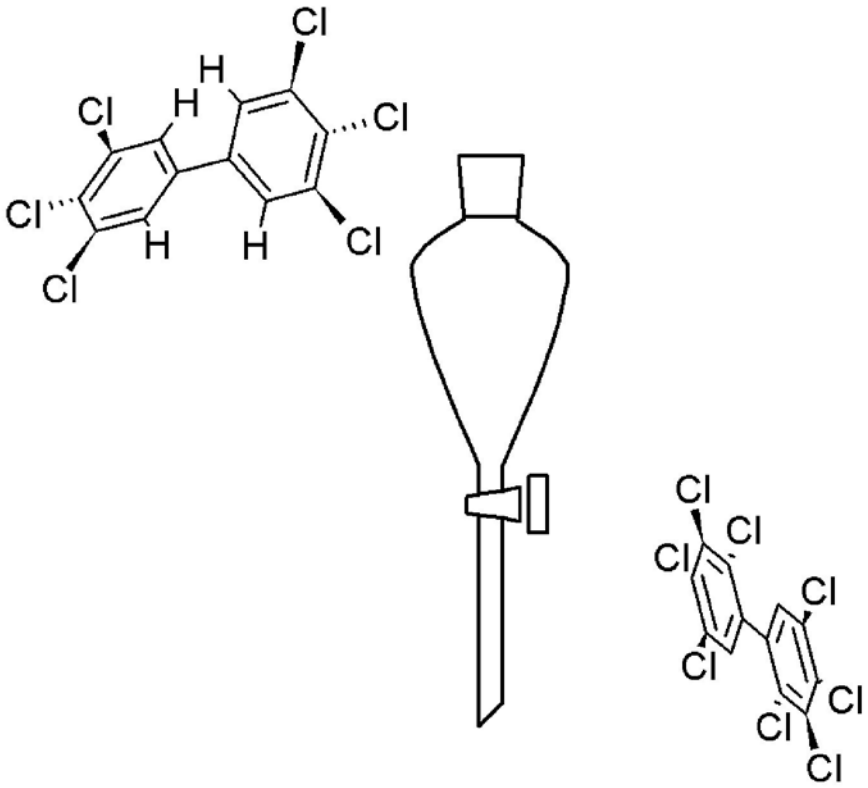
Method 1668A Interlaboratory Validation Study Report

November 2008



U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
Engineering and Analysis Division (4303T)
1200 Pennsylvania Avenue, NW
Washington, DC 20460

EPA-821-R-08-021

Executive Summary

This report presents the results of EPA's interlaboratory validation of EPA Method 1668A *Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS*. This study was conducted in 2003-2004 to validate the performance of EPA Method 1668A in municipal wastewater, fish tissue, and biosolids matrices.

EPA used the results of the study to evaluate and revise Method 1668A quality control (QC) acceptance criteria for initial precision and recovery, ongoing precision and recovery, and labeled compound recovery from real world samples. These interlab criteria (Table 5-1) replace the single-lab criteria, and are published in Table 6 of the revised version of this PCB-congener method, EPA Method 1668B.

Acknowledgments

This report was written under contract for EPA by CSC Systems & Solutions, LLC, and Interface, Inc. EPA acknowledges the volunteer laboratories that participated in the study and, in particular, those laboratories that took the extra effort to comment on EPA Method 1668A and to provide suggestions for improvements.

Disclaimer

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Contacts

Richard Reding, Ph.D., Chief
Engineering & Analytical Support Branch
Engineering and Analysis Division (4303T)
Office of Science and Technology, Office of Water
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue NW
Washington, DC 20460
http://www.epa.gov/waterscience
ostcwamethods@epa.gov

Table of Contents

List of Tables

List of Figures

Section 1
Introduction and Background

1.1 Introduction

This report describes the interlab validation study of EPA Method 1668A that EPA conducted in 2003 - 2004 on municipal wastewater, biosolids and fish tissue matrices. The study was conducted according to the *Study Plan for Interlaboratory Validation of EPA Method 1668A for Determination of Chlorinated Biphenyl Congeners in Water, Biosolids, and Tissue by HRGC/HRMS, November 2003*, which is an appendix to this report. A draft of this report was peer reviewed, and the following changes were made as a result of this review:

- Rounded all numbers to 3 significant figures maximum.
- Expanded discussion of how QC acceptance criteria were generated
- Moved the definition of "Youden pair" from Section 2.5.1 to its first use in this paragraph.
- Table 4-4: Truncated numbers at the decimal point in the "# pairs" column.

EPA used the results of the study to revise Method 1668A and publish, in 2008, Method 1668B. Quality control (QC) acceptance criteria for initial precision and recovery, ongoing precision and recovery, and labeled compound recovery from real world samples are in Table 5-1 of this report. These interlab criteria replace the single-lab criteria, and are published in Table 6 of EPA Method 1668B.

1.2 Background

Method 1668A is for determination of chlorinated biphenyl congeners (PCBs) in water, soil, sediment, biosolids, and tissue by high resolution (capillary column) gas chromatography combined with high resolution mass spectrometry (HRGC/HRMS). These 209 PCB-congeners are the individual chemicals that comprise a class of pollutants known as Aroclors. Since publication in 1999, Method 1668A has been used to measure PCBs in biosolids in EPA's 2001 National Sewage Sludge Survey, and fish tissue in EPA's four year National Study of Chemical Residues in Lake Fish Tissue. Additional background on the nature and determination of PCBs and on the history of development, validation, and peer-review of EPA Method 1668A is in the study plan.

1.3 2003 Revision to Method 1668A

Minor revisions to Method 1668A were made in August 2003 in preparation for the study. The changes corrected technical and typographical errors and reflected practice of the method by laboratories based on comments received. The August 2003 revision was used for the study and is the revision distributed by EPA at the time of writing of this report.

Section 2
Study Management, Objectives, Design, and Implementation

2.1 Study Management

This study was designed and managed by the Engineering and Analytical Support Branch (EASB, formerly the Statistics and Analytical Support Branch) of the Engineering and Analysis Division in the

Office of Science and Technology within EPA's Office of Water. Day-to-day coordination of study activities was performed by the contractor-operated Sample Control Center (SCC).[1]

Preliminary results of this study were presented at the 2004 National Environmental Monitoring Conference in Washington, DC, July 20, 2004. Since that presentation, the results have been further evaluated and presented in this report. Therefore, this report supersedes any material previously presented or published.

2.2 Study Objectives and Design

Objectives of this study were to 1) characterize the performance of Method 1668A in multiple laboratories and matrices, and 2) evaluate and, if appropriate, revise the QC acceptance criteria in the method.

EASB designed the study in accordance with guidelines published by EPA and ASTM International (ASTM).[2,3] These guidelines recommend a minimum of six complete data sets for evaluation of a method. To allow for some loss of data due to error, lost samples, outlier removal, or other unforeseen causes, EPA included 14 participant laboratories in the study. The study design is detailed in an appendix to this report.

2.3 Laboratory Selection

EPA used volunteer laboratories for participation in the study. Each interested laboratory was asked to demonstrate that it had recent experience in using HRGC/HRMS to determine chlorinated pollutants in environmental samples and confirm that it would determine all 209 congeners using an SPB-Octyl column, as described in Method 1668A. The intent was to ensure that Study participants already possessed the facilities, equipment, and trained staff necessary to implement the method.

Fourteen (14) volunteer laboratories were selected to participate in this study. The laboratories were notified of their selection at least two weeks before the study began, so that they would have time to review the method and study-specific instructions. Of the 14 laboratories selected, 11 were commercial laboratories and 3 were EPA Regional laboratories. Laboratories were not required to validate the method in all three matrices; as a result, the number of participant laboratories varied, depending on the matrix tested. As discussed in section 3 of this report, because of scheduling problems 3 of these 14 volunteer labs did not submit data.

To offset costs to the laboratories, EPA provided each laboratory with a set of analytical standards necessary to identify and measure the 209 PCB congeners targeted by Method 1668A. EPA also provided the laboratories sets of standard solutions containing native and carbon-13 labeled compounds necessary to calibrate their instruments and to conduct all analyses. The packaged sets of standards were purchased from Cambridge Isotope Laboratories (CIL) and AccuStandard, Inc. Laboratories were provided with detailed instructions for combining and diluting standards to preclude injudicious use of standards. The instructions were based on procedures given in Method 1668A.

In addition to the 14 volunteer participant laboratories, a sample processing laboratory was contracted to perform all activities necessary to ensure that the participant laboratories received homogenized, spiked, and aliquoted samples. Homogenization of bulk sample volume was necessary to

[1] The Sample Control Center (SCC) is operated by CSC Systems & Solutions, LLC under contract to EPA.
[2] *Guidelines for Selection and Validation of US EPA's Measurement Methods,* U.S. EPA Office of Acid Deposition, Environmental Monitoring and Quality Assurance (OADEMQA), Office of Research and Development, U.S. Environmental Protection Agency, August 1987 DRAFT.
[3] ASTM Standard D2777-98, "Standard Practice for Determination of Precision and Bias of Methods of Committee D-19 on Water," *Annual Book of ASTM Standards, Vol. 11.01,* ASTM International, West Conshohocken, PA 19428.

prepare replicate samples for analysis by participant laboratories. The participant and sample processing laboratories are listed in Table 2-1.

Table 2-1 Laboratories Participating in the Method 1668A Validation Study

Alta Analytical Laboratory Inc. 1104 Windfield Way El Dorado Hills, CA 95762 Phone: 916-933-1640	Battelle-Columbus Laboratories 505 King Avenue Columbus, OH 43201 Phone: 614-424-7884	Philip Analytical Services Corporation 5555 North Service Road Burlington, ON L7L 5H5 CANADA Phone: 800-668-0639
EPA Region 7 300 Minnesota Ave. Kansas City, KS 66101 Phone: 913-551-5120	Columbia Analytical Services 10655 Richmond Avenue, Suite 130A Houston, TX 77042 Phone: 713-266-1599	Enviro-Test Laboratories 9936-67th Avenue Edmonton, AB T6E OP5 CANADA Phone: 780-413-6481
Axys Analytical Services, Ltd. 2045 Mills Road West Sidney, BC V8L 3S8 Canada Phone: 250-655-5800	Severn Trent Laboratories – Knoxville 5815 Middlebrook Pike Knoxville, TN 37921 Phone: 865-291-3000	EPA Region 3 701 Mapes Road Fort Meade, MD 20755-5350 Phone: 410-305-2606
Pace Analytical Services 1700 West Albany Broken Arrow, OK 74012 Phone: 918-251-2858	Paradigm Analytical Laboratories, Inc. 5500 Business Drive Wilmington, NC 28405 Phone: 910-350-1903	Severn Trent Laboratories – Sacramento 880 Riverside Parkway West Sacramento, CA 95605 Phone: 916-374-4433
Pacific Analytical, Inc. 6056 Corte del Cedro Carlsbad, CA 92009 Phone: 760-496-2200	Data Analysis Technologies, Inc. 7715 Corporate Blvd. Plain City, OH 43064 Phone: 800-733-8644	EPA Region 4 980 College Station Rd. Athens, GA 30605-2720 Phone: 706-355-8807

Note: The primary purpose of this study was to evaluate the performance of Method 1668A. While results obtained by individual laboratories were used relative to this purpose, no attempt was made to assess performance of individual laboratories. No endorsement of these laboratories is implied, nor should any be inferred. To preserve confidentiality, laboratories that volunteered for this study, including three that did not submit lab data, were assigned numbers randomly from 1 to 14. The lab identities and that of the sample processing laboratory are not revealed in the data or lists in this report.

2.4 Sample Selection

To minimize burden on volunteer laboratories, the study was designed so that no more than two samples of each matrix type would be analyzed, with each sample containing varying concentrations of the target PCB congeners. EPA provided existing (archived) fish tissue and biosolids samples to the sample processing laboratory to prepare Study samples representing these matrices. No archived sample volume was available for wastewater, therefore, the sample processing laboratory prepared the wastewater samples. In preparing Study samples, EPA's objective was to ensure that the congeners present in each sample matrix would span the anticipated measurement range of Method 1668A, from the upper end of the calibration range down to "not detected."

