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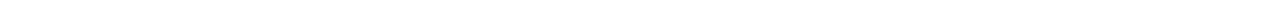
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Report on the Multi-laboratory Validation of Clean Water Act Method 1628 for PCB Congeners

U.S. Environmental Protection Agency
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Office of Science and Technology
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Disclaimer

This report was prepared by Adrian Hanley of the Engineering and Analysis Division within EPA's Office of Water, with assistance from Harry McCarty and Mirna Alpizar of CSRA, a General Dynamics Information Technology company. Mention of company names, trade names, or commercial products in this report does not constitute endorsement or recommendation for use.

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Executive Summary

The goal of this project was to develop and validate a polychlorinated biphenyl (PCB) congener method for use in Clean Water Act compliance monitoring. Currently, Method 608.3 is the commonly used EPA method that is approved at 40 CFR Part 136 for compliance monitoring of PCBs, and it only measures seven common Aroclor mixtures, not the total amount of PCB contamination, nor the specific PCB congeners designated as toxic by the World Health Organization (WHO). To address these shortcomings of Method 608.3, a new PCB congener method should meet the following criteria:

1. Identifies and quantifies PCB contamination using individual congeners, rather than attempting to recognize and quantify the patterns generated from Aroclor mixtures.
2. Is more sensitive than the currently approved Method 608.3, but is not so sensitive that it is adversely affected by typical laboratory background contamination.
3. Can be implemented at a typical mid-sized full-service environmental laboratory.

In response to this need, the US Environmental Protection Agency (EPA) Office of Water convened a workgroup of EPA, laboratory, and utility staff, supported by contractors. After examining a suite of candidate procedures, the workgroup prioritized an unpublished laboratory procedure and a specific set of congeners for further development and validation efforts, designed the study described in this report, and reviewed all the study products (see Section 1).

The newly validated Method 1628 detects all 209 PCB congeners, and quantifies them either directly or indirectly. A total of 29 carbon-13 labeled congeners are used as isotope dilution quantification standards. An additional 19 congeners are quantified by an extracted internal standard procedure, using one of the isotope dilution standards. The remaining 144 congeners are quantified against a labeled standard in the same homolog by assuming that it has a similar response (see Section 4). The method requires the laboratory to run standards containing all 209 congeners to establish retention times and method detection limits, but not during routine analysis. This approach strikes a balance between enabling the laboratory to detect and quantify all 209 congeners, while not making the method too arduous. Based on the results of matrix spike samples (see Section 8), method performance was similar across all the congeners, regardless of the quantification approach.

The primary focus of this validation study was on wastewater compliance monitoring, but there is a need for testing biosolids, soils/sediments, and fish tissue as well. Therefore, the multi-laboratory study tested:

- Nine aqueous sample types, including wastewater effluents and influents collected from publicly owned treatment works and indirect industrial dischargers
- Three sediments collected from the Great Lakes region
- Three biosolids collected from municipal wastewater treatment plants
- Fish tissues from three species collected from the Great Lakes

The multi-laboratory validation study of Method 1628 met all of the goals that EPA set for this study. The study generated initial precision and recovery data for aqueous, solid, and tissue matrices. Over 95% of the spike recoveries for both the aqueous and tissue samples fell between 50 - 150%, while 87% of the biosolids samples and 63% of the sediment samples had recoveries between 50 - 150%. The percentage of false negatives for aqueous samples was less than 0.2%, for solids samples (sediments and biosolids) it was less than 3%, and for tissue samples it was less than 0.1%. While a particularly difficult matrix may cause an interference that invalidates the results for one congener, it is unlikely that the matrix will cause the same type of interference for all the PCB congeners in the sample.

The performance of Method 1628 and the quality control requirements incorporated into it make it more sensitive and accurate compared to the currently approved Method 608.3, as outlined in Section 11.

Table of Contents

Disclaimer	i
Acknowledgements.....	ii
Executive Summary.....	iii
1. Introduction	1
Background	1
EPA Workgroup.....	2
SOP Selection.....	2
Congener Prioritization	3
Quantification.....	3
Summary of the Results of the Single-laboratory Study	4
Multi-laboratory Study Goals and Design.....	5
2. Identification and Selection of Laboratories	6
3. Study Samples	8
Wastewater Matrices	8
Biosolids Matrices	9
Sediment Matrices.....	9
Fish Tissue Matrices.....	10
Reconnaissance Analyses.....	10
4. Approaches to Calibration and Quantification	12
Multi-point Initial Calibration	16
Response Ratios and Response Factors.....	18
Mass-to-charge Ratios Monitored for Each Analyte.....	19
Area Subtraction of Higher Homolog Interference	24
Calibration Linearity and Stability	24
5. Initial Precision and Recovery.....	27
Aqueous IPR/OPR Criteria.....	29
Solids IPR and OPR Results	33
Tissue IPR and OPR Results	37
6. Method Detection Limits.....	42
Aqueous Sample Pooled MDL Determinations	43
Soil/Sediment and Biosolids Sample MDL Determinations	49
Tissue Sample MDL Determinations	55
7. Unspiked Sample Analyses	61
Wastewater Samples.....	61
Sediment Samples	63
Biosolids Samples	67
Tissue Samples	70
8. Matrix Spike Analyses	75
Aqueous Sample MS/MSD Results.....	76
Sediment Sample MS/MSD Results.....	82
Biosolids Sample MS/MSD Results.....	86
Tissue Sample MS/MSD Results	89

9.	Labeled Compound Results.....	92
	Aqueous Sample Labeled Compound Results.....	92
	Sediment Sample Labeled Compound Results.....	95
	Biosolids Sample Labeled Compound Results.....	97
	Tissue Sample Labeled Compound Results	99
10.	Data Review and Validation.....	102
11.	Conclusions	104
	Study Goals	104
	Method Performance	105
	Summary	108
12.	References	109

Appendices

- Appendix A Labeled PCB Congeners to be used as Quantitation Standards
- Appendix B Interim Quality Control Acceptance Criteria Arising from the Method Validation Study
- Appendix C Study Plan for Multi-laboratory Validation of the EAD PCB Congener Method

List of Tables

Table 1.	List of Participating Laboratories	6
Table 2.	Bulk Wastewater Matrix Sources	8
Table 3.	Water Quality Characteristics of the Study Samples (in mg/L)	9
Table 4.	Reconnaissance Analyses Results for Study Samples	11
Table 5.	Quantification Reference and Calibration Approach for the 65 Congeners with Direct Calibration Data.....	12
Table 6.	Congeners Not Calibrated Directly	14
Table 7.	Composition of the Initial Calibration Standards	16
Table 8.	Ions Monitored for each Congener with Direct Calibration Data and the Labeled Compounds, in Retention Time Order.....	20
Table 9.	Ions Monitored for the 144 Other Congeners, in Retention Time Order.....	21
Table 10.	Ions Monitored for Correcting for Interferences from Higher Homologues	24
Table 11.	Summary of Response Ratios, Response Factors, and Relative Standard Deviations for 32 Initial Calibrations	25
Table 12.	Aqueous IPR and OPR Calculated QC Acceptance Criteria for Target Analytes.....	29
Table 13.	IPR and OPR QC Acceptance Criteria for Labeled Compounds in Aqueous Matrices	31
Table 14.	Observed Labeled Compound Recovery Failure Rates for Two Potential Acceptance Criteria for Aqueous Matrix IPR and OPR.....	32
Table 15.	IPR and OPR QC Acceptance Criteria for Target Analytes in Solid Matrices.....	34
Table 16.	IPR and OPR QC Acceptance Criteria for Labeled Congeners in Solid Matrices	35
Table 17.	Observed Labeled Compound Recovery Failure Rates for Two Potential Acceptance Criteria for Solid Matrix IPR and OPR	36
Table 18.	IPR and OPR QC Acceptance Criteria for Target Analytes in Tissue Matrices.....	37
Table 19.	IPR and OPR QC Acceptance Criteria for Labeled Compounds in Tissue Matrices	39
Table 20.	Observed Labeled Compound Recovery Failure Rates for Two Potential Acceptance Criteria for Tissue Matrix IPR and OPR	40
Table 21.	Aqueous Sample Pooled MDL and ML Results (ng/L)	43
Table 22.	Frequency of Aqueous MDL _b values by Lab	47

Table 23. Solid Sample Pooled MDL and ML Results (ng/g).....	49
Table 24. Frequency of Solid MDL _b values by Lab	53
Table 25. Tissue Sample Pooled MDL and ML Results (ng/g).....	55
Table 26. Frequency of Tissue MDL _b values by Lab	58
Table 27. Summary of Wastewater Sample Cleanups.....	61
Table 28. Unspiked Wastewater Sample Results in ng/L.....	61
Table 29. Summary of Sediment Sample Cleanups	63
Table 30. Unspiked Sediment Sample Results in ng/g (dw)	64
Table 31. Summary of Biosolids Sample Cleanups	67
Table 32. Unspiked Biosolids Sample Results in ng/g (dw)	67
Table 33. Unspiked Tissue Sample Results in ng/g.....	70
Table 34. Results for Congeners Detected by Four Labs for Tissue Sample #1 in ng/g	73
Table 35. Results for Congeners Detected by Five Labs for Tissue Sample #2 in ng/g.....	73
Table 36. Results for Congeners Detected by Five Labs for Tissue Sample #3 in ng/g.....	74
Table 37. Composition of Matrix Spiking Solutions.....	75
Table 38. Matrix Spike Recoveries for Wastewater Samples #3 to #6 (%)	77
Table 39. Matrix Spike Recoveries for Wastewater Samples #7 to #10 (%)	79
Table 40. Matrix Spike Recoveries for Sediment Samples	82
Table 41. Matrix Spike Recoveries for Biosolids Samples	86
Table 42. Matrix Spike Recoveries for Tissue Samples	89
Table 43. Observed Aqueous Labeled Compound Recoveries and Calculated Acceptance Criteria.....	92
Table 44. Observed Aqueous Labeled Compound Recovery Failure Rates for Potential Acceptance Criteria	94
Table 45. Observed Sediment Labeled Compound Recoveries and Calculated Acceptance Criteria.....	95
Table 46. Observed Sediment Labeled Compound Recovery Failure Rates for Potential Acceptance Criteria.....	96
Table 47. Observed Biosolids Labeled Compound Recoveries and Calculated Acceptance Criteria.....	97
Table 48. Observed Biosolids Labeled Compound Recovery Failure Rates for Potential Acceptance Criteria.....	98
Table 49. Observed Tissue Labeled Compound Recoveries and Calculated Acceptance Criteria.....	99
Table 50. Observed Tissue Labeled Compound Recovery Failure Rates for Potential Acceptance Criteria	100
Table 51. Comparison of Method 608.3 and Method 1628 for Aqueous Samples.....	105
Table 52. Estimated Aroclor 1242 Concentrations Using 5 Most Prevalent Congeners in Aqueous Matrices	106

List of Figures

Figure 1. PCB Structure and Congener Counts	1
Figure 2. Pooled Aqueous MDL _s Values in Elution Order.....	48
Figure 3. Pooled Solid MDL _s Values in Elution Order.....	54
Figure 4. Pooled Tissue MDL _s Values in Elution Order.....	59
Figure 5. Mean Matrix Spike Wastewater Recoveries by Sample, in Elution Order (without Sample #2)	81
Figure 6. Mean Matrix Spike Sediment Recoveries by Sample, in Elution Order	85
Figure 7. Mean Matrix Spike Biosolids Recoveries by Sample, in Elution Order	88
Figure 8. Mean Matrix Spike Tissue Recoveries by Sample, in Elution Order	91

1. Introduction

The goal of this project is to complete multi-laboratory validation of a polychlorinated biphenyl (PCB) congener method that is a significant improvement to Method 608.3, the only approved PCB method for wastewater. Method 608.3 only measures the seven common Aroclor mixtures, not the total amount of PCB contamination, nor the specific PCB congeners known to be most toxic. PCB production has been banned since 1977, therefore, most PCB contamination in the environment is more than 40 years old. Environmental PCB contamination is usually made up of weathered PCBs that do not resemble the original Aroclor mixtures. Furthermore, not all PCB contamination is from Aroclor mixtures. This leads to false negatives and artificially low results. A new PCB congener method must meet the following criteria:

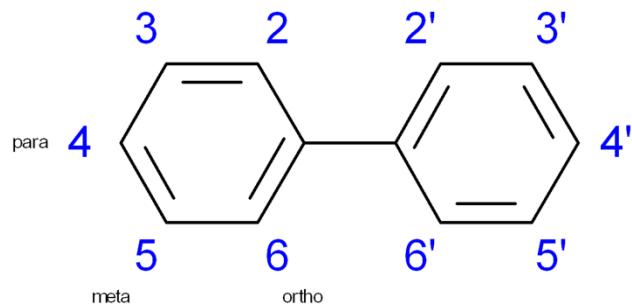
1. The method identifies and quantifies PCB contamination using individual congeners, not an estimated quantity based off patterns generated from Aroclor mixtures.
2. The method is more sensitive than the currently approved Method 608.3, but is not so sensitive that it is adversely affected by typical laboratory background contamination.
3. The method can be implemented at a typical mid-sized full-service environmental laboratory.

This multi-laboratory validation study was a follow-on to the successful single-laboratory study conducted in 2017 (CSRA 2018).

Background

PCB contamination and its properties are well documented, so only a brief summary is provided here. A biphenyl is 12 carbon atoms, made up of two 6 carbon aromatic rings that are bonded together at one location (Figure 1). Chlorinated biphenyls are a group of compounds made up of a biphenyl with one to ten chlorine atoms attached to the 10 possible bond locations. There are 209 possible molecules, depending on the number and the placement of the chlorine atoms. Each of these 209 possibilities is called a congener. All of the congeners that contain the same number of chlorine atoms are called a homolog (e.g., all of the congeners that contain 4 chlorine atoms are one homolog, this homolog is often referred to as the tetrachlorobiphenyls or the tetrachloro homolog).

Figure 1. PCB Structure and Congener Counts



# Chlorine Atoms - Homolog	# of Congeners	Congener Numbers
1 - Monochloro	3	1 - 3
2 - Dichloro	12	4 - 15
3 - Trichloro	24	16 - 39
4 - Tetrachloro	42	40 - 81
5 - Pentachloro	46	82 - 127
6 - Hexachloro	42	128 - 169
7 - Heptachloro	24	170 - 193
8 - Octachloro	12	194 - 205
9 - Nonachloro	3	206 - 208
10 - Decachloro	1	209

Most PCBs were produced in the U.S. by a batch process of exposing biphenyl to chlorine gas until the desired percentage of chlorinated compounds were produced. The commercial mixtures were sold under the name Aroclor. There were seven commonly produced Aroclors: 1016, 1221, 1232, 1242, 1248, 1254, and 1260, and many other less common mixtures. The last two digits of the Aroclor mixture indicate the percent chlorine by weight (except 1016, which is 41% chlorine by weight). The higher the number, the

higher the chlorine content. Aroclors occurred as oily liquids or waxy solids, depending on the specific Aroclor mixture. PCBs are known to occur from other sources aside from the Aroclor mixtures and are sometimes produced as unintentional by-products of various combustion processes involving chlorine-containing materials (Erickson, 1997).

Historically, PCBs were used for many industrial applications, most commonly in electrical capacitors and transformers. Domestic manufacture of PCBs occurred from 1927 until 1977, when production was banned. PCBs are very stable in the environment, nonflammable, insulating, and thermally stable. They can be destroyed by incineration, but only at very high temperatures. PCBs are relatively non-volatile and insoluble in water. Most PCBs found in water are believed to be attached to particulate matter. They primarily exist in air as particulates, which can wash out during rain events and end up in surface waters. PCBs bioaccumulate as they move up the food chain, but some congeners bioaccumulate more than others.

EPA Workgroup

Prior to beginning the single-laboratory validation study, the Engineering and Analysis Division, which is part of the Office of Science and Technology within the Office of Water (OW/OST/EAD), assembled an EPA workgroup. The workgroup included representatives of all three divisions of OW/OST, the Office of Research and Development (ORD), OW's Office of Wastewater Management, the Office of Land and Emergency Management, EPA Regions 3, 4, 5, and 10, two subject matter experts from outside of EPA, and EAD's support contractor staff, CSRA.

SOP Selection

Prior to the single-laboratory validation study, the workgroup met to review available laboratory standard operating procedures (SOPs) for analysis of PCB congeners. The SOPs and published articles reviewed can be summarized into three categories.

Dual-Column Gas Chromatography Electron Capture Detector (GC-ECD): This is the same analytical technique used for Method 608.3. EPA and other organizations have developed methods and SOPs that measure Aroclor mixtures and specific congeners using GC-ECD. The addition of the specific congeners makes the technique better at detecting weathered PCB congeners, but the overall sensitivity is not improved dramatically. Because ECD is not a selective detector and has the ability to detect many types of organic compounds (halogens, organometallic compounds, nitriles, or nitro compounds), matrices that are high in organic contamination can cause significant interference, and often require extensive sample cleanup. Since many municipal wastewater treatment facilities treat high organic content wastewater with chlorine, which can produce interfering background peaks when using an ECD, the workgroup did not recommend these GC-ECD SOPs for further testing.

Gas Chromatography with Tandem Mass Spectrometers (GC-MS-MS): Some literature and instrument SOPs exist for both GC-MS-MS and GC with triple quadrupole MS. Most of these are instrument SOPs and no published SOP was found for environmental samples. The testing that has been done on PCBs is with very small lists of congeners (22 or fewer of the 209). The European Union (EU) published guidance on this type of testing, and some laboratories in Europe run methods with this technology, but it is mainly used for environmental and feed screening. The EU guidance only mentions 22 congeners. This technology looks promising and appears to have excellent sensitivity, but there are not any fully developed lab SOPs currently available to EAD. The workgroup did not recommend this technology due to a lack of any documented laboratory SOPs and because very few commercial environmental labs own this instrumentation, making it difficult to implement on a national scale in the next few years.

Gas Chromatography with Mass Spectrometry and Select Ion Monitoring (GC-MS-SIM): Two promising laboratory SOPs were reviewed that use GC-MS-SIM. Both addressed all 209 congeners, but

some of the congeners co-eluted into one peak. One SOP resolved all 209 congeners into 189 peaks (22 co-eluting peaks), while the other resolved all 209 congeners in 160 peaks (49 co-eluting peaks). The workgroup agreed to pursue the SOP with more co-elutions. The rationale was that this SOP had a run time that was 20 minutes shorter (45 minutes instead of 65 minutes), which was better for implementation, and had better quantitation and quality control. It used 12 Carbon-13 (^{13}C) labeled standards (one for each PCB homolog and 2 additional surrogates), while the other SOP only had 2 labeled standards and 4 internal standards (oddly, some were unlabeled target analytes – albeit congeners that are almost never detected in the environment). The SOP selected by the workgroup has been run for well over 10 years at the laboratory that submitted the SOP in a wide variety of matrices, and it requires equipment that most environmental laboratories already own.

