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HIGH RESOLUTION MASS SPECTROMETRY **METHOD 1668 for Solid/Air/Aqueous/Tissue Matrices**			
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1. PURPOSE

   1. **This procedure describes the analytical techniques used for extraction and analysis of aqueous, solid, oil, wipe, waste, air and tissue samples for polychlorinated biphenyls (PCBs) by EPA Methods 1668A, 1668B, and 1668C.**

2. APPLICABLE MATRICES

   1. This method is applicable to all matrices. .

   2. The limits of detection and quantitation in various matrices are available in the Performance Commitment document.

3. SCOPE AND APPLICATION

4.1 This method is for the determination of coplanar polychlorinated biphenyls (PCBs), mono-ortho substituted PCB congeners, the 10 PCB homologue groups as well as the 209 PCB congeners at the quantitation limits listed in Table 1 for water, soil, sediment, sludge, ash, tissue, and other sample matrices such as air by gas chromatography/high-resolution mass spectrometry/selective ion monitoring (GC/HRMS/SIM). The method is based upon the combined features of EPA Method 1668B to extract, cleanup sample extracts, and measure toxic PCB congener target compounds.

4.2 Twenty-seven $^{13}C_{12}$-PCB congeners, including the 12 WHO congeners, are added to each sample as Extraction Standards. Following a matrix-specific extraction, the extracts are fortified with a group of three $^{13}C_{12}$-PCB congeners as Cleanup Standards. Once cleaned up, the residue is fortified with a mixture of five $^{13}C_{12}$-PCB congeners as Injection Standards. For air samples, the role of the CS is modified so that they can be used as Sampling Standards; i.e., they are added to the sampling module before the sampling session. A five-point ICAL is used to derived the analytes and labeled compounds RRFs. For assays requiring an OPR, a front-end CS3 is analyzed while the BCS_3 system – developed for Method 8290B – is applied for assays not mandating an OPR (e.g., air samples, R&D, SW…).

4. SUMMARY OF THE TEST METHOD

   1. AQUEOUS SAMPLES (<1% solids) - Stable isotopically labeled analogs of PCB target congeners are spiked into a one-liter sample and either vacuum-filtered (if particulates are present) through a glass-fiber filter, toluene-SDS the filter and liquid-liquid extract the filtrate with methylene chloride. If the sample does not show visible particulates, a liquid-liquid partitioning is carried out with methylene chloride.
   2. SOLID, SEMI-SOLID, AND MULTI-PHASE SAMPLES (except tissue) - The labeled compounds are spiked into the sample containing 10 g dry-weight equivalent. Samples containing multiple phases are filtered and any aqueous liquid discarded. Coarse solids are ground or homogenized. Any non-aqueous liquid from multi-phase samples is combined with the solids and extracted in a Soxhlet/Dean Stark (SDS) extractor. The extract is concentrated for cleanup. Results are reported on a dry-weight basis.
   3. FISH AND TISSUE- A 25-g aliquot of sample is homogenized, and spiked with the labeled isotope compounds. The sample is mixed with Hydromatrix™ and extracted for 16 hours using hexane in a Soxhlet Dean-Stark extractor over acid-coated silica gel (see Method 8290 for tissue for details). The extract is evaporated to dryness. The lipid content is determined using a separate aliquot of the tissue as per the procedure described for Method 8290.
   4. AIR SAMPLES – For Modified Method Five and ambient air samples, the sampling sorbent is fortified with a mixture of Sampling Standards before sampling. Upon return to the laboratory, the various components of the train are processed as per the M23 diagram (see Method 23). The extraction starts with hexane SDS for 16 hours followed by toluene for 16 hours. This approach is applicable to PCBs whether in conjunction with PCDD/Fs or not, whether in conjunction with PAHs or not, and whether selected PCB congeners are assayed or not. The combination of solvent is necessary to ensure the recovery of low- and high-molecular weight congeners as well as coplanar and mono-ortho congeners. Alternatively, it is permitted to perform an 8-H hexane SDS followed by an 8-H toluene SDS.
   5. The extract is fortified with Cleanup Standards and fractionated using the ASECS procedure (see Method 8290 or Fractionation SOP). For air samples, do not add the CS because the Cleanup Standards are actually playing the role of Sampling Standards and were introduced inside the sampling module before the sampling session. Collect the PCB fraction. After cleanup, the extract is concentrated to near dryness. Injection Standards are added to each extract and an aliquot of the extract is injected. The analytes are separated by the GC and detected by gas chromatography/high resolution mass spectrometry/selective ion monitoring (GC/HRMS/SIM) to achieve the required quantitation limits of the method. Two exact m/z's are monitored for each homologue.
      A. For the measurement of the 209 PCB congeners, the GC/MS system is calibrated using the native and labeled PCB congeners listed in Table 1.
   6. An individual PCB congener is identified by comparing the GC retention time and ion abundance ratio of two exact m/z's with the corresponding retention time of an authentic standard and the theoretical ion-abundance ratio of the two exact m/z's. Isomer specificity for the toxic PCBs is achieved using GC columns that resolve these congeners from the other PCBs. For this SOP, the SB Octyl 60-m column is used as described in Method 1668B. A 60-m DB-5MS is also used for WHO 29 target analytes where the SB-Octyl co-eluting pair of iso-TEF isomers PCB-156/157 can be resolved. Data validating such isomer-specificity were supplied to NELAC by comparing the analyses of a mixture containing all 209 congeners for the two co-eluting isomers on the SB-Octyl column and the DB-5MS column (click here for details).
   7. Quantitative analysis is performed using selected ion current profile (SICP) areas, in one of two ways:
      A. For PCB congeners with labeled analogs, the GC/MS system is calibrated, and the concentration of each compound is determined using the isotope dilution technique.
      B. For PCB congeners without labeled isotopes, and for PCB homologues, the GC/MS system shall be calibrated and the concentration of each compound determined using internal standard technique.
      C. Note that different relationships are used as permitted by the method (Table 3). The selection is based on the rationale discussed in the attachment, which can be viewed by clicking here.
      D. The effects from quantitative interferences, a common occurrence observed with PCB analyses targeting such a wide range of compounds, are mitigated by the use of a custom-developed and validated program known under the name of "Equalizer". The program is considered intellectual property and no written details will be provided until a peer-reviewed publication of the concept and the demonstration of its benefits for this type of assays is completed. The concept is also considered proprietary, and we request any third parties made aware of this unique, innovative and powerful tool to respect the confidential aspects until the paper is accepted for publication. The raw data shows the file name appended with the letters "EQ" to indicate that the data underwent the special treatment with the ©Equalizer.
      E. For dichlorinated congeners, a single ion corresponding to the $M^{+\cdot}$ is used for the quantitations and to determine S/N. Indeed, the M+2 ion is interfered by background ions originating from the reference compound PFK. The latter increases the chemical noise and leads to unrealistically high detection limits. The letters "SI" appear on the raw data to indicate when such feature is turned on at the data generation stage. Retention time matching, detection of the molecular ion and other aspects associated with the number of possible dichlorinated congeners are considered sufficient to provide reliable data.
   8. The quality of the analysis is assured through reproducible calibration and testing of the extraction, cleanup, and GC/MS systems. Under the BCS_3 system, such performance assessment can be obtained. However, under the OPR system, we cannot make the same statement.

5. DEFINITIONS

    1. Definitions (and more thorough explanations) of concepts introduced by Analytical Perspectives may be found in the Performance Commitment document.

6. INTERFERENCES

    1. All materials used in the analysis shall be demonstrated to be free from interferences by running reference matrix method blanks with each sample batch. Note that with PCBs, background contributions are very common in a laboratory environment. Method 1668B introduced the EML concept in recognition of this fact. However, AAP has adopted a policy to "beat" these EMLs as much as possible by investigating the sources of PCB background contributions and ultimately achieve the same results as currently achieved with PCDD/Fs (i.e., no detectable signals). This effort might take some time to achieve. EMLs constitute a "nice" protection that should not take away our determination to succeed in this endeavor.
        A. The reference matrix should simulate, as closely as possible, the sample matrix under test. For IPAs/OPRs, reagent water can be used to simulate water samples; playground sand or white quartz sand can be used to simulate soils; filter paper can be used to simulate papers or similar materials; and corn oil or cod fish can be used to simulate tissues. However, for Method Blanks, use only the materials that are part of the field samples processing. For instance, if Hydromatrix^{TM} is used to prepare a serum sample, than, the same amount of Hydromatrix^{TM} from the same lot number should be used for the Method Blank. Adding to the MB some oil, which is not added to the field samples, is an action that ignores the purpose and function of the MB.
    2. Interferences co-extracted from samples will vary from source to source, depending on the site being sampled. The cleanup steps can be used to reduce or eliminate such interferences. It is worth mentioning that assaying for such a broad range of target compounds, complications will results. Indeed, certain types of interferences (a.k.a. quantitative interferences, QI) cannot be removed without removing the target analytes. Appropriate actions will be taken and considered on a case-by-case basis. These can range from additional cleanup, dilutions, to using the ©Equalizer and the application of adequate data qualifiers and interpretation/discussion in the case narrative.