Tissue and biosolids samples were generated from excess sample collected during EPA's *National Study of Chemical Residues in Fish Tissues* and EPA's *2001 National Sewage Sludge Survey*, respectively. These samples had been stored in freezers at an EPA sample repository. So that a sufficient amount of each sample was available to support the study, EPA identified several samples of each matrix type that could be combined to produce large volumes of Youden pairs with the desired congener distribution. (Youden pairs are defined as two samples of the same matrix containing similar, but not exact, concentrations of the analytes of interest.) Once these stored samples were identified, they were forwarded on ice to the sample processing laboratory. Although PCBs are stable and do not require preservation, ice was used to prevent decomposition of fish tissue and to retard gas production in the biosolids. For wastewater, amounts of effluent grab samples were collected from a publicly owned treatment works (POTW) that were sufficient to provide enough samples for all of the participant

laboratories, and excess sample in case of breakage, spillage, or other problems. Bulk wastewater was collected in polyethylene carboys and shipped overnight to the sample processing laboratory for spiking and distribution.

2.5 Preparation of Study Samples

The sample processing laboratory was provided with a detailed set of instructions for:

- Combining and homogenizing the biosolids samples
- Combining and homogenizing fish tissues
- The number of aliquots to be prepared from each combined/homogenized matrix
- Aliquoting and spiking the wastewater samples
- Labeling and shipping the prepared sample aliquots.

2.5.1 Biosolids and Tissues

Because the biosolids and tissue samples used in this study were already known to contain PCBs at levels sufficient to cover the analytical range of Method 1668A, the sample processing laboratory did not have to spike these matrices with PCBs. This eliminated concerns about how well spiked constituents would be incorporated into these matrices and whether spiked samples were representative of real-world samples. The goal of the mixing and aliquoting scheme for biosolids and tissues was to obtain Youden pairs for each matrix of interest (*i.e.*, composites A and B). As described in ASTM Practice D2777, the concentrations of Youden pairs should differ by no more than 20%. Because the available "excess" volumes of the biosolids and fish tissues were limited and the number of laboratory participants was relatively large, the Youden pairs were prepared in a multi-step process. For the biosolids, the first step was to combine and homogenize five biosolids samples to form a composite. This composite was then divided approximately in half. One half of the composite was designated as biosolids sample "A" while the other half was used to prepare biosolids sample "B." Biosolids sample "B" was prepared by adding material from a sixth existing biosolids sample, plus some clean sand, to produce a composite with PCB congener concentrations that were approximately 20 % different from those in sample "A." For the tissue samples, two existing tissue samples were homogenized. The composite was then divided approximately in half, with one half being designated as tissue sample "A." Tissue sample "B" was prepared by adding tissue from a third existing sample to the remaining half of the initial composite.

The sample processing laboratory was required to perform background and homogeneity analyses of both the biosolids and tissue matrices. The laboratory was instructed to analyze one 10-g dry weight aliquot of sample "A" as the background analysis, and two 10-g dry weight aliquots of sample "B" as the homogeneity aliquots for both the biosolids and tissue matrices. Because of the mixing scheme for both of these matrices, it was assumed that if the homogeneity for sample "B" is found to be acceptable, the homogeneity of sample "A" would also be acceptable. This approach was used to preserve sample mass. Results of tissue and biosolids background and homogeneity analyses are discussed in Section 4.1.

2.5.2 Wastewater

Based on previous experience, municipal wastewater discharges would be unlikely to contain PCB congeners at concentrations sufficient to adequately test the capabilities of the method. Thus, the sample processing laboratory was instructed to first analyze an aliquot of wastewater from a publicly owned treatment works (POTW) to determine background PCB congener levels. Following a review of the background results by SCC, EPA defined the spiking levels, and provided the sample processing laboratory with detailed instructions to divide the unspiked POTW matrix into the required number of aliquots and spike each aliquot separately (rather than spiking a bulk volume of wastewater and then subdividing the spiked sample into replicate aliquots) to the appropriate concentrations. Spiking each aliquot separately avoids problems with "wall effects," whereby organic pollutants spiked into a bulk

sample tend to adhere to the walls of the container, making it difficult to divide a bulk sample into multiple aliquots containing the same analyte concentrations.

Because of the difficulty that would be encountered in preparing custom spiking solutions, wastewater samples were spiked with varying amounts of "individual native CB congener solutions" A2 through E2 listed in Table 4 of EPA Method 1668A. Concentrations of the congeners in the wastewater samples, by level of chlorination, are given in Table 2-2.

Table 2-2 Spiked Congener Concentrations in Wastewater Samples (by Level of Chlorination)

Congeners	Concentration (pg/L)	
	Youden Pair #1	Youden Pair #2
24 Mono- through Trichlorinated biphenyl congeners	900	750
6 Mono- through Dichlorinated biphenyl congeners	1200	1000
9 Mono- through Trichlorinated biphenyl congeners	1500	1250
74 Tetra- through Heptachlorinated biphenyl congeners	1800	1500
38 Tetra- through Heptachlorinated biphenyl congeners	2400	2000
42 Tetra- through Heptachlorinated biphenyl congeners	3000	2500
13 Octa- through Decachlorinated biphenyl congeners	2700	2250
3 Octachlorinated biphenyl congeners	4500	3750

The sample processing laboratory analyzed two random aliquots of one concentration level for homogeneity determination. Results of the homogeneity analyses are discussed in Section 4.1.

2.5.3 Labeling and Shipping

SCC provided the sample processing laboratory with a unique 5-digit sample number for each sample. After the aliquots were prepared, the sample processing laboratory labeled each sample container and cap with the corresponding unique sample number. The sample processing laboratory then shipped the prepared, numbered samples to the participant laboratories via air courier. Although PCBs are persistent, and thus do not require preservation, biosolids and tissue samples were shipped on ice to hinder decomposition of the tissues and gas formation in the biosolids. The sample processing laboratory notified SCC of the shipping date, and SCC notified participant laboratories of the shipping and scheduled arrival dates. Table 2-3 lists the numbers of wastewater, biosolids, and tissue samples that were prepared for distribution to the 14 participant laboratories.

Table 2-3 Sample Pairs for Distribution to 14 Participant Laboratories

Matrix	Samples per Laboratory	Number of Aliquots Distributed
Wastewater	2 (1 Youden Pair)	28
Biosolids	2 (1 Youden Pair)	28
Tissue	2 (1 Youden Pair)	28
All Three Matrices	6	84

2.6 Sample Analysis and Data Reporting

Participant laboratories did not know the concentration of PCBs in the samples received, and were instructed to prepare and analyze the samples according to Method 1668A procedures, except where stated otherwise in the participant's scope of work. In addition to the analysis of study samples, laboratories also were required to prepare and analyze two ongoing precision and recovery (OPR) samples in reagent water, one reagent water blank, and one solids/tissue blank (playground sand mixed with corn oil).

Because study results were to be used to evaluate or further develop QC acceptance criteria, laboratories were prohibited from performing multiple analyses to improve results. Laboratories were,

however, allowed to implement corrective action and reanalyses for QC failures attributable to analyst error, instrument failure, or identified contamination. The laboratories also were instructed that any deviations from the method and SOW must be pre-approved by EPA.

Laboratories were required to submit electronic and hard copies of summary sample results, and hard copies of all supporting raw data, run chronologies, chromatograms, example equations, and case narratives to SCC for review and data validation. Additionally, laboratories were asked to provide a detailed narrative describing any problems or recommendations and a description of any modifications to procedures specified in the method. All submitted data were reviewed against the study and method requirements prior to use for evaluation of method performance. Laboratories were asked to adhere to the following rules in reporting results:

- Report results to the lowest level possible, using a signal-to-noise ratio of 3 as the sample-specific detection limit.
- For congeners that are not detected, report as "<nn", where nn is an estimate of the detection limit at S/N=3. Do not use the terms "zero," "trace," or "ND" (not detection).
- Report PCB congener concentrations in pg/L for aqueous samples or in ng/kg for biosolids and tissue samples.
- Report individual values, including results for congeners found in blanks.
- Do not average or perform other data manipulations unless required by the method or study-specific instructions. Report data to three significant figures, rounding or truncating the data only after all calculations have been completed.
- Report data in the electronic format provided to the laboratory by SCC.
- If data are reported in hardcopy form, paginate all data packages.

2.7 Deviations from the Method or Study design

Although Method 1668A explicitly allows use of a five-point calibration for less-sensitive instruments (e.g., VG70) and a six point calibration for more-sensitive instruments (e.g., Micromass Autospec Ultima), laboratories interpreted this option differently. This, and other deviations from the study design are described below. Most of these deviations involved use of smaller sample volumes and/or diluted extracts.

2.7.1 Instrument Calibration

Section 10.4 of Method 1668A states that the relative response (RR) (labeled to native) vs. concentration in the calibration solutions should be determined using a five-point calibration for less-sensitive HRMS instruments and a five- or six-point calibration for more-sensitive instruments. Laboratories used the following calibration approaches in this study:

- Laboratories 7 and 8 used a six-point calibration (CS-0.2 through CS-5).
- Laboratories 2, 10, and 13 used a five-point calibration (CS-1 through CS-5).
- Laboratory 4 used a six-point calibration with a CS-5 standard at 1/4 the concentration given in the method to prevent saturation of their HRMS instrument.
- Laboratory 12 performed two sets of calibrations, a high and a low. This laboratory applied a high calibration range (CS-1 through CS-5), except in cases where a signal was observed below the CS-1 point, in which case it applied a low calibration range (CS-0.2 through CS-4).
- Laboratory 6 did not provide calibration data.

Provided the instruments were calibrated using a consistent injection volume, these differences in the calibrations used by the laboratories had little or no effect on the results of study samples.

2.7.2 Biosolids

Some laboratories submitted results for the analysis of biosolids samples that: used a smaller sample size than suggested in the method, analyzing more dilute extracts than suggested in the method, or both.

- Laboratory 2 used a 15-g (wet weight) sample as opposed to the 30-g sample suggested by the method, resulting in a two-fold dilution.
- Laboratory 12 used a 6-g (wet weight) sample as opposed to the 30-g sample, resulting in a five-fold dilution.
- Laboratory 7 used a 10-g (wet weight) sample as opposed to the 30-g sample and concentrated the extract to a final volume of 100 μL, as opposed to 20 μL, resulting in a 15-fold dilution.
- Laboratory 8 used the full sample size, but concentrated the extract to a final volume of 200 μL as opposed to 20 μL, resulting in a ten-fold dilution.
- Laboratory 13 used a 1-g (wet weight) sample as opposed to the 30-g sample, and concentrated the extract to a final volume of 100 μL as opposed to 20 μL, resulting in a 150-fold dilution. Discussions with this laboratory revealed no attempt to analyze a 30-g sample. Instead, based on past experience with GC/HRMS analyses, the laboratory used a 1-g sample, and concentrated the extract to 100 μL. Their general experience has been that using a 30-g sample results in difficulties during instrumental analysis (lock-mass problems). Based on their GC/HRMS experience, Laboratory 13 also did not use the prescribed sample amounts for the fish tissue and wastewater samples.

Two laboratories (4 and 10) did not submit biosolids data because of difficulties encountered with clean-up and analysis. Both of these laboratories attempted analyses on 30-g samples, as suggested in the method.

Laboratory 4 reported difficulties with the cleanup of both the biosolids samples. In one of the biosolids samples, upon the first acid wash, the sample appeared black in color and the phases could not be distinguished. The laboratory proceeded with the addition of sodium chloride in an attempt to mitigate the problem. During the subsequent acid wash steps (second, third and fourth) no color appeared in the aqueous layer. The extract layer contained suspended particles and had a tar-like appearance and viscosity. The sample was then put through an acid/base silica column before the gel permeation chromatography (GPC) step in hopes that the extract would then not plug the filter used in the GPC. In the case of the second biosolids sample, an emulsion resulted during back-extraction with base (Section 12.5 in the method). The laboratory unsuccessfully attempted to break the emulsion by adding sodium chloride and cooling, and tried diluting the extract with sodium chloride solution and hexane, followed by hexane rinses, and addition of sulfuric acid. The extract was drained into a round bottom flask and concentrated by heating mantle. The sample was then washed with the maximum number (4) of acid washes suggested in the method.