Congener Prioritization

Prior to the single-laboratory validation study, the workgroup met to discuss and prioritize which congeners were a high priority for this method. There was general consensus that the more important congeners to monitor have the following characteristics:

- Most common in the environment
- Congeners known to be most prevalent in human tissue
- Congeners present in the largest quantities within the manufactured Aroclor mixtures
- The 12 toxic congeners identified by the World Health Organization (WHO)

There is significant overlap between the first three of these four categories.

OW/OST/EAD assembled several databases of PCB congener data prior to the meeting. Databases were selected that had PCB congener data from Method 1668, a highly sensitive high-resolution gas chromatography mass spectrometry (HRGC/HRMS) method.

- Wastewater data from the Delaware River Basin Commission (DRBC) (2005 - 2013)
- EPA National Lake Fish Tissue Survey data (2000 - 2004)
- EPA National Sewage Sludge Survey data (2001)
- Upper Trenton Channel sediment data from the Great Lakes National Program Office

ORD provided a list of the most common congeners detected in tissues. The workgroup also consulted the Aroclor formulation data compiled by Frame, G. M., Cochran, J. W., and Boewadt, S. S. in the *Journal of High Resolution Chromatography*, Vol. 19, pp 657-668 (1996).

The selection process for how EPA chose which congeners to be used as isotope dilution standards and internal standards is detailed in Appendix A, “Labeled PCB Congeners to be used as Quantitation Standards.” Through that selection process, EPA identified 48 congeners that are a high priority for the new PCB method because of their prevalence in environmental samples, high concentrations in Aroclors, and their toxicities (see the introduction to that report for further details). To facilitate proper identification of the congeners in each homolog, EPA included the first and last eluting congeners in each homolog in the target analytes list for the procedure. Using common GC columns and conditions, some of those 48 targeted congeners coelute with 17 other congeners. Therefore, EPA included those 17 other congeners in the calibration process of the draft procedure, and for the purposes of this report, EPA is referring to these 65 congeners as the congeners with direct calibration data.

Quantification

In order to quantify those 65 congeners, EPA selected commercially available $^{13}\text{C}_{12}$ -labeled analogs of 29 of those congeners that are to be used as isotope dilution standards. Three additional $^{13}\text{C}_{12}$ -labeled standards are used as “recovery standards” that are spiked after extraction and used to calculate the

recoveries of the isotope dilution standards. The details of calibration and quantification approach are described in Section 4 of this report.

Each laboratory will still need to run standards of all 209 congeners on occasion to establish retention times and method detection limits, but not during routine analysis. This strikes a balance between enabling the laboratory to detect and quantify all 209 congeners, without making the method too arduous.

If a laboratory or discharger wants to quantify one or more specific congeners more accurately, the method allows the laboratory to expand the list of congeners used for calibration and/or the list of isotope dilution standards. The method flexibility codified at 40 CFR Part 136.6 allows a laboratory to add analytes and quantification standards to an approved wastewater method if they can demonstrate adequate performance.

Summary of the Results of the Single-laboratory Study

The single-laboratory validation was performed by SGS AXYS Analytical, the developer of the original laboratory SOP that was selected by the EPA workgroup and that study was deemed a success. As noted in the report on that study (CSRA 2018), the single-laboratory validation study of the draft PCB congener method met two of EPA's three criteria.

- 1. The method identifies and quantifies PCB contamination using individual congeners, not an estimated quantity based off patterns generated from Aroclor mixtures.*

The study generated initial precision and recovery data for aqueous, solid, and tissue matrices. Of the over two hundred matrix spike samples analyzed during the single-laboratory study:

- Almost all of the sediment samples achieved recoveries between 60 - 115% (1,243 out 1,248 results, or 99.6%).
- Almost all of the biosolid samples achieved recoveries between 75 - 150% (1,334 out of 1,344 results, or 99.3%).
- All of the fish tissue samples achieved recoveries between 88 - 115%.
- A majority of the wastewater samples extracted by separatory funnel achieved recoveries between 60 - 130% (3588 out of 3648 results, or 98.4%)
- Almost all of the wastewater samples extracted by disk-based SPE achieved recoveries between 75 - 130% (3448 out of 3456 results, or 99.8%).
- A majority of the wastewater samples extracted by cartridge-based SPE achieved recoveries between 60 - 130% (3,267 out of 3,360 results, or 97.2%).

The single-laboratory validation results demonstrate that this method can identify and quantify individual PCB congeners.

- 2. The method is more sensitive than the currently approved Method 608.3.3, but is not so sensitive that it is adversely affected by typical laboratory background contamination.*

The only published sensitivity data for Method 608.3.3 are for Aroclor 1242, which has an MDL of 65 ng/L in aqueous samples. According to the 1996 Frame *et al.* data, the main constituents of Aroclor 1242 are PCB Congeners 8 (7.05%), 18 (8.53%), 28 (6.86%), 31 (7.34%), and 33 (5.01%). The highest aqueous MDL calculated in the single-laboratory study for PCB-18, the largest component of Aroclor 1242, was 0.96 ng/L. Assuming that all of the PCB-18 came from unweathered Aroclor 1242, and the other congeners were present at the proportions described by Frame *et al.*, the PCB-18 MDL suggests that Aroclor 1242 could be present at approximately 11 ng/L (0.96 ng/L/0.0853).

The method detection limits generated during the single-laboratory study demonstrate that this method is more sensitive than Method 608.3 and is not subject to any significant blank contamination.

Given that the single-laboratory study was conducted by the laboratory that developed the method, one of the major goals of the multi-laboratory study was to evaluate EPA's third criterion for a new PCB congener method.

3. The method can be implemented at a typical mid-sized full-service environmental laboratory.

The remainder of this report includes information that allowed EPA to address that goal. All of the required instrumentation for this method is available in any typical full-service environmental laboratory.

Multi-laboratory Study Goals and Design

The goals of the multi-laboratory validation study were to:

- Obtain data from matrices that are representative of the method's intended use
- Obtain data from laboratories that are representative of those likely to use the approved method, but that were not directly involved in its development
- Obtain feedback from laboratory users on the specifics of the draft method (e.g., is it clear and easy to understand, or are changes to the method text needed?)
- Use study data to characterize performance of the method
- Develop statistically derived QC acceptance criteria that will reflect method performance capabilities in real-world situations

The design of the multi-laboratory study is described in a formal study plan that is included as Appendix B to this report. The design is based on the specifications in EPA's *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program* (USEPA 2018). Briefly, the design involved:

- At least nine laboratories, with a goal of complete wastewater data from at least six laboratories
- Nine wastewater samples from a variety of sources
- Determination of retention times for all 209 PCB congeners and labeled analytes
- Multi-point calibration of the target analytes
- Initial demonstration of capability (IDC) by each laboratory
- Determination of method detection limits (MDLs) by each laboratory
- Analyses of matrix spike and matrix spike duplicate (MS/MSD) samples prepared from each of the nine wastewater samples

In addition, the study involved similar analyses of three samples each of sediment, biosolids, and fish tissue. Because these matrices are not subject to the same requirements for analyses by methods approved at 40 CFR Part 136 as are wastewater samples, fewer samples and laboratories were required for those portions of the study.

2. Identification and Selection of Laboratories

Prior to completing the single-laboratory study, potential participants in the anticipated multi-laboratory study were identified. By March 2018, through a combination of established relationships, review of an EPA database of laboratory capabilities, internet searches, and telephone calls, over 100 potential participants were identified, including commercial environmental laboratories, state laboratories, and utility laboratories. EPA identified potential Regional and emergency response laboratories within the Agency as well. Additional interest in the study was generated through poster and platform presentations by EPA at national and regional meetings.

Between June and October 2018, EPA conducted teleconference calls and a webinar with the potential participants to firm up the list of laboratories. Many of the potential participants decided that they would not be interested in participating, either because of time and staff constraints, unavailability of suitable instrumentation for the duration of the study, or for the utility laboratories in particular, an inability to contract with EPA's support contractor, CSRA, for the study. Ultimately, EPA decided to target a maximum of 20 participant laboratories, including those contracted and those who could volunteer.

CSRA developed a lengthy contractual statement of work (SOW) covering all aspects of the study, sent a formal solicitation to 12 commercial laboratories, many with multiple locations, received bids from 9 of those, and ultimately selected 8 commercial laboratories to receive purchase orders for participation. EPA arranged for 4 volunteer participants and used the CSRA SOW as the basis for a memorandum of understanding with each of the volunteer laboratories. The list of all 12 original participants is provided in Table 1.

Table 1. List of Participating Laboratories

Alpha Analytical Inc. Mansfield, MA	Southwest Research Institute San Antonio, TX
Apex Laboratories Tigard, OR	Weck Laboratories Industry, CA
Agriculture and Priority Pollutant Laboratories Clovis, CA	Department of Toxic Substances Control Pasadena, CA
Battelle Memorial Institute Norwell, MA	US EPA/OSWER OEM/CBRN CMAT Edison, NJ
Eurofins Lancaster Laboratories Lancaster, PA	CSS Castle Rock, CO
SGS North America Inc. Wilmington, NC	US EPA Region 4 Athens, GA

The primary focus of the study was on the analyses of wastewater samples and all 12 laboratories agreed to perform those analyses, by either separatory funnel extraction procedures, or solid-phase extraction procedures. A few laboratories agreed to perform wastewater analyses using both extraction procedures.

The other three matrix types (sediment, biosolids, and fish tissue) were the secondary focus of the study. Of the 12 laboratories:

- 8 agreed to analyze sediments by Soxhlet extraction, and 3 of those agreed to also analyze the sediments by another extraction procedure,
- 6 agreed to analyze the biosolids by Soxhlet extraction, and 3 of those agreed to also analyze the biosolids by another extraction procedure,
- 6 agreed to analyze the fish tissues by Soxhlet extraction, and 2 of those agreed to also analyze the fish tissues by another extraction procedure,

Immediately prior to the start of the study, EPA held kick-off calls with all of the participating laboratories to discuss the specifics on the study as a group. In order to accommodate the schedules, two kick-off calls were held in early February 2019. A summary of both calls, including answers to any questions raised by the participants, was circulated to all of the laboratories after the second call.

EPA subsequently held biweekly conference calls from February 2019 through October 2019. Because not all of the participants were able to attend every call, the discussion and any critical points were summarized and circulated by email to all of the participants after each call.

Unfortunately, despite EPA's efforts, not all twelve laboratories completed the study, or completed all of the analyses that they originally agreed to perform. In the end, only seven laboratories provided full data sets for all aspects of the wastewater portion of the study. The other five laboratories provided some data for the initial start-up phases of the study, but did not analyze any of the actual study samples. These five laboratories all cited time and resource constraints as the reason for dropping out of the study. None of the five laboratories dropped out of the study because they were unable to perform the analysis. Where practical, the data from those five laboratories were considered for use in the study. However, having data from seven laboratories met EPA's study design goals of acquiring data from at least six laboratories for the wastewater matrices.

Other than the list of laboratories in Table 1 above, the remainder of this report does not associate specific results with a named laboratory. Rather, each laboratory that completed any portion of the study was randomly assigned an identifying number between 1 and 12.

3. Study Samples

Wastewater Matrices

The wastewater samples used in the study were selected to meet the specifications in EPA's new method protocol (USEPA 2018), namely, that at least one of the wastewater matrix types should have one of the following characteristics, such that each criterion below is represented by at least one wastewater:

- Total suspended solids (TSS) greater than 40 mg/L
- Total dissolved solids (TDS) greater than 100 mg/L
- Oil and grease greater than 20 mg/L
- NaCl greater than 120 mg/L
- CaCO₃ greater than 140 mg/L

EPA worked to obtain large volumes of actual wastewaters and sufficient masses of soils/sediments, biosolids, and fish tissues. EPA contacted three major wastewater treatment operations: Massachusetts Water Resources Authority (MWRA), Los Angeles Sanitation, and the Hampton Roads Sanitation District (HRSD) to obtain bulk volumes of wastewater effluents and influents, as well as bulk samples of aqueous matrices from indirect dischargers to those systems. EPA also worked with the National Council for Air and Stream Improvement (NCASI) and the Delaware River Basin Commission (DRBC) to obtain bulk wastewaters representing a pulp and paper discharge and an industrial discharge.

EPA planned to prepare enough samples for up to 20 laboratories to participate in the study, so approximately 140 liters each of 10 aqueous matrices were collected by those organizations and shipped to ERA, a commercial preparer of performance testing samples, that was contracted to homogenize and aliquot the bulk samples into study-specific sizes and distribute them to each laboratory. During transit, containers of one of the bulk samples developed leaks. As a result, that wastewater matrix did not have enough volume to be used in the study. Fortunately, the other 9 bulk wastewaters were sufficient in quantity and characteristics to meet EPA's study design specifications. Table 2 contains a list of the bulk wastewater samples provided. (Because the numbering scheme for the wastewaters was assigned ahead of time and the samples were collected and shipped over the course of several weeks, when Matrix 1 did not provide sufficient volume because of leakage, the original numbering scheme was retained.)

Table 2. Bulk Wastewater Matrix Sources

Industry Type	Wastewater Matrix
Landfill leachate	2
Metal finisher	3
POTW Effluent	4
Hospital	5
POTW Influent	6
Bus washing station	7
Power Plant	8
Pulp and paper effluent	9
POTW Effluent	10

Once received at ERA, the bulk samples were homogenized and tested for the five water quality characteristics listed above. Three replicate analyses of each wastewater were performed and the mean result for each of the parameters was calculated. Each of those characteristic specifications were met by at least one of the nine bulk samples, and EPA deemed the nine samples suitable for use in the study. A summary of the characteristics is provided in Table 3. The shaded cells in Table 3 indicate that the sample met the requirements for that parameter. All of the bulk samples met the requirements for TDS and Conductivity. Four of the bulk samples met the requirements for TSS, and one each met the requirements for oil and grease, and hardness, respectively.

Table 3. Water Quality Characteristics of the Study Samples (in mg/L)

Wastewater	TSS	TDS	O&G	NaCl, as Conductivity	Hardness, as CaCO₃
2	168	4564.3	0.6667	7163	330
3	188	3681	1.07	4530	20.4
4	244	403	10.9	838	23.5
5	5.51	384	0.967	777	93.6
6	72	772	3.93	1708	67.5
7	29.0	509	23	688	23.2
8	8.97	143	0.333	256	31.8
9	37	1992	187	2815	205
10	9.69	893	0.0	1839	127

Following EPA's acceptance, the bulk samples were homogenized by placing the entire volume of each matrix lot in a 200-L bulk tank with a mechanical stirrer and thoroughly mixed for 30 minutes. The samples were aliquoted into 1-L glass containers and stored at ERA until they were shipped to the laboratories. Each laboratory received four 1-L bottles of each of the nine wastewaters, providing enough volume for analyses of the unspiked sample, an MS/MSD pair, and a spare bottle in case of issues in transit or during preparation at the laboratory. Additional samples were shipped to the laboratories which had agreed to extract the wastewater samples by both separatory funnel and solid-phase procedures. The remaining bottles were stored at ERA in case they were needed at a later date.

Biosolids Matrices

Two of the municipal treatment facilities provided bulk quantities of the finished biosolids from their treatment operations. Two biosolids were wet and one was in the form of dried pellets that are sold as a soil amendment. The bulk samples were also sent to ERA, where they were homogenized by placing the entire bulk volume of each matrix lot into a large Pyrex dish and stirred to mix. ERA determined the percent solids using a portion of the three bulk samples and aliquoted the samples into 2-oz screw cap jars with the required mass based on the determined dry weight (at least 5 grams dry weight). Biosolid sample #2 required augmentation with Ottawa sand to meet the volume demand. This sample was re-homogenized by mixing and % solids was re-performed prior to aliquoting. The jars were stored under refrigeration at ERA until shipment to the laboratories.

The percent solids data for each bulk sample were provided to the participating laboratories to be used to report dry-weight concentrations of the PCB congeners. That approach eliminated the need to ship additional material to each laboratory just for the solids determination, and reduced the variability in the study results that would have occurred by using the different solids contents determined in each individual laboratory in the study.

Sediment Matrices

The sediment samples used for the study were prepared from excess archived material maintained by CSRA. The samples were collected in 2011 as grab samples in the Raisin River in Monroe County, Michigan, as part of an EPA Great Lakes National Program Office (GLNPO) remedial assessment effort under the Great Lakes Legacy Act. GLNPO collected samples from a large area, sent some for analysis immediately after collection and had CSRA store other samples in the event that additional analyses were required.

CSRA had stored the archived samples in their original 1-gallon self-sealing plastic bags frozen at approximately -20 °C since 2011. After GLNPO determined that all the archived samples could be sent for disposal, CSRA retained a small number for possible use in studies such as this one. Aroclor 1242 was reported as present in many of the other samples in the original remedial assessment effort, and therefore, CSRA proposed to use a number of the samples for this PCB method validation study. Using

the results from the other samples collected near the archived samples, CSRA grouped the available archived samples into those likely to contain low, medium, and high levels of PCBs.

As with the biosolids, the bulk sediment samples were sent to ERA, where they were processed and stored as described above. In the case of the presumed low-level sediment sample (sediment #3), EPA agreed to augment that sample with Ottawa sand to provide a sufficient number of 10-g (dry weight) aliquots for 20 possible laboratories in the study. As with the biosolids samples, the percent solids data for each sediment sample were provided to participating laboratories to be used to report dry-weight concentrations of the PCB congeners.

Fish Tissue Matrices

The fish tissue samples used for the study were prepared from excess archived material maintained by CSRA. The samples were field duplicate samples collected as part of the National Lake Fish Tissue Study between 2000 and 2004, homogenized and stored, but never analyzed. CSRA had stored the archived samples in their original 500-mL glass jars, frozen at approximately -20 °C. As with the sediment samples, when EPA released these field duplicate samples for disposal, CSRA retained a selection of the excess jars for other uses.

CSRA selected samples representing three common freshwater species: white sucker, largemouth bass, and common carp, and used PCB congener results generated for the National Lake Fish Tissue Study from the samples of these species collected from the same site, to group the samples together by concentration level. Ultimately:

- Four jars of homogenized fillet tissue from white sucker specimens that were collected from four sites around the U.S. were composited to create a low-level PCB sample,
- Four jars of homogenized fillet tissue from largemouth bass specimens that were collected from four other sites around the U.S. were composited to create a medium-level PCB sample, and
- Three jars of homogenized fillet tissue from common carp specimens that were collected from three sites around the U.S. were composited to create a high-level PCB sample.

These three fish species represent both predator species (large-mouth bass) and bottom-dwelling species (white sucker and common carp), as well as a range of lipid contents (~1 to 7%).

The jars of fish tissue were sent to ERA for compositing and further homogenization. The individual jars of the matrix lot were combined into one large glass dish and stirred to mix. Each homogenized study sample was divided into 10-g (wet weight) aliquots in 2-oz screw-top jars and stored frozen until shipped to a participating laboratory.

