCBs are hydrophobic they are likely to be adsorbed on suspended solids. If suspended solids are present in the source, a representative portion of solids must be included in the water sample.

8.1.3 When sampling from a water tap, open the tap and allow the system to flush until the water temperature has stabilized (about 10 minutes). Adjust the flow to about 1 L/min and collect the duplicate samples from the flowing stream.

8.1.4 When sampling from an open body of water, fill a 1 g wide-mouth bottle from a representative area. Carefully fill the duplicate sample bottles from the 1 g bottle.

8.2 Sample Preservation -- No chemical preservation reagents are recommended. Store the samples at 4°C to retard microbial action until analysis.

8.3 Sample Storage -- Extract samples within 14 days of collection[7]. Extracts and perchlorinated extracts may be stored for up to 30 days if protected from solvent volatilization.

9.0 **CALIBRATION** -- Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed and is required intermittently throughout sample analyses as dictated by results of continuing calibration checks. After initial calibration is successfully performed, a continuing calibration check is required at the beginning and end of each set of samples or eight-hour period during which analyses are performed.

9.1 Initial Calibration

9.1.1 Inject duplicate aliquots (1-3 µL) of each calibration solution into the GC. (Autoinjectors are preferred, especially with splitless injectors.) Inject five additional aliquots of the 0.10 ng/µL standard.

9.1.2 Accurately determine the DCB retention time (RT) and peak area or peak height for each injection.

9.1.3 Determine the average RT and the standard deviation (SD) of RTs for all 15 injections. To be acceptable, the RSD of the RTs should be less than 0.2%.

9.1.4 Determine the response factor (RF) for each of the injections by dividing the amount (ng) injected into the resulting area or peak height or integrator units.

9.1.5 Determine the average RF and its SD and RSD for the seven injections at the 0.10 ng/µL level.

9.1.6 The RSD of the RF should be less than 6% for the seven injections at the 0.1 ng/µL level.

9.1.7 Compare the RF determined for the 0.01, 0.08, 0.25, and 1.0 ng standards to the average RF calculated in Section 9.1.5 ±3 SD. If any value falls outside of this range, then the instrument is not being operated within an acceptable linear range and the sample volume injected must be adjusted accordingly. Alternatively, the linear dynamic range can be clearly defined by injecting standards at other concentrations. To be marginally acceptable, the system should function from 0.08-0.25 ng injected.

Table II shows typical values obtained during method developmnt.

9.2 For an acceptable continuing calibration check, the 0.1 ng/µL calibration standard must be analyzed before and after a series of samples or at least once after each eight hours of operation. The RF must be within ±20% of the mean value determined in Section 9.1.5, or a new calibration curve must be generated. Additionally, the RT must fall within the mean value ±3 SD determined in Section 9.1.3, or a new calibration curve must be generated or the reason for the RT variance must be found and rectified.

9.3 Extract Matrix Effect Evaluation -- It has been found that there may be a matrix effect from the perchlorinated extract which can bias the response on certain GC systems. Until this problem is understood, an extract matrix effect evaluation should be performed on each gas chromatographic system to determine if the system can be used for this procedure. This test should be repeated each time a modification or change is made to the system.

9.3.1 Extract, perchlorinate, and cleanup duplicate drinking water samples or laboratory reagent blanks according to the procedure halting at step Section 11.2.13.

9.3.2 Combine the two extracts together in a 25 mL beaker or flask and mix.

9.3.3 Immediately place 5.0 mL in a volumetric flask and seal. Place the remaining solution in a second hermetically sealed container and label MS-1 (Mixed Sample 1).

9.3.4 Analyze MS-1 in duplicate. If the value for the DCB is ≤0.05 ng/µL, proceed to Section 9.3.5. If ≥0.05 ng/µL, proceed to Section 9.3.6.

9.3.5 Fortify the contents of the volumetric flask with 10.0 µL of the 50.0 ng/µL extract matrix evaluation solution (Section 7.5.5) and label SE-1 (Fortified Extract 1). Analyze SE-1 in duplicate, then proceed to Section 9.3.