Laboratory 10 reported difficulties with the cleanup and extraction of both biosolids samples and reported that, despite having made two separate attempts to cleanup and extract the biosolids samples, they were not able to obtain reportable results. The samples were initially extracted using approximately 22 grams of each sample (dry weight basis). A total of six cleanup steps were applied to each sample. According to the laboratory narrative, even after these measures, the final extracts contained significant amounts of white crystals. The remaining liquid portions of the extracts were separated from the crystals and injected. These extracts did not yield reportable results. The laboratory attempted to extract the samples a second time, this time using 2 grams each. Two cleanup steps were applied to these samples. No crystals were present in the final extracts; however the laboratory was still unable to obtain reportable results.

Laboratories 7 and 12 reported biosolids results on a wet weight basis whereas laboratories 8 and 13 reported biosolids results on a dry weight basis. The dry weight data for laboratory 8 were corrected to wet weight based on percent solids data provided by laboratory 8 (33.3% solids for Youden 1 and 39.3%

for Youden 2). Because laboratory 13 did not provide percent solids data, the laboratory 13 dry weight data were corrected to wet weight based on the mean of the percent solids data provided by the: sample preparation laboratory, laboratory 2, and laboratory 8. These three laboratories were the only labs that provided percent solids data (33.3% solids for Youden 1 and 35.9% for Youden 2).

The laboratory narratives suggest that many laboratories lacked experience extracting and cleaning up a biosolids matrix. The resulting deviations from the method and study-specific instructions for analysis of biosolids samples by different laboratories resulted in some unusable and inconsistent data. Thus, EPA excluded some biosolids results, as described in Section 3 of this report.

2.7.3 Tissue

Two of the seven laboratories that submitted usable tissue data used a smaller sample size than that suggested in the method, or analyzed a more dilute extract than suggested in the method.

- Laboratory 7 used a 5-g (wet weight) sample as opposed to the 10-g sample suggested in the method, resulting in a two-fold dilution.
- For reasons similar to their deviation in biosolid sample volume (i.e., previous experience with GC/HRMS analyses), Laboratory 13 concentrated the extract to a final volume of 100 μL as opposed to the 20-μL volume suggested in the method, resulting in a 5-fold dilution.

Laboratory 6 did not submit tissue data, and reported difficulties with the analysis of this matrix due to interferences from lipids. The laboratory reported unsuccessful use of an acid-base wash extraction, and two rounds of silica gel cleanup.

2.7.4 Wastewater

One of the eight laboratories that submitted usable wastewater data analyzed a more dilute extract than suggested in the method. Specifically Laboratory 13, for reasons explained previously (GC/HRMS experience), concentrated the extract to a final volume of 100 μL as opposed to the 20-μL volume suggested in the method, resulting in a 5-fold dilution.

Section 3
Data Review and Validation

Three of the 14 volunteer laboratories that were selected to participate in this study failed to submit data despite repeated requests and offers to extend the submission deadlines. In all three cases, the laboratories cited scheduling conflicts as the reason for their inability to complete the study.

Data from the 11 laboratories that submitted results were reviewed and validated by SCC as soon as possible after receipt. Data packages included sample tracking logs, summary results, QC summaries, raw data, sample calculations, laboratory narratives (including descriptions of any problems encountered, corrective actions taken, and comments on method procedures), and electronic data reporting spreadsheets. Data were reviewed against requirements in the study plan and the method to ensure that results from each laboratory were complete (i.e., that all required data were present, including results of all required tests, sample lists, run chronologies, summaries of analytical results, raw data, example questions). This included verification that: all samples were analyzed properly; appropriate spike levels were used; the analytical systems were properly calibrated; results calculation procedures were followed correctly; and that raw data supported the results. A fundamental objective of this review was to maximize data use, and every attempt was made to resolve data discrepancies with laboratories. This review disclosed the following facts:

- Data from Laboratories 3 and 11 failed to meet one or more of the chromatographic resolution requirements in Section 6.9.1.1.2 of Method 1668A. This section specifies that the SPB-Octyl GC column must resolve congener pairs 34/23 and 187/182, and that congener pair 156/157 *must* coelute.

- Laboratory 3 data showed coelutions across several chlorination levels, inability to detect many of the congeners in the low (CS-1) calibration standard, and high baseline noise that made integration difficult. Laboratory 3 also reported loss of sensitivity, column deterioration, and expressed general dissatisfaction with the method. Laboratory 3 reported results for only 1 wastewater sample, 1 blank sample, and no other QC or sample results.

- Laboratory 11 data indicated an inability to recover 25 of the 34 labeled compounds spiked into the biosolids samples without an acknowledgment or explanation of the difficulties, and incomplete raw supporting data. For example, selected ion current profiles for samples in which the laboratory reported very high recoveries of some labeled compounds (e.g., 572%) were not provided. SCC contacted the laboratory repeatedly, but did not receive an explanation.

- Laboratory 2 submitted only summary level sample results, and provided little or no calibration data. During attempts to obtain details and raw supporting data, SCC learned that the laboratory manager was no longer with the company and that the laboratory was closing. Without sufficient information to support the summary level results submitted, it was not possible to investigate potential causes of the observed low recoveries. Sample results for this laboratory were consistently below those for all other laboratories, and the labeled compound recoveries were generally low in both study and QC samples.

- Laboratory 6 did not submit tissue sample results and did not provide OPR results associated with the wastewater samples. In e-mail correspondence, the laboratory indicated that the lighter PCB congeners were lost during final transfer, and therefore, results were not submitted. This laboratory provided some raw data (e.g., selected ion current profiles) for some QC samples, but only summary level data for the results of calibration, calibration verification, and field samples. SCC was unable to obtain additional information or supporting data. The laboratory reported results for 167 peaks containing one or more congeners. This is more than most other laboratories, and more than the 159 peaks described in the method, making a side-by-side comparison with data from other laboratories difficult.

- Mean relative response (RR) and response factor (RF) values reported by Laboratory 14 were reported inconsistently across the laboratory's report forms. For example, page 318 of the laboratory's data package lists the RR for ^{13}C-labeled PCB congener 81 as 2.7956 and page 319 has RR values ranging from 2.01 to 2.28 (with a mean of 2.09). Many of the congeners have only 5 RR values, while many others appear to have 6 RR values. Conversely, for congener 77L, SCC could reproduce the mean RR value of 2.16 reported on page 319, but this value does not match the value of 2.8026 on report Form 3B. Results differ most for the early-eluting labeled congeners. SCC examined the calibration data for these congeners and compared them to calibration data from other laboratories in the study. Although some differences in the responses are expected between different GC/MS instruments, results from Laboratory 14 were inconsistent with results from the other laboratories.

Of the remaining six laboratories:

- Four laboratories (7, 8, 11, and 13) provided usable data for all three of the matrices used in this study, and
- Two laboratories (4 and 10) provided usable data for wastewater and tissue matrices only.

Thus, this validation study using volunteer labs yielded six usable data sets for wastewater and tissue matrices, and four for biosolids. Data obtained from these laboratories followed the requirements of the study plan and the method and included results for the required accompanying QC analyses; i.e., calibration, calibration verification (where submitted), OPR, reagent water blank, and solids/tissue blank (playground sand mixed with corn oil). Table 3-1 summarizes the status of results from the laboratories.

Table 3-1 Summary of Data Received from Participant Laboratories

Laboratory	Submitted data?		
	Wastewater	Tissue	Biosolids
1	No	No	No
2	Yes, but unusable	Yes, but unusable	Yes, but unusable
3	Yes, but unusable	No	No
4	Yes	Yes	No
5	No	No	No
6	Yes, but unusable	No	Yes, but unusable
7	Yes	Yes	Yes
8	Yes	Yes	Yes
9	No	No	No
10	Yes	Yes	No
11	Yes, but unusable	Yes, but unusable	Yes, but unusable
12	Yes	Yes	Yes
13	Yes	Yes	Yes
14	Yes, but unusable	Yes, but unusable	Yes, but unusable
Total usable data packages	**6**	**6**	**4**

Study samples were assessed for outlying results using Grubbs' outlier test, performed in accordance with *Standard Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D-19 on Water (ASTM D2777-98)*. Details on the outlier analyses are presented in Appendix A.

Section 4
Results and Discussion

4.1 Background and Homogeneity testing

As described in Section 2.3 of this report, the sample processing laboratory was required to perform background analyses of the wastewater matrix, and homogeneity analyses of the tissue and biosolids matrices.

4.1.1 Wastewater Sample Homogeneity

Wastewater samples were prepared: by determining the background concentration to select appropriate spike levels, by spiking and aliquoting the samples as described in Section 2.3, and analyzing two random aliquots for homogeneity verification. Relative percent differences (RPDs) for all congener concentrations between wastewater homogeneity test aliquots B1 and B2 were less than 16%, and all but five were less than 10%, confirming the adequacy of the homogenization and aliquoting process.

4.1.2 Tissue and Biosolids Sample Homogeneity

For tissue and biosolids, the sample processing laboratory was instructed to analyze one 10-g dry weight aliquot of sample "A" as the background analysis, and two 10-g dry weight aliquots of sample "B" as the homogeneity aliquots for both the biosolids and tissue matrices. Because of the mixing scheme for the tissue matrix it was assumed, if the homogeneity for sample "B" was found acceptable, that the homogeneity of sample "A" would be acceptable. This approach was used to preserve mass of sample for the study itself by taking the two homogeneity aliquots from the larger aliquot (sample "B"). Relative percent differences (RPDs) between tissue homogeneity test aliquots B1 and B2 were calculated to verify the homogenization and aliquoting scheme. All but five RPD values were $\leq$20%; the remaining five were associated with sample concentrations below the sample-specific ML, where greater uncertainty is expected.

4.2 Congener Concentrations in Samples

The frequency of detection and the mean, median, and maximum concentrations of the congeners found in tissue, wastewater, and biosolids samples by level of chlorination (LOC) are in Table 4-1. The total number of congeners analyzed reflects the total number of congeners or coeluted congener groups analyzed by all labs in both samples for the given chlorination level. For example, 12 congeners were analyzed in water for LOC 10. This equates to one congener reported by six labs in two samples (12 = 1 x 6 x 2). Although the same six labs provided usable data for tissue and water, there are differences between these matrices in the number of congeners analyzed for a given LOC. For example for LOC 4, a total of 356 tetrachlorinated congeners (or co-eluting congeners) were analyzed in the wastewater Youden pairs, but only 352 tetrachlorinated congeners were analyzed in the in tissue Youden pair. The difference is attributable to the removal of outliers. The next two columns in the table provide information on the number of detected congeners in each LOC, and the percentage of analyzed congeners that were detected. Finally, the mean, median, and maximum concentrations in each LOC represent all congeners within that level; when coelutions of two or more congeners occurred, the combined value of those co-eluted congeners was used.

In wastewater samples, all congeners at LOCs 4 and higher were detected by all laboratories. Only LOC 1 had a rate of detection below 90%. The rate of congener detection across laboratories was generally consistent for the different LOCs in biosolids and tissue samples, ranging between 69% and 100% for tissue and between 70% and 100% for biosolids. With the exception of LOCs 9 and 10 (which include only congeners 3 and 1, respectively), at least one congener was not detected in the solids matrices by at least one laboratory for each LOC. The reason that all laboratories do not detect the same congeners in each sample is likely due to differences in coelutions and because some laboratories concentrated extracts to 100 or 50 μL instead of 20 μL as required by EPA Method 1668A. Those laboratories that did not concentrate extracts to 20 μL would not measure to as low a level as laboratories that did, and low concentration congeners would, therefore, not be detected by these laboratories.