Reconnaissance Analyses

As noted earlier, the study design called for analyses of MS/MSD samples. In order to provide information to each participant lab about the concentrations at which to spike those MS/MSD pairs of each sample, EPA sent single aliquots of each study sample to SGS AXYS Analytical, the developer of the original laboratory SOP used as the basis for the draft method. The results from those analyses were used by EPA and CSRA to develop spiking concentrations for the MS/MSD aliquots of each of the study samples. Those results that passed the identification criteria in the draft method are summarized in Table 4 and they became the basis for study-specific instructions that were distributed to each laboratory before sample analyses began. Additional peaks were present in many of those reconnaissance analyses that met most, but not all, of the identification criteria. Those peaks are not counted in Table 4, but the results were used to guide the spiking levels for the congeners involved (i.e., the instructions considered that those congeners might be present and were candidates for the spiking instructions).

The reconnaissance results of the sediment and tissue samples also confirmed CSRA's characterization of the components of each of the composite samples to create study samples that contained low, medium, and high concentration of PCBs. The reconnaissance results were *not* used to assess performance of the participant laboratories in this study, nor were they used as "true values" in any way.

Additional aliquots of each of the study samples were sent to another commercial laboratory and analyzed for Aroclors, using EPA Method 608.3, a GC/ECD method that is currently approved at 40 CFR Part 136.3 for NPDES compliance monitoring of PCBs as Aroclors. The purpose of those analyses was to be able to contrast the results for samples in which Aroclors were not originally reported with the PCB congener results from the draft procedure.

The laboratory contracted for the Aroclor analyses had method detection limits (MDLs) for Aroclor 1016 and Aroclor 1260 in aqueous matrices at 4.8 ng/L and 3.9 ng/L, and in solid matrices at 0.41 ng/g and 0.36 ng/g. Those aqueous MDLs are well below the published Method 608.3 Aroclor MDL of 65 ng/L. Although Method 608.3 does not address solid samples directly, the laboratory's solid MDLs are well below the value one would obtain by converting the method's aqueous MDL into an estimate of the detection limit in a solid sample.

Table 4. Reconnaissance Analyses Results for Study Samples

Study Sample	# Peaks Detected*	Sum of Detected Analyte Concentrations (ng/L or ng/g)		Gross Characterization
		as PCB congeners	as Aroclors	
Wastewater 2	40	84.9	0	NA
Wastewater 3	0	0	0	NA
Wastewater 4	0	0	0	NA
Wastewater 5	0	0	0	NA
Wastewater 6	5	2.3	0	NA
Wastewater 7	0	0	0	NA
Wastewater 8	0	0	0	NA
Wastewater 9	0	0	0	NA
Wastewater 10	0	0	0	NA
Sediment 1	135	1208	210	Medium
Sediment 2	92	248	12	Low
Sediment 3	135	1454	460	High
Biosolids 1	86	99.2	25	NA
Biosolids 2	12	6.7	0	NA
Biosolids 3	119	246	110	NA
Tissue 1	46	2.7	0.9	Low
Tissue 2	76	15.4	2.5	Medium
Tissue 3	105	98.6	41	High

*Peaks in the congener analysis that met the identification criteria. Some peaks represent more than one congener.

NA = Not applicable. No information was available with which to characterize the wastewater or biosolids samples prior to the start of the study.

4. Approaches to Calibration and Quantification

The draft procedure calibrates and quantifies 65 target PCB congeners by one of three different approaches:

- True isotope dilution quantification (ID), whereby the response of the target congener is compared to the response of its $^{13}\text{C}_{12}$ -labeled analog. 23 target congeners are quantified in this way.
- Modified isotope dilution (mID), when one or more congeners in the same level of chlorination (LOC) coelute with a congener that has a $^{13}\text{C}_{12}$ -labeled analog. 14 target congeners are quantified in this way (6 with $^{13}\text{C}_{12}$ -labeled analogs and 8 that coelute with one of those 6).
- Extracted internal standard quantification (EIS), whereby the response of the target congener (or one or more congeners in the same level of chlorination that coelute) is compared to the response of the $^{13}\text{C}_{12}$ -labeled analog of another congener in the same level of chlorination (LOC) with which it coelutes. 28 target congeners are quantified in this way.

Of these 65 congeners, 48 are those that EPA chose as high priorities for the procedure because of their retention times (e.g., first and last eluting congeners in a LOC), prevalence in environmental samples, high concentrations in Aroclors, and their toxicities (see the introduction to this report for further details). The other 17 congeners coelute with one of the 48 high priority congeners.

The remaining 144 congeners are quantified indirectly using isotope dilution standards of similar congeners with the same level of chlorination. The response factor is assumed to be the same as the reference isotope dilution standard. This approach may produce less accurate results for these congeners than using any of the three approaches described above, but calibrating all of the congeners would make the level of effort more burdensome for the laboratories that are the intended users of this procedure. These congeners were seen less often and/or at lower concentrations in the environmental databases surveyed and the original Aroclor formulations. For the purposes of this report, EPA is referring to these 144 congeners as the congeners without direct calibration data.

Note: If any of those 144 congeners are a priority to a specific data user, the laboratory is welcome and encouraged to calibrate additional congeners using any of the three approaches listed above. This is allowed under the flexibility of the method.

During analysis of samples, the labeled compound is added to the sample before any other processing or analysis steps and the final result for the target congener is corrected for any losses (or apparent gains) of the labeled analogue during the entire analytical process. This recovery correction is inherent in the calculations and is applied to the results for all of the congeners, regardless of the specific calibration and quantification approach described above.

Table 5 provides the list of the 65 congeners with direct calibration data as well as the approach to calibration and quantification used for each.

Table 5. Quantification Reference and Calibration Approach for the 65 Congeners with Direct Calibration Data

Target Congener	LOC	Quantification Reference	Calibration Approach
<i>Isotope Dilution and Modified Isotope Dilution Quantification</i>			
PCB-1	Mono	$^{13}\text{C}_{12}$ -PCB-1	ID
PCB-3		$^{13}\text{C}_{12}$ -PCB-3	ID
PCB-4+10	Di	$^{13}\text{C}_{12}$ -PCB-4	mID
PCB-11		$^{13}\text{C}_{12}$ -PCB-11	ID
PCB-15		$^{13}\text{C}_{12}$ -PCB-15	ID

Table 5. Quantification Reference and Calibration Approach for the 65 Congeners with Direct Calibration Data

Target Congener	LOC	Quantification Reference	Calibration Approach
PCB-19	Tri	$^{13}\text{C}_{12}\text{-PCB-19}$	ID
PCB-28		$^{13}\text{C}_{12}\text{-PCB-28}$	ID
PCB-37		$^{13}\text{C}_{12}\text{-PCB-37}$	ID
PCB-52+73	Tetra	$^{13}\text{C}_{12}\text{-PCB-52}$	mID
PCB-54		$^{13}\text{C}_{12}\text{-PCB-54}$	ID
PCB-70		$^{13}\text{C}_{12}\text{-PCB-70}$	ID
PCB-77		$^{13}\text{C}_{12}\text{-PCB-77}$	ID
PCB-85+120	Penta	$^{13}\text{C}_{12}\text{-PCB-85}$	mID
PCB-89+90+101		$^{13}\text{C}_{12}\text{-PCB-101}$	mID
PCB-104		$^{13}\text{C}_{12}\text{-PCB-104}$	ID
PCB-106+118		$^{13}\text{C}_{12}\text{-PCB-118}$	mID
PCB-126		$^{13}\text{C}_{12}\text{-PCB-126}$	ID
PCB-138+163+164	Hexa	$^{13}\text{C}_{12}\text{-PCB-138}$	mID
PCB-153		$^{13}\text{C}_{12}\text{-PCB-153}$	ID
PCB-155		$^{13}\text{C}_{12}\text{-PCB-155}$	ID
PCB-169		$^{13}\text{C}_{12}\text{-PCB-169}$	ID
PCB-180	Hepta	$^{13}\text{C}_{12}\text{-PCB-180}$	ID
PCB-188		$^{13}\text{C}_{12}\text{-PCB-188}$	ID
PCB-189		$^{13}\text{C}_{12}\text{-PCB-189}$	ID
PCB-202	Octa	$^{13}\text{C}_{12}\text{-PCB-202}$	ID
PCB-205		$^{13}\text{C}_{12}\text{-PCB-205}$	ID
PCB-206	Nona	$^{13}\text{C}_{12}\text{-PCB-206}$	ID
PCB-208		$^{13}\text{C}_{12}\text{-PCB-208}$	ID
PCB-209	Deca	$^{13}\text{C}_{12}\text{-PCB-209}$	ID
Extracted Internal Standard Quantification			
PCB-5+8	Di	$^{13}\text{C}_{12}\text{-PCB-11}$	EIS
PCB-18	Tri	$^{13}\text{C}_{12}\text{-PCB-28}$	EIS
PCB-31		$^{13}\text{C}_{12}\text{-PCB-28}$	EIS
PCB-41+64	Tetra	$^{13}\text{C}_{12}\text{-PCB-70}$	EIS
PCB-44		$^{13}\text{C}_{12}\text{-PCB-52}$	EIS
PCB-66+80		$^{13}\text{C}_{12}\text{-PCB-70}$	EIS
PCB-61+74		$^{13}\text{C}_{12}\text{-PCB-70}$	EIS
PCB-93+95	Penta	$^{13}\text{C}_{12}\text{-PCB-101}$	EIS
PCB-99		$^{13}\text{C}_{12}\text{-PCB-101}$	EIS
PCB-105+127		$^{13}\text{C}_{12}\text{-PCB-118}$	EIS
PCB-110		$^{13}\text{C}_{12}\text{-PCB-118}$	EIS
PCB-132+168	Hexa	$^{13}\text{C}_{12}\text{-PCB-153}$	EIS
PCB-147		$^{13}\text{C}_{12}\text{-PCB-153}$	EIS
PCB-139+149		$^{13}\text{C}_{12}\text{-PCB-153}$	EIS
PCB-156		$^{13}\text{C}_{12}\text{-PCB-153}$	EIS
PCB-166		$^{13}\text{C}_{12}\text{-PCB-153}$	EIS
PCB-177	Hepta	$^{13}\text{C}_{12}\text{-PCB-180}$	EIS
PCB-182+187		$^{13}\text{C}_{12}\text{-PCB-180}$	EIS
PCB-199	Octa	$^{13}\text{C}_{12}\text{-PCB-202}$	EIS

ID = Isotope dilution quantitation

mID = Modified isotope dilution quantitation

EIS = Extracted internal standard quantitation

Table 6 provides the lists of the congeners not calibrated directly and the quantification references that are used to estimate their concentrations in samples.

Table 6. Congeners Not Calibrated Directly

Congener	LOC	Quantification Reference
PCB-2	Mono	PCB-3
PCB-6	Di	PCB-11
PCB-7+9		PCB-11
PCB-12+13		PCB-11
PCB-14		PCB-11
PCB-16+32		PCB-18
PCB-17	Tri	PCB-18
PCB-20+21+33		PCB-28+31 (Average) ¹
PCB-22		PCB-28+31 (Average) ¹
PCB-23+34		PCB-28+31 (Average) ¹
PCB-24+27		PCB-28+31 (Average) ¹
PCB-25		PCB-28+31 (Average) ¹
PCB-26		PCB-28+31 (Average) ¹
PCB-29		PCB-28+31 (Average) ¹
PCB-30		PCB-28+31 (Average) ¹
PCB-35		PCB-28+31 (Average) ¹
PCB-36		PCB-28+31 (Average) ¹
PCB-38		PCB-28+31 (Average) ¹
PCB-39		PCB-28+31 (Average) ¹
PCB-40	Tetra	PCB-44
PCB-42		PCB-44
PCB-43+49		PCB-44
PCB-45		PCB-44
PCB-46		PCB-44
PCB-47+48+75		PCB-52+73
PCB-50		PCB-52+73
PCB-51		PCB-52+73
PCB-53		PCB-52+73
PCB-55		PCB-70
PCB-56+60		PCB-70
PCB-57		PCB-70
PCB-58		PCB-70
PCB-59		PCB-41+64
PCB-62		PCB-41+64
PCB-63		PCB-70
PCB-65		PCB-41+64
PCB-67		PCB-70
PCB-68		PCB-70
PCB-69		PCB-41+64
PCB-71	Penta	PCB-41+64
PCB-72		PCB-70
PCB-76		PCB-70
PCB-78		PCB-70
PCB-79		PCB-70
PCB-81		PCB-77
PCB-82		PCB-89+90+101
PCB-83+109		PCB-89+90+101
PCB-84		PCB-93+95
PCB-86+97		PCB-89+90+101
PCB-87+115+116		PCB-89+90+101
PCB-88+121		PCB-93+95
PCB-91		PCB-93+95
PCB-92		PCB-89+90+101
PCB-94		PCB-93+95

Table 6. Congeners Not Calibrated Directly

Congener	LOC	Quantification Reference
PCB-96	Penta	PCB-104
PCB-98+102		PCB-93+95
PCB-100		PCB-93+95
PCB-103		PCB-93+95
PCB-107+108		PCB-106+118
PCB-111+117		PCB-110
PCB-112		PCB-110
PCB-113		PCB-110
PCB-114		PCB-106+118
PCB-119		PCB-110
PCB-122		PCB-106+118
riPCB-123		PCB-106+118
PCB-124		PCB-106+118
PCB-125		PCB-110
PCB-128		PCB-132+168
PCB-129		PCB-132+168
PCB-130		PCB-132+168
PCB-131+142	Hexa	PCB-132+168
PCB-133		PCB-132+168
PCB-134		PCB-132+168
PCB-135+144		PCB-147
PCB-136		PCB-153
PCB-137		PCB-132+168
PCB-140		PCB-147
PCB-141		PCB-132+168
PCB-143		PCB-147
PCB-145		PCB-153
PCB-146		PCB-153
PCB-148		PCB-147
PCB-150		PCB-153
PCB-151		PCB-147
PCB-152		PCB-153
PCB-154		PCB-147
PCB-157		PCB-156
PCB-158+160	Hepta	PCB-166
PCB-159		PCB-156
PCB-161		PCB-166
PCB-162		PCB-156
PCB-165		PCB-153
PCB-167		PCB-156
PCB-170+190		PCB-180
PCB-171		PCB-181
PCB-172+192		PCB-180
PCB-173		PCB-181
PCB-174		PCB-181
PCB-175		PCB-180
PCB-176		PCB-188
PCB-178		PCB-181
PCB-179		PCB-188
PCB-181		PCB-180
PCB-183		PCB-180
PCB-184		PCB-188
PCB-185		PCB-181
PCB-186		PCB-188

Table 6. Congeners Not Calibrated Directly

Congener	LOC	Quantification Reference
PCB-191	Hepta	PCB-189
PCB-193		PCB-189
PCB-194	Octa	PCB-199
PCB-195		PCB-199
PCB-196+203		PCB-199
PCB-197		PCB-202
PCB-198		PCB-199
PCB-200		PCB-202
PCB-201		PCB-202
PCB-204		PCB-202
PCB-207	Nona	PCB-208

¹ The quantification reference for these 12 congeners is the average of the response ratio for PCB-28 and the response factor for PCB-31, which are calibrated as individual congeners by isotope dilution and extracted internal standard, respectively. In contrast, other congeners in this table use the single response factor of a coeluting pair of congeners that are calibrated as that pair (e.g., PCB-52+73).

The 29 ¹³C₁₂-labeled analogs themselves are present in each standard at a constant concentration that reflects the concentration of the label that is added to each sample. All of the ¹³C₁₂-labeled analogs that are added to the samples before extraction are quantified by the traditional EPA non-extracted internal standard (NIS) approach, whereby three other labeled compounds (¹³C₁₂-PCB-8, ¹³C₁₂-PCB-79, and ¹³C₁₂-PCB-162) are added to each sample *extract* shortly before GC/MS analysis and the responses of those three compounds are used to quantify the other ¹³C₁₂-labeled analogs. In some procedures, those last three labeled compounds may be referred to as “recovery standards” because they are used to determine the recovery of the other labeled compounds. In Table 7 below, they are referred to as the non-extracted internal standards.

Multi-point Initial Calibration

The GC/MS instrument was calibrated using a series of six calibration standards designated as CS1 to CS6. The concentrations of six calibration standards are shown in Table 7 below, along with the approach used for calibration.

Table 7. Composition of the Initial Calibration Standards

Analyte	Calibration Standards (ng/mL)						Coeluting Congeners	Calibration Approach
	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6		
Target Congeners								
PCB-1	10	20	40	160	400	2000		ID
PCB-3	10	20	40	160	400	2000		ID
PCB-4	10	20	40	160	400	2000	PCB-10 ¹	mID
PCB-8	10	20	40	160	400	2000	PCB-5 ²	EIS
PCB-11	10	20	40	160	400	2000		ID
PCB-15	10	20	40	160	400	2000		ID
PCB-18	10	20	40	160	400	2000		EIS
PCB-19	10	20	40	160	400	2000		ID
PCB-28	10	20	40	160	400	2000		ID
PCB-31	10	20	40	160	400	2000		EIS
PCB-37	10	20	40	160	400	2000		ID
PCB-44	10	20	40	160	400	2000		EIS
PCB-52	10	20	40	160	400	2000	PCB-73 ¹	mID
PCB-54	10	20	40	160	400	2000		ID
PCB-64	10	20	40	160	400	2000	PCB-41 ²	EIS
PCB-66	10	20	40	160	400	2000	PCB-80 ²	EIS
PCB-70	10	20	40	160	400	2000		ID

Table 7. Composition of the Initial Calibration Standards

Analyte	Calibration Standards (ng/mL)						Coeluting Congeners	Calibration Approach
	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6		
PCB-74	10	20	40	160	400	2000	PCB-61 ²	EIS
PCB-77	10	20	40	160	400	2000		ID
PCB-85	10	20	40	160	400	2000	PCB-120 ¹	mID
PCB-95	10	20	40	160	400	2000	PCB-93 ²	EIS
PCB-99	10	20	40	160	400	2000		EIS
PCB-101	10	20	40	160	400	2000	PCB-89+0 ¹	mID
PCB-104	10	20	40	160	400	2000		ID
PCB-105	10	20	40	160	400	2000	PCB-127 ²	EIS
PCB-110	10	20	40	160	400	2000		EIS
PCB-118	10	20	40	160	400	2000	PCB-106 ¹	mID
PCB-126	10	20	40	160	400	2000		ID
PCB-132	10	20	40	160	400	2000	PCB-168 ²	EIS
PCB-138	10	20	40	160	400	2000	PCB-163+164 ¹	mID
PCB-147	10	20	40	160	400	2000		EIS
PCB-149	10	20	40	160	400	2000	PCB-139 ²	EIS
PCB-153	10	20	40	160	400	2000		ID
PCB-155	10	20	40	160	400	2000		ID
PCB-156	10	20	40	160	400	2000		EIS
PCB-166	10	20	40	160	400	2000		EIS
PCB-169	10	20	40	160	400	2000		ID
PCB-177	10	20	40	160	400	2000		EIS
PCB-180	10	20	40	160	400	2000		ID
PCB-187	10	20	40	160	400	2000	PCB-182 ²	EIS
PCB-188	10	20	40	160	400	2000		ID
PCB-189	10	20	40	160	400	2000		ID
PCB-199	10	20	40	160	400	2000		EIS
PCB-202	10	20	40	160	400	2000		ID
PCB-205	10	20	40	160	400	2000		ID
PCB-206	10	20	40	160	400	2000		ID
PCB-208	10	20	40	160	400	2000		ID
PCB-209	10	20	40	160	400	2000		ID
Labeled Congeners								
¹³ C ₁₂ -PCB-1	400	400	400	400	400	400	NIS	NIS
¹³ C ₁₂ -PCB-3	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-4	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-11	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-15	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-19	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-28	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-37	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-52	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-54	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-70	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-77	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-85	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-101	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-104	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-118	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-126	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-138	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-153	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-155	400	400	400	400	400	400		NIS

Table 7. Composition of the Initial Calibration Standards

Analyte	Calibration Standards (ng/mL)						Coeluting Congeners	Calibration Approach
	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6		
¹³ C ₁₂ -PCB-169	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-180	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-188	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-189	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-202	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-205	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-206	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-208	400	400	400	400	400	400		NIS
¹³ C ₁₂ -PCB-209	400	400	400	400	400	400		NIS
Non-extracted Internal Standards								
¹³ C ₁₂ -PCB-8	400	400	400	400	400	400		NA
¹³ C ₁₂ -PCB-79	400	400	400	400	400	400		NA
¹³ C ₁₂ -PCB-162	400	400	400	400	400	400		NA

¹ These coeluting congeners are not included in the calibration standard, but the responses in the samples for all of the congeners that elute together at a given retention time are quantified by modified isotope dilution, based on the response ratio derived for the single congener in the calibration standard and its corresponding labeled analogue.