7. SAFETY

    1. Standard sample preparation laboratory safety precautions apply, as outlined in AP's Health and Safety SOPs.

8. EQUIPMENT AND SUPPLIES

    1. CTC Autosampler
    2. Alpha Station
    3. Water Cooler.
    4. Magnetic Sector High Resolution Mass Spectrometer.
    5. Food Slicer.
    6. Pipets, disposable, serological, 10 mL.
    7. Pipets, Pasteur.
    8. Amber glass bottles, 1 liter (Teflon-lined screw cap).
    9. Two-liter separatory funnels.
    10. Teflon boiling chips.
    11. glass chromatographic column (see ASECS in Method 8290)
    12. N-Evaporator
    13. Conical vials, 2 mL.
    14. Pyrex fiber glass, 8µm sliver (glass wool plug).
    15 . Funnels, 100 mL.
    16. Dean-Stark Trap, condenser and flask.
    19. Rotary Evaporator.
    20. Round-bottom flasks, 500 mL.
    21. Top-Loader Balance.
    22. Injection vials.
    23 Electrothermal electromantles.
    24. Drying oven.
    25. Whatman glass microfibre filters.

9. REAGENTS, STANDARDS AND SOLVENTS

    1. Reagents

        1. Sulfuric acid, concentrated.

        2. Silica gel. Highest purity grade.

        3. Sodium Thiosulfate, 80 mg/L

        4. Water, distilled.

        5. Florisil.

        6. Prepurified nitrogen gas.

        7. Anhydrous sodium sulfate.

    2. Solvents

        1. Methylene chloride. Highest available purity.

        2. Hexane. Highest available purity.

        3. Tetradecane. Highest available purity.

        4. Toluene. Highest available purity.

        5. Methanol. Highest available purity.

    3. Standards

1. PCB Analytical standards (Cambridge Isotope Laboratory, Wooburn, MA).
2. 2. For details on the preparation and independent verification, see Standards SOP in the Preparation of Supplies Section.
3. 3. The NS (native spike) solution used for the BCS_3 must originate from an independent source

10. COLLECTION, PRESERVATION, AND HANDLING

1. Aqueous Samples
   A. At minimum, collect one liter of sample. If residual chlorine is present, add 80mg/L of sodium thiosulfate.
   B. Adjust sample pH 2-3 with sulfuric acid.
   C. Store aqueous samples in the dark at 2-6°C.

7.2 Solid Samples
   A. Solid samples are collected as grab samples using wide-mouth jars.
   B. Store solid, semi-solid, oily, and mixed-phase samples in the dark at 2-6°C.

7.3 Fish and Tissue Samples
   A. Fish may be cleaned, filleted, or processed in other ways such that the sample is received as whole fish, fish fillets or other tissues for analysis.
   B. Samples must be frozen and maintained in the dark at <-18-20°C.

7.4 Holding Times
   A. If stored in the dark at 2-6°C and preserved as described in Section 7.3, aqueous samples may be stored for up to one year.
   B. If stored in the dark at <-10°C, solid, semi-solid, oily, multi-phase, fish and tissue samples may be stored for up to one year. If stored at 2-4°C, the holding time for the extraction becomes 30 days from collection.
   C. Sample extracts should be stored at 4°C until analyzed. If stored in the dark at <-10°C, sample extracts may be stored for up to one year

11. QUALITY CONTROL

Quality control is demonstrated by an initial demonstration of laboratory capability, analysis of spiked samples with labeled compounds to evaluate and document data quality, and the analyses of standards and blanks as a test of continued performance.

1, Initial Precision and Recovery (IPA): Four aliquots of the diluted precision and recovery standard are analyzed to establish the ability to generate acceptable precision and accuracy in reference matrix. Alternatively, AAP's initial demonstration is performed while conducting the MDL studies using a spike level that is 20 times lower than what the method's calls for. Recently, AAP has adopted a policy to use a mixture containing the 209 congeners for IPAs and future MDLs.
   A. An IPA is performed prior to the first time this method is used and any time the method or instrumentation is modified.
   B. Using the results of the set of four analyses, calculate the average concentration (x) and the standard deviation (s) for each compound.
   C. Compare x and s for each native and labeled compound with the limits for IPA in Table 6 of method 1668B. If any individual s exceeds the precision limit or any individual x fall outside the range for accuracy, the test must be repeated. For congeners that are not listed in the method table, refer to the limits posted in the spreadsheet summarizing the data.

2. Method Blank (MB): Method Blank is a sand, distilled water or other appropriate matrix preparation that is free from native analyte that has been prepared and analyzed using the same procedures followed for the rest of the sample batch.
   A. A MB is run with every analytical batch or 20 samples, whichever is less, per matrix type.
   B. Analytical data is accepted (with a data qualifier) if the amount found in the MB is less than one tenth of the level found in the associated samples. Otherwise, the samples are re-extracted and analyzed. Use the EMLs in Method 1668B for guidance only. Use the "B" data qualifier when a specific congener is found at a level above the RL or when at a level that is not "significantly" different then the one found in the field sample even if below the RL.

3. Ongoing Precision and Recovery (OPR): An ongoing precision and recovery sample is prepared by adding a known quantity of native standard to an interferant-free matrix and used to assess method performance (precision and recovery).
   A. Spike the native compounds into the sample at a level corresponding to the CS3.
   B. An OPR is analyzed with every analytical batch or 20 samples (whichever is less) per matrix type.
   C. For each native and labeled compound, compare the concentration with the limits for ongoing accuracy in Table 6 in Method 1668B.

4. Matrix Spike (MS/MSD): A matrix spike sample is prepared by adding a known quantity of native standard to a sample matrix prior to extraction. The MS/MSD are only prepared when requested by the clientele. Although, it is customarily to use only the unlabeled compounds present in the ICAL, AAP might consider using the 209 congeners once every other year to assess its ability to determine the full list of congeners.
   A. Spike the native compounds into the sample at a level corresponding to the CS3.
   B. The relative percent difference between MS/MSD samples should be ≤50%.

5 Batch CS_3: For air samples and other sample projects not requiring an OPR and/or MS/MSD, a Batch CS_3 is prepared and analyzed at the beginning and at the end of each 12-H analytical sequence according to the descriptions provided in Method 8290. The concentrations of the Batch CS_3 are the same as the CS_3 of the ICAL. As indicated earlier, a database of Batch CS_3's will become the basis for developing control limits. Until such time, the control limits currently proposed for Method 8290 will be considered. For more details on the BCS_3 concept, refer to the Performance Commitment document.

6 Solvent Blank Spike (SBS): To ensure that no carry-overs are operating, an SBS injection is performed right after the analysis of the BCS_3 (or CS3, or OPR). The goal is to verify that no peaks from the previous run are found with a S/N>2.5. However, in order to distinguish the absence of carry-overs from a "miss-injection", the SBS is used instead of just nonane by itself. The SBS contains a number of PCB congeners ranging from Cl_3 to Cl_7 (see list below). A second function fulfilled by the SBS is verification of the overall instrument sensitivity. The various PCB congeners present in the SBS are present at a concentration that is ½ the lowest point on the calibration curve (or ML; 0.5 pg/µL).

PCB Congeners in the SBS

Tri-CB:	36
TCB:	72, 79, 78
PeCB:	96, 98, 89, 107, 106
HxCB:	152, 166
HpCB:	184, 182
OCB:	204

12. CALIBRATION AND STANDARDIZATION

1. Initial Calibration
   A. Inject the reference compound perfluorokerosene (PFK). PFK provides the required lock masses and is used for tuning the mass spectrometer.
   B. Using a PFK molecular leak, tune the instrument to meet the minimum required resolving power of 10,000. For each mass descriptor, monitor and record the resolution and exact m/z of three to five reference peaks covering the range of the descriptor.
   C. An appropriate lock mass will be monitored for each descriptor and shall not vary by more than ± 220% throughout the respective retention time window.
   D. For the measurement of PCBs, the exact m/z's to be monitored in each descriptor are listed in Table 6 of the method.

E. Inject 1 µL of the Window Defining Mix (CPSM) or Batch CS_3.
   i. The first and last eluters are verified to be within the appropriate retention time windows for each chlorination level.
F. Under the same conditions, inject 1 µLL of each of the five calibration solutions containing all PCB isomers. Calibration standard solutions are presented in Table 2.
   i. The signal to noise ratio (S/N) must exceed 10:1 for all ions monitored,
   ii. The ion abundance ratio measurements must be within ± 15% of the theoretical ratio.
G. Calibration by Isotope Dilution: Isotope dilution calibration is used for the native PCBs for which labeled compounds are added to samples prior to extraction.
   i. If the relative response for any compound is less than 20% coefficient of variation over the 5 point calibration range, an averaged relative response is used for that compound; otherwise, the complete calibration curve for that compound is used over the 5 point range.
H. Calibration by Internal Standard: Internal standard method is used for the determination of native PCBs for which a labeled compound is not available.
   i. If the response factor for any compound is less than 35% coefficient of variation over the 5 point calibration range, an averaged response factor may be used for that compound; otherwise, the complete calibration curve for that compound shall be used over the 5 point range.

1. Continuing Calibration

   1. Follow the BCS_3 requirements. Using the same operating conditions as established for the ICAL, inject 1 mL of the batch control spike (BCS_3)) at the beginning and end of each 12-hour period during which samples are analyzed in order to demonstrate adequate GC resolution and sensitivity, response factor reproducibility, to establish the PCB retention time windows and isomer-specificities, and to validate the standards and the spiking technique. When true 1668 requirements are required, follow the procedures below.