Table 4-1 Congener Detection Rates and Concentrations in Study Samples (by Matrix and Level of Chlorination)

Matrix	LOC	# Labs	# Congeners Analyzed	# Congeners Detected	% Congeners Detected	Concentration (Detects Only)[1,2]		
						Mean	Median	Maximum
Biosolids	1	4	24	23	96	142	159	281
	2		88	64	73	494	265	2780
	3		160	134	84	972	482	7130
	4		240	195	81	1270	372	12400
	5		237	166	70	2070	742	13400
	6		254	196	77	1120	407	12300
	7		169	129	76	665	344	4810
	8		81	72	89	377	259	1750
	9		24	23	96	280	230	821
	10		8	8	100	313	299	493
Tissue	1	6	36	26	72	4	3	12
	2		131	90	69	47	27	188
	3		232	181	78	267	150	1610
	4		352	288	82	402	130	3330
	5		347	258	74	418	128	15700
	6		362	270	75	429	108	10700
	7		240	182	76	276	120	3560
	8		114	105	92	157	108	709
	9		35	35	100	162	137	390
	10		12	12	100	200	201	236
Water	1	6	36	25	69	27	20	106
	2		128	118	92	533	505	1460
	3		233	223	96	1100	946	3430
	4		356	356	100	2850	2170	15300
	5		344	344	100	2660	1750	21800
	6		362	362	100	2190	1660	11800
	7		235	235	100	1750	1420	7370
	8		116	116	100	241-	1740	9560
	9		35	35	100	1760	1520	3350
	10		12	12	100	1740	1510	3170

[1]Biosolids and tissue concentration in ng/kg (pg/g); water concentration in pg/L
[2]Concentrations in each congener level represent all congeners within that level, and when coelutions of two or more congeners occurred, the combined value of those co-eluted congeners was used.

4.3 Congener Concentrations in Blanks

Table 4-2 gives mean, medium, and maximum congener concentrations found in the water and sand/corn oil blanks, by level of chlorination. PCBs can be ubiquitous in the laboratory environment. Congener detection rates in blank samples ranged from 8-33%, with most of the detected congeners being reported at very low concentrations relative to the concentrations reported in samples. The relatively low frequency of detection of congeners in blanks by all laboratories is thought to be attributable to the failure by some laboratories to concentrate extracts to 20 μL and to lesser PCB backgrounds in some laboratories.

Table 4-2 Congener Detection Rates Concentrations in Blanks (by Matrix and Level of Chlorination)

Matrix	LOC	# Labs	# Congeners Analyzed	# Congeners Detected	% Congeners Detected	Concentration (Detects Only)[1,2]		
						Mean	Median	Maximum
Sand/oil	1	10[2]	50	14	28	4.0	3.5	9.6
	2		130	20	15	5.3	5.1	12.9
	3		227	61	27	2.5	1.4	12.3
	4		328	77	23	4.4	2.0	29.0
	5		370	70	19	6.7	3.3	37.7
	6		355	100	28	5.7	0.4	60.8
	7		245	65	27	3.3	0.6	20.0
	8		122	30	25	1.3	0.2	5.6
	9		50	4	8	2.0	2.1	3.0
	10		20	3	15	0.7	0.1	1.9
Water	1	6	30	10	33	25.8	15.1	82.1
	2		79	9	11	34.8	21.3	113
	3		135	33	24	17.3	11.0	57.7
	4		197	43	22	79.5	10.0	2280
	5		220	29	13	23.9	20.2	74.2
	6		213	52	24	12.2	2.2	85.0
	7		146	35	24	6.7	2.5	39.2
	8		74	15	20	9.4	9.9	28.4
	9		30	3	10	25.4	32.0	33.2
	10		12	2	17	13.2	13.2	26.1

[1]Sand/oil concentration in ng/kg (pg/g); water concentration in pg/L
[2]Six labs provided usable data for sand/oil blanks. Four of the six labs (Labs 7, 8, 10, and 13) analyzed two sand/oil blanks, yielding a total of ten sand/oil blanks.

4.4 Wastewater Sample Recovery and Precision

Table 4-3 summarizes the laboratories' ability to recover congeners from the wastewater samples, presenting the recovery and precision of congener determination by level of chlorination.

The mean and median recoveries of nearly all congeners were in the 60 - 110 percent range, typical for recovery of organic compounds extracted from wastewater. Excluding data at LOCs 1 and 2, the median recovery across all congeners and all labs is approximately 75%, and the median RSD is approximately 10%. Low recoveries at LOCs 1 and 2 (Table 4-3) may be due to loss during transport from the sample preparation laboratory to the participant laboratories. (Data reported by the sample prep laboratory indicate that the congeners were present immediately after shipment.) The exact reason for this loss is not known. Possible causes may be loss by evaporation into the headspace of the sample container during shipment, with subsequent release to the atmosphere when the container is opened, or to biological or other degradation during transit, although selective degradation of congeners at LOCs 1 and 2 only would be unusual. In Figure 4-1, low recoveries for congeners in the 750 - 900 pg/L range are, almost exclusively, attributable to these partial losses of the mono- and dichloro- congeners.

Table 4-3 Wastewater Sample Recovery and Precision by Level of Chlorination										
	Percent Recovery (%)					Within-pair Relative Standard Deviations (%)				
LOC	# Results	Mean	Median	Min.	Max.	# Pairs	Pooled*	Median	Min.	Max.
1	25	3.15	2.71	0.49	11.8	11	29.7	17.5	5.80	80.8
2	118	54.2	44.6	2.63	162	57	12.2	4.42	0.17	62.4
3	223	89.5	82.8	34.2	164	111	7.62	5.06	0.02	24.0
4	356	95.6	91.4	38.1	201	178	7.36	6.16	0.00	20.6
5	344	81.4	72.2	30.6	182	170	10.8	7.99	0.06	40.5
6	362	75.3	68.8	8.14	196	178	12.1	8.64	0.16	46.8
7	235	72.3	64.4	10.4	155	114	9.63	5.24	0.14	39.3
8	116	68.0	59.3	18.1	135	56	11.3	7.26	0.12	32.3
9	35	70.8	57.8	44.4	126	17	8.91	6.66	0.57	18.5
10	12	70.0	59.1	49.3	118	6	5.67	4.23	0.37	9.05

* Pooled RSD calculated as the square root of the mean of the squared within-pair RSDs

Recovery as a function of concentration is plotted in Figure 4-1. (Plots of absolute and relative precision as a function of concentration are addressed with precision for biosolids and tissue samples in Section 4.5.) The spike concentrations displayed in Figure 4-1 do not match exactly the concentrations that were spiked (see Table 2-2) because coelutions result in combined concentrations.

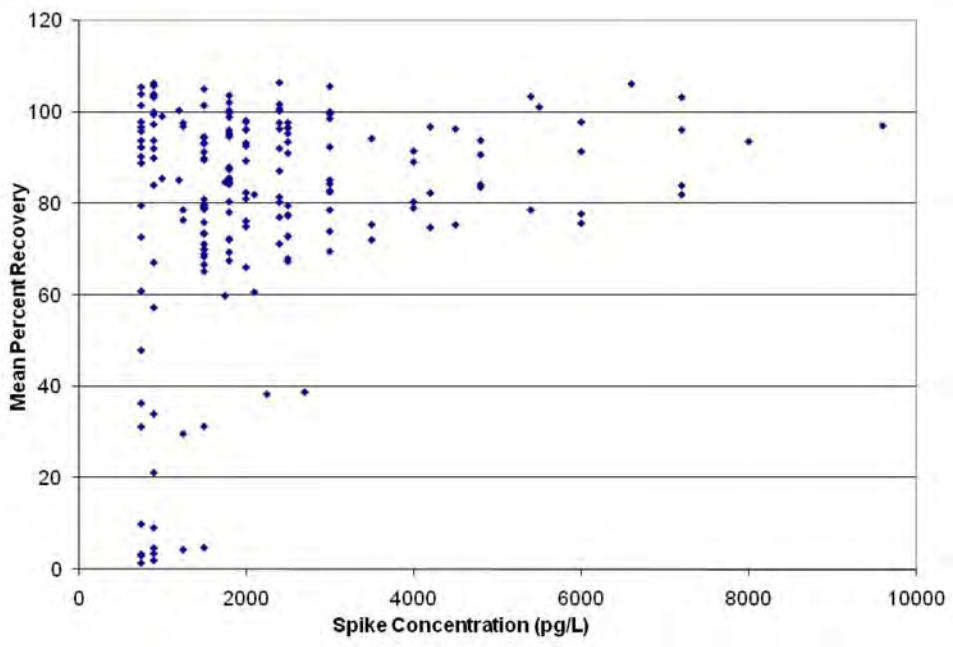


Figure 4-1 Mean Recovery vs. Spike Concentration, PCB Congeners in Wastewater

4.5 Variability as a Function of Concentration

Because true congener concentrations in the tissue and biosolids samples were not known, it was not possible to calculate recoveries of congeners from tissue and biosolids. Variability (precision) vs. concentration was determined for wastewater, soil, and, tissue matrices. The following subsections present plots of absolute precision (as standard deviation of the determined concentrations) and relative precision (as relative standard deviation) as functions of congener concentration for each of these matrices. For the three matrices, standard deviation increased approximately linearly with increasing concentration. It was expected that, at the very low concentrations in the tissue and biosolids samples, standard deviation would become constant and the plots would resemble a "hockey stick." (The wastewater sample was not spiked at low enough concentrations to demonstrate this effect.) The lack of a hockey stick appearance for the tissue and biosolids; i.e., the lack of constant standard deviation at low

concentrations, is good because this indicates that measurements are being made in the quantitative range for the congeners. This is not surprising because the rigorous congener identification criteria in Method 1668A are that the signal-to-noise ratio must be greater than 3 and ratio of the peak heights or areas for the 2 exact m/zs must be within 15% of theoretical, in addition to the requirement that the relative retention time of the congener must be within a specified window based on a calibration or calibration verification standard. Thus, the identification criteria raise the lowest level of congeners that are determined to levels above the region of constant standard deviation.

4.5.1 Variability vs. Concentration for Wastewater

4.5.1.1 Absolute variability vs. concentration for wastewater

Figure 4-2 is a plot of the standard deviation as a function of concentration for the congeners spiked into wastewater. The congener concentrations are defined by the spiking solutions, as described in Section 2.5.2. Results appear slightly skewed to lower standard deviation at low concentration. The skewed appearance is likely due to the higher concentrations of the coeluted congeners.

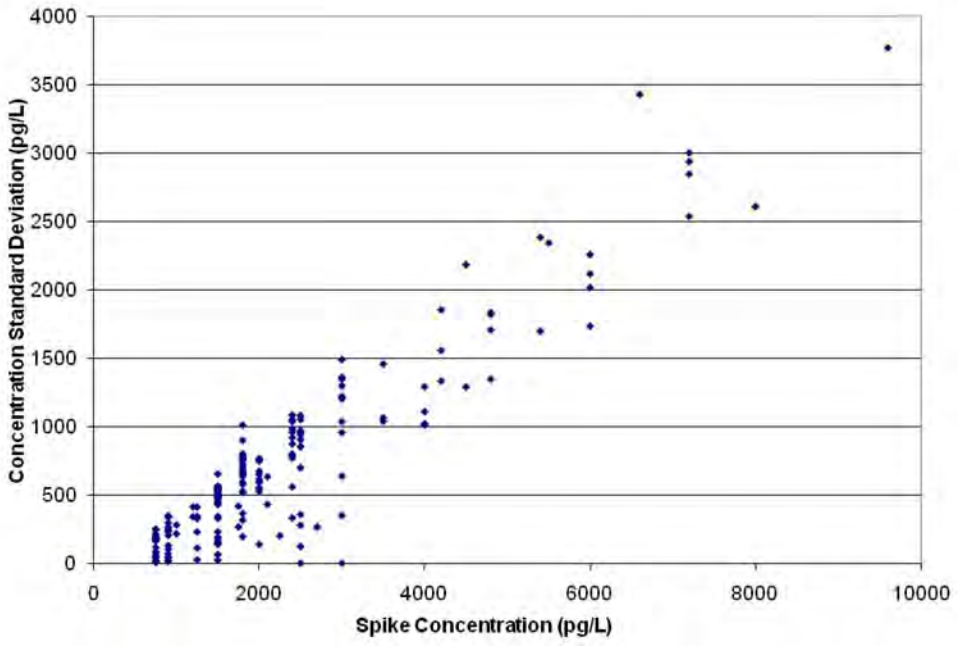


Figure 4-2 Concentration Standard Deviation vs. Spike Concentration, PCB Congeners in Wastewater

4.5.1.2 Relative variability vs. concentration for wastewater

Figure 4-3 is a plot of RSDs as a function of concentration for the congeners spiked into wastewater. The congener concentrations are defined by the spiking solutions, as described in Section 2.5.2. The variability is somewhat higher than expected at the higher concentrations, with RSDs of approximately 40%. The reason for these higher than expected RSDs is not known.