² These coeluting congeners are not included in the calibration standard, but the responses in the samples for all of the congeners that elute together at a given retention time are quantified by extracted internal standard, based on the response factor derived for the single congener in the calibration standard and the labeled analogue for another congener in the same level of chlorination.

ID = Isotope dilution quantitation

mID = Modified isotope dilution quantitation

EIS = Extracted internal standard quantitation

NIS = Non-extracted internal standard quantitation

NA = Not applicable - these congeners are not quantified

For the purpose of the multi-laboratory study, EPA procured full sets of all of the standards employed in the method from commercial vendors. These standards were ordered as custom mixtures of the six calibration standards (CS1 to CS6), as well as a spiking solution of the 65 native congeners of primary interest, the non-extracted internal standard solution, and a series of commercially available mixtures of all 209 native PCB congeners that was used to establish the retention time of each congener in each laboratory.

By providing these standards to all of the laboratories, EPA reduced the variability in the study results that would have resulted from having each laboratory prepare all of the standards from neat materials. This approach also reduced the direct costs to each laboratory for their participation, allowing more laboratories to participate. It also expanded the pool of potential participants because not all commercial laboratories are willing or able to prepare standards from neat materials.

EPA anticipates that if this method comes into routine use, the vendor community will continue to provide these method-specific standards as routine commercial products, as they do now for many other EPA monitoring methods.

Response Ratios and Response Factors

The response ratio (RR) for each congener calibrated by isotope dilution is calculated according to the equation below, separately for each of the calibration standards, using the areas of the ions with the mass-to-charge ratios (*m/z*) shown in Table 8.

$$RR = \frac{Area_n C_l}{Area_l C_n}$$

where:

Area_n = The measured area of the primary *m/z* for the native (unlabeled) PCB
 Area_l = The measured area at the primary *m/z* for the labeled PCB

- C_l = The concentration of the labeled compound in the calibration standard
 C_n = The concentration of the native compound in the calibration standard

This response ratio is used for the 23 congeners quantified by true isotope dilution and the 14 congeners quantified by modified isotope dilution.

Similarly, the response factor (RF) for each unlabeled congener calibrated by extracted internal standard is calculated according to the equation below.

$$RF = \frac{Area_s C_{eis}}{Area_{eis} C_s}$$

where:

- $Area_s$ = The measured area of the primary m/z for the target (unlabeled) PCB
 $Area_{eis}$ = The measured area at the primary m/z for the labeled PCB used as the extracted internal standard
 C_{ris} = The concentration of the labeled compound used as the extracted internal standard in the calibration standard
 C_s = The concentration of the target compound in the calibration standard

This response factor is used for the 28 congeners quantified by extracted internal standard.

Similarly, a response factor is calculated for each labeled compound added before extraction using the following equation:

$$RF = \frac{Area_l C_{nis}}{Area_{nis} C_l}$$

where:

- $Area_l$ = The measured area of the primary m/z for the labeled PCB standard added to the sample before extraction
 $Area_{nis}$ = The measured area at the primary m/z for the labeled PCB used as the non-extracted internal standard
 C_{nis} = The concentration of the labeled compound used as the non-extracted internal standard in the calibration standard
 C_l = The concentration of the labeled PCB standard added to the sample before extraction

This response factor is used for the 29 labeled congeners quantified by non-extracted internal standard.

Mass-to-charge Ratios Monitored for Each Analyte

The equations above for the response ratio and the response factor are based on the area of the more intense of the two characteristic ions produced by each congener under the mass spectrometer electron impact (EI) operating conditions described in the draft method. For the purposes of this method, the “primary ion” is the ion with the most intense response and the “confirmation ion” is the next most intense response. For all of the analytes, the mass difference between the two m/zs is 2 Daltons and represents the presence or absence of an atom of the less common isotope ^{37}Cl in the m/z versus the more common ^{35}Cl isotope. For some congeners, the higher m/z is the primary ion, but in most cases, the lower m/z provides the most intense response.

The draft method employs “single-ion quantitation,” whereby the area response of the primary m/z for each analyte is used to calculate a response ratio (RR) or response factor (RF) for each calibration standard. The response of the confirmation m/z is not used to determine the RR or RF value, or to quantify the analyte in a sample. However, the confirmation ion must be present and the ratio of the abundance of the primary m/z to the confirmation m/z must meet an acceptance limit centered around the theoretical abundance of all of the atoms making up the analyte in order to confirm the identification of the analyte.

Table 8 presents the ions monitored for the 65 congeners with direct calibration data and all of the labeled compounds, while Table 9 presents similar data for the 144 other congeners that are analyzed by the draft method using the indirect calibration approach described above.

Table 8. Ions Monitored for each Congener with Direct Calibration Data and the Labeled Compounds, in Retention Time Order

Congener	Primary ¹	Confirmation ²	Expected Ratio (%) ³
¹³ C ₁₂ - PCB-8 (NIS) ⁴	234	236	65.6
¹³ C ₁₂ - PCB-1	200	202	33.2
PCB-1	188	190	33.2
¹³ C ₁₂ - PCB-3	200	202	33.2
PCB-3	188	190	33.2
¹³ C ₁₂ - PCB-4	234	236	65.6
PCB-4+10	222	224	65.6
¹³ C ₁₂ -PCB-11	234	236	65.6
PCB-8+5	222	224	65.6
PCB-11	222	224	65.6
¹³ C ₁₂ -PCB-15	234	236	65.6
PCB-15	222	224	65.6
¹³ C ₁₂ -PCB-19	268	270	98.0
PCB-19	256	258	98.0
¹³ C ₁₂ -PCB-28	268	270	98.0
PCB-18	256	258	98.0
PCB-31	256	258	98.0
PCB-28	256	258	98.0
¹³ C ₁₂ -PCB-37	268	270	98.0
PCB-37	256	258	98.0
¹³ C ₁₂ -PCB-79 (NIS) ⁴	304	302	76.7
¹³ C ₁₂ -PCB-54	304	302	76.7
PCB-54	292	290	76.7
¹³ C ₁₂ -PCB-52	304	302	76.7
PCB-18	292	290	76.7
PCB-52+73	292	290	76.7
PCB-44	292	290	76.7
¹³ C ₁₂ -PCB-70	304	302	76.7
PCB-41+64	292	290	76.7
PCB-74+61	292	290	76.7
PCB-70	292	290	76.7
PCB-66+80	292	290	76.7
¹³ C ₁₂ -PCB-77	304	302	76.7
PCB-77	292	290	76.7
¹³ C ₁₂ -PCB-162 (NIS) ⁴	372	374	81.5
¹³ C ₁₂ -PCB-104	338	340	65.3
PCB-104	326	328	65.3
¹³ C ₁₂ -PCB-101	338	340	65.3
PCB-95+93	326	328	65.3
PCB-90+101+89	326	328	65.3
PCB-99	326	328	65.3
¹³ C ₁₂ -PCB-118	338	340	65.3
PCB-110	326	328	65.3
PCB-118+106	326	328	65.3
PCB-105+127	326	328	65.3
¹³ C ₁₂ -PCB-85	338	340	65.3
PCB-85+120	326	328	65.3
¹³ C ₁₂ -PCB-126	338	340	65.3
PCB-126	326	328	65.3

Table 8. Ions Monitored for each Congener with Direct Calibration Data and the Labeled Compounds, in Retention Time Order

Congener	Primary ¹	Confirmation ²	Expected Ratio (%) ³
¹³ C ₁₂ -PCB-155	372	374	81.5
PCB-155	360	362	81.5
¹³ C ₁₂ -PCB-153	372	374	81.5
PCB-147	360	362	81.5
PCB-149+139	360	362	81.5
PCB-153	360	362	81.5
PCB-132+168	360	362	81.5
PCB-166	360	362	81.5
PCB-156	360	362	81.5
¹³ C ₁₂ -PCB-138	372	374	81.5
PCB-138+163+164	360	362	81.5
¹³ C ₁₂ -PCB-169	372	374	81.5
PCB-169	360	362	81.5
¹³ C ₁₂ -PCB-188	406	408	97.7
PCB-188	394	396	97.7
¹³ C ₁₂ -PCB-180	406	408	97.7
PCB-187+182	394	396	97.7
PCB-177	394	396	97.7
PCB-180	394	396	97.7
¹³ C ₁₂ -PCB-189	406	408	97.7
PCB-189	394	396	97.7
¹³ C ₁₂ -PCB-202	442	440	87.8
PCB-202	430	428	87.8
PCB-199	430	428	87.8
¹³ C ₁₂ -PCB-205	442	440	87.8
PCB-205	430	428	87.8
¹³ C ₁₂ -PCB-208	476	474	76.9
PCB-208	464	462	76.9
¹³ C ₁₂ -PCB-206	476	474	76.9
PCB-206	464	462	76.9
¹³ C ₁₂ -PCB-209	510	512	86.7
PCB-209	498	500	86.7

¹ The primary ion is the more intense ion of the two ions monitored for each analyte. Its area is used in calculating the RR or RF values and for calculating the concentration of the analyte in samples.

² The confirmation ion is the less intense ion of the two ions monitored for each analyte. Its area is not used in the calculation of RR or RF values, or calculating the concentration of the analyte in samples. However, it is used as part of the qualitative identification criteria for demonstrating that the analyte is present.

³ The expected ratio is the area of the confirmation ion divided by the area of the primary ion. All values are shown in percent and are less than 100%, indicating that the primary ion has the more intense response.

⁴ Labeled congeners 8, 79, and 162 are added to the final extract immediately before injection and their responses are used to quantify the other labeled compounds added to the sample prior to extraction. These are termed “non-extracted internal standard” or “NIS”

Table 9. Ions Monitored for the 144 Other Congeners, in Retention Time Order

Congener	Primary ¹	Confirmation ²	Expected Ratio (%) ³
PCB-2	188	190	33.2
PCB-7+9	222	224	65.6
PCB-6	222	224	65.6
PCB-14	222	224	65.6
PCB-12+13	222	224	65.6
PCB-30	256	258	98.0
PCB-17	256	258	98.0
PCB-24+27	256	258	98.0

Table 9. Ions Monitored for the 144 Other Congeners, in Retention Time Order

Congener	Primary ¹	Confirmation ²	Expected Ratio (%) ³
PCB-16+32	256	258	98.0
PCB-34+23	256	258	98.0
PCB-29	256	258	98.0
PCB-26	256	258	98.0
PCB-25	256	258	98.0
PCB-33+20+21	256	258	98.0
PCB-22	256	258	98.0
PCB-36	256	258	98.0
PCB-39	256	258	98.0
PCB-38	256	258	98.0
PCB-35	256	258	98.0
PCB-50	292	290	76.7
PCB-51	292	290	76.7
PCB-45	292	290	76.7
PCB-46	292	290	76.7
PCB-49+43	292	290	76.7
PCB-47+48+75	292	290	76.7
PCB-42	292	290	76.7
PCB-40	292	290	76.7
PCB-69	292	290	76.7
PCB-65	292	290	76.7
PCB-62	292	290	76.7
PCB-59	292	290	76.7
PCB-72	292	290	76.7
PCB-71	292	290	76.7
PCB-68	292	290	76.7
PCB-57	292	290	76.7
PCB-67	292	290	76.7
PCB-58	292	290	76.7
PCB-63	292	290	76.7
PCB-76	292	290	76.7
PCB-55	292	290	76.7
PCB-56+60	292	290	76.7
PCB-79	292	290	76.7
PCB-78	292	290	76.7
PCB-81	292	290	76.7
PCB-96	326	328	65.3
PCB-103	326	328	65.3
PCB-100	326	328	65.3
PCB-94	326	328	65.3
PCB-98+102	326	328	65.3
PCB-88+121	326	328	65.3
PCB-91	326	328	65.3
PCB-92	326	328	65.3
PCB-84	326	328	65.3
PCB-83+109	326	328	65.3
PCB-97+86	326	328	65.3
PCB-87+115+116	326	328	65.3
PCB-82	326	328	65.3
PCB-113	326	328	65.3
PCB-119	326	328	65.3
PCB-112	326	328	65.3
PCB-125	326	328	65.3
PCB-111+117	326	328	65.3

Table 9. Ions Monitored for the 144 Other Congeners, in Retention Time Order

Congener	Primary ¹	Confirmation ²	Expected Ratio (%) ³
PCB-124	326	328	65.3
PCB-107+108	326	328	65.3
PCB-123	326	328	65.3
PCB-114	326	328	65.3
PCB-122	326	328	65.3
PCB-150	360	362	81.5
PCB-152	360	362	81.5
PCB-145	360	362	81.5
PCB-148	360	362	81.5
PCB-136	360	362	81.5
PCB-154	360	362	81.5
PCB-151	360	362	81.5
PCB-144+135	360	362	81.5
PCB-140	360	362	81.5
PCB-143	360	362	81.5
PCB-134	360	362	81.5
PCB-133	360	362	81.5
PCB-131+142	360	362	81.5
PCB-165	360	362	81.5
PCB-146	360	362	81.5
PCB-161	360	362	81.5
PCB-141	360	362	81.5
PCB-137	360	362	81.5
PCB-130	360	362	81.5
PCB-158+160	360	362	81.5
PCB-129	360	362	81.5
PCB-166	360	362	81.5
PCB-159	360	362	81.5
PCB-162	360	362	81.5
PCB-128	360	362	81.5
PCB-167	360	362	81.5
PCB-156	360	362	81.5
PCB-157	360	362	81.5
PCB-184	394	396	97.7
PCB-179	394	396	97.7
PCB-176	394	396	97.7
PCB-186	394	396	97.7
PCB-178	394	396	97.7
PCB-175	394	396	97.7
PCB-183	394	396	97.7
PCB-185	394	396	97.7
PCB-174	394	396	97.7
PCB-181	394	396	97.7
PCB-171	394	396	97.7
PCB-173	394	396	97.7
PCB-172+192	394	396	97.7
PCB-170+190	394	396	97.7
PCB-193	394	396	97.7
PCB-191	394	396	97.7
PCB-201	430	428	87.8
PCB-204	430	428	87.8
PCB-197	430	428	87.8
PCB-200	430	428	87.8
PCB-198	430	428	87.8

Table 9. Ions Monitored for the 144 Other Congeners, in Retention Time Order

Congener	Primary ¹	Confirmation ²	Expected Ratio (%) ³
PCB-196+203	430	428	87.8
PCB-195	430	428	87.8
PCB-194	430	428	87.8
PCB-207	464	462	76.9

¹ The primary ion is the more intense ion of the two ions monitored for each analyte. Its area is used in the calculating the RR or RF values and for calculating the concentration of the analyte in samples.

² The confirmation ion is the less intense ion of the two ions monitored for each analyte. Its area is not used in the calculation of RR or RF values, or calculating the concentration of the analyte in samples. However, it is used as part of the qualitative identification criteria for demonstrating that the analyte is present.

³ The expected ratio is the area of the confirmation ion divided by the area of the primary ion. All values are shown in percent and are less than 100%, indicating that the primary ion has the more intense response.

Area Subtraction of Higher Homolog Interference

Six congeners: PCB-35, PCB-77, PCB-81, PCB-123, PCB-126, and PCB-157, coelute with congeners from higher homologues when using a DB-5 capillary column and the AXYS SOP GC instrument parameters. Those higher homologue congeners can lose one or two chlorines during mass fragmentation, producing the same ions that are used as the primary quantification ion for one of these five congeners. This results in an artificial increase for the areas of the quantification ions of the six congeners. The quantification ion areas for PCB-35, PCB-77, PCB-81, PCB-123, PCB-126, and PCB-157 are corrected by multiplying the area of the quantification ion (Q1) of the higher (interfering) homologue by an experimentally determined correction factor (see Table 10) and subtracting the product from the area of Q1 of the co-eluting lower homologue congener. The recalculation is performed by the quantification software. Therefore, the areas provided in the raw data (quantitation report) are post-corrected areas. Of the six congeners exhibiting interferences, only PCB-77 and PCB-126 are congeners with direct calibration data.

Table 10. Ions Monitored for Correcting for Interferences from Higher Homologues

Congener	Q1 of Congener	HH Interference	Q1 of HH interference	Correction Factor
PCB-35	256	PCB-104	326	0.4971
PCB-81	292	PCB-87+115+116	326	0.0141
PCB-77	292	PCB-110	326	0.0567
PCB-123	326	PCB149+139	360	0.0460
PCB-126	326	PCB-178	394	0.5122
PCB-157	360	PCB-201	430	0.451

Note: The ions listed as the Q1 for the higher homologue interference are not necessarily those that produce the interference with the five congeners, but they are the ions used for the correction calculation. For example, PCB-110 fragments to produce an ion that is the same mass as the primary quantitation ion for PCB-77. The difference between the quantitation ions for the two congeners is 34 Daltons (326 minus 292). Given that chlorine has a mass of 35 Daltons, the interfering peak probably did not originate from the quantification ion peak for PCB-110 at 326 Daltons, but from another mass fragment produced by PCB-110. The PCB-77 interference is known to be caused by PCB-110, because the interference is seen when PCB-110 is spiked into a reference solution.

Calibration Linearity and Stability

One of the tasks for each laboratory during the earliest portion of the study was to perform an initial calibration of their instrument using the six calibration standards provided by EPA for the study. All 12 of the original laboratories submitted initial calibration data during that early phase. The 7 laboratories

that completed the study performed another 20 initial calibrations during the course of their analyses of the actual study samples. The results of those 32 calibrations of the target congeners (over 8,900 observations) are summarized in Table 11, in terms of the mean response ratio (RR), mean response factor (RF), and the relative standard deviation (RSD) of the RR or RF values *within each calibration*. For example, the low Mean RR of 0.799 for PCB-1 was the lowest mean RR from all 32 calibrations.