9.3.6 Fortify the contents of the volumetric flask at three to ten times the concentration found in Section 9.3.4. If the fortified value plus the MS-1 value found in Section 9.3.4 exceeds the linear dynamic range of the detector (Section 9.1.7), then terminate the test and select another sample. Do not dilute extract matrices to perform this test.

9.3.7 Determine the extract matrix bias according to the following calculation:

$$\frac{\text{(SE-1 ng/µL) - (MS-1 ng/µL) x 100}}{\text{(Fortified value ng/µL)}} = \text{\% recovery}$$

Recoveries between 80 and 120% are acceptable. If the recovery is <80%, the test should be repeated. If the recovery remains <80%, then another GC system should be used.

10.0 QUALITY CONTROL

10.1 Laboratory Reagent Blank (LRB) -- Perform all steps in the analytical procedure (Section 11.0) using all glassware, reagents, standards, equipment, apparatus, and solvents that would be used for a sample analysis using 1 L of reagent water.

10.1.1 Prepare and analyze a LRB before any samples are extracted and analyzed.

10.1.2 Prepare and analyze additional LRB whenever new batches or sources of reagents are introduced into the analysis scheme.

10.1.3 Prepare a LRB each time samples are perchlorinated. If large batches of samples are perchlorinated, then prepare and analyze 1 LRB per 10 samples.

10.1.4 An acceptable LRB contains ≤0.025 ng/µL of DCB.

10.1.5 Corrective action for unacceptable LRB -- Systematically check solvents, reagents (particularly the $SbCl_5$ and methylene chloride), apparatus and glassware to locate and eliminate the source of contamination before any samples are extracted, perchlorinated, and analyzed. Purify or discard contaminated reagents and solvents.

10.2 Calibration -- Included among initial and continuing calibration procedures are numerous QC checks to ensure that valid data are being acquired (See Section 9.0). Continuing calibration checks are accomplished with results from analysis of one solution, the 0.10 ng/µL calibration solution.

10.2.1 If some criteria are not met for a continuing calibration check after an eight-hour period or after a series of samples are analyzed, then those samples must be reanalyzed. Those criteria include the RF criteria and the RT criteria described in Section 9.2.

10.3 All sample concentrations must be bracketed by the calibration curve and must be within the linear dynamic range of the detector. (See Section 9.1.7.)

10.3.1 Samples that fall outside the linear dynamic range due to excessive concentration must be reanalyzed after appropriate dilution if accurate values for DCB are required.

10.4 All GC systems must be evaluated for extract matrix effect bias according to Section 9.3.

10.4.1 Systems that exhibit a bias in excess of ±20% should not be used for this determination.

10.5 Initial demonstration of laboratory capability for water analysis.

10.5.1 Prepare one or more solutions containing representative PCB mixtures at a concentration that falls within the linear dynamic range of the instrument. Reagent water fortified with Aroclor 1260 is recommended for this test.

10.5.2 Fortify four to seven 1 L portions of reagent water with 10.0 µL of the 50 ng/µL PCB solution (Section 7.5.3). Extract and analyze the fortified water samples according to the procedure (Section 11.0).

10.5.3 Calculate the recovery according to the following formula:

$$\% \text{ Recovery} = \frac{(\text{Total ng found in extract}) \times 100}{691}$$

where $691 = 500 \text{ ng} \dfrac{\text{mw DCB (499)}}{\text{mw Aroclor 1260 (361)}^{a}}$

[a]See Table 1 for the molecular weights of other Aroclors.

10.5.4 Determine the average concentration and the relative SD of the five measurements. Average recovery should be 100% ±20 with a RSD of <10%.

10.6 Fortify reagent water with varying quantities of the 50 ng/µL PCB solution (Section 7.5.3). Analyze at least one fortified sample for each batch of 20 samples. Calculate recovery according to Section 10.5.3. Maintain QC charts of these data.

Until interlaboratory data are available, the recovery of the fortified sample should be equivalent to that determined in Section 10.5.4.

10.7 Sample matrix effects have been observed with this procedure and they are significant. Check for sample matrix effects by analyzing one laboratory fortified sample matrix (LFM) for every 20 samples.