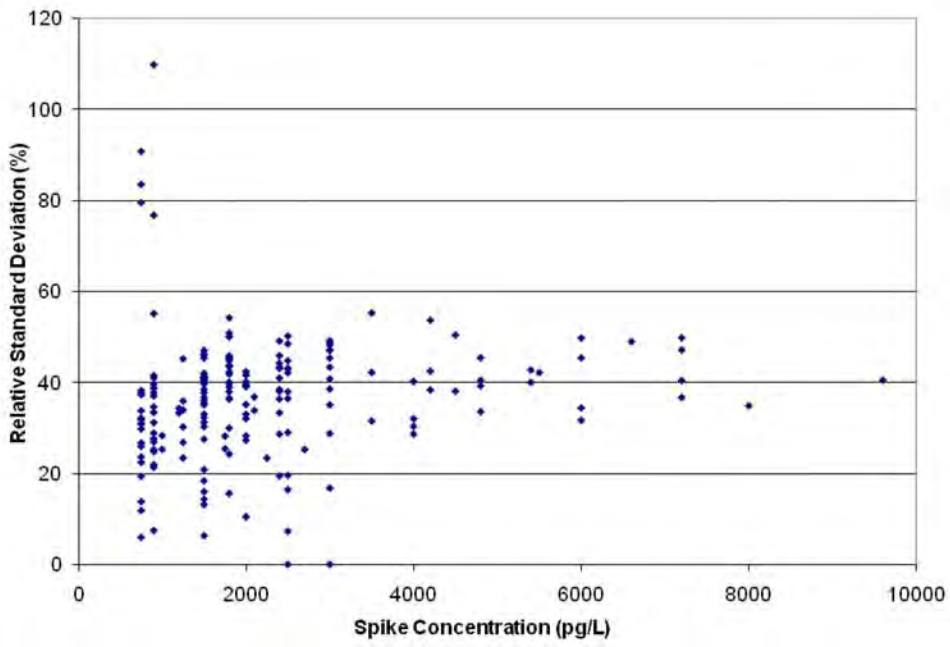


Figure 4-3 Relative Standard Deviation vs. Spike Concentration, PCB Congeners in Wastewater

4.5.2 Variability vs. Concentration for Tissue

4.5.2.1 Absolute variability vs. concentration for tissue

Figure 4-4 is a plot of standard deviation as a function of concentration for congeners detected in tissue. Congeners were detected in tissue from as low as a few parts-per-trillion (ppt; pg/g) to well into the part-per-billion (ppb; ng/g) range.

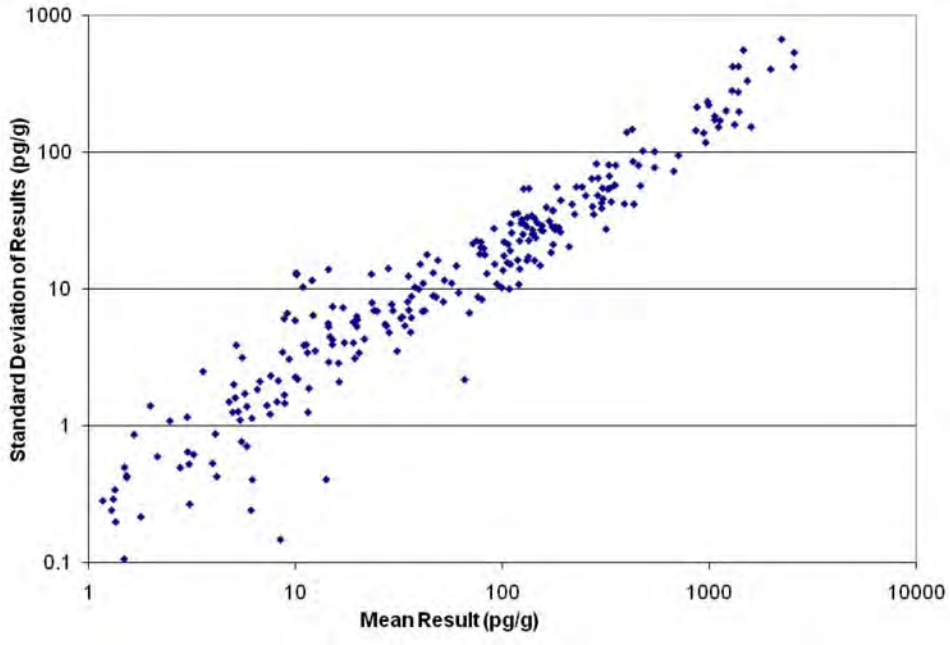


Figure 4-4 Mean vs. Standard Deviation of Measured Tissue Results

4.5.2.2 Relative Variability vs. Concentration for Tissue

Figure 4-5 is a plot of RSD as a function of concentration for the congeners detected in tissue. RSDs are mostly between 10 and 30 percent, as expected, with a few outlying high values. The unusually high RSDs occurred in congeners that are only rarely detected (2-3 laboratories.)

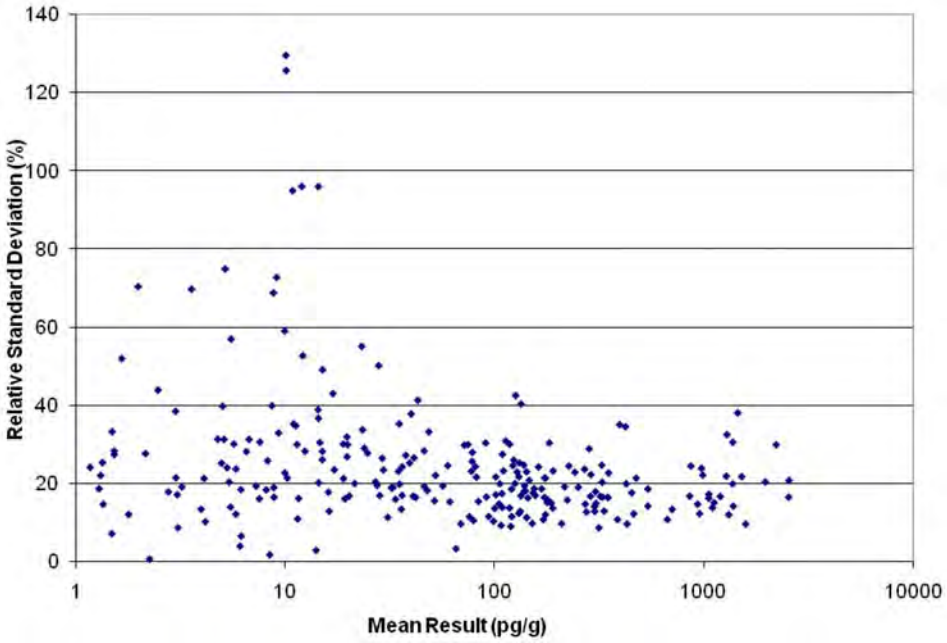


Figure 4-5 Mean vs. Relative Standard Deviation of Measured Tissue Results

4.5.3 Variability vs. concentration for biosolids

4.5.3.1 Absolute Variability vs. Concentration for Biosolids

Figure 4-6 is a plot of standard deviation as a function of concentration for congeners detected in biosolids. Congeners were detected in biosolids from as low as a few ppt to well into the ppb range.

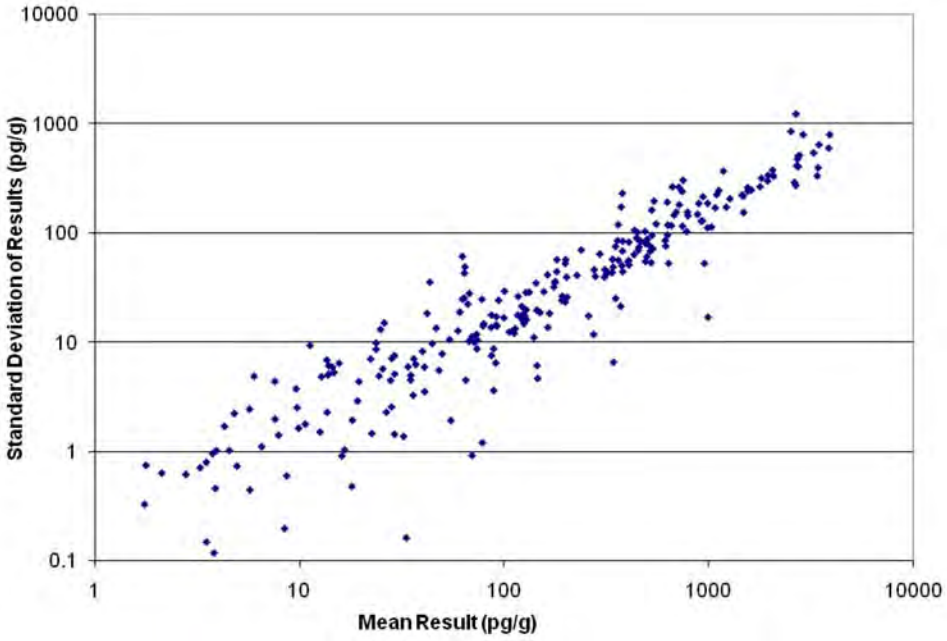


Figure 4-6 Mean vs. Standard Deviation of Measured Biosolids Results

4.5.3.2 Relative Variability vs. Concentration for Biosolids

Figure 4-7 is a plot of RSDs as a function of concentration for the congeners detected in biosolids. Unlike the plots of relative variability for tissue and wastewater samples, this plot does not suggest a strong relationship between variability and concentration. RSDs are mostly between the expected ranges of 10 to 30 percent, with a few outlying high values.

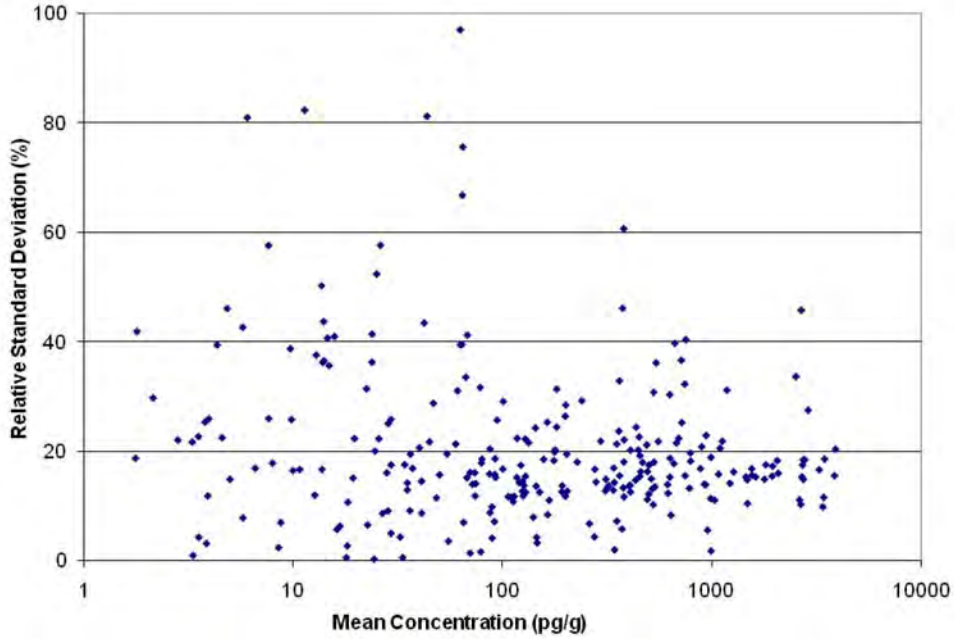


Figure 4-7 Mean vs. Relative Standard Deviation of Measured Biosolids Results

4.6 Labeled Compound Recovery and Precision

Table 4-4 lists labeled compound recovery and precision across all three matrices as a function of chlorination level. Except for LOC 1, median recoveries ranged from 62 to 85%, and pooled RSDs ranged from 8 to 20%.