Table 11. Summary of Response Ratios, Response Factors, and Relative Standard Deviations for 32 Initial Calibrations

Target Congener	LOC	Quantification Reference	Mean RR or RF*		RSD (%)*	
			Low	High	Low	High
<i>Isotope Dilution and Modified Isotope Dilution Quantification</i>						
PCB-1	Mono	$^{13}\text{C}_{12}$ -PCB-1	0.799	1.302	0.5	7.2
PCB-3		$^{13}\text{C}_{12}$ -PCB-3	0.781	1.300	0.6	5.5
PCB-4	Di	$^{13}\text{C}_{12}$ -PCB-4	0.762	1.208	0.3	7.1
PCB-11		$^{13}\text{C}_{12}$ -PCB-11	0.794	1.230	0.3	5.8
PCB-15		$^{13}\text{C}_{12}$ -PCB-15	0.768	1.203	0.3	6.8
PCB-19	Tri	$^{13}\text{C}_{12}$ -PCB-19	0.775	1.223	0.7	5.3
PCB-28		$^{13}\text{C}_{12}$ -PCB-28	0.503	1.272	1.0	19.7
PCB-37		$^{13}\text{C}_{12}$ -PCB-37	0.851	1.234	0.8	5.4
PCB-52	Tetra	$^{13}\text{C}_{12}$ -PCB-52	0.797	1.178	0.5	5.0
PCB-54		$^{13}\text{C}_{12}$ -PCB-54	0.823	1.250	0.3	5.7
PCB-70		$^{13}\text{C}_{12}$ -PCB-70	0.850	1.190	0.5	26.0
PCB-77		$^{13}\text{C}_{12}$ -PCB-77	0.893	1.215	0.3	5.7
PCB-85	Penta	$^{13}\text{C}_{12}$ -PCB-85	0.772	1.092	0.4	6.7
PCB-101		$^{13}\text{C}_{12}$ -PCB-101	0.852	1.135	0.6	7.4
PCB-104		$^{13}\text{C}_{12}$ -PCB-104	0.805	1.139	0.3	4.1
PCB-118		$^{13}\text{C}_{12}$ -PCB-118	0.860	1.624	0.6	6.4
PCB-126		$^{13}\text{C}_{12}$ -PCB-126	0.869	1.133	0.5	5.8
PCB-138	Hexa	$^{13}\text{C}_{12}$ -PCB-138	0.744	1.116	0.6	6.2
PCB-153		$^{13}\text{C}_{12}$ -PCB-153	0.881	1.187	0.4	11.8
PCB-155		$^{13}\text{C}_{12}$ -PCB-155	0.720	1.069	0.3	4.6
PCB-169		$^{13}\text{C}_{12}$ -PCB-169	0.705	1.210	0.6	9.2
PCB-180	Hepta	$^{13}\text{C}_{12}$ -PCB-180	0.698	1.149	0.4	8.1
PCB-188		$^{13}\text{C}_{12}$ -PCB-188	0.715	1.139	0.3	11.1
PCB-189		$^{13}\text{C}_{12}$ -PCB-189	0.696	1.227	0.5	9.3
PCB-202	Octa	$^{13}\text{C}_{12}$ -PCB-202	0.636	1.173	0.3	9.6
PCB-205		$^{13}\text{C}_{12}$ -PCB-205	0.831	1.337	0.8	27.0
PCB-206	Nona	$^{13}\text{C}_{12}$ -PCB-206	0.496	1.208	0.5	28.4
PCB-208		$^{13}\text{C}_{12}$ -PCB-208	0.512	1.297	0.5	19.8
PCB-209	Deca	$^{13}\text{C}_{12}$ -PCB-209	0.207	1.156	0.6	18.5
<i>Extracted Internal Standard Quantification</i>						
PCB-8	Di	$^{13}\text{C}_{12}$ -PCB-11	0.722	1.218	2.3	10.7
PCB-18	Tri	$^{13}\text{C}_{12}$ -PCB-28	0.437	0.736	0.7	7.6
PCB-31		$^{13}\text{C}_{12}$ -PCB-28	0.716	1.399	0.8	16.1
PCB-41	Tetra	$^{13}\text{C}_{12}$ -PCB-70	0.741	1.095	0.4	11.4
PCB-44		$^{13}\text{C}_{12}$ -PCB-52	0.641	1.008	1.0	7.8
PCB-66		$^{13}\text{C}_{12}$ -PCB-70	0.807	1.179	0.6	7.0
PCB-74		$^{13}\text{C}_{12}$ -PCB-70	0.739	1.139	1.0	7.1
PCB-95	Penta	$^{13}\text{C}_{12}$ -PCB-101	0.746	1.037	1.2	11.7
PCB-99		$^{13}\text{C}_{12}$ -PCB-101	0.830	1.174	1.1	7.6
PCB-105		$^{13}\text{C}_{12}$ -PCB-118	0.787	1.558	1.4	8.2
PCB-110		$^{13}\text{C}_{12}$ -PCB-118	0.816	1.540	0.9	6.8
PCB-132	Hexa	$^{13}\text{C}_{12}$ -PCB-153	0.424	0.912	1.1	21.6
PCB-147		$^{13}\text{C}_{12}$ -PCB-153	0.636	0.876	1.4	10.9
PCB-149		$^{13}\text{C}_{12}$ -PCB-153	0.672	0.938	1.3	12.1
PCB-156	Hexa	$^{13}\text{C}_{12}$ -PCB-153	0.722	1.590	2.5	25.7

Table 11. Summary of Response Ratios, Response Factors, and Relative Standard Deviations for 32 Initial Calibrations

Target Congener	LOC	Quantification Reference	Mean RR or RF*		RSD (%)*	
			Low	High	Low	High
PCB-166	Hepta	¹³ C ₁₂ -PCB-153	0.705	1.223	1.9	15.0
PCB-177		¹³ C ₁₂ -PCB-180	0.598	0.894	1.5	10.9
PCB-187		¹³ C ₁₂ -PCB-180	0.681	1.200	0.7	14.8
PCB-199		¹³ C ₁₂ -PCB-202	0.434	1.023	0.0	20.2

* The mean RR, mean RF, and RSD values are those calculated within a single initial calibration, and not across calibrations, nor across laboratories.

The RR and RF values varied across the 12 laboratories, but within a given calibration at each laboratory, the instrument responses were generally quite consistent. The mean RR values within each calibration ranged from roughly 0.750 to 1.250 for most of the congeners quantified by isotope dilution. Four of those congeners had mean RR values in a calibration that ranged much lower than 0.750 (PCBs 28, 206, 208, and 209), but those lower RR values tended to occur consistently in several calibrations from a given laboratory for each congener, suggesting that those low values are not a pervasive concern and not issue of a random variation in the response in a single standard among the six calibration points. This observation is supported by the fact that the RSD values for those congeners in the laboratories with the lower than expected response ratios are not noticeably different from the RSDs for other congeners in that calibration, nor from the RSDs for those congeners in other laboratories. Whatever may be responsible for the lower response ratios for those four congeners in certain calibrations, it is occurring consistently across all six standards, such that the calibration still meets the linearity criterion in the draft procedure.

The ranges of mean RF values tend to have lower upper limits than the ranges of the mean RR values, which is expected, because these congeners are not calibrated using isotope dilution, where the native analyte and its label have identical structures and fragmentation patterns.

Table 11 also contains the range of RSD values for all 32 calibrations, which are a measure of the variability in the actual RR or RF values for the analyte in each initial calibration. The RSD is used as a metric of linearity and assumes that the calibration relationship can be represented by a straight line that runs through the origin. EPA methods that employ the RSD as a linearity metric generally specify QC limits on the order of 15% to 25%. The lower RSD values in Table 11 are all below 2%, with two exceptions at 2.3% and 2.5%. The upper RSD values are below 20%, with six exceptions that range as high as 28.4%. Four of those six exceptions are for congeners that are quantified by the extracted internal standard approach, while the other two are quantified by isotope dilution. For two of the six exceptions, the high RSD value was driven by the RR or RF value in either the lowest or highest of the six calibration standards. In both of those cases, the laboratory could have dropped the offending standard from the calibration, met a 20% criterion, and adjusted their calibration range accordingly.

Overall, the study data demonstrate that calibration standards specified in the draft procedure exhibit excellent linearity for the target analytes and do not require the use of more involved calibration models such as a linear regression that does not pass through the origin, or a quadratic equation. Moreover, the commonly used linearity metric of RSD \leq 20% is appropriate for the target analytes in this procedure.

A similar examination of the calibration data was performed for the 29 labeled compounds. The mean RF values ranged from 0.145 to 1.933 across all of the calibrations. Across all 32 calibration, the RSDs for the labeled compounds in each laboratory were below 20%, with one exception at 34.8% for ¹³C₁₂-PCB-209 in the only calibration performed by one of the volunteer laboratories that did not complete the study. The low RSD values are not unexpected, because the labeled compounds are present in the calibration standards at a single concentration (400 ng/mL) across all six solutions (see Table 7).

5. Initial Precision and Recovery

EPA required that each laboratory perform initial precision and recovery (IPR) studies in each of the matrix types that they agreed to analyze: aqueous, solids (e.g., sediment and biosolids), and tissue. For each IPR study, four aliquots of a clean reference matrix were spiked with the 65 PCB congeners of primary interest for the method. The reference matrices and aliquot sizes were:

- 1 liter of reagent water for water matrices
- 10 grams of clean sand for solid matrices
- 10 grams of a 10:90 w/w mixture of canola oil and Ottawa sand

The mass of the 48 native PCB congeners added to the IPR study samples was 16 ng per sample and was equivalent to the on-column concentration of the CS-4 standard in the 6-point initial calibration range. The native compound spiking solution described in the method and provided to the laboratories by EPA was used for spiking. For the aqueous IPR samples, the concentration of each congener was 16 ng/L. For the solid and tissue IPR samples, the concentration of each congener was 1.6 ng/g. The labeled compounds were spiked into the IPR aliquots separately, at the level used for all sample analyses, 40 ng of each labeled compound in each sample.

For each set of four IPR aliquots, each laboratory calculated and reported the mean concentration and mean recovery, the standard deviation of the recoveries of each target analyte, and the relative standard deviations (RSDs) of the recoveries.

Each laboratory also prepared a single ongoing precision and recovery (OPR) sample with each batch of study samples prepared and analyzed. The OPR aliquots were spiked at the same concentrations as the IPR aliquots. Each laboratory calculated and reported the recovery of the spiked analytes in each OPR aliquot.

The IPR and OPR results from the laboratories were used to calculate quality control (QC) acceptance criteria for target as well as labeled compounds, using the statistical procedures described in the study plan and EPA's new method protocol (USEPA 2018). Separate QC acceptance criteria were calculated for the aqueous, solid, and tissue matrix types. Those criteria are presented in Tables 12 through 17 below.

One of the EPA Office of Water's objectives in conducting a multi-laboratory validation study is to generate data from which the Office of Water can derive multi-laboratory QC acceptance criteria for the various performance tests in the method, or to evaluate the ability of the method to meet commonly applied acceptance criteria for some performance tests. In this study, the Office of Water calculated QC acceptance criteria for initial precision and recovery (IPR) tests, ongoing precision and recovery (OPR) tests, and labeled compound recoveries. The derivations of those limits were based on the processes and equations in Appendix G of the *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program* (USEPA 2018), with modifications to account for the actual number of laboratories, samples and replicates in the study. To yield a more complete dataset, IPR and OPR data were combined when calculating the criteria, using different formulas to generate IPR- and OPR-specific criteria that account for how they would be evaluated in practice (i.e., mean and RSD of four IPR replicates, and OPRs evaluated on an individual basis). Labeled congener recovery in samples was calculated by combining recoveries for the unspiked and spiked samples for the given matrix, to ensure that within-laboratory variability could be distinguished from between-sample variability for each laboratory. Briefly:

- The QC acceptance criteria for recovery in the IPR test is calculated by constructing a prediction interval around the mean percent recovery, using a Student's *t* value, with the degrees of freedom

determined using the Satterthwaite estimation procedure (Satterthwaite, 1946), using the between- and within-laboratory variance components calculated for that congener, weighted based on future IPR usage (assuming means of four replicates per laboratory).

- The maximum acceptable RSD for the four IPR aliquots is calculated by an upper confidence limit around the observed RSD of the results from all of the laboratories. The RSD_{IPR} (computed as s_w divided by \bar{X}) is multiplied by the square root of a 95th percentile F value with 3 degrees of freedom in the numerator and $n_T \cdot m$ degrees of freedom in the denominator, where m = the number of laboratories, and n_T is the number of data points across all laboratories for the given congener.
- The QC acceptance criteria for recovery in the OPR test is calculated by constructing a prediction interval around the mean percent recovery, using a Student's t value, with the degrees of freedom determined using the Satterthwaite estimation procedure, using the between- and within-laboratory variance components calculated for that congener, weighted based on future OPR usage (assuming a single replicate per laboratory).
- The QC acceptance criteria for labeled sample recovery is calculated by constructing a prediction interval around the mean percent recovery, using a Student's t value with the degrees of freedom determined using the Satterthwaite estimation procedure, using the between-laboratory, between-sample, and within-laboratory variance components calculated for that congener, weighted based on future criterion usage (assuming a single replicate per laboratory).

Generally, these criteria would be calculated at the 95% confidence level (a 95th percentile F -statistic for the one-sided RSD upper bound, and a 97.5th percentile t -statistic for the two-sided IPR and OPR recovery bounds). This means that a laboratory performing the method properly would be assumed to have a 5% probability of failing that criterion merely due to chance. However, the probability would be 5% for each of the 48 individual target and 29 labeled PCB congeners being evaluated, and as a result, the probability of at least one of those congeners failing just by chance would be much higher. To ensure an overall 5% probability of any of the congeners failing the criteria, each congener criterion was calculated using t - and F -statistics with much more stringent confidence levels (using 99.9th percentile F -statistics and 99.95th percentile t -statistics).

Given the multi-analyte correction and because these criteria are designed to assess performance in multiple laboratories, the calculations often result in acceptance limits that are fairly wide. Historically, the Office of Water has been willing to accept the fact that limits derived from multi-laboratories studies are wider than those that would be derived in a single-laboratory study, or from long-term data within any given laboratory. However, in this study, many of the calculated lower limits for the IPR, OPR, and labeled compound recoveries were negative numbers. As noted repeatedly in the body of this report, negative recovery values have no physical meaning. (Even in the unlikely scenario that something in the samples was actively destroying the analytes or that they were irreversibly removed from the sample extracts, the calculated recoveries would bottom out at zero percent.)

The Office of Water's challenge in such situations is to balance its desire for practical acceptance criteria that can be applied across all laboratories against the time and expense that would be required to collect much more data from laboratories that have had significant time to practice the method before the study begins. The solution that the Office of Water has successfully utilized in the past is a hybrid approach that employs statistically calculated limits where such limits appear reasonable to most analysts, and rely on simpler "consensus-style" round number limits in place of calculated limits that include negative lower limits and/or exceptionally high upper limits.

The remainder of this section includes the results of such a hybrid approach. The Office of Water anticipates including the limits in these tables into the draft method as an interim starting point. If practical, the Office of Water may solicit additional performance data at a later date and revise these limits accordingly.

Aqueous IPR/OPR Criteria

The aqueous IPR and OPR data from eight of the nine laboratories that completed that phase of the study were used to determine QC acceptance criteria for IPRs and OPRs. The data from the ninth laboratory (Lab 5) were excluded from the statistical analyses because they prepared their IPR samples using a volume of only 750 mL, instead of 1 L, but spiked the same mass of the PCB congeners, resulting in a higher concentration in the final samples. A total of 45 sets of IPR and OPR data were provided by those eight laboratories, and were used to calculate the acceptance criteria shown in Table 12, rounded to no decimal places. The criteria are listed for each of the 48 spiked congeners, in congener order number. Some of these congeners coelute with other congeners (as illustrated in Table 5), but because only these 48 congeners are spiked in the IPR and OPR aliquots, only the spiked congeners are listed.

Table 12. Aqueous IPR and OPR Calculated QC Acceptance Criteria for Target Analytes

Congener	# Labs	# Results	Calculated Acceptance Criteria		
			IPR Range (%)	Max RSD (%)	OPR Range (%)
PCB-1	8	45	78 - 130	18	71 - 136
PCB-3	8	45	74 - 117	14	71 - 120
PCB-4	8	45	77 - 112	14	72 - 117
PCB-8	8	45	42 - 120	18	43 - 119
PCB-11	8	45	62 - 125	9	63 - 124
PCB-15	8	45	70 - 111	10	69 - 111
PCB-18	8	45	60 - 107	17	57 - 111
PCB-19	8	45	77 - 107	12	73 - 111
PCB-28	8	45	18 - 184	17	21 - 180
PCB-31	8	45	46 - 129	19	46 - 129
PCB-37	8	45	67 - 112	9	68 - 111
PCB-44	8	45	44 - 131	13	46 - 130
PCB-52	8	45	61 - 128	8	62 - 127
PCB-54	8	45	67 - 112	8	68 - 111
PCB-64	8	45	74 - 108	10	73 - 110
PCB-66	8	45	64 - 118	8	65 - 117
PCB-70	8	45	55 - 127	8	57 - 126
PCB-74	8	45	74 - 102	8	73 - 103
PCB-77	8	45	58 - 118	9	59 - 116
PCB-85	8	45	68 - 106	7	69 - 105
PCB-95	8	45	63 - 117	12	63 - 117
PCB-99	8	45	66 - 107	10	66 - 107
PCB-101	8	45	64 - 118	9	65 - 117
PCB-104	8	45	64 - 117	8	65 - 116
PCB-105	8	45	64 - 120	10	65 - 119
PCB-118	8	45	61 - 119	10	62 - 118
PCB-110	8	45	63 - 106	12	62 - 107
PCB-126	8	45	63 - 113	7	64 - 112
PCB-132	8	45	51 - 133	11	53 - 131
PCB-138	8	45	61 - 117	11	61 - 116
PCB-147	8	45	61 - 117	12	62 - 117
PCB-149	8	45	57 - 120	11	58 - 119
PCB-153	8	45	46 - 134	16	48 - 132
PCB-155	8	45	64 - 116	10	65 - 115
PCB-156	8	45	46 - 149	23	45 - 150
PCB-166	8	45	34 - 157	9	36 - 156
PCB-169	8	45	50 - 122	10	52 - 121
PCB-177	8	45	47 - 130	10	49 - 128
PCB-180	8	45	52 - 124	11	53 - 123

Table 12. Aqueous IPR and OPR Calculated QC Acceptance Criteria for Target Analytes

Congener	# Labs	# Results	Calculated Acceptance Criteria		
			IPR Range (%)	Max RSD (%)	OPR Range (%)
PCB-187	8	45	36 - 138	17	38 - 136
PCB-188	8	45	57 - 122	11	58 - 121
PCB-189	8	45	56 - 119	11	58 - 118
PCB-199	8	45	-100 - 281	59	-93 - 273
PCB-199 (w/o Lab 8)	7	39	42 - 164	57	14 - 193
PCB-202	8	45	55 - 121	12	56 - 120
PCB-205	8	45	52 - 118	18	51 - 119
PCB-206	8	45	35 - 135	17	37 - 133
PCB-208	8	45	44 - 125	15	45 - 124
PCB-209	8	45	31 - 130	27	30 - 131

The statistically determined recovery ranges for the IPRs and OPRs reflect the variability within each laboratory, as well as the variability across all eight laboratories that completed that portion of the study using 1-L samples. Generally speaking, the congeners that are quantified by isotope dilution have narrower ranges than the congeners that are quantified by extracted internal standard. For example, the observed IPR recoveries for PCB-4, quantified by isotope dilution, ranged from 86 to 103%, while, the recoveries for PCB-8, quantified by extracted internal standard, ranged from 68 to 92%. The observed OPR results were also affected by fact that some laboratories submitted more OPR data than others (e.g., there were 13 sets of OPR results for aqueous samples, from 8 labs) because some laboratories analyzed the wastewater samples in more batches than other laboratories, and each sample preparation batch contained an OPR aliquot.