10.8 At least quarterly, analyze a quality control sample (QCS) from an external source. If measured analyte concentrations are not of acceptable accuracy (Section 10.5.4), check the entire analytical procedure to locate and correct the problem source.

10.9 Qualitative identification of DCB in the samples is based on the average RT for DCB determined in Section 9.1.3. For a positive identification, the DCB peak must elute within the window bracketed by the average retention ±3 SD. If DCB appears to fall outside of this window, then further analyses of samples should be halted and Section 9.2 initiated.

10.10 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to assess the precision of the environmental measurements. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

11.0 PROCEDURE

11.1 Sample Extraction

11.1.1 Mark the sample meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2 L separatory funnel.

11.1.2 Add 60 mL of methylene chloride (See Section 5.6) to the sample bottle, seal, and shake 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Wait at least 10 minutes to allow the organic layer to separate from the water phase. If the emulsion interface between layers is more than one-third the volume of the solvent layer, use mechanical techniques (such as stirring, filtration of emulsion through glass wool, or centrifugation) to complete phase separation. Collect the methylene chloride extract in a 250 mL Erlenmeyer flask. Add a second 60 mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

11.1.3 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10 mL concentrator tube to a 500 mL evaporative flask.

11.1.4 Pour the combined extract into a solvent-rinsed drying column containing about 10 cm of anhydrous sodium sulfate. Rinse the Erlenmeyer flask with a 20-30 mL portion of methylene chloride adding the rinse to the drying column. Collect the combined extract in the K-D concentrator.

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

11.1.6 Remove the 10 mL concentrator tube from the 500 mL evaporative flask and attach a three-ball micro Snyder column. After wetting the column with about 0.5 mL of methylene chloride, continue concentrating the extract down to about 2 mL.

11.1.7 Determine the original sample volume by refilling the sample bottle with water to the mark and transferring the liquid to a 1000 mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11.2 Perchlorination[8,9]

11.2.1 Quantitatively transfer the extract to a 100 mm x 13 mm i.d. screw cap test tube. Rinse the KD ampul three times with 250 µL of chloroform adding the rinse to the test tube.

11.2.2 Concentrate the extract to about 0.1 mL (0.1 mL is about the volume of one drop of water) by directing a stream of nitrogen flowing at about 100 mL/m into the test tube while warming the base of the test tube in a 50°C water bath.

11.2.2.1 Do not allow to go to dryness.

11.2.2.2 Disposable pipettes are a convenient means of directing the nitrogen into the test tube. In an effort to minimize cross contamination, a new pipette should be used for each sample.

11.2.3 Add an additional 2 mL of chloroform and again concentrate to 0.1 mL using the nitrogen blow-down technique.

11.2.4 Add 100 mg of iron powder to the extract.

11.2.5 Using a disposable pipette, carefully add 25 drops of $SbCl_5$ to the extract. (See Section 5.3). Seal immediately.

11.2.6 Heat to 205°C ±5°C for a minimum of 30 minutes but do not exceed 45 minutes. Perform the reaction in the hood behind an explosion shield.

11.2.7 Allow the mixture to cool to room temperature.

11.2.8 Carefully open in a hood. (The extract will be under a slight pressure.)

11.2.9 Slowly add 0.5 mL of 1+1 diluted hydrochloric acid to the perchlorinated extract in a hood. Caution: The remaining $SbCl_5$ will react exothermally with the HCl. If a white precipitate is present, add additional hydrochloric acid solution until it dissolves.

11.2.10 Add 2.0 mL of hexane to the contents of the test tube. Seal and shake for two minutes. Allow the two phases to separate. Decant the top layer into a 5.0 mL volumetric flask. Reextract the mixture two additional times: First with 2.0 mL of hexane, then with 1.0 mL of hexane, adding the extracts to the 5.0 mL volumetric flask. Carefully adjust the volume to 5.0 mL using hexane.

11.2.11 Add 4 mL of 0.1 N $NaHCO_3$ to a 15 mL test tube with a Teflon-lined screw cap. Pour the contents of the 5 mL volumetric flask into the test tube.

NOTE: Do not rinse the volumetric flask with additional solvent.

Seal and shake for one minute. Allow the two phases to separate.