Table 4-4 Interlaboratory Recovery and Precision of Labeled Samples, by Level of Chlorination

LOC	Percent Recovery (%)					Within-pair Relative Standard Deviations (%)				
	# Results	Mean	Median	Min.	Max.	# pairs	Pooled*	Median	Min.	Max.
1	64	56.6	57.0	13.0	107	32	20.5	20.5	0.81	47.6
2	64	62.8	62.3	25.0	120	32	15.3	15.3	0	31.6
3	96	71.7	71.8	4.48	118	48	11.3	11.3	0.52	33.3
4	93	69.8	65.0	25.5	129	45	9.48	9.48	0.87	22.0
5	220	82.3	85.1	41.0	123	108	10.5	10.5	0	28.7
6	131	81.3	77.5	30.5	216	65	12.2	12.2	0	37.5
7	94	83.2	85.4	5.00	136	46	20.3	20.3	0	126
8	63	83.6	84.9	38.4	145	31	8.54	8.54	0.25	20.0
9	64	80.6	84.3	49.0	129	32	8.45	8.45	0	19.8
10	32	78.7	79.8	47.7	110	16	9.16	9.16	2.23	18.9

* Pooled RSD calculated as the square root of the mean of the squared within-pair RSDs

Section 5
Revision of Quality Control Acceptance Criteria

Interlaboratory quality control (QC) acceptance criteria were developed for initial precision and recovery (IPR), on-going recovery (OPR; laboratory control sample, LCS), and for recovery of labeled compounds from samples. These revised criteria are in Table 5-1 of this report, and Table 6 of the successor method, 1668B. The statistical details for development of these criteria are in an appendix to this report. The tests to which these criteria are applied are discussed in this Section of this report.

5.1 Calibration

The study plan and study-specific instructions suggested a 5- or 6- point calibration. Laboratories did not provide enough calibration data to permit revision of the QC acceptance criterion for calibration linearity. Therefore, the criterion for which an average relative response may be used for a given congener remains at 20%, as stated in Section 10.4.4 of EPA Method 1668A; otherwise, a calibration curve must be used for that congener. This calibration linearity criterion applies to congeners determined by isotope dilution only (i.e., the "toxics," "level of chlorination," and "GC window-defining" congeners) because all other congeners are calibrated at a single point.

5.2 Calibration Verification

The study plan and study-specific instructions suggest a single calibration verification after calibration. Because only two laboratories submitted calibration verification data, EPA did not revise the calibration verification QC acceptance criteria. The calibration verification QC acceptance criteria in Table 5-1 remain unchanged from previous revisions of EPA Method 1668A. If EPA receives calibration verification data from enough laboratories, EPA may revise these criteria in future versions of 1668.

5.3 Initial Precision and Recovery

To minimize resource burden on volunteer participants, laboratories were not required to prepare and analyze IPR samples. Instead, EPA used the OPR data gathered in the study to develop revised IPR and OPR QC acceptance criteria. In addition, results from the aqueous and solids (sand/corn oil) OPRs were combined to yield a single set of OPR QC acceptance criteria that would be applicable to aqueous, solids, and tissue samples. Two laboratories resolved labeled and native congeners 156 and 157, while the other laboratories reported these congeners as coeluting pairs. Similarly, one laboratory reported coelution of congeners 4 and 10, one laboratory reported coelution of congeners 114 and 122, and two laboratories reported congener 106 coeluting with either congener 107 or 109. Because calculations of IPR/OPR QC acceptance criteria were based on recoveries, coelution was ignored when generating the revised criteria.

Data from Laboratories 2, 3, 6, 11, and 14 were excluded for the reasons described in Section 3 of this report. The remaining dataset yielded a total of 15 usable reagent water and solid matrix OPR samples. After performing Grubbs' outlier tests on these OPRs, a total of 13 individual data points were identified as outliers and removed from the dataset prior to development of revised IPR/OPR QC acceptance criteria. Table 5-1 presents revised IPR QC acceptance criteria. When compared to QC acceptance criteria in Method 1668A, recoveries windows are generally narrower than those in the method. Recoveries for low molecular weight congeners are centered lower than for the other congeners and for recoveries of low molecular weight congeners in Method 1668A. These lower recovery windows reflect that these congeners are partially lost in the solvent evaporation step(s).

Table 5-1. Revised QC Acceptance Criteria for IPR, OPR, and Labeled Compounds in Samples						
Congener	Congener number[1]	Test conc. (ng/mL)[2]	IPR		OPR Recovery (%)[3]	Labeled Compound Recovery in Samples and Blanks (%)[3]
			RSD (%)	Recovery (%)[3]		
2-MoCB	1	50	25	84 – 119	71 – 132	NA
4-MoCB	3	50	22	83 – 112	72 – 123	
2,2'-DiCB	4	50	18	82 – 105	73 – 114	
4,4'-DiCB	15	50	17	85 – 107	76 – 116	
2,2'6-TrCB	19	50	13	86 – 103	79 – 109	
3,4,4'-TrCB	37	50	26	77 – 109	64 – 122	
2,2'6,6'TeCB	54	50	17	84 – 106	76 – 114	
3,3',4,4'-TeCB	77	50	20	81 – 106	71 – 116	
3,4,4',5-TeCB	81	50	20	81 – 106	70 – 116	
2,2',4,6,6'-PeCB	104	50	19	83 – 107	74 – 117	
2,3,3',4,4'-PeCB	105	50	19	83 – 107	73 – 117	
2,3,4,4',5-PeCB	114	50	18	83 – 105	74 – 113	
2,3',4,4',5-PeCB	118	50	13	88 – 105	81 – 112	
2',3,4,4',5-PeCB	123	50	16	82 – 102	74 – 109	
3,3',4,4',5-PeCB	126	50	17	82 – 104	74 – 113	
2,2',4,4',6,6'-HxCB	155	50	15	86 – 105	79 – 112	
2,3,3',4,4',5-HxCB 4	156	50	16	87 – 108	78 – 117	
2,3,3',4,4',5'-HxCB 4	157	50	16	87 – 108	78 – 117	
2,3',4,4',5,5'-HxCB	167	50	13	85 – 101	79 – 107	
3,3',4,4',5,5'-HxCB	169	50	16	80 – 100	73 – 108	
2,2',3,4',5,6,6'-HpCB	188	50	14	88 – 106	81 – 113	
2,3,3',4,4',5,5'-HpCB	189	50	16	85 – 106	77 – 114	
2,2',3,3',5,5',6,6'-OcCB	202	50	17	82 – 104	74 – 112	
2,3,3',4,4',5,5',6-OcCB	205	50	15	87 – 107	79 – 115	
2,2',3,3',4,4',5,5',6-NoCB	206	50	17	85 – 106	76 – 115	
2,2',3,3,'4,5,5',6,6'-NoCB	208	50	17	86 – 108	77 – 116	
DeCB	209	50	20	81 – 106	71 – 116	
Labeled Compounds						
$^{13}C_{12}$-2-MoCB	1L	100	78	21 – 100	2 – 100	4 – 100
$^{13}C_{12}$-4-MoCB	3L	100	63	31 – 100	13 – 100	11 – 106

Table 5-1. Revised QC Acceptance Criteria for IPR, OPR, and Labeled Compounds in Samples						
Congener	Congener number[1]	Test conc. (ng/mL)[2]	IPR		OPR Recovery (%)[3]	Labeled Compound Recovery in Samples and Blanks (%)[3]
			RSD (%)	Recovery (%)[3]		
$^{13}C_{12}$-2,2'-DiCB	4L	100	56	35 – 100	18 - 100	14 – 107
$^{13}C_{12}$-4,4'-DiCB	15L	100	70	34 – 100	10 – 118	19 – 107
$^{13}C_{12}$-2,2',6-TrCB	19L	100	68	32 – 100	10 – 106	1 – 108
$^{13}C_{12}$-3,4,4'-TrCB	37L	100	57	47 – 104	24 – 128	25 – 123
$^{13}C_{12}$-2,2',6,6'-TeCB	54L	100	62	37 – 100	16 – 111	13 – 105
$^{13}C_{12}$-3,3',4,4'-TeCB	77L	100	35	57 – 100	43 – 105	31 – 109
$^{13}C_{12}$-3,4,4',5-TeCB	81L	100	33	57 – 100	44 – 102	14 – 127
$^{13}C_{12}$-2,2',4,6,6'-PeCB	104L	100	48	49 – 100	30 – 115	36 – 115
$^{13}C_{12}$-2,3,3',4,4'-PeCB	105L	100	31	66 – 101	52 – 116	50 – 111
$^{13}C_{12}$-2,3,4,4',5-PeCB	114L	100	41	57 – 100	39 – 117	41 – 121
$^{13}C_{12}$-2,3',4,4',5-PeCB	118L	100	33	65 – 102	51 – 117	49 – 111
$^{13}C_{12}$-2',3,4,4',5-PeCB	123L	100	32	66 – 103	52 – 118	49 – 116
$^{13}C_{12}$-3,3',4,4',5-PeCB	126L	100	29	67 – 100	54 – 113	50 – 106
$^{13}C_{12}$-2,2',4,4',6,6'-HxCB	155L	100	42	58 – 103	40 – 121	25 – 124
$^{13}C_{12}$-2,3,3',4,4',5 –HxCB5	156L	100	35	61 – 100	46 – 115	40 – 120
^{13}C--2,3,3',4,4',5'-HxCB5	157L	200	35	61 – 100	46 – 115	40 – 120
$^{13}C_{12}$-2,3',4,4',5,5'-HxCB	167L	100	24	74 – 103	63 – 115	45 – 118
^{13}C--3,3',4,4',5,5'-HxCB	169L	100	33	66 – 103	51 – 117	37 – 117
$^{13}C_{12}$-2,2',3,4',5,6,6'-HpCB	188L	100	47	53 – 102	33 – 121	23 – 125
$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB	189L	100	28	68 – 100	55 – 112	47 – 116
$^{13}C_{12}$-2,2',3,3',5,5',6,6'-OcCB	202L	100	50	56 – 113	33 – 136	31 – 134
$^{13}C_{12}$-2,3,3',4,4',5,5',6-OcCB	205L	100	21	70 – 100	61 – 103	46 – 115
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6-NoCB	206L	100	29	64 – 100	51 – 107	38 – 122
$^{13}C_{12}$-2,2',3,3',4,5,5',6,6'-NoCB	208L	100	32	62 – 100	48 – 111	31 – 126
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6,6'-DeCB	209L	100	30	65 – 100	52 – 111	43 – 115

Table 5-1. Revised QC Acceptance Criteria for IPR, OPR, and Labeled Compounds in Samples						
Congener	**Congener number[1]**	**Test conc. (ng/mL)[2]**	**IPR**		**OPR Recovery (%)[3]**	**Labeled Compound Recovery in Samples and Blanks (%)[3]**
			RSD (%)	**Recovery (%)[3]**		
Cleanup standards						
$^{13}C_{12}$-2,4,4'-TrCB	28L	100	63	43 – 106	18 – 131	14 – 131
$^{13}C_{12}$-2,3,3',5,5'-PeCB	111L	100	23	75 - 102	64 – 113	57 – 112
$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB	178L	100	30	78 - 117	62 – 133	57 – 125

1 Suffix “L” indicates labeled compound.
2 See Table 5 in EPA Method 1668A.
3 Where necessary, the limit was increased to include 100% recovery.
4 PCBs 156 and 157 are tested as the sum of the two concentrations.

QC acceptance criteria for IPR precision, as relative standard deviation (RSD) of recoveries, are also presented in Table 5-1. The RSDs generally are higher than those in Method 1668A for some of the low molecular weight congeners, and lower for some of the other congeners. The higher RSDs for the low molecular weight congeners reflect partial loss of these congeners in the solvent evaporation step(s) in Method 1668A, resulting in greater variability in results for these congeners.

5.4 Ongoing Precision and Recovery

Each participating laboratory was required to spike and analyze two reagent water OPR samples. These samples were used to evaluate laboratory and method performance and to update IPR and OPR QC acceptance criteria. Although not required by the study design, four laboratories analyzed at least one solids matrix OPR sample. In some cases, laboratories provided one solids matrix OPR and one reagent water OPR instead of two reagent water OPRs. In other cases, laboratories supplemented the two reagent water OPRs with one or more solids matrix OPRs.

Revised OPR QC acceptance criteria are in Table 5-1. As with the IPR QC acceptance criteria, OPR recovery windows are, generally, narrower than those in Method 1668A, and centered lower for some of the low molecular weight congeners.