   2. Inject a column performance standard mix (CPSM) to verify retention time windows.

      1. The first and last PCB eluters are verified to be within the homologue retention time windows.

   1. Inject a mid-range standard from the initial calibration curve (CS3) at the beginning and end of every 12 hours. The following criteria must be met:

      1. The relative response factors for the mid-range standard are within the limits established in Method 1668. That is, the percent RSD for the mean response factors must be within the method's tolerances.

      2. The ion ratios are within 15 percent of the theoretical.

      3. The signal to noise ratio (S/N) exceeds 10:1 for all ions monitored.

      4. The retention times must be within the criteria established in Method 1668.

13. EXTRACTION AND CLEANUP PROCEDURES

1. Determine the percent solids on all samples. Weigh 2-10g of sample, dry overnight in 110°C oven and re-weigh.
2. AQUEOUS OR SAMPLES WITH <1% SOLIDS: Extract sample using one of the following two extraction procedures:
   Using 1 L of the sample, spike 2 ng of the Extraction Standards into the sample. Into the OPR, spike the native compounds at a level consistent with the CS3 concentration. If particulates are present, filter (0.45 µm) and perform a dual extraction (liq-liq with MC on the filtrate and toluene SDS on the filter/solid). SDS Soxhlet extract the filter with toluene for 16 hours. Combine the extracts and solvent exchange into hexane. Proceed with cleanup procedures. In the absence of particulates, liquid-liquid partition with MC (3x60 mL in separatory funnel or using the CLL). Allow the organic layer to separate and collect by passing through a funnel with cleaned Na_2SO_4 into a 500mL round bottom. Concentrate and solvent-exchange into hexane. Proceed with appropriate cleanup procedures.
3. SOIL, SEDIMENT, SLUDGE OR SAMPLES WITH >1% SOLIDS: Spike 2 ng of the Extraction Standards into the thimble containing 10-20 g equivalent dry weight of sample mixed with Hydromatrix™ .. SDS Soxhlet extract for 16 hours hexane followed by 16 H with toluene. Concentrate, combine and solvent-exchange into hexane. Proceed with cleanup procedures. It is permitted to perform an 8-H hexane SDS followed by an 8-H toluene SDS extraction.
4. MULTI-PHASE SAMPLES: Using the percent solids, determine the volume of sample that will provide 10-20 g of solids, up to 1L of sample. Pressure filter the amount of sample determined through glass-fiber filter paper. If necessary, separate the phases and/or settle the solids, centrifuge the aliquots before filtration. Discard any aqueous phase, if present. Remove any non-aqueous liquid present and reserve the maximum amount filtered from the sample or 10 g, whichever is less, for combination with the solid phase.
5. ASH SAMPLES: Spike 2 ng of the Extraction Standards into the thimble containing 5 g of sample mixed with Hydromatrix™.. SDS Soxhlet extract for 16 hours with hexane, followed by 16 H with toluene. Concentrate, combine and solvent-exchange into hexane. Proceed with cleanup procedures. For ash matrices, do not use the two 8-H SDS extraction option.
6. AIR SAMPLES: See Methods 23/8290B for procedural details. Depending on whether or not PCBs are analyzed in conjunction with PCDD/Fs and/or PAHs, the spike amount ranges from 2 ng to 4 ng. Custom-made charts are used to help with the description of the spike profiles, the sample handling and extractions. Because these charts are designed on a per project basis, see the project folder for the charts. The resin/PUF sorbents are pre-spiked with three Sampling Standards (i.e., the CS in 1668B).
7. FISH AND TISSUE SAMPLES: Mix 25 g of well ground fish with Hydromatrix™.. Stir frequently to remove any lumps. Transfer the mixture to a thimble containing 50 g of acid-coated silica gel. Spike 2 ng of the Extraction Standards. Soxhlet extract for 16 hours with hexane. Proceed with cleanup procedures. Calculate the % lipids on a separate aliquot.
   A. % Lipids
      i. Using a 5-to-10-g separate aliquot, extract inside a VOA vial using MC and sonication.
      ii. Filter through a glasswool plug containing sodium sulfate.
      iii. Allow the extract to air dry completely and then place in a vacuum oven overnight at room temperature.
      iv. Weigh the residue on the balance and record the weight. Calculate the % lipids using the following equation:

$$\% \text{ lipids} = \frac{\text{lipid residue wt.}}{\text{sample wt.}} \times 100$$

1. Cleanups **(note: refer to specific SOPs for the latest version; this Core Method SOP may not contain the same level of details)**.

   1. If not done, add the Cleanup Standards. Rotovap to **C_{14}**. Proceed to first cleanup.

   2. ABP-Acid/base partitioning ("dirty" samples only)

   1. CAcid/Base Silica Column (see SOP AP-SP-CU1 current revision)

   2. D**Florisil Column** (see SOP AP-SP-CU1 current revision)

14. GC/MS Analysis

A. Establish the necessary operating conditions. The GC conditions may be optimized for compound separation and sensitivity. Once optimized, the same GC conditions must be used for the analysis of all standards, blanks, OPR aliquots, BCS_3 and samples. Use the SB-Octyl GC column described in Method 1668B. For isomer specific determination of the PCB-156 and PCB-157, use the WHO 2 SOP.

B. The reference perfluorokerosene (PFK) provides the required lock masses and is used for tuning the mass spectrometer.

i. An appropriate lock mass will be monitored for each descriptor and shall not vary by more than ± 20% throughout the respective retention time window.

ii. Using a PFK molecular leak, tune the instrument to meet the minimum required resolving power of 10,000. For each descriptor, monitor and record the resolution and exact m/z of three to five reference peaks covering the range of the descriptor. The mass resolution check is achieved before any analysis is performed and at the end of each 12-hour shift.

C Set up the analytical run following this sequential injection pattern: Window Defining Mix or CPSM, CS3, OPR, BCS_3, Solvent Blank, Method Blank, Samples, closing BCS_3. Note that under the BCS_3 system, no CS3 or OPR are required. And similarly, under the OPR system, no BCS_3 are required. However, it is important to emphasize that AAP has been operating in dual mode for many months before adopting this policy.

D. Inject 1µL of the Window Defining Mix.

i. The first and last eluters are verified to be within the appropriate retention time windows for each chlorination level.

E. Inject either a mid-range standard from the initial calibration curve (CS3), or a BCS_3 created for the samples to be analyzed. The following criteria must be met:

i. Calculate the concentration of each native compound either by isotope dilution or internal standard technique. Each compound must be within the verification limits established in Method 1668B.

ii. The ion ratios must be within 15% of theoretical.

iii. The signal to noise ratio (s/n) must exceed 10:1 for all ions monitored.

iv. Criteria for BCS_3 are available from M8290B SOP (i.e., in addition to the RT, S/N, ion-abundance ratio, a PD of $\leq$20 percent for Ax, PD$\leq$50 percent for ES/CS/SS; RPD $\leq$10 percent for Ax; RPD $\leq$20 percent for ES/CS/SS).

1. Qualitative Determination

1. To identify a chromatographic peak as a PCB (either an unlabeled or a labeled compound). It must meet the following criteria:

1. The signals for the two exact m/z being monitored must be present and must maximize within ±2 seconds of one another.

2. The signal-to-noise ratio (S/N) of each of the two exact m/z must be greater than or equal to 2.5:1 for a sample extract, and greater than or equal to 10:1 for a calibration standard.

3. The ion abundance ratios must have a ratio within the limits established for the homologous series.

4. The relative retention times of the peak for a toxic PCB must be within 5% of the relative retention times listed in Method 1668. The retention times of peaks representing PCBs other than the toxic PCBs must be within the retention time windows established

2. Quantitative Determination

1. For peaks that meet the criteria listed above, quantitate the PCB peaks from the mean RRF relative to the appropriate Extraction Standard established in BCS_3 or the initial calibration, as appropriate.

2. Any peaks representing the other congeners are quantitated using the response factors from specified labeled PCBs isomers at the same level of chlorination (Table 3)

3. Report results in picogram per gram, picogram per liter or picogram per sample.

15. DATA ANALYSIS AND CALCULATIONS

1. The concentrations for the PCB compounds are calculated by using the formula:

$$C_X = \frac{A_X \times Q_{is}}{A_{is} \times W \times RRF}$$

Where

C_X = Concentration of unlabeled PCB congeners (or group of coeluting isomers within an homologous series) in pg/g.

A_X = Sum of the integrated ion abundances of the quantitation ions

A_{is} = Sum of the integrated ion abundances of the quantitation ion

Q_{is} = Quantity, in pg, of the Extraction Standard added to the sample before extraction.

W = Weight of the sample (solid or liquid).

RRF = Calculated relative response factor for the analyte.

2. The detection limits for each absent PCB can be calculated using the following formula:

$$DL = \frac{2.5 \times H_N \times Q_{is}}{H_{IS} \times W \times RRF}$$

DL = Estimated detection limit for homologous PCBs.

H_N = Noise height (peak to peak).

H_{IS} = Peak height of the Extraction Standard.