The initial statistical calculations for PCB-199 resulted in exceptionally wide ranges that included a negative lower value, as shown in Table 12. Those results were driven by the wildly variable results for this congener in only one laboratory (Lab 8). CSRA staff examined the results for those four IPR aliquots and two OPR aliquots in detail, as did the laboratory staff. When no obvious errors were identified through either review, the statistical calculations were rerun without the results from Lab 8 for that one congener, yielding the much more reasonable ranges for PCB-199 shown in red in Table 12 for the IPR, although the OPR calculations still result in a fairly high upper limit of 193%.

The calculated maximum RSD values for target analytes in Table 12 are below 20% for 45 of the 48 target analytes. The exceptions are PCB-156 at 23%, PB-209 at 27%, and PCB-199 at 57% even after removal of the results from Laboratory 8 for that congener. Except for the calculated maximum RSD for PCB-199, all of the values in Table 12 are well within reasonable expectations.

The labeled compound data from the same eight laboratories were used to calculate the IPR and OPR acceptance criteria. The range of the *observed* mean recoveries of the labeled compounds in the IPRs was from 35 to 81%. The *observed* mean RSD was less than 20% for 26 of the 29 labeled congeners. The mean RSDs for the two monochlorinated labeled congeners and the first dichlorinated labeled congener (labeled PCBs 1, 3, and 4) were 29%, 28%, and 27%, respectively.

The calculated ranges for IPR/OPR labeled compound recoveries and RSD are presented in Table 13, rounded to no decimal places. All of the calculated ranges for the IPR and OPR are much wider than the observed values from the eight laboratories in the study. This is a function of the statistical calculations, which incorporate not only the variability *within* a given laboratory, but the variability *across* all of the laboratories, and an allowance for testing multiple analytes at the same time.

Table 13. IPR and OPR QC Acceptance Criteria for Labeled Compounds in Aqueous Matrices

Congener	Calculated Acceptance Criteria (%)			Interim Acceptance Criteria (%)		
	IPR Mean	Max RSD	OPR	IPR (each aliquot)	Max RSD	OPR
¹³ C ₁₂ -PCB-1	-39 - 110	91	-40 - 111	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-3	-27 - 111	75	-29 - 113	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-4	-18 - 102	72	-22 - 105	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-11	-24 - 136	47	-22 - 133	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-15	-29 - 144	44	-25 - 140	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-19	-16 - 116	53	-16 - 115	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-28	-21 - 143	39	-18 - 139	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-37	-38 - 179	36	-33 - 174	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-52	-3 - 117	34	-1 - 115	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-54	-4 - 103	43	-4 - 102	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-70	-9 - 138	30	-6 - 135	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-77	-12 - 151	29	-9 - 148	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-85	5 - 129	31	7 - 127	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-101	11 - 97	40	8 - 99	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-104	8 - 118	34	8 - 118	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-118	-1 - 141	30	1 - 138	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-126	-13 - 159	32	-9 - 155	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-138	17 - 98	39	13 - 102	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-153	14 - 122	30	15 - 122	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-155	3 - 142	28	6 - 139	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-169	-92 - 253	39	-85 - 246	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-180	17 - 110	36	15 - 113	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-188	7 - 139	27	9 - 136	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-189	-5 - 157	30	-2 - 153	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-202	10 - 120	28	11 - 118	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-205	-9 - 153	37	-6 - 151	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-206	-12 - 147	35	-9 - 144	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-208	-18 - 156	41	-15 - 154	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-209	-30 - 165	39	-26 - 161	15 - 130	40	15 - 130

As can be seen in Table 13, the lower limits of the IPR acceptance criteria are less than zero for 20 of the 29 labeled analytes, and for the OPR criteria, 19 of the 29 labeled analytes have lower limits less than zero. Negative recovery values have no physical basis. Rather, as noted here, those calculated limits are a function of the variability of the data from the study. Had more laboratories chosen to complete the study, or had all of the laboratories had more time to practice the method, one would expect that the observed recoveries and their precision would have improved, yielding narrower ranges for the IPR and OPR acceptance criteria.

In contrast, to the calculated lower limits, the calculated upper limits for the IPR ranged from 97% to 253%, with 21 of 29 labeled analytes having calculated upper limits less than or equal to 150%. Likewise, the calculated upper limits for the OPR ranged from 99% to 246%, with 22 of 29 labeled analytes having calculated upper limits less than or equal to 150%. Recoveries well over 100% are a function of the uncertainty in the quantitation of the labeled compounds added prior to sample extraction using the internal standards injected into the final extract immediately prior to the instrumental analysis. Other isotope dilution methods from EPA have used 150% as an upper limit for labeled compound recovery, and many non-isotope dilution methods allow the areas of the injected internal standards used in those procedures to range up to 200% of their corresponding areas in the most recent calibration verification standard. Therefore, the upper limits of IPR and OPR ranges in Table 13 for many of the labeled compounds are not unprecedented by any means.

The criterion for the maximum RSD for the labeled compounds in the IPR analyses ranged from 27% to 91%. The calculated maximum RSD values for 21 of the 29 labeled compounds are at or below 40%. The highest calculated RSD values are for the mono- and dichlorinated labeled compounds. Those values may reflect the known concerns with potential loss of these lightest of the labels during extract concentration.

Given the nature of isotope dilution quantitation, and the fact that the labeled compounds are not regulated parameters under the Clean Water Act, there is much merit to using simpler consensus-style acceptance limits for the recoveries of the labeled compounds in the IPR and OPR aliquots. After EPA reviewed the results of the validation study and the calculated QC acceptance criteria, the decision was made to instead compile the interim acceptance criteria for the draft method shown (in green) in Table 13 above. The draft method used in the study employed limits of 15 - 130% for the recovery of the labeled analogs of PCB-1, PCB-3, PCB-4, PCB-11, PCB-15, and PCB-19, and limits of 40 - 130% for all of the other 23 labeled analogs (e.g., labeled PCB-28 to labeled PCB-209). However, those limits were based on data from the single laboratory that developed the original procedure, and as such, are not expected to be representative of multi-laboratory performance. Therefore, EPA examined the failure rates for the labeled compound recoveries in the IPR and OPR analyses from the study using two sets of consensus-style limits: 15 - 130% and 25 - 150%, as shown Table 14. These failures represent instances where the mean IPR result from all four IPR aliquots or the single OPR aliquot fell outside of the stated limits.

Table 14. Observed Labeled Compound Recovery Failure Rates for Two Potential Acceptance Criteria for Aqueous Matrix IPR and OPR

Congener	Aqueous IPR (mean of 4 aliquots)				Aqueous OPR (1 aliquot)					
	Total # Mean Results	Observed Failure Rate (%)				Total # Results	Observed Failure Rate (%)			
		LL 15%	UL 130%	LL 25%	UL 150%		LL 15%	UL 130%	LL 25%	UL 150%
¹³ C ₁₂ PCB-1	8	25.0	0.0	50.0	0.0	13	7.7	0.0	15.4	0.0
¹³ C ₁₂ PCB-3	8	0.0	0.0	25.0	0.0	13	0.0	0.0	15.4	0.0
¹³ C ₁₂ PCB-4	8	0.0	0.0	25.0	0.0	13	0.0	0.0	7.7	0.0
¹³ C ₁₂ PCB-11	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-15	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-19	8	0.0	0.0	12.5	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-28	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-37	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-52	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-54	8	0.0	0.0	3.1	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-70	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-77	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-85	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-101	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-104	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-118	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-126	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-138	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-153	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-155	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-169	8	0.0	12.5	0.0	0.0	13	7.7	0.0	7.7	0.0
¹³ C ₁₂ PCB-180	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-188	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-189	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-202	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0

Table 14. Observed Labeled Compound Recovery Failure Rates for Two Potential Acceptance Criteria for Aqueous Matrix IPR and OPR

Congener	Aqueous IPR (mean of 4 aliquots)					Aqueous OPR (1 aliquot)				
	Total # Mean Results	Observed Failure Rate (%)				Total # Results	Observed Failure Rate (%)			
		LL 15%	UL 130%	LL 25%	UL 150%		LL 15%	UL 130%	LL 25%	UL 150%
¹³ C ₁₂ PCB-205	8	0.0	0.0	0.0	0.0	13	0.0	7.7	0.0	0.0
¹³ C ₁₂ PCB-206	8	0.0	0.0	0.0	0.0	13	0.0	7.7	0.0	0.0
¹³ C ₁₂ PCB-208	8	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-209	8	0.0	0.0	0.0	0.0	13	0.0	7.7	0.0	0.0

No labeled congeners failed the 150% upper limit for either the IPR or OPR analyses. Only one labeled congener failed the 130% upper limit for the mean of the IPR and only three labeled congeners failed the 130% upper limit in the OPR. The labeled analogs of PCB-205, PCB-206, and PCB-209 each had one failure out of 13 sets of OPR results (e.g., 7.7% of 13 = 1 failure). All three of those labeled analogs failed in the same OPR from one laboratory (a second aqueous OPR run by that laboratory had all three congeners pass the 130% upper limit). The mean recovery of the labeled analog of PCB-169 exceeded the 130% upper limits for the IPR results once in the 8 sets of IPR results (e.g., 12.5% of 8 = 1 failure).

There were more failures of the lower limits for the IPR and OPR data. The labeled analogs of PCB-1, PCB-3, PCB-4, and PCB-19 failed the 25% lower limit for the mean 12.5% to 50% of the time for the sets of IPR data. The labeled analogs of PCB-1, PCB-3, and PCB-4 failed the 25% lower limit from 7.7% to 15.4% for OPR results (e.g., 1 and 2 failures respectively). The labeled analog of PCB-169 also failed in the OPR one time at the 25% lower limit.

Using a 15% lower limit reduced or eliminated all but one of the failures observed at the 25% IPR limit, for the labeled analog of PCB-1, which still failed 25% of the time. This labeled congener and its native counterpart have the lowest molecular weights of all of the congeners and are known to be susceptible to evaporative losses during extract concentration. Such losses can be overcome by employing appropriate care during extract concentration.

Therefore, based on the results in Table 14, EPA recommends the use of a single set of limits, namely 15 - 130%, for all of the labeled compound recoveries in the IPR and OPR aliquots.

The maximum RSD final criteria are based on the mean of the calculated RSDs from all 29 labeled compounds, which was 40% RSD.

Solids IPR and OPR Results

The solids IPR and OPR data from all six laboratories that completed the sediment portion of the study were used to determine QC acceptance criteria for IPRs and OPRs. The solids IPR samples were prepared in Ottawa sand. (While tissues are a “solid,” as opposed to a “liquid,” in the method and this report, the term “solid” refers to soils, sediments, or biosolids.) A total of 31 sets of IPR and OPR data were provided by those six laboratories and were used to calculate the acceptance criteria shown in Table 15, rounded to no decimal places. The criteria are listed for each of the 48 spiked congeners, in congener order number. Some of these congeners coelute with other congeners (as illustrated in Table 5), but because only these 48 congeners are spiked in the IPR and OPR aliquots, only the spiked congeners are listed.

Table 15. IPR and OPR QC Acceptance Criteria for Target Analytes in Solid Matrices

Congener	Calculated Acceptance Criteria (%)			Interim Acceptance Criteria (%)		
	IPR Mean	Max RSD	OPR	IPR Mean	Max RSD	OPR
PCB-1	61 - 154	59	19 - 196	61 - 154	59	19 - 196
PCB-3	40 - 156	42	29 - 167	40 - 156	42	29 - 167
PCB-4	48 - 144	61	13 - 179	48 - 144	61	13 - 179
PCB-8	-21 - 196	42	-11 - 187	35 - 150	40	25 - 160
PCB-11	-35 - 255	35	-20 - 239	35 - 150	40	25 - 160
PCB-15	36 - 150	44	25 - 162	36 - 150	44	25 - 162
PCB-18	20 - 148	40	18 - 149	20 - 148	40	18 - 149
PCB-19	26 - 157	32	28 - 156	26 - 157	32	28 - 156
PCB-28	-5 - 202	41	2 - 195	25 - 150	35	30 - 150
PCB-31	38 - 147	37	32 - 153	38 - 147	37	32 - 153
PCB-37	38 - 147	38	31 - 155	38 - 147	38	31 - 155
PCB-44	23 - 153	34	24 - 151	23 - 153	34	24 - 151
PCB-52	57 - 138	42	36 - 159	57 - 138	42	36 - 159
PCB-54	56 - 132	56	21 - 167	56 - 132	56	21 - 167
PCB-64	29 - 153	29	31 - 150	29 - 153	29	31 - 150
PCB-66	50 - 138	25	49 - 140	50 - 138	25	49 - 140
PCB-70	43 - 144	27	42 - 144	43 - 144	27	42 - 144
PCB-74	41 - 135	30	38 - 138	41 - 135	30	38 - 138
PCB-77	42 - 134	40	30 - 145	42 - 134	40	30 - 145
PCB-85	57 - 121	27	50 - 128	57 - 121	27	50 - 128
PCB-95	55 - 125	29	47 - 133	55 - 125	29	47 - 133
PCB-99	33 - 140	34	30 - 143	33 - 140	34	30 - 143
PCB-101	57 - 125	26	51 - 132	57 - 125	26	51 - 132
PCB-104	52 - 128	44	32 - 148	52 - 128	44	32 - 148
PCB-105	65 - 122	17	63 - 124	65 - 122	17	63 - 124
PCB-118	48 - 133	19	50 - 131	48 - 133	19	50 - 131
PCB-110	31 - 142	20	36 - 137	31 - 142	20	36 - 137
PCB-126	48 - 129	14	52 - 124	48 - 129	14	52 - 124
PCB-132	42 - 146	18	47 - 141	42 - 146	18	47 - 141
PCB-138	60 - 123	19	58 - 125	60 - 123	19	58 - 125
PCB-147	58 - 126	25	53 - 132	58 - 126	25	53 - 132
PCB-149	51 - 129	28	46 - 134	51 - 129	28	46 - 134
PCB-153	76 - 109	25	61 - 124	76 - 109	25	61 - 124
PCB-155	60 - 122	37	41 - 140	60 - 122	37	41 - 140
PCB-156	76 - 119	25	62 - 133	76 - 119	25	62 - 133
PCB-166	71 - 122	21	64 - 128	71 - 122	21	64 - 128
PCB-169	56 - 130	55	23 - 164	56 - 130	55	23 - 164
PCB-177	71 - 114	29	55 - 130	71 - 114	29	55 - 130
PCB-180	72 - 112	25	58 - 125	72 - 112	25	58 - 125
PCB-187	64 - 114	23	56 - 122	64 - 114	23	56 - 122
PCB-188	61 - 118	27	52 - 128	61 - 118	27	52 - 128
PCB-189	67 - 117	24	58 - 126	67 - 117	24	58 - 126
PCB-199	62 - 126	22	58 - 130	62 - 126	22	58 - 130
PCB-202	51 - 127	24	49 - 129	51 - 127	24	49 - 129
PCB-205	54 - 116	31	44 - 126	54 - 116	31	44 - 126
PCB-206	52 - 129	49	27 - 154	52 - 129	49	27 - 154
PCB-208	45 - 131	21	47 - 129	45 - 131	21	47 - 129
PCB-209	67 - 111	19	62 - 117	67 - 111	19	62 - 117

As with the aqueous sample portion of the study, the statistically determined recovery ranges for the solids IPRs and OPRs reflect the variability within each laboratory, as well as the variability across all six laboratories that completed that portion of the study. The observed mean IPR recoveries for the 48

congeners ranged from about 86 to 114%. The calculated IPR ranges, while wider than the ranges for the sample congeners in the aqueous IPR samples, are generally reasonable for solid samples.

As shown in Table 15, the IPR ranges for 45 of the 48 target analytes have calculated lower limits well above zero. The three target analytes with calculated lower limits that are negative values are PCB-8, PCB-11, and PCB-28 (**in red**). The calculated upper limits of those three congeners are also well above 150% (**in red**). The OPR ranges for those three congeners (**in red**) are also notably wider than for the other 45 target analytes. Except for those three congeners, although generally wider than the calculated limits in the aqueous matrix, the study data for the solid IPR and OPR analyses demonstrate reasonable reproducibility.

EPA examined the failure rates for PCB-8, PCB-11, and PCB-28 for various consensus-style acceptance lower and upper limits, including 15%, 25%, 35%, 130%, 150%, and 160%. None of the mean IPR results from the study failed at any of those potential lower limits. All three of those congeners had failures of the upper limit at 130%, with failure rates of 4% to 20% for the IPR results. Relative to the 150% upper limit, only PCB-11 had any failures, with 4% of the study results above that limit (e.g., 1 mean IPR). Based on those failure rates, EPA replaced the calculated criteria for PCB-8 and PCB-11 with interim acceptance criteria (**in green**) based on the calculated results for PCB-15, a closely eluting dichlorobiphenyl, but rounded to multiples of 5. PCB-28 is a trichlorobiphenyl, and the final criteria (**in green**) are based on the results for PCB-18 and PCB-19, similarly rounded. The final method will encourage each laboratory to employ control charts and to develop in-house statistical quality control limits, as long as those limits are no wider than the limits in the published method.

The labeled compound data from the six laboratories that completed the solid sample portion of the study were used to calculate the IPR and OPR acceptance criteria presented in Table 16. The derivation of the interim acceptance criteria is described after the table.