5.5 Labeled Compound Recovery from Samples, Blanks, and IPR and OPR standards

Labeled compound recovery data from samples were used to construct revised QC acceptance criteria for labeled compound recoveries. Results from a total of 24 analyses were used to develop the labeled compound recovery QC acceptance criteria (Table 5-1.) The IPR and OPR recovery windows are centered lower, for the low molecular weight congeners.

Section 6
Conclusions

This study demonstrated that PCB congeners can be measured in water, biosolids, and tissue in multiple laboratories using EPA Method 1668A. Results show that recovery is nearly constant as a function of concentration, and that precision is proportional to concentration. Of significance with this method is the benefit that measured concentrations are corrected by the isotope dilution technique, even when the recovery of the labeled compounds is low.

The results of this interlab study met our objectives to characterize the performance of Method 1668A in several laboratories and matrices, and use the results to replace the single-lab QC acceptance criteria in 1668A with interlab criteria. These new interlab QC criteria are in Table 6 of the successor EPA method, 1668B.

Appendix
Statistical Procedures Used to Develop QC Acceptance Criteria

1.0 Initial Precision and Recovery (IPR) and Ongoing Precision and Recovery (OPR)

IPR and OPR QC acceptance criteria were calculated using OPR results for all matrix types for each given congener. The acceptance criteria were calculated as prediction limits for mean and individual recoveries, set at the 95% confidence level.

Prior to calculation of QC acceptance criteria, Grubbs' outlier test, as described in ASTM E178-02, was first run on the individual OPR sample recoveries. Based on Grubbs' test, a single outlying recovery was removed for 13 of the native or labeled congeners. These results were not included in the subsequent IPR and OPR QC acceptance criteria calculations.

Upper and lower limits for IPR samples were calculated as:

$$\overline{X} \pm t_{(0.975,n-1)} * s * \sqrt{\frac{1}{4} + \frac{1}{n}}$$

where: $\overline{X}$ *is the overall mean of all OPR recoveries for the given congener,*
s is the standard deviation of all OPR recoveries for the given congener, and
n is the number of OPR recoveries for the given congener.

Upper and lower limits for OPR samples were calculated as:

$$\overline{X} \pm t_{(0.975,n-1)} * s * \sqrt{1 + \frac{1}{n}}$$

The maximum RSD for IPR samples was calculated as:

$$\frac{s}{\overline{X}} * \sqrt{F_{(0.95,n-1)}} * 100\%$$

2.0 Labeled Compound Recovery from Samples and Blanks

QC acceptance criteria for the recovery of labeled compound from samples and blanks were calculated using all labeled sample results for all matrix types for the given congener. The acceptance criteria were calculated as prediction limits for mean and individual recoveries, set at the 95% confidence level.

Prior to calculation of QC acceptance criteria, the Grubbs' outlier test, described in ASTM E178-02, was first run on the individual labeled sample recoveries. Based on Grubbs' test, two outlying recoveries were each removed for two of the native or labeled congeners. These results were not included in the subsequent labeled sample recovery QC acceptance criteria calculations.
Upper and lower limits for IPR samples were calculated as:

$$\overline{X} \pm t_{(0.975,n-1)} * s * \sqrt{1 + \frac{1}{n}}$$

where: $\overline{X}$ *is the overall mean of all labeled sample recoveries for the given congener,*
s is the standard deviation of all labeled sample recoveries for the given congener, and
n is the number of labeled sample recoveries for the given congener.

Appendix

Study Plan for Interlaboratory Validation of EPA Method 1668A for Determination of Chlorinated Biphenyl Congeners in Water, Biosolids, and Tissue by HRGC/HRMS

Prepared for:

William A. Telliard, Director of Analytical Methods
Engineering and Analysis Division (4303T)
Office of Science and Technology
Office of Water
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue NW
Washington, DC 20460

Prepared by:

DynCorp Systems & Solutions LLC
6101 Stevenson Avenue
Alexandria, VA 22304

May 2003

Acknowledgments

This study plan was prepared under the direction of William A. Telliard of the Engineering and Analysis Division within the U.S. Environmental Protection Agency (EPA) Office of Water.

Disclaimer

This study plan has been reviewed and approved by EPA's Engineering and Analysis Division. Mention of company names, trade names, or commercial products does not constitute endorsement or recommendation for use.

1.0 INTRODUCTION

This study plan is for interlaboratory validation of EPA Method 1668, Revision A: *Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS* ("Method 1668A"). Method 1668A is for determination of the 12 polychlorinated biphenyl (PCB) congeners designated as "toxic" by the World Health Organization (WHO), and the remaining 197 chlorinated biphenyl (CB) congeners, either as individual congeners or as congener groups.[4]

2.0 BACKGROUND

From the 1940s into the early 1980s, PCBs were manufactured under several trade names, most predominantly "Aroclor" in the U.S. The Aroclor name was accompanied by a four-digit number indicating the degree of chlorination of the commercial mixture (e.g., Aroclor 1016, Aroclor 1260, etc.). In general, the higher the number, the higher the degree of chlorination.

From the late 1950s through the 1970s, PCBs were determined as Aroclors by low resolution (packed column) GC with an electron capture detector (ECD). In the late 1970s and early 1980s, heightened interest in PCBs and ambiguities in PCB identification led several researchers to separate and identify all 209 PCB congeners using high resolution (open tubular capillary) GC columns coupled with low resolution mass spectrometry (LRMS). In the early to mid-1990s, researchers began to investigate use of high resolution mass spectrometry (HRMS) more intensely as a means to reduce or eliminate interferences that compromise measurement of PCBs by ECD or LRMS.

In 1995, EPA developed Method 1668, which uses high resolution GC (HRGC) combined with HRMS for determination of 13 dioxin-like PCBs that the World Health Organization (WHO) designated as "toxic" in 1994. Method 1668 was based on data from studies conducted at Pacific Analytical, Inc., Carlsbad, CA. In 1997, interest in additional congeners led EPA to investigate determination of as many congeners as possible in a single HRGC/HRMS run. This led to draft Revision A of EPA Method 1668. At about the same time that Method 1668A was drafted, WHO modified the list of dioxin-like congeners by adding congener 81 and deleting congeners 170 and 180, resulting in the current list of 12 PCBs that exhibit “dioxin-like” toxicity.

Method 1668A was validated in a single-laboratory study at Axys Analytical Services Ltd., Sidney, BC, Canada. Axys Analytical produced a report that was subsequently published in March, 2000, in two parts titled: *Development of a Full-Congener Version of EPA Method 1668 and Application to Determination of 209 CB Congeners in Aroclors* (Part I) and *Development of Method 1668A* (Part II).

Draft Method 1668A was subjected to formal peer review in September-October of 1999. The peer review was conducted in accordance with EPA's *Science Policy Council Peer Review Handbook* (EPA 100-B-98-001, January 1998). Based on the peer review, EPA revised and published Method 1668A without the word "Draft" in December of 1999 (EPA 821-R-00-002). EPA also published a report titled *Peer Review of Draft EPA Method 1668, Revision A: Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS* in February 2000.

[4]Although some congeners have only a single chlorine atom, the entire suite of 209 chlorinated biphenyl congeners will be referred to as “PCBs” in the remainder of this study plan for consistency with common usage.

3.0 STUDY OBJECTIVES

The objectives of this study are to 1) characterize the performance of EPA Method 1668A in multiple laboratories and matrices and 2) evaluate and, if appropriate, revise the quality control (QC) acceptance criteria in the method. The ultimate objective is to propose and promulgate Method 1668 at 40 CFR part 136 for use in EPA's Clean Water Act programs.

To ensure that these study objectives are met, EPA will require that:

- Each laboratory follow all analytical and quality control procedures in EPA Method 1668A and study-specific instructions,
- Any laboratory that wishes to deviate from the procedures in Method 1668A or the study-specific instructions obtain prior approval of the changes and document those approved changes in detail
- All data produced be capable of being verified by an independent person reviewing the analytical data package
- Each laboratory have a comprehensive quality assurance (QA) program in place and operating throughout the study. This QA program will ensure that the data produced are of appropriate and documented quality.

4.0 STUDY MANAGEMENT

The study will be managed by the Statistics and Analytical Support Branch (SASB) in the Engineering and Analysis Division within EPA's Office of Science and Technology. Day-to-day management and coordination of study activities will be performed by the contractor-operated Sample Control Center (SCC) under SASB guidance.[5] SCC will coordinate the purchase of standards, sample collection, sample and data tracking, and monitor day-to-day study activities. SCC also will establish schedules for activities given in this study plan and will keep SASB informed as to the status of the study. SASB will draw conclusions from the study and produce a report presenting study results. If appropriate, SASB will revise Method 1668A as necessary to reflect study findings.

5.0 STUDY DESIGN

The design of this study is intended to provide EPA with a sufficient amount of data to evaluate method performance in accordance with the guidelines published by EPA, AOAC-International, and ASTM International.[6,7,8] These guidelines recommend a minimum of six data sets for evaluation of a

[5]The Sample Control Center (SCC) is operated by DynCorp Systems & Solutions LLC under EPA Contract No. 68-C-01-091. All SCC activities are performed under the direction and guidance of EPA SASB.

[6]*Guidelines for Selection and Validation of US EPA's Measurement Methods*, U.S. EPA Office of Acid Deposition, Environmental Monitoring and Quality Assurance (OADEMQA), Office of Research and Development, U.S. Environmental Protection Agency, August 1987 DRAFT.

[7]"Report of the Committee on Collaborative Interlaboratory Studies," *J. Assoc. Office. Anal. Chem.*, *67*, (2), 1984

method. In order to allow for some loss of data due to error, lost samples, outlier removal, or other unforeseen causes, EPA plans to identify at least nine laboratories willing and able to participate in the study. However, given the relatively limited number of laboratories with the equipment and experience necessary to analyze for PCBs using HRGC/HRMS, it may not be possible to identify nine laboratories willing to participate as volunteers or to obtain at least six usable sets of data. If it is not possible to obtain at least six usable data sets, EPA may utilize any Method 1668A data available to assess method performance, develop revised QC acceptance criteria, and for other purposes.

Due to budget limitations, EPA intends to seek as much volunteer participation as possible in this study. To help offset study costs, EPA will provide volunteer laboratories with a set of analytical standards necessary to implement Method 1668A. Even so, it is not reasonable to expect laboratories to donate tens of thousands of dollars worth of analyses. Therefore, the number of analyses will be balanced against the need to obtain a sufficient number of participant laboratories.

An interlaboratory study designed in accordance with ASTM standard D-2777 would involve spikes of all 209 congeners at multiple and replicate concentrations in multiple matrices, plus initial and batch QC. The total number of analyses per laboratory could be upwards of 75 if calibration, QC, and a method detection limit (MDL) study are included. Given that a single HRGC/HRMS analysis costs $750 - 1200, the cost for such a study in a minimum of nine laboratories would exceed available EPA resources and be impractical for volunteers.

To address these cost concerns, EPA intends to include no more than two samples of each matrix type, with each sample containing varying concentrations of the target PCB congeners. EPA anticipates validating the method in wastewater, biosolids, and fish tissue. To further reduce study costs, EPA plans to use excess sample volume collected from previous studies of biosolids and fish. Biosolid samples or sample locations will be selected based on results of EPA's 2001 National Sewage Sludge Survey; tissue samples or sample locations will be selected based on results of EPA's ongoing National Study of Chemical Residues in Fish Tissue. EPA does not have a similar supply of stored wastewater sample volume. Therefore, EPA plans to collect and spike wastewater samples with PCBs.

Given the above considerations, EPA believes that the study can be conducted with a total of 10 analyses per laboratory (in addition to 5 runs necessary to determine the absolute and relative retention time for each congener, and an initial 6-point instrument calibration) as follows:

- 2 reagent water samples,
- 2 biosolid samples,
- 2 tissue samples,
- 2 wastewater samples,
- 1 reagent water blank, and
- 1 solids/tissue blank (playground sand spiked with corn oil).

[8]ASTM Standard D2777-98, "Standard Practice for Determination of Precision and Bias of Methods of Committee D-19 on Water," *Annual Book of ASTM Standards, Vol. 11.01*, ASTM International, West Conshohocken, PA 19428.