Q_{is} = Quantity, in pg, of the Extraction Standard added to the sample before extraction.

W = Weight of the sample (solid or liquid).

RRF = Calculated relative response factor for the analyte.

16. METHOD PERFORMANCE

1. The method performance is thoroughly documented in our Statement of Performance, which includes the results of performance testing studies, audit samples, and international interlaboratory calibration studies.

17. POLLUTION PREVENTION

1. See Pollution Prevention Basics

18. DATA ASSESSMENT AND ACCEPTANCE CRITERIA

1. This method is performance-based. Thus, the acceptance criteria are ideally determined by the project needs through a process of negotiation with the client and any other ultimate end-user of the data. These criteria should ensure that the performance of the method is adequate for the purposes for which it is intended. The criteria will likely not resemble those used in the past. In the absence of such criteria, those outlined elsewhere within this SOP and within the published methods may be used.

2. 2. A failure to meet all criteria does not necessarily result in a particular course of action (see next section for details). Data assessment includes a thinking process that assesses the impact of a particular analytical problem and develops the most appropriate response, in the context of the data users needs (when known). Data that do not meet all criteria for all target analytes may still be reported with relevant qualifiers and notes in the case narrative, if appropriate.

19. CORRECTIVE ACTIONS

1. Corrective actions are taken whenever needed, regardless of acceptance criteria. It is not always the case that data meeting all acceptance criteria should be accepted nor that data not meeting all criteria should be rejected.

2. 2. When the BCS3 fails, it is important to discern the following:

    1. The fundamental objective of the BCS_3 is to "validate" the standard solutions and spiking technique and the RRFs used to quantitatively characterize the analytes in the samples **at the time** the standards are used to prepare and analyze the samples. They are four types of standards involved in the preparation of the BCS_3 that provide various probes into assessing this "validation" procedure. They are the NS (symbolized as A_x in expressions or tables), ES, SS or CS, and JS. The question becomes how can one "extract" the information needed to complete the validation, or how does one "filter" out the irrelevant information to help with the distinction between a critical error and a minor one. A critical error means erroneous data resulting from a seriously flawed spiking technique (e.g., wrong amount of ES added under the OPR/LCS/ICAL system, or the NS under the BCS_3 system) while other minor errors can provide useful information or feedback on the measurement step (e.g., instrumentation variation). The interpretation of the information obtained from the analysis of the BCS_3 is best handled when done contextually. This analytical protocol does not claim that it offers a comprehensive analysis but merely puts forward guidelines to help the analyst in assessing the quality and reliability of the data.

    2. A failure on the "**PD**" requirements may be indicative of an instrumentation difficulty or spiking error. The latter can be of Level PD-1 (i.e., at the standard solution level) or Level PD-2 (i.e., at the spiking operation level). A third Level PD-3 is associated with instrumentation. An error at the NS standard solution level (Level PD-1) constitutes, under the BCS_3 system, the most serious failure and requires that a new set of standard solutions be prepared and independently validated before repeating the sample extraction and analysis, if called for by the decision flow chart ("*Thinking Method*"). Note that the decision flow charts make a distinction between short- and long-term actions. When appropriate, a new initial calibration may be required before analyzing the BCS_3 and the samples. However, if the error is a Level PD-2 error, a re-extraction and analysis is the most suitable action after correcting the flawed spiking technique. As customarily done, a new BCS_3 is prepared with a Level PD-2 error. Here again, the decision flow charts should be used since the text herein is only for illustration purposes. Distinction between Levels PD-1 and PD-2 can be accomplished contextually by examination of the initial independent validation study and control charts (showing for instance a trend suggesting a degradation of the ES solution), behavior over time charts, and/or using the matrices shown in Table Inserts 3 or 4. The Level PD-3 error is associated with instrumentation when an out-of-calibration situation is present or a temporary or localized instrumentation variation is operative. Depending on the severity of the Level PD-3 error, a new calibration (either ICAL or rerunning the BCS_3 and all the affected samples) following a new "tuning" of the instrumentation may be required (see Decision Flow Charts for "*Thinking Method*").

    3. A failure on the "**RPD**" requirements may be indicative of instrumentation instability or inability to sustain the instrumentation's performance over a 12-H period. Again, two levels are possible. Level RPD-1 is strictly associated with instrumentation difficulties that are unrelated to the samples under analysis. A re-analysis (i.e., re-injection) of the BCS_3 **and** of the samples can be considered as a corrective action after correction of the source of the instrumentation's shortfall (see Decision Flow Charts for "*Thinking Method*"). If

however, the re-analysis of the BCS_3 fails again, and there are indications that the spiking procedure is questionable (Level PD-1 or PD-2), the associated extraction batch may need to undergo re-extraction and analysis with the preparation of a new associated BCS3 as discussed above for the BCS_3 PD deviations. A Level RPD-2 BCS_3 failure may be found with the analysis of samples presenting special challenges (i.e., highly complex matrices that do not cleanup well under the various options offered by this protocol). Depending on the severity of the deviation, additional cleanup or other appropriate actions may be required before re-analysis of the samples and associated BCS_3. If such action proves to be ineffective, the data should be qualified accordingly.

4. The BCS_3 "PD Requirements" are summarized in the four table inserts below. In addition to the traditional RRFs, another set of "pseudo-RRFs" is computed from the BCS3 data to help with the validation of the ES and RRFs used to report the sample analytes. The pseudo-RRFs are used to further differentiate the various "A" to "C" types PD Requirements. Use Table Insert 3 (non air) or Table Insert 4 (air) for departing-from-the-norm groups of analytes (e.g., all 17 A x or the five SS show a deviation similar in "sign" and "amplitude"). It is also recommended to examine the data contextually (e.g., using QC charts)

Table Insert 1:

Batch CS_3 PD Requirements

Based on Traditional RRFs

("A" to "C"Types)

3.

Type	Analytes	Requirement	Failure Possible Cause[a,b]	Failure Level	Suggested Corrective Action
A	**A_X** vs **ES**	+ 30%	1. Calibration out 2. Spiking error	1. PD-3 2. PD-1/PD-2	3. New Calibration 4. New Standards/New Extraction
B	**ES** vs **JS**	+ 50% non air + 50% for air [c]	1. Calibration out 2. Spiking error	5. PD-3 6. PD-1/PD-2	7. New Calibration 8. New Standards/New Extraction
C_1	**CS** vs **JS**	+ 50%	1. Calibration out 2. Spiking error	9. PD-3 10. PD-1/PD-2	11. New Calibration 12. Affects other Types
C_2	**SS** vs **ES** (air)	+ 50%	1. Calibration out 2. Spiking error	13. PD-3 14. PD-1/PD-2	15. New Calibration 16. New Standards/New Extraction/New Sampling[d]

a. Calibration out = usually when one (localized) or several/all analytes are affected; instrumental source.
b. Spiking error = when all analytes are affected with the same "sign" and "amplitude"; must be considered contextually; i.e., using historical data or other information on the set of standards such as the "pseudo-RRFs". Situations when selected analytes degrade are rare but should not be excluded from consideration.
c. By design for air matrices, the amounts of ES and JS added during the preparation of the Batch CS 3 are different. Thus, an additional error is introduced, which can deceive the analyst's interpretation. In this case, the QC emphasis is shifted towards the "C2" type PD requirement.
d. Because of the nature of an "air" sample, there is no additional sample volume available to repeat the extraction. The laboratory is required to qualify the data by estimating and documenting accordingly the "error" associated with the reported measurements. If such documentation is not possible, and/or the information points toward a seriously flawed ES addition (as opposed to a spiking error associated with the SS), the data can be rejected and re-sampling efforts may be necessary. See the "Air Spiking Related Error Matrix" tables for an alternative approach whereby the A_x vs SS RRFs are used to determine the analyte's concentrations (Table Insert 4).

Table Insert 2 :

Batch CS_3 PD Requirements

Based on Pseudo-RRFs

("D" to "G"Types)

Type	Analytes[a]	Requirement
D	**A_x** vs **CS/SS**	+/- 25%

E_1	**A_x** vs **JS** (non air)	+/- 35%
E_2	**A_x** vs **JS** (air)	+/- 35%
F_1	**ES** vs **CS** (non air)	+/- 20%
G_2	**SS** vs **JS** (air)	+/- 50%

a. Pseudo-RRFs are limited to analytes, for which an analogous/homologous standard is available:

Table Insert 3:

"Non-Air" Spiking Related PD Errors

(departing-from-the-norm group of analytes)

"PD Requirements Decision Matrix" – Normal Configuration
(use BCS_3 RRFs)

	Ax	ES	CS	JS
Ax	-	Y	Y	Y
ES	-	-	Y	Y
CS	-	-	-	Y

"PD Requirements Decision Matrix" – Defective A_x Spiking = Fatal
(If A_x spiking is shown to be in error, and if no additional or replacement sample is available, use ICAL RRFs if CS_3 is acceptable)

	Ax	ES	CS	JS
Ax	-	N	N	N
ES	-	-	Y	Y
CS	-	-	-	Y

"PD Requirements Decision Matrix" – Defective JS Spiking
(use BCS_3 RRFs)
percent recovery measurements for CS & ES affected, not the analytes