Table 16. IPR and OPR QC Acceptance Criteria for Labeled Congeners in Solid Matrices

Congener	Calculated Acceptance Criteria (%)			Interim Acceptance Criteria (%)		
	IPR Mean	Max RSD	OPR	IPR (each aliquot)	Max RSD	OPR
¹³ C ₁₂ -PCB-1	-105 - 186	89	-90 - 171	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-3	-81 - 170	83	-68 - 158	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-4	-71 - 163	81	-60 - 153	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-11	-34 - 151	70	-31 - 148	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-15	-29 - 145	73	-29 - 145	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-19	-46 - 152	71	-39 - 145	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-28	-18 - 146	72	-23 - 151	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-37	0 - 142	71	-14 - 156	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-52	-41 - 140	58	-32 - 132	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-54	-27 - 142	53	-21 - 136	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-70	-16 - 144	50	-11 - 140	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-77	-7 - 148	52	-6 - 147	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-85	-31 - 141	51	-23 - 133	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-101	-14 - 139	49	-9 - 135	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-104	-12 - 143	47	-8 - 138	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-118	-9 - 145	48	-5 - 142	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-126	-10 - 155	51	-8 - 153	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-138	-18 - 139	51	-13 - 134	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-153	-2 - 139	49	-1 - 139	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-155	3 - 141	50	1 - 142	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-169	-16 - 158	61	-17 - 159	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-180	-7 - 142	50	-5 - 141	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-188	-2 - 151	50	-2 - 151	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-189	-22 - 168	44	-14 - 160	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-202	-1 - 143	52	-2 - 145	15 - 130	60	15 - 130

Table 16. IPR and OPR QC Acceptance Criteria for Labeled Congeners in Solid Matrices

Congener	Calculated Acceptance Criteria (%)			Interim Acceptance Criteria (%)		
	IPR Mean	Max RSD	OPR	IPR (each aliquot)	Max RSD	OPR
¹³ C ₁₂ -PCB-205	2 - 149	49	1 - 150	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-206	5 - 136	53	1 - 141	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-208	-11 - 155	51	-8 - 153	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-209	4 - 138	52	1 - 141	15 - 130	60	15 - 130

In marked contrast to the calculated ranges for the target analytes, the vast majority of the calculated IPR and OPR ranges for the labeled compounds extend below zero. Only five of the labeled compounds have non-negative calculated lower IPR limits, and those five range from 0 to 5%. Somewhat surprisingly, the upper IPR limits for the labeled compound are not as extreme. The upper limits for 19 of the labeled compounds are at or below 150%, and six of the ten labeled compounds are between 151% and 160%.

The calculated OPR ranges for the labeled compounds are similarly affected, with only 4 labeled compounds with calculated lower limits above zero (all four at 1%), and the upper OPR limits are generally below 150%.

EPA used a similar approach in evaluating potential labeled compound acceptance criteria as was used for the aqueous IPR and OPR results. Table 17 presents the IPR and OPR failure rates relative to the same two consensus-style acceptance criteria of 15% to 130% and 25% to 150%.

Table 17. Observed Labeled Compound Recovery Failure Rates for Two Potential Acceptance Criteria for Solid Matrix IPR and OPR

Congener	Solid IPR (mean of 4 aliquots)				Solid OPR (1 aliquot)					
	Total # Mean Results	Observed Failure Rate (%)				Total # Results	Observed Failure Rate (%)			
		LL 15%	UL 130%	LL 25%	UL 150%		LL 15%	UL 130%	LL 25%	UL 150%
¹³ C ₁₂ PCB-1	6	0.0	0.0	33.3	0.0	7	28.6	0.0	28.6	0.0
¹³ C ₁₂ PCB-3	6	0.0	0.0	0.0	0.0	7	28.6	0.0	28.6	0.0
¹³ C ₁₂ PCB-4	6	0.0	0.0	0.0	0.0	7	28.6	0.0	28.6	0.0
¹³ C ₁₂ PCB-11	6	0.0	0.0	0.0	0.0	7	14.3	0.0	28.6	0.0
¹³ C ₁₂ PCB-15	6	0.0	0.0	0.0	0.0	7	14.3	0.0	28.6	0.0
¹³ C ₁₂ PCB-19	6	0.0	0.0	0.0	0.0	7	28.6	0.0	28.6	0.0
¹³ C ₁₂ PCB-28	6	0.0	0.0	0.0	0.0	7	14.3	0.0	28.6	0.0
¹³ C ₁₂ PCB-37	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-52	6	0.0	0.0	0.0	0.0	7	0.0	0.0	14.3	0.0
¹³ C ₁₂ PCB-54	6	0.0	0.0	0.0	0.0	7	14.3	0.0	28.6	0.0
¹³ C ₁₂ PCB-70	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-77	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-85	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-101	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-104	6	0.0	0.0	0.0	0.0	7	0.0	0.0	14.3	0.0
¹³ C ₁₂ PCB-118	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-126	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-138	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-153	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-155	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-169	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-180	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-188	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0

Table 17. Observed Labeled Compound Recovery Failure Rates for Two Potential Acceptance Criteria for Solid Matrix IPR and OPR

Congener	Solid IPR (mean of 4 aliquots)					Solid OPR (1 aliquot)				
	Total # Mean Results	Observed Failure Rate (%)				Total # Results	Observed Failure Rate (%)			
		LL 15%	UL 130%	LL 25%	UL 150%		LL 15%	UL 130%	LL 25%	UL 150%
¹³ C ₁₂ PCB-189	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-202	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-205	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-206	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-208	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-209	6	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0

As can be seen in Table 17, neither upper acceptance limit was exceeded by the study IPR or OPR results. The smaller number of IPR and OPR data sets for the solid matrices, compared to the aqueous matrices, means that a 14.3% failure rate for the OPR represents 1 of the 7 OPR sets and 28.6% represents 2 of the 7 OPR sets.

Only the IPR mean for one labeled congener, PCB-1, failed the 25% lower recovery limit and using a lower limit of 15% recovery for the labeled compounds in the IPR analyses eliminates that failure. The use of the 15% lower limit for the OPR data is not as effective, but it still reduces the overall number of failures across all of the labeled congeners from 10 labeled analogs to 8, 4 of which only had one failure each at 15%.

Based on these failure rates, EPA compiled the interim acceptance criteria shown in Table 16 (in green) for the draft method. Because the same issues of negative lower limits and very high upper limits arose with the calculated limits for the labeled compounds in the solid sample IPR and OPR aliquots as occurred for labeled compounds in the aqueous matrix IPR and OPR aliquots, the solution was to apply the same 15 - 130% limits for each labeled compound. In the case of the maximum RSD, the value was based on rounding the mean of the calculated RSDs from all 29 labeled compounds of 57% RSD to 60%.

Tissue IPR and OPR Results

The tissue IPR and OPR data from all four laboratories that completed the tissue portion of the study were used to determine the QC acceptance criteria for IPRs and OPRs. The tissue IPR samples were prepared in a 10:90 mixture of canola oil and sand, which EPA used as a simulant for fish tissues containing 10% lipids. A total of 20 sets of IPR and OPR data were provided by those four laboratories and were used to establish the acceptance criteria shown in Table 18, rounded to no decimal places. The criteria are listed for each of the 48 spiked congeners, in congener order number. Some of these congeners coelute with other congeners (as illustrated in Table 5), but because only these 48 congeners are spiked in the IPR and OPR aliquots, only the spiked congeners are listed. The derivation of the interim acceptance criteria is described after the table.

Table 18. IPR and OPR QC Acceptance Criteria for Target Analytes in Tissue Matrices

Congener	Calculated Acceptance Criteria (%)			Interim Acceptance Criteria (%)		
	IPR Range	Max RSD	OPR Range	IPR Mean	Max RSD	OPR Range
PCB-1	-28 - 227	22	6 - 194	25 - 150	25	25 - 150
PCB-3	-62 - 253	24	-23 - 214	25 - 150	25	25 - 150
PCB-4	-602 - 876	110	-405 - 679	25 - 150	25	25 - 150
PCB-8	-34 - 220	29	0 - 186	25 - 150	25	25 - 150
PCB-11	-18 - 212	21	12 - 182	25 - 150	25	25 - 150
PCB-15	-40 - 225	21	-7 - 193	25 - 150	25	25 - 150
PCB-18	-38 - 215	26	-4 - 181	25 - 150	25	25 - 150

Table 18. IPR and OPR QC Acceptance Criteria for Target Analytes in Tissue Matrices

Congener	Calculated Acceptance Criteria (%)			Interim Acceptance Criteria (%)		
	IPR Range	Max RSD	OPR Range	IPR Mean	Max RSD	OPR Range
PCB-19	-56 - 240	22	-20 - 204	25 - 150	25	25 - 150
PCB-28	-82 - 292	19	-45 - 254	25 - 150	25	25 - 150
PCB-31	-62 - 239	36	-22 - 199	25 - 150	25	25 - 150
PCB-37	12 - 179	23	33 - 157	25 - 150	25	25 - 150
PCB-44	-60 - 234	25	-23 - 197	25 - 150	25	25 - 150
PCB-52	-11 - 199	20	17 - 171	25 - 150	25	25 - 150
PCB-54	11 - 177	20	33 - 155	25 - 150	25	25 - 150
PCB-64	-49 - 232	22	-14 - 198	25 - 150	25	25 - 150
PCB-66	-30 - 213	18	0 - 184	25 - 150	25	25 - 150
PCB-70	-27 - 207	21	3 - 177	25 - 150	25	25 - 150
PCB-74	-39 - 215	14	-16 - 192	25 - 150	25	25 - 150
PCB-77	-4 - 182	13	17 - 161	25 - 150	25	25 - 150
PCB-85	6 - 171	12	25 - 152	25 - 150	25	25 - 150
PCB-95	5 - 175	13	25 - 155	25 - 150	25	25 - 150
PCB-99	15 - 160	16	15 - 160	25 - 150	25	25 - 150
PCB-101	-1 - 185	15	23 - 162	25 - 150	25	25 - 150
PCB-104	-33 - 214	17	-5 - 185	25 - 150	25	25 - 150
PCB-105	11 - 172	17	33 - 151	25 - 150	25	25 - 150
PCB-118	-6 - 181	13	15 - 161	25 - 150	25	25 - 150
PCB-110	20 - 156	13	38 - 138	25 - 150	25	38 - 138
PCB-126	20 - 157	15	38 - 139	25 - 150	25	38 - 139
PCB-132	32 - 156	15	48 - 140	32 - 156	25	48 - 140
PCB-138	27 - 150	13	43 - 134	27 - 150	25	43 - 134
PCB-147	-2 - 182	14	20 - 160	25 - 150	25	25 - 150
PCB-149	-23 - 204	17	4 - 177	25 - 150	25	25 - 150
PCB-153	33 - 142	14	47 - 127	33 - 142	25	47 - 127
PCB-155	-15 - 194	13	8 - 171	25 - 150	25	25 - 150
PCB-156	-13 - 206	22	17 - 177	25 - 150	25	25 - 150
PCB-166	-3 - 191	18	23 - 165	25 - 150	25	25 - 150
PCB-169	14 - 167	14	34 - 147	25 - 150	25	25 - 150
PCB-177	16 - 164	14	36 - 144	25 - 150	25	25 - 150
PCB-180	42 - 137	18	52 - 127	42 - 137	25	52 - 127
PCB-187	39 - 137	19	49 - 126	39 - 137	25	49 - 126
PCB-188	7 - 170	13	27 - 150	25 - 150	25	25 - 150
PCB-189	-30 - 201	17	-3 - 175	25 - 150	25	25 - 150
PCB-199	34 - 153	24	45 - 142	34 - 153	25	45 - 142
PCB-202	33 - 145	16	47 - 131	33 - 145	25	47 - 131
PCB-205	22 - 158	25	37 - 144	25 - 150	25	37 - 144
PCB-206	4 - 166	20	26 - 144	25 - 150	25	25 - 150
PCB-208	-23 - 191	16	1 - 167	25 - 150	25	25 - 150
PCB-209	-198 - 396	25	-150 - 348	25 - 150	25	25 - 150

The calculated limits for the target analytes in the tissue matrix IPR and OPR aliquots exhibited significantly more issues with negative lower limits and very high upper limits than either the aqueous matrix or the solid matrix. This may be due, in part to the smaller number of laboratories that completed that portion of the study, but also to the challenges and potential interferences in the tissue matrix. The calculated tissue limits also included many positive lower limits that were below 25%.

EPA used a similar approach in evaluating potential target analyte acceptance criteria as was used for the aqueous IPR and OPR results, evaluating the IPR and OPR failures rates in tissues relative to the same two consensus-style acceptance criteria of 15% to 130% and 25% to 150%. The failures for the IPR and

OPR in tissues were quite limited for the target analytes. No target analytes had IPR or OPR failures at 15% and 25%. One laboratory exceeded both the 130% and 150% upper limits for PCB-4+10 in all of their IPR aliquots, equating to a 25% failure rate for the four laboratories in the tissue phase of the study. That same laboratory has three IPR aliquot failures for PCB-209 at the 130% upper limit and one failure at the 150% upper limit, which equates to 18.75% and 6.25% rates respectively. However, the mean recovery of PCB-209 in the four IPR aliquots was 135%, which would pass relative to an upper limit of 150%. The only OPR failure was in that same laboratory, for PCB-209, coincidentally with a recovery of 135%. Given that those failures were limited to one laboratory, the EPA recommendation for the IPR limits for the target analytes in tissues is a range of 25 - 150%. Therefore, any of the calculated limits that began below 25% were replaced with 25 -150% (in green above), and any calculated limits that began above 25% were retained and shown in black above.

In contrast to the overly wide recovery limits for many of the target analytes, the calculated maximum RSD values were at or below 25% for 45 of the 48 target analytes, and the mean of all of the calculated maximum RSD values was 21%. That suggests that while the recoveries across all of the labs varied greatly, within each laboratory, the recoveries were more consistent. However, for simplicity, a maximum RSD limit of 25% was applied to all of the target analytes.

As with the other matrices, the labeled compound data from the four laboratories that completed the tissue portion of the study were used to calculate the IPR and OPR acceptance criteria presented in Table 19. The derivation of the interim acceptance criteria is described after the table.

Table 19. IPR and OPR QC Acceptance Criteria for Labeled Compounds in Tissue Matrices

Congener	Calculated Acceptance Criteria (%)			Interim Acceptance Criteria (%)		
	IPR Mean	Max RSD	OPR	IPR (each aliquot)	Max RSD	OPR
¹³ C ₁₂ -PCB-1	-198 - 291	76	-138 - 251	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-3	-218 - 313	73	-157 - 208	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-4	-160 - 261	63	-107 - 204	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-11	-145 - 257	71	-91 - 182	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-15	-115 - 226	78	-71 - 207	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-19	-152 - 261	65	-97 - 190	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-28	-113 - 234	74	-69 - 170	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-37	-59 - 183	90	-46 - 240	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-52	-186 - 289	51	-137 - 229	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-54	-170 - 283	53	-117 - 218	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-70	-154 - 274	60	-98 - 222	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-77	-158 - 279	65	-100 - 226	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-85	-166 - 284	62	-107 - 216	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-101	-157 - 272	62	-101 - 215	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-104	-162 - 267	57	-110 - 211	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-118	-145 - 265	64	-91 - 220	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-126	-159 - 278	68	-101 - 219	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-138	-150 - 275	63	-93 - 214	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-153	-157 - 269	62	-102 - 214	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-155	-148 - 269	61	-93 - 204	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-169	-146 - 258	72	-92 - 242	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-180	-171 - 300	54	-112 - 215	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-188	-150 - 270	61	-95 - 263	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-189	-191 - 318	46	-135 - 208	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-202	-139 - 261	61	-86 - 385	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-205	-312 - 446	47	-252 - 293	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-206	-222 - 346	45	-168 - 385	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-208	-315 - 459	54	-241 - 630	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-209	-600 - 758	85	-472 - 630	15 - 130	60	15 - 130

The same issues of negative lower limits and very high upper limits arose with the calculated limits for the labeled compounds in the tissue sample IPR and OPR aliquots as occurred for labeled compounds in the other matrices, only with negative lower IPR limits for all of the labeled compounds, and negative lower OPR limits for all but three of the labeled compounds.

As with the IPR/OPR data for the other matrices, EPA examined the labeled compound failures rates relative to the same two potential sets of acceptance criteria. Table 20 presents the IPR and OPR failures rates relative to the same two consensus-style acceptance criteria of 15% to 130% and 25% to 150%.

Table 20. Observed Labeled Compound Recovery Failure Rates for Two Potential Acceptance Criteria for Tissue Matrix IPR and OPR

Congener	Tissue IPR (mean of 4 aliquots)				Total # Results	Tissue OPR (1 aliquot)				
	Total # Mean Results	Observed Failure Rate (%)				Total # Results	Observed Failure Rate (%)			
		LL 15%	UL 130%	LL 25%	UL 150%		LL 15%	UL 130%	LL 25%	UL 150%
¹³ C ₁₂ PCB-1	4	0.0	0.0	25.0	0.0	4	0.0	0.0	25.0	0.0
¹³ C ₁₂ PCB-3	4	0.0	0.0	25.0	0.0	4	0.0	0.0	25.0	0.0
¹³ C ₁₂ PCB-4	4	0.0	0.0	0.0	0.0	4	0.0	0.0	25.0	0.0
¹³ C ₁₂ PCB-11	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-15	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-19	4	0.0	0.0	0.0	0.0	4	0.0	0.0	25.0	0.0
¹³ C ₁₂ PCB-28	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-37	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-52	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-54	4	0.0	0.0	0.0	0.0	4	0.0	0.0	25.0	0.0
¹³ C ₁₂ PCB-70	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-77	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-85	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-101	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-104	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-118	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-126	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-138	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-153	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-155	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-169	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-180	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-188	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-189	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-202	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-205	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-206	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-208	4	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0
¹³ C ₁₂ PCB-209	4	0.0	25.0	0.0	25.0	4	0.0	0.0	25.0	0.0

None of the means for the labeled compounds in the IPR aliquots failed a lower limit of 15%. Only two labeled congener means failed at 25%, the labeled analogs for PCB-1 and PCB-3 each failed once. On the upper end of the IPR recovery ranges, the label for PCB-209 failed the 130% upper limit once (e.g., 25% of the 4 IPR sets) and it still failed using a 150% upper limit. Both of those failures of labeled PCB-209 were in a single laboratory.

For the four sets of OPR data, six labeled congeners failed once each at the 25% lower recovery limit. Labeled congener PCB-209 failed in one laboratory and the other five labeled compounds all failed in a second laboratory. All six of those failures were eliminated using the 15% lower recovery limit.

Based on these failure rates, EPA compiled the interim acceptance criteria for the draft method shown (**in green**) in Table 19. The solution was to apply the same 15 - 130% limits for each label. In the case of the maximum RSD, the average RSD (which was 60%) was used for all 29 labeled compounds.

6. Method Detection Limits

EPA required that each laboratory determine the method detection limits (MDLs) for all 209 PCB congeners in each of the matrix types that they agreed to analyze: aqueous, solids (e.g., sediment and biosolids), and tissue. MDLs were determined using the newly revised MDL procedure promulgated by EPA in 2017.

The revised procedure defines the MDL as:

“... the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results.”

The procedure consists of two parts: determination of the MDL based on method blanks (called MDL_b), and determination of the MDL based on spiked samples (called MDL_s). Both MDL_b and MDL_s are determined in a reference matrix, using at least seven replicates prepared and analyzed on three non-consecutive days.

The MDL_b is calculated as:

$$MDL_b = \bar{X} + t_{(n-1, 1-\alpha=0.99)} S_b$$

where:

- \bar{X} = mean of the method blank results (use zero in place of the mean if the mean is negative)
 $t_{(n-1, 1-\alpha=0.99)}$ = the Student's t -value appropriate for the single-tailed 99th percentile t statistic and a standard deviation estimate with $n-1$ degrees of freedom
 S_b = sample standard deviation of the replicate method blank sample analyses

Note: The equation above is used when all of the method blanks for an individual analyte give numerical results. If some (but not all) of the method blank results give numerical results, then the MDL_b is set to be equal to the highest method blank result.