EPA believes that increasing the number of samples beyond the numbers described above would significantly limit the number of laboratories willing to participate in the study, even with enticements offered by the recognition gained through participation in the study and EPA-provided standards.

6.0 STUDY IMPLEMENTATION

The study will be conducted in four phases: (1) identifying and selecting the participant laboratories; (2) collecting, preparing, and shipping standards and samples; (3) sample analysis and data reporting; and (4) data review and assessment. Details of each phase are summarized below.

6.1 Phase 1 - Laboratory Identification and Selection

The study will involve one sample processing laboratory and a group of participant laboratories. The total number of participant laboratories will depend upon laboratory capability, availability, cost, and scheduling constraints. Participant laboratories may include commercial laboratories, academic laboratories, State laboratories, EPA laboratories, and/or municipal laboratories. EPA will also request participation by international laboratories so that study results reflect worldwide application of EPA Method 1668A. EPA recognizes international environmental concerns and abilities to implement laboratory analytical techniques targeting PCBs, and successfully included international participation in validating EPA Method 1613 (chlorinated dibenzo-*p*-dioxins and dibenzofurans), EPA Method 1622 (*Cryptosporidium*), EPA Method 1623 (*Cryptosporidium* and *Giardia*), and EPA Method 1631 (mercury). As noted in Section 5 above, EPA 1) plans to identify at least nine laboratories willing and able to participate in the study and 2) intends to seek as much volunteer participation as possible.

All laboratories that participate in the study will be required to demonstrate that they have recent experience in analyzing for chlorinated pollutants in environmental samples by HRGC/HRMS with selected ion monitoring (SIM). This is intended to ensure that study participants already have the facilities, equipment, and trained staff necessary to implement Method 1668A. Once qualified participant laboratories have been identified, they will be provided with at least two weeks notice of their selection to participate in the study before the study begins. This is intended to provide study participants with a reasonable amount of time to review any study-specific instructions.

Note: Given the relatively limited number of laboratories with HRGC/HRMS instrumentation, and EPA's desire to obtain volunteer support, it may not be possible to achieve a sufficient number of laboratories to meet the study design. If a sufficient number of volunteer laboratories are not identified, EPA may consider issuing contracts with one or more qualified laboratories through a competitive bidding process.

6.2 Phase 2 - Collection, Preparation, and Shipment of Samples and Standard Solutions

6.2.1 Sample Identification and Collection

Biosolid samples will be generated from excess sample volume collected during EPA's 2001 National Sewage Sludge Survey. Tissue samples will be generated from excess sample volume collected during EPA's National Study of Chemical Residues in Fish Tissues. Excess sample volume from both studies is currently stored in freezers at an EPA sample repository. As described in Section 5, EPA will examine biosolids and fish tissue data from these studies to identify samples that contain PCB congeners at concentrations of interest. In selecting the samples, EPA's objective will be to maximize the number of congeners represented and ensure that the congeners span the anticipated measurement range of the method, ranging from the upper end of the calibration range down to "not detected." In order to ensure that a sufficient volume of each sample is available to support the needs of this study, EPA will identify several samples of each matrix type that can be combined to produce large volumes of Youden pairs with the desired congener distribution. Once these frozen, stored samples are identified, they will be forwarded on ice to the Sample Processing laboratory. (Although PCBs are stable and do not require preservation, ice will be used to prevent decomposition of the fish and retard gas production in the biosolids.)

Because EPA does not have a stored supply of excess wastewater sample volume, wastewater will be collected for this study from a publicly owned-treatment works (POTW) located near the sampling organization. Based on previous experience, EPA believes that municipal wastewater discharges are unlikely to contain a sufficient number of PCB congeners at concentrations to adequately test the capabilities of the method. Depending on available resources and the selected site location, these wastewater samples may be collected by SCC, the Sample Processing Laboratory, States, or EPA Regional staff. Samples will be collected by individuals trained in appropriate sample collection and handling procedures. The sampling team will collect a sufficient volume to allow for testing in all laboratories and to provide extra volume in case of sample breakage, lost shipment, or other unforeseen problems. Samples will be collected into pre-cleaned bottles (e.g., from a bottle-manufacturing process that includes high-temperature annealing or that have been cleaned by a laboratory experienced in the determination of PCBs by HRGC/HRMS). Because PCBs are known to be persistent in the environment, wastewater samples will not be stored on ice.

Immediately after sample shipment (i.e., as soon as samples are in the custody of the carrier), the sample repository or sampling team will call SCC and provide information on the shipment, including sample numbers, numbers of coolers, and courier and air bill number. SCC will notify the processing laboratory of the scheduled shipment and confirm that samples have arrived in good condition and as scheduled. If necessary, SCC will implement tracking activities to locate any lost shipment(s).

6.2.2 Sample Processing at the Processing Laboratory

Each set of tissue and biosolid samples sent to the sample processing laboratory will be accompanied by a detailed set of instructions concerning combination and homogenization of sample volumes, the number of aliquots to be prepared from each combined/homogenized sample, and instructions for labeling the prepared sample aliquots. These instructions will reflect the considerations described in Section 6.2.1 (i.e., creating sufficient volume of samples that contain a large number of PCB congeners at a wide range of concentrations). The sample processing laboratory will combine and homogenize the tissue and biosolid samples according to these instructions.

EPA also will direct the sample processing laboratory to divide the unspiked wastewater into the required number of aliquots and spike each aliquot separately (rather than spiking a bulk volume wastewater and then subdividing the spiked sample into replicate aliquots). Spiking each aliquot separately avoids the problems with "wall effects," whereby organic pollutants spiked into a bulk sample in a solvent tend to adhere to the walls of the container, making it difficult, if not impossible, to divide the bulk sample into multiple aliquots containing the same concentration. EPA will provide detailed instructions to the sample processing laboratory regarding the number of aliquots, the PCBs to be used for spiking, and spiking concentrations. In developing those instructions, EPA will assume that any background concentration of PCB congeners in the wastewater samples is minimal.

Because PCBs are ubiquitous in the environment, including laboratories, the sample processing laboratory must judiciously guard against sample contamination. To minimize contamination, the processing facility will homogenize the samples and divide the homogenized samples into replicate aliquots under controlled conditions.

Note: It is not necessary that the exact congener concentrations of each sample be known because 1) each sample will have been designed to ensure that a wide variety of congeners and concentrations are present, 2) the purpose of the study is to compare interlaboratory measurements rather than to definitively characterize specific samples, and 3) spikes of labeled compounds into these matrices will be used to measure recovery.

6.2.3 Sample Shipment

Once the study samples have been prepared, aliquotted, and labeled, the sample processing laboratory will ship the samples to the participant laboratories via air courier. Because of the stability of PCBs, the samples will not require preservation. Biosolids and tissue samples will be shipped on ice, however, to hinder decomposition of tissues and gas formation in the biosolids. The processing laboratory will notify SCC of the shipping date so that SCC can notify all participant laboratories of the shipping and scheduled arrival dates, and if necessary, implement tracking procedures for any lost shipments.

Note: If overseas laboratories are included in the study, biosolids and tissues may be freeze dried so that they can be shipped without concern that ice may melt during extended transit times.

6.2.4 Standards Acquisition, Packaging, and Shipment

To reduce the cost to volunteer laboratories, EPA will provide each volunteer laboratory with a single set of standards sufficient to calibrate their instrumentation and conduct all analyses. Sets of standards solutions will be acquired from suppliers of native and carbon-13 labeled compounds. If possible, a single supplier will aggregate all standards solutions into a set, and package a set of standards for shipment to each laboratory. To preclude injudicious use of standards, EPA will remind laboratories of the instructions given in Method 1668A for combining and diluting standards.

6.3 Phase 3 - Sample Analysis and Data Reporting

6.3.1 Sample Analysis

Participant laboratories will be required to analyze samples in a timely fashion in accordance with the study schedule, and will follow procedures for preparation, handling, and analysis of standards solutions and samples provided in EPA Method 1668A.

If analytical results appear unreasonable, laboratories will be instructed to investigate possible causes, first by checking for transcription and calculation mistakes, and then by reanalysis. Although laboratories will be prohibited from performing multiple analyses to improve results, they will be allowed to implement corrective action and reanalysis for QC failures that are attributable to instrument failure or to analyst error (e.g., incorrect spiking levels).

6.3.2 Data Reporting

Specific reporting requirements will be provided in detailed instructions to the laboratories. Gathering data from analyses of 209 congeners in IPR, OPR, blank, and the study sample(s) could represent a formidable challenge because of the multiplicity of possible data reporting formats. To simplify data evaluation, EPA will provide an electronic spreadsheet template and request that laboratories submit data in this suggested format.

Each laboratory will be asked to report the following:

- Summary level data in spreadsheet format;
- Summary level and raw data in hardcopy format;
- Individual results, including results for all congeners found in all blanks. (**Note**: Laboratories will not be allowed to average results or perform other data manipulations beyond those described in Method 1668A. When results are below the minimum level of quantitation but are detected, laboratories will be required to report the actual calculated result, regardless of its value);
- A list of the composition and concentrations of PCB congeners in the calibration, IPR, blank(s), OPR, samples analyzed, and a run chronology;

- Copies of all raw data, including chromatograms, quantitation reports, spectra, bench sheets, and laboratory notebooks showing weights, volumes, and other data that will allow verification of the calculations performed and will allow the final results reported to be traced to the raw data. Each data element must be clearly identified in the laboratory's data package;
- A written report that details any problems associated with analysis of samples or standard solutions. The written report also must provide comments on the performance of any part of Method 1668A; and
- A detailed description of any modifications to the procedures specified in Method 1668A. Details and raw data from all runs will be reviewed for determination as to whether further testing is required.

Laboratories also will be instructed to use the following rules in reporting results:

- Quantitative results above or at the MDL - report value;
- Quantitative results below the MDL - report value but flag with footnote giving the MDL;
- Nonquantitative results - report as less than the MDL value and state the MDL value; and
- The terms “zero,” “trace,” or “ND (not detected)” are not to be used.

EPA will request that laboratories submit analytical results within 45 days of receipt of samples.

6.4 Phase 4 - Data Review and Assessment

Upon receipt of laboratory data packages, SCC will review the data to ensure all results were generated in accordance with the method and with the requirements of this study plan and any associated laboratory instructions. An objective of this review will be to maximize data use. If a discrepancy occurs, it will be resolved with the laboratory, where possible. Data and laboratory comments and recommendations will be assessed in the context of the objectives of the study and the ultimate uses of EPA Method 1668A under the Clean Water Act.

The objective of this assessment will be to evaluate the precision, recovery, and comparability of results obtained by multiple laboratories employing the method, and to determine if the QC acceptance criteria in Method 1668A should be revised based on study results. Results of this assessment will be published in a study report.

EPA plans to perform a statistical analysis of the data to determine acceptability and suitability for use. This statistical analysis will be performed in accordance with *Standard Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D-19 on Water* (ASTM D2777) or other accepted statistical practice.

7.0 LIMITATIONS

The study design does not include a requirement that each laboratory perform an MDL or IPR study in each reference matrix. In order to ensure that the MDL and IPR specifications published in the final method can be achieved in these matrices by multiple laboratories, EPA intends to supplement this study with MDL and IPR data gathered from at least three sources. One of these sources will include existing MDL data generated in reference tissue, solids, and aqueous matrices. EPA already has such data from Axys Analytical Services Ltd., and will contact other laboratories to determine if such existing data are available.

Given the cost of Method 1668A analyses, EPA believes it is neither feasible nor necessary to validate the method in each and every possible matrix or to validate each and every congener at low, medium and high concentrations. This study design focuses on representative matrices and concentrations. EPA believes that application of a method to one or more matrices in multiple laboratories usually can reflect the performance of the method across multiple matrices. EPA also believes that PCBs are extremely stable and are not subject to adsorption and other processes that cause percent recovery to vary as a function of concentration for some analytes (e.g., nitrophenols). If EPA is able to gather data from other matrices and concentrations not tested in this study, EPA will make such data available to interested parties, either upon request or as an addendum to the final study report. EPA also is willing to consider expanding the study if the additional analyses can be justified in terms of the additional information that they will provide, and if external funding can be found to support the additional analyses.