	Ax	ES	CS	JS
Ax	-	Y	Y	N
ES	-	-	Y	N
CS	-	-	-	N

"PD Requirements Decision Matrix" – Defective CS Spiking
(use BCS_3 RRFs)
percent recovery measurements for CS affected, not the analytes

	Ax	ES	CS	JS
Ax	-	Y	N	Y
ES	-	-	N	Y
CS	-	-	-	N

"PD Requirements Decision Matrix" – Defective ES Spiking
(use BSC_3 RRFs; see Levels PD-1 or PD-2)

	Ax	ES	CS	JS
Ax	-	N	Y	Y
ES	-	-	N	N
CS	-	-	-	Y

Table Insert 4:

"Air" Spiking Related PD Errors

(departing-from-the-norm group of analytes)

"PD Requirements Decision Matrix" – Normal Configuration
(use BCS_3 RRFs)

	Ax	ES	SS	JS
Ax	-	Y	Y	Y
ES	-	-	Y	Y
SS	-	-	-	Y

"PD Requirements Decision Matrix" – Defective A_x Spiking = **Fatal**
(If Ax spiking is shown to be in error, and if no additional or replacement)

	Ax	ES	SS	JS
Ax	-	N	N	N
ES	-	-	Y	Y
SS	-	-	-	Y

"PD Requirements Decision Matrix" – Defective JS Spiking
(use BCS_3 RRFs)
percent recovery measurements for ES affected, not the analytes or the SS

	Ax	ES	SS	JS
Ax	-	Y	Y	N
ES	-	-	Y	N
SS	-	-	-	N

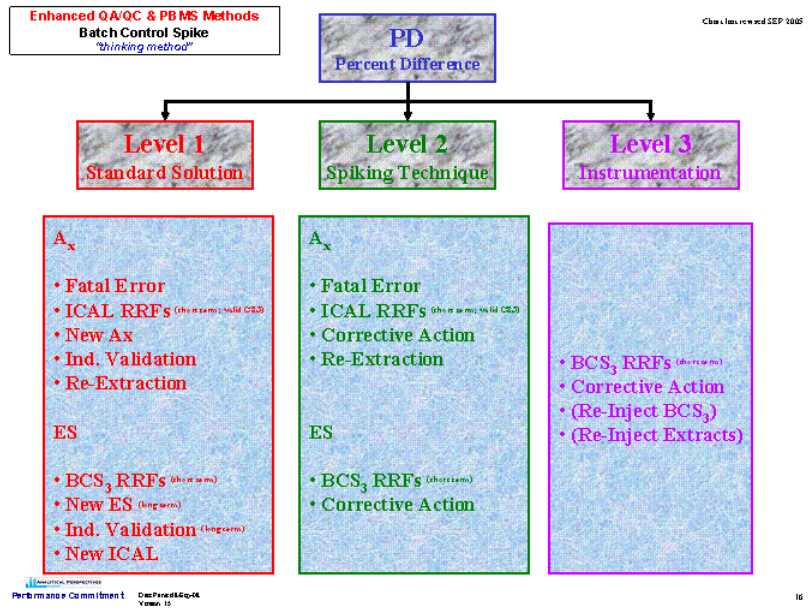
"PD Requirements Decision Matrix" – Defective SS Spiking
(use BCS_3 RRFs)
percent recovery measurements for SS affected, not the analytes

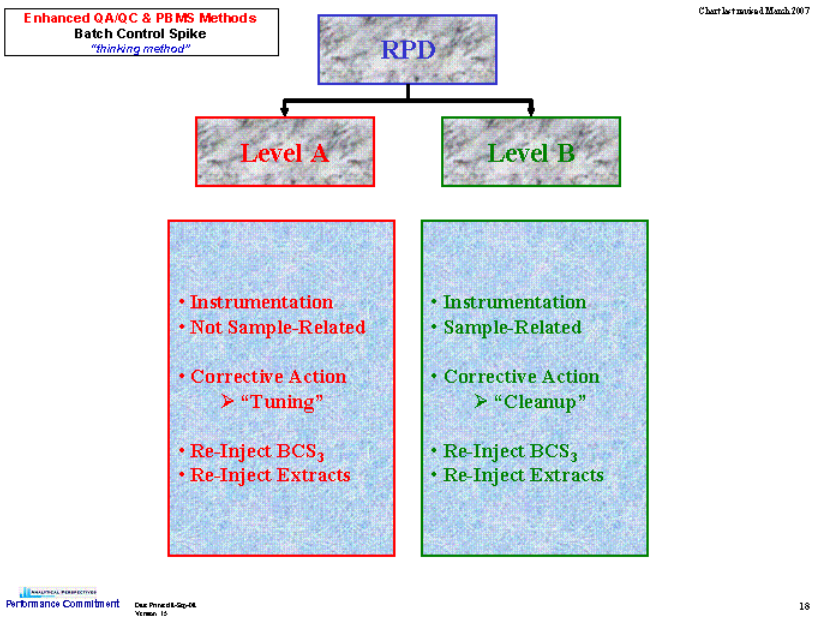
	Ax	ES	SS	JS
Ax	-	Y	N	Y
ES	-	-	N	Y
SS	-	-	-	N

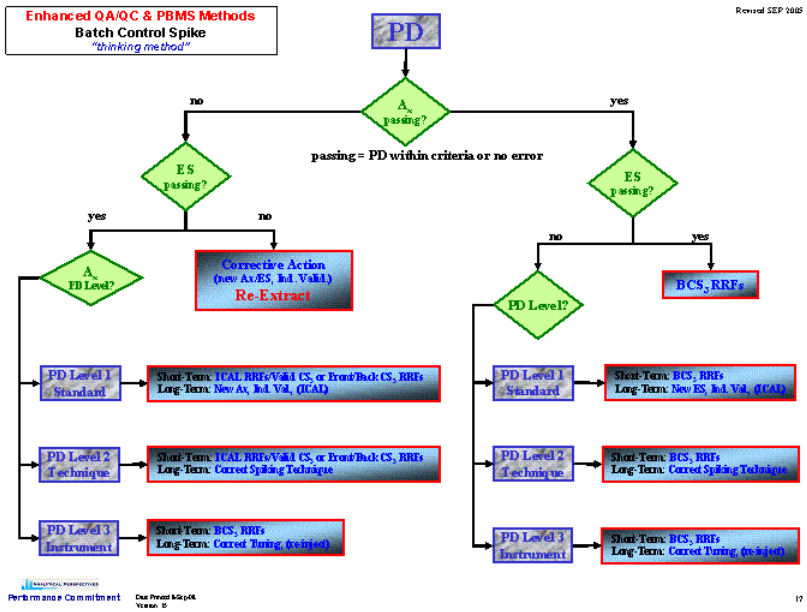
"PD Requirements Decision Matrix" – Defective ES Spiking
(use BCS_3 RRFs; see Levels PD-1 or PD-2; for air samples only, consider using the A_x vs SS RRFs)

	Ax	ES	SS	JS
Ax	-	N	Y	Y
ES	-	-	N	N
SS	-	-	-	Y

Decision Flow Charts for "*Thinking Method*"


Enhanced QA/QC & PBMS Methods
Batch Control Spike
"thinking method"
PD
Percent Difference
Level 1
Standard Solution
Level 2
Spiking Technique
Level 3
Instrumentation
A_x
• Fatal Error
• ICAL RRFs
• New Ax
• Ind. Validation
• Re-Extraction
ES
• BCS_3 RRFs
• New ES
• Ind. Validation
• New ICAL
A_x
• Fatal Error
• ICAL RRFs
• Corrective Action
• Re-Extraction
ES
• BCS_3 RRFs
• Corrective Action
• BCS_3 RRFs
• Corrective Action
• (Re-Inject BCS_3)
• (Re-Inject Extracts)
Performance Commitment
16


Enhanced QA/QC & PBMS Methods
Batch Control Spike
"thinking method"
RPD
Level A
Level B
• Instrumentation
• Not Sample-Related
• Corrective Action
➢ "Tuning"
• Re-Inject BCS_3
• Re-Inject Extracts
• Instrumentation
• Sample-Related
• Corrective Action
➢ "Cleanup"
• Re-Inject BCS_3
• Re-Inject Extracts
Performance Commitment
18



20. CONTINGENCIES FOR HANDLING OUT -OF-CONTROL OR UNACCEPTABLE DATA

1. Should data be found unacceptable after a thorough assessment, the appropriate corrective action will be taken. In the case that the appropriate corrective action requires reextraction of exhausted samples, the client will be contacted for consultation with regard to the next steps to be taken.

21. WASTE MANAGEMENT

1. The storage and disposal of wastes generated by this method is covered in our Health and Safety SOPs.

22. REFERENCES

Method 1668A. Measurement of Toxic PCB Congeners By Isotope Dilution HRGC/HRMS, Prepared by Analytical Methods Staff, Engineering and Analysis Division, Office of Science and Technology, Office of Water, U. S. Environmental Protection Agency, Washington, DC, December, 1999.

Method 1668B. Measurement of Toxic PCB Congeners By Isotope Dilution HRGC/HRMS, Prepared by Analytical Methods Staff, Engineering and Analysis Division, Office of Science and Technology, Office of Water, U. S. Environmental Protection Agency, Washington, DC, November 2008.