11.2.12 Decant the top layer into a second 15 mL test tube. Add 4 mL of reagent water. Seal and shake for one minute.

11.2.13 Decant the top layer and store in a hermetically sealed container for GC analysis.

11.3 GC -- Packed: on-column injection ECD, capillary: on-column injection electron capture and capillary splitless injection ECD GC systems have been evaluated and found to generate acceptable data for DCB as long as Section 10.4 criteria are met. The following conditions were used to generate the single-laboratory accuracy and precision data listed in Section 13.0. The values given are for guidance because slight modifications may be necessary to optimize specific GC systems.

11.3.1 The packed column GC was operated with a glass column 3 m long with an i.d. of 2 mm. The column was packed with 3% OV-1 coated on 80-100 mesh Supelcoport. Three µL volumes of each sample was injected directly on column using an autosampler. The injection port was held at 200°C while the column was maintained isothermally at 235°C with an Argon +5% methane carrier gas flowing at 50 mL/min. The ECD was maintained at 300°C with no auxiliary make-up gas.

Under these conditions, the average RT for DCB was 9.49 minutes with a SD of 0.014. DCB was adequately resolved from other perchlorination reaction byproducts to generate accurate data for drinking water samples. Highly contaminated raw source water generated complex chromatograms with late eluting components that interfered with DCB measurements.

11.3.2 The capillary column on-column GC was operated with a DB-5 fused silica column 30 m long with a 0.32 mm i.d. and a 0.25 µm film thickness. The helium carrier gas was adjusted to flow at 29 cm/sec at 60°C. Three microliter sample volumes were injected on-column into a 0.5 mm i.d. x 10 cm fused silica retention gap using an autoinjector. The retention gap was maintained at 60°C during injection.

The capillary column was maintained at 60°C until one minute after injection, then programmed at 20°C/min to 180°C. After a two minute hold, the column was again programmed at 20°C/min to 290°C and held there until all compounds eluted. The ECD was operated at 300°C with an Argon +5% methane makeup gas flowing at 20 mL/min.

Under these conditions the average RT for DCB was 21.85 minutes with a SD of 0.021. DCB was adequately resolved from other perchlorination byproducts to generate accurate data for both finished drinking water and raw source water samples.

11.3.3 The capillary column splitless injection GC was operated with a DB-5 fused silica column 30 m long with an i.d. of 0.32 mm and a 0.25 µm film thickness. The helium carrier gas was adjusted to flow at 29 cm/sec at 180°C. Three µL injection volumes were delivered by an autoinjector into the splitless injector operated at 250°C. The splitless time was set for 30 seconds.

The capillary column was maintained at 180°C until one minute after injection, then programmed at 20°C/min to 290°C and held for 20 minutes or until all late eluting compounds eluted. The electron capture was operated at 300°C with an argon + 5% methane makeup gas flowing at 20 mL/min.

Under these conditions the average RT for DCB was 24.75 minutes with a SD of 0.009. DCB was adequately resolved from other perchlorination

byproducts to generate accurate data for both finished drinking water and raw source water samples.

12.0 CALCULATIONS

12.1 Calculate the concentration of the DCB found in each extract using an automated data system or according to the formula.

12.1.1 $$\text{Extract concentration ng/µL} = \frac{\frac{\text{Area Sample}}{\text{µL Injected}}}{\text{RF}}$$

12.1.2 $$\text{Sample concentration ng/L} = \frac{\text{(Concentration ng/µL) (5000)}}{\text{volume of sample (L)}}$$

where: area sample = area, peak height or integrator units.
µL injected = volume of sample injected into GC.
5000 = final volume of extract in µL (Section 11.2.10).
Volume of sample (L) = volume of sample extracted in liters (Section 11.1.7).
RF = average RF (Section 9.1.4) for the 0.1 ng/µL standard.

12.1.3 Calculations should utilize all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (one digit of uncertainty). Experience indicates that three significant figures may be used for concentrations above 99 µg/L, two significant figures for concentrations between 0.1-99 µg/L, and one significant figure for lower concentrations.