Method 1668C. Measurement of Toxic PCB Congeners By Isotope Dilution HRGC/HRMS, Prepared by Analytical Methods Staff, Engineering and Analysis Division, Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency, Washington, DC, April 2010.

23. TABLES AND NOTES

1. Table 1. Concentration of PCBs in Calibration and Calibration Verification Solutions

1668A ICAL
units: pg / µL

Individual Standard	IUPAC	CS0	CS-1	CS-2	CS-3	CS-4	CS-5
					VER		
2-MoCB	1	0.5	1.0	5.0	50	400	2000
4-MoCB	3	0.5	1.0	5.0	50	400	2000
2,2'-DiCB	4	0.5	1.0	5.0	50	400	2000
4,4'-DiCB	15	0.5	1.0	5.0	50	400	2000
2,2',6-TrCB	19	0.5	1.0	5.0	50	400	2000
3,4,4'-TrCB	37	0.5	1.0	5.0	50	400	2000
2,2',6,6'-TeCB	54	0.5	1.0	5.0	50	400	2000
3,3',4,4'-TeCB	77	0.5	1.0	5.0	50	400	2000
3,4,4',5-TeCB	81	0.5	1.0	5.0	50	400	2000
2,2',4,6,6'-PeCB	104	0.5	1.0	5.0	50	400	2000
2,3,3',4,4'-PeCB	105	0.5	1.0	5.0	50	400	2000
2,3,4,4',5-PeCB	114	0.5	1.0	5.0	50	400	2000
2,3',4,4',5-PeCB	118	0.5	1.0	5.0	50	400	2000
2',3,4,4',5-PeCB	123	0.5	1.0	5.0	50	400	2000
3,3',4,4',5-PeCB	126	0.5	1.0	5.0	50	400	2000
2,2',4,4',6,6'-HxCB	155	0.5	1.0	5.0	50	400	2000
2,3,3',4,4',5-HxCB	156	0.5	1.0	5.0	50	400	2000
2,3,3',4,4',5'-HxCB	157	0.5	1.0	5.0	50	400	2000
2,3',4,4',5,5'-HxCB	167	0.5	1.0	5.0	50	400	2000
3,3',4,4',5,5'-HxCB	169	0.5	1.0	5.0	50	400	2000
2,2',3,4',5,6,6'-HpCB	188	0.5	1.0	5.0	50	400	2000
2,3,3',4,4',5,5'-HpCB	189	0.5	1.0	5.0	50	400	2000
2,2',3,3',5,5',6,6'-OcCB	202	0.5	1.0	5.0	50	400	2000
2,3,3',4,4',5,5',6-OcCB	205	0.5	1.0	5.0	50	400	2000
2,2',3,3',4,4',5,5',6-NoCB	206	0.5	1.0	5.0	50	400	2000
2,2',3,3',4,5,5',6,6'-NoCB	208	0.5	1.0	5.0	50	400	2000
DeCB	209	0.5	1.0	5.0	50	400	2000

1668A ICAL

Individual Standard	IUPAC	CS0	CS-1	CS-2	CS-3 VER	CS-4	CS-5
Extraction Standards							
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
13C12-3,3',4,4'-TeCB	77 L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
13C12-3,4,4',5-TeCB	81 L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
13C12-2,3,3',4,4'-PeCB	105 L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
13C12-2,3,4,4',5-PeCB	114 L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
13C12-2,3',4,4',5-PeCB	118 L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
13C12-2',3,4,4',5-PeCB	123 L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
13C12-3,3',4,4',5-PeCB	126 L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
13C12-2,3,3',4,4',5-HxCB	156 L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
13C12-2,3,3',4,4',5'-HxCB	157 L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
13C12-2,3',4,4',5,5'-HxCB	167 L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
13C12-3,3',4,4',5,5'-HxCB	169 L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
13C12-2,3,3',4,4',5,5'-HpCB	189 L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
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Cleanup or Sampling Standards							
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
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Injection Standards							
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[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 2. Theoretical Ion Abundance Ratios and QC Limits

Number of Chlorine Atoms	Ion Type	Theoretical Ratio	Control Limits(1)	
			Lower	Upper
1	M/M+2	3.13	2.66	3.60
2	M/M+2	1.57	1.33	1.81
3	M/M+2	1.04	0.88	1.20
4	M/M+2	0.77	0.65	0.89
5	M+2/M+4	1.56	1.32	1.78
6	M/M+2	0.51	0.43	0.59
6	M+2/M+4	1.25	1.05	1.43
7	M/M+2	0.45	0.37	0.51
7	M+2/M+4	1.04	0.88	1.20
8	M+2/M+4	0.89	0.76	1.02
9	M/M+2	1.34	1.14	1.54
9	M/M-2	0.78	0.66	0.90
10	M/M+2	1.17	0.99	1.35

(1) Represents ± 15% windows around the theoretical ion abundance ratios.

(Initial Calibration used for High-Level Samples Requiring 20-Fold Dilution of the Extract)

Table 3: Quantitation Relationships (Ax vs ES)

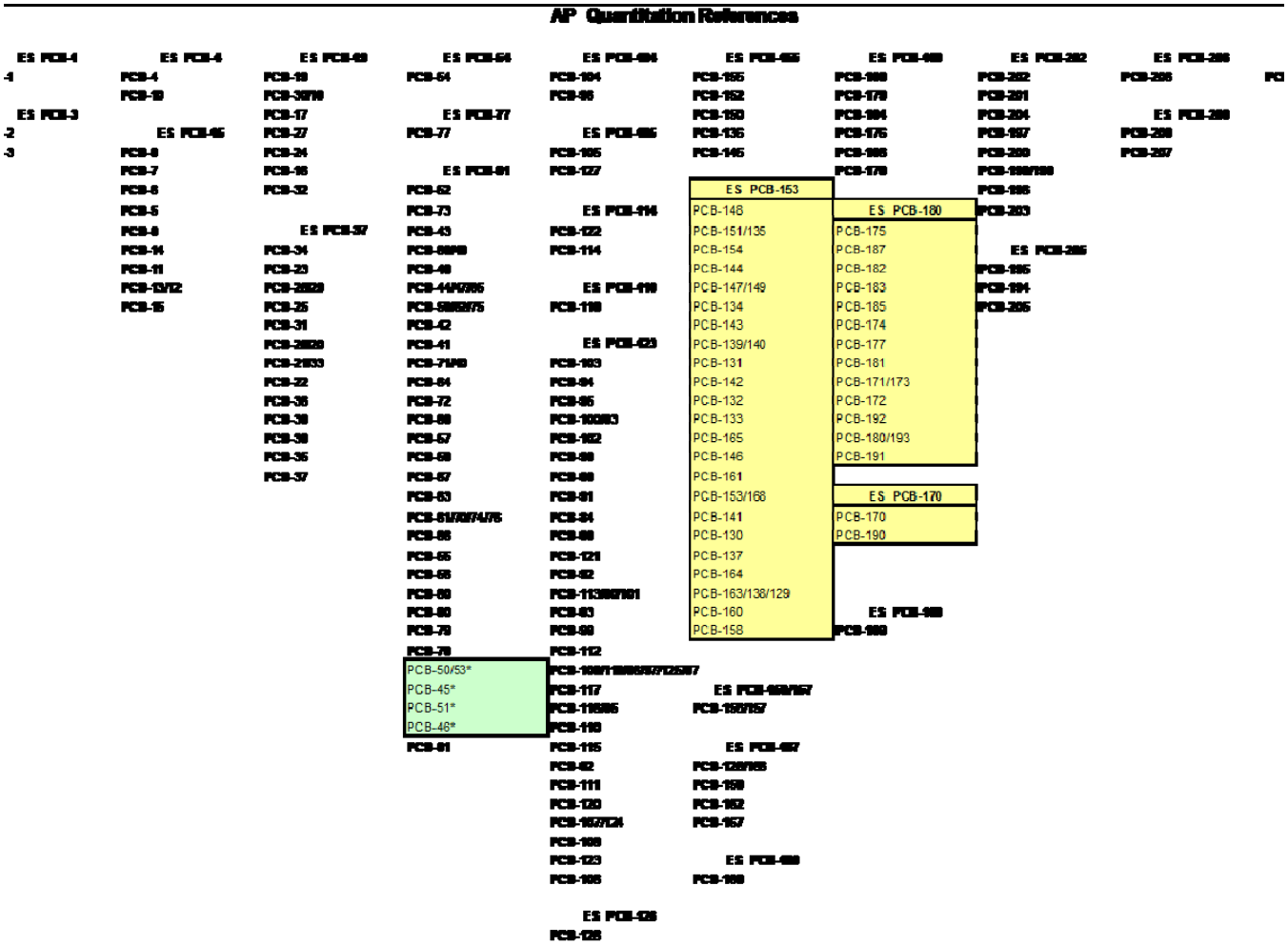

Table 4: Quantitation Relationships (ES vs JS ; CS vs JS)

ES/JS Quantitation References					CS/JS Quantitation References		
ES 188 & 202 were originally quantitated against JS 194 - based on analytical observations JS 138 was determined a better choice							
JS PCB-9	JS PCB-52	JS PCB-101	JS PCB-138	JS PCB-194	CS PCB-28	CS PCB-111	CS PCB-170
ES PCB-1	ES PCB-37	ES PCB-104	ES PCB-166		JS PCB-62	JS PCB-101	JS PCB-138
ES PCB-3	ES PCB-54	ES PCB-105	ES PCB-156/157	ES PCB-189			
ES PCB-4	ES PCB-77	ES PCB-114	ES PCB-167				
ES PCB-15	ES PCB-81	ES PCB-118	ES PCB-189	ES PCB-195			
ES PCB-19		ES PCB-123	ES PCB-188*	ES PCB-198	SS PCB-28	SS PCB-111	SS PCB-170
		ES PCB-126	ES PCB-202*	ES PCB-200	JS PCB-37	JS PCB-123	JS PCB-189
				ES PCB-208			

Table 5A-C: QC Criteria for EPA Methods 1668A,B,C

The following criteria are used for the purpose of qualifying data when it is processed as having been run with Method 1668A.

Congener	IUPAC number[2]	Test conc (ng/mL)[3]	VER[4] (%)	IPR RSD (%)	IPR X (%)	OPR (%)	Labeled compound recovery in samples (%)
2-MoCB	1	50	70-130	40	60-140	50-150	
4-MoCB	3	50	70-130	40	60-140	50-150	
2,2'-DiCB	4	50	70-130	40	60-140	50-150	
4,4'-DiCB	15	50	70-130	40	60-140	50-150	
2,2'6-TrCB	19	50	70-130	40	60-140	50-150	
3,4,4'-TrCB	37	50	70-130	40	60-140	50-150	
2,2'6,6'TeCB	54	50	70-130	40	60-140	50-150	
3,3',4,4'-TeCB	77	50	70-130	40	60-140	50-150	
3,4,4',5-TeCB	81	50	70-130	40	60-140	50-150	
2,2',4,6,6'-PeCB	104	50	70-130	40	60-140	50-150	

2,3,3',4,4'-PeCB	105	50	70-130	40	60-140	50-150	
2,3,4,4',5-PeCB	114	50	70-130	40	60-140	50-150	
2,3',4,4',5-PeCB	118	50	70-130	40	60-140	50-150	
2',3,4,4',5-PeCB	123	50	70-130	40	60-140	50-150	
3,3',4,4',5-PeCB	126	50	70-130	40	60-140	50-150	
2,2',4,4',6,6'-HxCB	155	50	70-130	40	60-140	50-150	
2,3,3',4,4',5-HxCB[5]	156	50	70-130	40	60-140	50-150	
2,3,3',4,4',5'-HxCB[5]	157	50	70-130	40	60-140	50-150	
2,3',4,4',5,5'-HxCB	167	50	70-130	40	60-140	50-150	
3,3',4,4',5,5'-HxCB	169	50	70-130	40	60-140	50-150	
2,2',3,4',5,6,6'-HpCB	188	50	70-130	40	60-140	50-150	
2,3,3',4,4',5,5'-HpCB	189	50	70-130	40	60-140	50-150	
2,2',3,3',5,5',6,6'-OcCB	202	50	70-130	40	60-140	50-150	
2,3,3',4,4',5,5',6-OcCB	205	50	70-130	40	60-140	50-150	
2,2',3,3',4,4',5,5',6-NoCB	206	50	70-130	40	60-140	50-150	
2,2',3,3,'4,5,5',6,6'-NoCB	208	50	70-130	40	60-140	50-150	
DeCB	209	50	70-130	40	60-140	50-150	
$^{13}C_{12}$-2-MoCB	1L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-4-MoCB	3L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2'-DiCB	4L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-4,4'-DiCB	15L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',6-TrCB	19L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-3,4,4'-TrCB	37L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',6,6'-TeCB	54L	100	50-150	50	35-135	30-140	25-150
13C12-3,3',4,4'-TCB	77L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-3,4,4',5-TeCB	81L	100	50-150	50	35-135	30-140	25-150

				IPR			Labeled compound recovery in samples
Congener	**IUPAC number[2]**	**Test conc (ng/mL)[3]**	**VER[4] (%)**	**RSD (%)**	**X (%)**	**OPR (%)**	**(%)**
$^{13}C_{12}$-2,2',4,6,6'-PeCB	104L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3,3',4,4'-PeCB	105L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3,4,4',5-PeCB	114L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3',4,4',5-PeCB	118L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2',3,4,4',5-PeCB	123L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-3,3',4,4',5-PeCB	126L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',4,4',6,6'-HxCB	155L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3,3',4,4',5 -HxCB[5]	156L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3,3',4,4',5'-HxCB[5]	157L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3',4,4',5,5'-HxCB	167L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-3,3',4,4',5,5'-HxCB	169L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',3,4',5,6,6'-HpCB	188L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB	189L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',3,3',5,5',6,6'-OcCB	202L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3,3',4,4',5,5',6-OcCB	205L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6-NoCB	206L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',3,3',4,5,5',6,6'-NoCB	208L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6,6'-DeCB	209L	100	50-150	50	35-135	30-140	25-150
Cleanup standard							
$^{13}C_{12}$-2,4,4'-TrCB	28L	100	60-130	45	45-120	40-125	30-135
$^{13}C_{12}$-2,3,3',5,5'-PeCB	111L	100	60-130	45	45-120	40-125	30-135
$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB	178L	100	60-130	45	45-120	40-125	30-135

1. QC acceptance criteria for IPR, OPR, and samples based on a 20 FL extract final volume
2. Suffix "L" indicates labeled compound.
3. See Table 5.
4. Section 15.3.
5. PCBs 156 and 157 are tested as the sum of two concentrations

Table 4B:
The following criteria are used for the purpose of qualifying data when it is processed as having been run with Method 1668B.

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Table 6. QC acceptance criteria for VER, IPR, OPR, and labeled compounds in samples [1,2]

Name	Congener Number [3]	Test conc (ng/mL) [4]	VER (%) [5]	IPR RSD (%)	IPR Recovery (%)	OPR recovery (%)	Labeled compound recovery in samples (%)
2-MoCB	1	50	70 – 130	25	84 – 119	71 – 132	
4-MoCB	3	50	70 – 130	22	83 – 112	72 – 123	
2,2'-DiCB	4	50	70 – 130	18	82 – 105	73 – 114	
4,4'-DiCB	15	50	70 – 130	17	85 – 107	76 – 116	
2,2'6-TrCB	19	50	70 – 130	13	86 – 103	79 – 109	
3,4,4'-TrCB	37	50	70 – 130	26	77 – 109	64 – 122	
2,2'6,6'TeCB	54	50	70 – 130	17	84 – 106	76 – 114	
3,3',4,4'-TeCB	77	50	70 – 130	20	81 – 106	71 – 116	
3,4,4',5-TeCB	81	50	70 – 130	20	81 – 106	70 – 116	
2,2',4,6,6'-PeCB	104	50	70 – 130	19	83 – 107	74 – 117	
2,3,3',4,4'-PeCB	105	50	70 – 130	19	83 – 107	73 – 117	
2,3,4,4',5-PeCB	114	50	70 – 130	18	83 – 105	74 – 113	
2,3',4,4',5-PeCB	118	50	70 – 130	13	88 – 105	81 – 112	
2',3,4,4',5-PeCB	123	50	70 – 130	16	82 – 102	74 – 109	N/A
3,3',4,4',5-PeCB	126	50	70 – 130	17	82 – 104	74 – 113	
2,2',4,4',6,6'-HxCB	155	50	70 – 130	15	86 – 105	79 – 112	
2,3,3',4,4',5-HxCB [6]	156	50	70 – 130	16	87 – 108	78 – 117	
2,3,3',4,4',5'-HxCB [6]	157	50	70 – 130	16	87 – 108	78 – 117	
2,3',4,4',5,5'-HxCB	167	50	70 – 130	13	85 – 101	79 – 107	
3,3',4,4',5,5'-HxCB	169	50	70 – 130	16	80 – 100	73 – 108	
2,2',3,4',5,6,6'-HpCB	188	50	70 – 130	14	88 – 106	81 – 113	
2,3,3',4,4',5,5'-HpCB	189	50	70 – 130	16	85 – 106	77 – 114	
2,2',3,3',5,5',6,6'-OcCB	202	50	70 – 130	17	82 – 104	74 – 112	
2,3,3',4,4',5,5',6-OcCB	205	50	70 – 130	15	87 – 107	79 – 115	
2,2',3,3',4,4',5,5',6-NoCB	206	50	70 – 130	17	85 – 106	76 – 115	
2,2',3,3,'4,5,5',6,6'-NoCB	208	50	70 – 130	17	86 – 108	77 – 116	
DeCB	209	50	70 – 130	20	81 – 106	71 – 116	
$^{13}C_{12}$-2-MoCB	1L	100	50 – 150	78	21 – 100	2 – 100	4 – 100
$^{13}C_{12}$-4-MoCB	3L	100	50 – 150	63	31 – 100	13 – 100	11 – 106
$^{13}C_{12}$-2,2'-DiCB	4L	100	50 – 150	56	35 – 100	18 - 100	14 – 107
$^{13}C_{12}$-4,4'-DiCB	15L	100	50 – 150	70	34 – 100	10 – 118	19 – 107
$^{13}C_{12}$-2,2',6-TrCB	19L	100	50 – 150	68	32 – 100	10 – 106	1 – 108
$^{13}C_{12}$-3,4,4'-TrCB	37L	100	50 – 150	57	47 – 104	24 – 128	25 – 123

Table 4C

The following criteria are used for the purpose of qualifying data when it is processed as having been run with Method 1668C.