12.1.4 Do not subtract method blanks from the sample data unless otherwise required in the procedure.

13.0 METHOD PERFORMANCE -- To obtain single-laboratory accuracy and precision data for method analytes, seven 1 L aliquots of chlorinated tap water, groundwater and river water were fortified with 500 ng of PCBs from several sources. The samples were extracted, perchlorinated and analyzed according to Section 11.0. Tables 3 and 4 list the resulting data.

14.0 REFERENCES

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TABLE 1. DECACHLOROBIPHENYL EQUIVALENT OF COMMON PCB SOURCES

Compound	Congener Number	Molecular Weight[a]	Concentration[b] (µg/L)	Decachloro-biphenyl Equivalent (%)[c]
2-Chlorobiphenyl	1	188.5	0.19	263
Aroclor 1221	N/A	188.5	0.19	263
Aroclor 1232	N/A	223	0.23	217
Aroclor 1242	N/A	257.5	0.26	192
Aroclor 1016	N/A	257.5	0.26	192
Aroclor 1248	N/A	292	0.30	167
Aroclor 1254	N/A	326.4	0.33	152
Aroclor 1260	N/A	361	0.36	139
Dichlorobiphenyl	209	499	0.50	100

[a]Values from Reference 10.
[b]µg/L of various PCBs required to generate a value of 0.50 µg/L DCB (assuming 100% method recovery).
[c]The decachlorobiphenyl produced by perchlorination will be this percentage greater than the original concentration of the PCB/Aroclor listed.

TABLE 2. CALIBRATION CURVE LINEARITY TEST AND RETENTION DATA

Standard Concentration (ng/µL)	Retention Time (min)	Response Factor (area/ng)		
0.01	24.74	48790		
0.01	24.74	50650		
0.08	24.74	48240		
0.08	24.73	47260		
0.1	24.75	48300	Average	48030
0.1	24.75	49550	Standard	
0.1	24.75	51170	Deviation	2500
0.1	24.75	49160		
0.1	24.75	43220	Relative	
0.1	24.74	47490	Standard	5.2%
0.1	24.74	47320	Deviation	
0.25	24.76	49960		
0.25	24.76	48240		
1.0	24.76	47230		
1.0	24.76	48410		

Average RT = 24.75
SD = 0.009
Relative Standard Deviation = 0.038%

TABLE 3. SPLITLESS CAPILLARY COLUMN SINGLE LABORATORY ACCURACY AND PRECISION FOR FORTIFIED TAP WATER

Source of PCBs	MDL[f1] (µg/L)	Concentration (µg/L)	Accuracy[ae] (%)	Precision[ae] RSD, (%)
2-Chlorobiphenyl	0.08	0.50	85; (96)[b]	5.0; (9.9)[b]
Aroclor 1221	0.14	0.50	99	8.4
Aroclor 1232	0.23	0.50	124	11.3
Aroclor 1242	0.21	0.50	82	13.1
Aroclor 1248	0.15	0.50	136	8.6
Aroclor 1254	0.14	0.50	122; (137)[c]	6.4; (7.6)[c]
Aroclor 1260	0.14	0.50	113; (96)[b]	6.5; (6.9)[b]
Biphenyl[d]		0.50	109; (75)[c]	4.8; (5.8)[c]

[a]Data corrected for source water background. Average value over study = 0.11 µg/L
[b]Data collected by on-column capillary column GC.
[c]Data collected by packed column GC.
[d]Potential method interference compound.
[e]Fortified matrix effect bias (See Section 9.3)

Splitless capillary column	103,	113
Packed column	93,	95
Splitless on-column (not performed)		

TABLE 4. SPLITLESS CAPILLARY COLUMN SINGLE LABORATORY ACCURACY AND PRECISION FOR RAW SOURCE WATERS

Raw Source Water	Source of PCBs Aroclor	Concen-tration (µg/L)	Extraction Solvent	Source Water Background (µg/L)	Accuracy (%)	Precision RSD (%)
Ohio River	1221	0.50	CH_2Cl_2	0.54	114	8.4
Spring	1260	0.50	CH_2Cl_2	0.19	101	7.9
Ohio River	1221	0.50	Hexane	0.16	123	7.5
Little Miami River	1260	5.0	Hexane	0.14	91	5.8
Ohio River	1260	5.0	Hexane	0.29	100	5.4