QC Acceptance Criteria for VER, IPR, OPR, and Labeled Compounds in Samples[1,2]

Congener Name	Congener No.[3]	Test Conc. (ng/mL)[4]	VER (%)[5]	IPR RSD (%)	IPR Mean Recovery (%)	OPR Recovery (%)	Labeled Compound Recovery in Samples (%)
2-MoCB	1	50	75 - 125	25	70 - 130	60 - 135	
4-MoCB	3	50	75 - 125	25	70 - 130	60 - 135	
2,2'-DiCB	4	50	75 - 125	25	70 - 130	60 - 135	
4,4'-DiCB	15	50	75 - 125	25	70 - 130	60 - 135	
2,2'6-TrCB	19	50	75 - 125	25	70 - 130	60 - 135	
3,4,4'-TrCB	37	50	75 - 125	25	70 - 130	60 - 135	
2,2'6,6'TeCB	54	50	75 - 125	25	70 - 130	60 - 135	
3,3',4,4'-TeCB	77	50	75 - 125	25	70 - 130	60 - 135	
3,4,4',5-TeCB	81	50	75 - 125	25	70 - 130	60 - 135	
2,2',4,6,6'-PeCB	104	50	75 - 125	25	70 - 130	60 - 135	
2,3,3',4,4'-PeCB	105	50	75 - 125	25	70 - 130	60 - 135	
2,3,4,4',5-PeCB	114	50	75 - 125	25	70 - 130	60 - 135	NA
2,3',4,4',5-PeCB	118	50	75 - 125	25	70 - 130	60 - 135	
2',3,4,4',5-PeCB	123	50	75 - 125	25	70 - 130	60 - 135	
3,3',4,4',5-PeCB	126	50	75 - 125	25	70 - 130	60 - 135	
2,2',4,4',6,6'-HxCB	155	50	75 - 125	25	70 - 130	60 - 135	
2,3,3',4,4',5-HxCB [6]	156	50	75 - 125	25	70 - 130	60 - 135	
2,3,3',4,4',5'-HxCB [6]	157	50	75 - 125	25	70 - 130	60 - 135	
2,3',4,4',5,5'-HxCB	167	50	75 - 125	25	70 - 130	60 - 135	
3,3',4,4',5,5'-HxCB	169	50	75 - 125	25	70 - 130	60 - 135	
2,2',3,4',5,6,6'-HpCB	188	50	75 - 125	25	70 - 130	60 - 135	
2,3,3',4,4',5,5'-HpCB	189	50	75 - 125	25	70 - 130	60 - 135	
2,2',3,3',5,5',6,6'-OcCB	202	50	75 - 125	25	70 - 130	60 - 135	

2,3,3',4,4',5,5',6-OcCB	205	50	75 - 125	25	70 - 130	60 - 135	
2,2',3,3',4,4',5,5',6-NoCB	206	50	75 - 125	25	70 - 130	60 - 135	
2,2',3,3,'4,5,5',6,6'-NoCB	208	50	75 - 125	25	70 - 130	60 - 135	
DeCB	209	50	75 - 125	25	70 - 130	60 - 135	
$^{13}C_{12}$-2-MoCB	1L	100	50 - 145	70	20 - 135	15 - 145	5 - 145
$^{13}C_{12}$-4-MoCB	3L	100	50 - 145	70	20 - 135	15 - 145	5 - 145
$^{13}C_{12}$-2,2'-DiCB	4L	100	50 - 145	70	20 - 135	15 - 145	5 - 145
$^{13}C_{12}$-4,4'-DiCB	15L	100	50 - 145	70	20 - 135	15 - 145	5 - 145
$^{13}C_{12}$-2,2',6-TrCB	19L	100	50 - 145	70	20 - 135	15 - 145	5 - 145
$^{13}C_{12}$-3,4,4'-TrCB	37L	100	50 - 145	70	20 - 135	15 - 145	5 - 145
$^{13}C_{12}$-2,2',6,6'-TeCB	54L	100	50 - 145	70	20 - 135	15 - 145	5 - 145

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QC Acceptance Criteria for VER, IPR, OPR, and Labeled Compounds in Samples[1,2]

Congener Name	Congener No.[3]	Test Conc. (ng/mL)[4]	VER (%)[5]	IPR		OPR Recovery (%)	Labeled Compound Recovery in Samples (%)
				RSD (%)	Mean Recovery (%)		
$^{13}C_{12}$-3,3',4,4'-TeCB	77L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-3,4,4',5-TeCB	81L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2,2',4,6,6'-PeCB	104L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2,3,3',4,4'-PeCB	105L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2,3,4,4',5-PeCB	114L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2,3',4,4',5-PeCB	118L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2',3,4,4',5-PeCB	123L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-3,3',4,4',5-PeCB	126L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2,2',4,4',6,6'-HxCB	155L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2,3,3',4,4',5 -HxCB[6]	156L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2,3,3',4,4',5'-HxCB[6]	157L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2,3',4,4',5,5'-HxCB	167L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-3,3',4,4',5,5'-HxCB	169L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2,2',3,4',5,6,6'-HpCB	188L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB	189L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2,2',3,3',5,5',6,6'-OcCB	202L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2,3,3',4,4',5,5',6-OcCB	205L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6-NoCB	206L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2,2',3,3',4,5,5',6,6'-NoCB	208L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6,6'-DeCB	209L	100	50 - 145	50	45 - 135	40 - 145	10 - 145
Cleanup standards							
$^{13}C_{12}$-2,4,4'-TrCB	28L	100	65 - 135	70	20 - 135	15 - 145	5 - 145
$^{13}C_{12}$-2,3,3',5,5'-PeCB	111L	100	75 - 125	50	45 - 135	40 - 145	10 - 145
$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB	178L	100	75 - 125	50	45 - 135	40 - 145	10 - 145

1. Reference 22 describes how interlaboratory results were pooled from analyses of wastewater, biosolids, and fish tissue samples.
2. QC acceptance criteria for IPR, OPR, and samples based on a 20-µL extract final volume
3. Suffix "L" indicates labeled compound.
4. See Table 5.
5. Section 15.3.
6. CBs 156/157 and 156L/157L are tested as the sum of the two congeners NA = Not applicable

Additional Notes: EPA Method 1668A has different retention times for PCB's 107, 108, and 109 versus EPA Method 1668B and 1668C. The appropriate retention times are used when processing data as per the method version being used.

Appendix

List of Abbreviations &Acronyms

1. AS = alternate standard
2. A_t = target analyte
3. BCS_n = batch control spike
4. CAA = Clean Air Act
5. COC = chain of custody
6. CPSM = column performance standard mixture
7. CS_n = calibration solution corresponding to the middle point of the initial calibration curve
8. CS = cleanup standard
9. CWA = Clean Water Act
10. *dbi* Technology = dioxin-furan array (fingerprinting reminiscent of DNA plates)
11. DOC = documentation of capability
12. DUP = duplicate
13. EDL = estimated detection limit (sample- and analyte-specific)
14. ES = extraction standard (isotopically labeled standard added before the extraction)
15. GC = gas chromatography
16. ICAL = initial calibration
17. ID-HRMS = comprehensive and stable isotope-dilution high-resolution mass spectrometry
18. Ind. Val. = independent validation
19. JS = injection standard
20. LOD = limit of detection
21. LOQ = limit of quantitation
22. MB = lab method blank
23. MDL = method detection limit (not sample-specific)
24. MRM = mass spectrometer resolving power
25. MIR = Methods Innovation Rule
26. ML = minimum level (equivalent to lowest point on the calibration curve)
27. MS/MSD = matrix spike/matrix spike duplicate
28. ND = not detected
29. NELAC = National Environmental Laboratory Accreditation Conference
30. OPR = on-going precision & recovery (equivalent to LCS or Lab Control Spike)
31. PAH = polynuclear aromatic hydrocarbon
32. PBMS = performance-based measurement system
33. PCB = polychlorinated biphenyl
34. PCDD/F = polychlorinated dibenzo-*p*-dioxin and dibenzofuran
35. PD = percent difference
36. PE = performance evaluation sample
37. QI = quantitative interference
38. QuanTIC = selected SVOAs by full-scan GC/MS (ID-HRMS)
39. RCRA = Resources Recovery Act
40. RL = reporting limit (usually represents the lowest point on the calibration curve or ML)
41. RPD = relative percent difference
42. RRF = relative response factor
43. RSD = relative standard deviation
44. RTW = retention time defining window solution
45. SDWA = Safe Drinking Water Act
46. SICP = selected ion current profile
47. S/N = signal-to-noise ratio
48. SRM = standard reference material
49. SS = sampling standard
50. TEQ = toxic equivalency quotient
51. TSCA = Toxic Substances Control Act
52. U-SVOA = ultra-semi volatile analyte (selected SVOAs by SIR by ID-HRMS)
53. VER = continuing calibration verification (equivalent to ConCal)
54. WHO-2 / WHO-2S = ID-HRMS assay for the 29 World Health Organization target analytes (S = serum)