The MDL_s is calculated as:

$$MDL_s = t_{(n-1, 1-\alpha=0.99)} S_s$$

where:

- $t_{(n-1, 1-\alpha=0.99)}$ = the Student's t -value appropriate for a single-tailed 99th percentile t statistic and a standard deviation estimate with $n-1$ degrees of freedom.
 S_s = sample standard deviation of the replicate spiked sample analyses.

For aqueous sample MDL determinations, the matrix was reagent water. For solid sample MDL determinations, the matrix was Ottawa sand. For tissue sample MDL determinations, the matrix was the same 10:90 mixture of canola oil and sand used for the IPR samples. After both an MDL_b and MDL_s have been determined, the laboratory sets their initial MDL as the greater of the MDL_b and MDL_s values.

The laboratories provided all of the results to CSRA and CSRA independently performed the calculations of MDL_b and MDL_s after subjecting all of the results to a formal data review process. The results from all of the laboratories were then pooled by matrix type and used to calculate pooled MDLs and pooled Minimum Levels (MLs), based on the statistical procedures outlined in EPA's new method protocol (USEPA 2018). The purpose of those pooled MDL and ML values is to provide data users with a conservative overall assessment of the sensitivity of the analytical method. In practice, each laboratory utilizing the method will determine its own MDL values for each matrix of interest and utilize those laboratory-specific MDLs in assessing method blanks and determining when an analyte is detected.

Aqueous Sample Pooled MDL Determinations

CSRA provided all of the laboratories with instructions for preparing their aqueous MDL samples based on the MDL results from the single-laboratory validation study. Using the sets of nine retention time standards provided by EPA, the laboratories prepared a spiking solution that contained 100 ng/mL of each of the 209 PCB congeners in a water-miscible solvent. Adding 20 µL of that solution to each 1-L reagent water aliquot yielded a mass of 2 ng of each congener in the 1-L sample. Given the known coelutions of some congeners, this means that some “analytes” were spiked at 4 ng, and some at 6 ng, but 130 of the 167 analytes were spiked a 2 ng. Laboratories were permitted to increase that concentration if their initial attempts to determine the MDL did not yield detectable results for each of the congeners.

CSRA determined the pooled MDLs and pooled MLs for aqueous samples using the data from 7 laboratories. The results are summarized in Table 21, below. The analytes listed in the shaded rows in Table 21 are the congeners and coeluting congeners that represent the analytes that have direct calibration data for this method.

Table 21. Aqueous Sample Pooled MDL and ML Results (ng/L)

Analyte	# Labs	Pooled MDL _s	Max. MDL _s	# MDL _b	Max. MDL _b	Pooled ML	Max. ML
PCB-1	7	1.75	5.97	1	0.53	5	20
PCB-2	7	0.71	2.33		0.00	2	5
PCB-3	7	0.69	2.23		1.14	2	5
PCB-4+10	7	1.90	6.10		0.00	5	20
PCB-8+5	7	1.00	2.73	1	0.12	2	10
PCB-6	7	0.57	1.55		1.05	2	5
PCB-7+9	7	1.17	2.87		0.07	5	10
PCB-11	7	0.72	2.34	1	0.14	2	5
PCB-12+13	7	1.11	3.56		0.08	5	10
PCB-14	7	0.64	1.99		0.06	2	5
PCB-15	7	0.44	1.34	1	0.02	1	5
PCB-16+32	7	0.80	1.58	1	0.02	2	5
PCB-17	7	0.49	1.01	1	0.18	2	2
PCB-18	7	0.46	0.80	1	0.09	1	2
PCB-19	7	0.63	1.90	1	0.01	2	5
PCB-33+20+21	7	1.11	3.15	1	0.52	5	10
PCB-22	7	0.39	0.96		0.00	1	2
PCB-34+23	7	1.00	2.88		0.00	2	10
PCB-24+27	7	0.64	1.24		0.08	2	5
PCB-25	7	0.46	1.37		0.23	1	5
PCB-26	7	0.43	1.30	1	0.02	1	5
PCB-28	7	0.69	1.71	1	0.08	2	5
PCB-29	7	0.49	1.40	1	0.02	2	5
PCB-30	7	0.61	1.38		0.00	2	5
PCB-31	7	0.50	1.36	1	0.30	2	5
PCB-35	7	0.89	2.54	1	0.69	2	10
PCB-36	7	0.54	1.57		0.15	2	5
PCB-37	7	0.44	1.27		0.20	1	5
PCB-38	7	1.66	3.64		0.00	5	10
PCB-39	7	0.53	1.47		0.29	2	5
PCB-40	7	1.12	3.84		0.43	5	10
PCB-41+64	7	0.97	1.97		0.00	2	5
PCB-42	7	0.73	1.38		0.14	2	5
PCB-49+43	7	1.06	3.11	1	0.01	2	10
PCB-44	7	0.40	0.75		0.30	1	2
PCB-45	7	0.31	0.66		0.00	1	2

Table 21. Aqueous Sample Pooled MDL and ML Results (ng/L)

Analyte	# Labs	Pooled MDL _s	Max. MDL _s	# MDL _b	Max. MDL _b	Pooled ML	Max. ML
PCB-46	7	0.36	0.79		0.21	1	2
PCB-47+48+75	7	1.71	5.39		0.00	5	20
PCB-50	7	0.58	1.42		0.15	2	5
PCB-51	7	0.48	1.26		0.08	2	5
PCB-52+73	7	0.97	2.60	1	0.08	2	10
PCB-53	7	0.33	0.72		0.11	1	2
PCB-54	7	0.58	1.80	1	0.01	2	5
PCB-55	7	0.39	1.02		0.53	1	2
PCB-56+60	7	0.74	1.62		0.17	2	5
PCB-57	7	0.47	1.30		0.00	1	5
PCB-58	7	0.46	1.20		0.00	1	5
PCB-59	7	0.60	1.19		0.22	2	5
PCB-74+61	7	0.96	2.68		0.00	2	10
PCB-62	7	0.49	0.99		0.09	2	2
PCB-63	7	0.38	1.11		0.13	1	5
PCB-65	7	0.57	0.89		0.16	2	2
PCB-66+80	7	0.91	2.33	1	0.02	2	5
PCB-67	7	0.45	1.34		0.17	1	5
PCB-68	7	0.66	1.88		0.00	2	5
PCB-69	7	0.53	1.18	1	0.08	2	5
PCB-70	7	1.32	4.20	2	0.10	5	10
PCB-71	7	1.09	3.65		0.00	2	10
PCB-72	7	0.50	1.37		0.17	2	5
PCB-76	7	0.53	1.31		0.14	2	5
PCB-77	7	0.50	1.40		0.16	2	5
PCB-78	7	0.51	0.93		0.00	2	2
PCB-79	7	0.48	1.28		0.00	2	5
PCB-81	7	0.49	1.43		0.10	2	5
PCB-82	7	0.61	1.31		0.12	2	5
PCB-83+109	7	0.76	1.47		0.07	2	5
PCB-84	7	2.53	8.66		0.16	10	20
PCB-85+120	7	1.19	3.08		0.00	5	10
PCB-97+86	7	1.67	4.07	1	0.16	5	10
PCB-87+115+116	7	2.23	5.08	1	0.15	5	20
PCB-88+121	7	0.93	2.23		0.20	2	5
PCB-90+101+89	7	3.36	11.03	1	0.23	10	50
PCB-91	7	0.39	0.92		0.00	1	2
PCB-92	7	0.53	1.63		0.00	2	5
PCB-95+93	7	2.01	5.20	1	0.10	5	20
PCB-94	7	0.32	0.56		0.00	1	2
PCB-96	7	0.37	0.78		0.00	1	2
PCB-98+102	7	0.77	1.38		0.00	2	5
PCB-99	7	1.30	4.39	1	0.09	5	10
PCB-100	7	0.50	1.09		0.00	2	2
PCB-103	7	0.48	0.85		0.00	2	2
PCB-104	7	0.51	1.31		0.06	2	5
PCB-105+127	7	1.23	3.90	1	0.11	5	10
PCB-118+106	7	3.21	11.04	1	0.27	10	50
PCB-107+108	7	0.86	2.56	1	0.03	2	10
PCB-110	7	3.94	13.18	1	0.49	10	50
PCB-111+117	7	1.33	2.54		0.15	5	10
PCB-112	7	0.34	0.74	1	0.30	1	2
PCB-113	7	0.34	0.74		0.10	1	2

Table 21. Aqueous Sample Pooled MDL and ML Results (ng/L)

Analyte	# Labs	Pooled MDL _s	Max. MDL _s	# MDL _b	Max. MDL _b	Pooled ML	Max. ML
PCB-114	7	0.28	0.68		0.00	1	2
PCB-119	7	0.42	0.87	1	0.16	1	2
PCB-122	7	0.19	0.35		0.14	0.5	1
PCB-123	7	0.31	0.84		0.19	1	2
PCB-124	7	0.35	0.76		0.11	1	2
PCB-125	7	0.81	2.17		0.08	2	5
PCB-126	7	0.42	1.07		0.00	1	2
PCB-128	7	1.27	4.19		0.00	5	10
PCB-129	7	0.33	0.68		0.19	1	2
PCB-130	7	0.35	0.67		0.00	1	2
PCB-131+142	7	1.46	4.73		0.00	5	20
PCB-132+168	7	1.91	5.83	2	0.63	5	20
PCB-133	7	0.39	0.77		0.00	1	2
PCB-134	7	0.75	2.33		0.12	2	5
PCB-144+135	7	1.26	3.75	1	0.09	5	10
PCB-136	7	1.39	4.62		0.12	5	10
PCB-137	7	0.38	0.68		0.19	1	2
PCB-138+163+164	7	3.95	11.56	2	0.26	10	50
PCB-149+139	7	4.98	16.27	2	0.77	20	50
PCB-140	7	4.00	14.06		0.21	10	50
PCB-141	7	1.35	4.42	1	0.23	5	10
PCB-143	7	0.40	0.82		0.00	1	2
PCB-145	7	0.43	0.82		0.00	1	2
PCB-146	7	0.57	1.28		0.00	2	5
PCB-147	7	0.30	0.61		0.00	1	2
PCB-148	7	0.44	0.81		0.00	1	2
PCB-150	7	0.46	0.86		0.00	1	2
PCB-151	7	1.97	6.70		0.00	5	20
PCB-152	7	0.50	1.05		0.00	2	2
PCB-153	7	3.90	12.59	6	1.51	10	50
PCB-154	7	0.42	0.90		0.00	1	2
PCB-155	7	0.43	1.00		0.08	1	2
PCB-156	7	0.37	0.61		0.24	1	2
PCB-157	7	0.60	1.67		0.46	2	5
PCB-158+160	7	0.73	1.55		0.00	2	5
PCB-159	7	0.51	1.38		0.12	2	5
PCB-161	7	0.43	1.20		0.00	1	5
PCB-162	7	0.60	1.60	2	0.17	2	5
PCB-165	7	1.51	5.20		0.06	5	20
PCB-166	7	0.37	0.74		0.16	1	2
PCB-167	7	0.94	3.11		0.00	2	10
PCB-169	7	0.34	0.93		0.00	1	2
PCB-170+190	7	1.95	6.68		0.00	5	20
PCB-171	7	0.60	1.78	1	0.10	2	5
PCB-172+192	7	0.59	1.09		0.00	2	2
PCB-173	7	0.33	0.56		0.21	1	2
PCB-174	7	3.12	10.92		0.00	10	20
PCB-175	7	0.33	0.56		0.00	1	2
PCB-176	7	0.56	1.58		0.09	2	5
PCB-177	7	1.57	5.44		0.18	5	20
PCB-178	7	0.50	1.03		0.14	2	2
PCB-179	7	1.54	5.27		0.10	5	20
PCB-180	7	0.37	0.92	1	0.07	1	2

Table 21. Aqueous Sample Pooled MDL and ML Results (ng/L)

Analyte	# Labs	Pooled MDL _s	Max. MDL _s	# MDL _b	Max. MDL _b	Pooled ML	Max. ML
PCB-181	7	3.10	10.86		0.09	10	20
PCB-187+182	7	2.28	7.75	1	0.25	5	20
PCB-183	7	0.92	3.03	2	0.25	2	10
PCB-184	7	0.49	1.16		0.00	2	5
PCB-185	7	0.36	0.78		0.00	1	2
PCB-186	7	0.35	0.74		0.00	1	2
PCB-188	7	0.39	0.90		0.00	1	2
PCB-189	7	0.26	0.54		0.00	1	2
PCB-191	7	0.22	0.48		0.00	0.5	2
PCB-193	7	0.39	1.11	1	0.13	1	5
PCB-194	7	3.16	10.31		0.00	10	20
PCB-195	7	0.43	0.81		0.36	1	2
PCB-196+203	7	1.13	3.51		0.00	5	10
PCB-197	7	0.43	0.90		0.06	1	2
PCB-198	7	0.80	2.27	1	0.34	2	5
PCB-199	7	0.88	2.84		0.20	2	10
PCB-200	7	0.44	1.30		0.00	1	5
PCB-201	7	0.59	1.81		0.23	2	5
PCB-202	7	0.26	0.46		0.00	1	1
PCB-204	7	0.65	1.81		0.08	2	5
PCB-205	7	0.75	2.49		0.00	2	10
PCB-206	7	0.64	1.92	1	0.09	2	5
PCB-207	7	0.62	1.97		0.00	2	5
PCB-208	7	0.90	3.05		0.00	2	10
PCB-209	7	0.50	1.34	2	3.12	2	5

The analytes listed in the shaded rows are the congeners and coeluting congeners that represent the analytes that have direct calibration data for this method.

The results from Lab 5 were not used in the pooled MDL calculations shown in Table 21 for two reasons. First, the laboratory only prepared 750-mL samples for their MDL studies, instead of a full 1 L. Second, their MDL results differed widely from those of the other seven laboratories and did not appear to be representative of the community as a whole.

Lab 8 was the only laboratory that completed the study that had agreed to analyze the aqueous samples using both separatory funnel extraction and solid-phase extraction (SPE). Their separatory funnel MDL results were included in the pooled MDLs shown in Table 21. However, their SPE MDL results were not included because they would be the only SPE results, which would not be representative of the MDLs that other laboratories might achieve.

For the 167 analytes, the separatory funnel results from Lab 8 accounted for 125 of the maximum MDL_s values in Table 21. The maximum MDL_s values for the remaining 42 analytes were contributed by five of the other six laboratories, with one laboratory never having the highest MDL_s value for any analyte.

Overall, the pooled MDL_s values ranged from 0.19 ng/L to 4.98 ng/L, while the maximum MDL_s values ranged from 0.35 ng/L to 16.27 ng/L. In total, 23 of the maximum MDL_s values in Table 20 are greater than 5 ng/L, and 10 of those are greater than 10 ng/L. However, as noted in Section 1, the published MDL value for Aroclor 1242 in EPA Method 608.3 is 65 ng/L. Thus, the highest MDL_s value in this study, 16.27 ng/L, is still 3.995 times lower than the MDL in Method 608.3. The majority of the pooled MDL values are far lower, with the highest pooled MDL of 4.98 for the coeluting pair of PCB-149+139 being 13 times lower. The pooled MDL_s values for all 167 analytes are presented graphically in Figure 2. The x-axis is in the same order as the analytes are listed in Table 21.

The pooled ML for each of the 167 analytes was calculated as a multiplier of 3.18 times the pooled MDL value, and then rounded to the nearest multiple of 1, 2, or 5, in order to facilitate future preparation of calibration standards at the ML. The 167 pooled ML values ranged from 0.5 ng/L to 20 ng/L. In total, 127 pooled ML values are less than or equal to 2 ng/L, and 156 pooled ML values are less than or equal to 5 ng/L. Another 10 pooled ML values are 10 ng/L, and only one analyte had a pooled ML value of 20 ng/L. The pooled MDL_s values for aqueous samples are presented in Figure 2.

Although the SPE MDL results from Lab 8 were not used in the pooled MDL calculations, CSRA did perform a gross comparison of those SPE MDLs to the pooled separatory funnel MDLs. In general, the SPE MDLs from Lab 8 were significantly higher than the pooled separatory funnel MDLs. Of the 167 analytes, 123 analytes had SPE MDLs that were more than 1.5 times the pooled MDLs, with 29 SPE MDLs lower than the pooled MDLs. Those 123 SPE MDLs ranged up to 160 times the pooled MDLs, with 8 SPE MDLs at least 25 times higher than the pooled MDLs. Lab 8 noted significant blank levels for several congeners that drove their use of MDL_b over MDL_s for 6 of the 167 analytes. Lab 8 also performed the SPE manually, rather than with an automated system, which appears to have introduced much larger variability into their MDL_s results and would explain their much higher MDL values, especially compared to the automated SPE MDLs from the single-laboratory study of the draft method.

Because of the nature of the MDL_b value, those were not used in the calculation of the pooled MDL_s values shown in Table 21 and Figure 2. However, Table 21 includes a column that provides the number of times that any one of the seven laboratories in the aqueous portion of the study reported a non-zero MDL_b value. EPA evaluated those MDL_b values and the frequencies at which they were reported. In total, aqueous MDL_b values were reported by one or more laboratories for 46 of the 167 analytes.

As anyone familiar with PCB analyses can attest, “blanks happen.” Therefore, the fact that a given laboratory reported non-zero MDL_b values is not surprising. Perhaps equally important is that reporting an MDL_b value itself is not evidence of problems in an individual laboratory. In fact, the reason that EPA promulgated the revised MDL procedure that includes the MDL_b concept was to acknowledge the role of method blanks in assessing the sensitivity (detection limit) of any method.

What is evident in the study data is that the necessity of reporting MDL_b values is decidedly a lab-specific issue. Table 22 includes a summary of the frequencies at which the seven laboratories reported aqueous MDL_b values.

Table 22. Frequency of Aqueous MDL_b values by Lab

Lab #	1	3	4	6	7	8	9
# MDL _b values	26	0	2	2	24	4	1

More specifically, of the 26 MDL_b values reported by Laboratory 1, 21 of them were exclusive to that laboratory (in other words, no other laboratory reported an MDL_b value for those 21 congeners). Of the 24 values reported by Laboratory 7, 16 of those were exclusive to that laboratory. In contrast, while Laboratory 4 reported MDL_b values for four analytes, at least one other laboratory also reported an MDL_b value for those four analytes.

The only congener where the MDL_b was prevalent was for PCB-153, where six of the seven laboratories reported an MDL_b. Even for this congener, the actual MDL_b values in those six laboratories ranged from 0.27 ng/L to 1.21 ng/L. The MDL_s value reported for PCB-153 by the seventh laboratory was 0.38 ng/L. The pooled MDL_s value in Table 20 and calculated from the results from all seven laboratories was 3.90 ng/L, reflecting the variability in the MDL_s values across all of the laboratories. None of those MDL values (whether an MDL_b or an MDL_s) was an impediment to analyzing the real-world samples in the study.

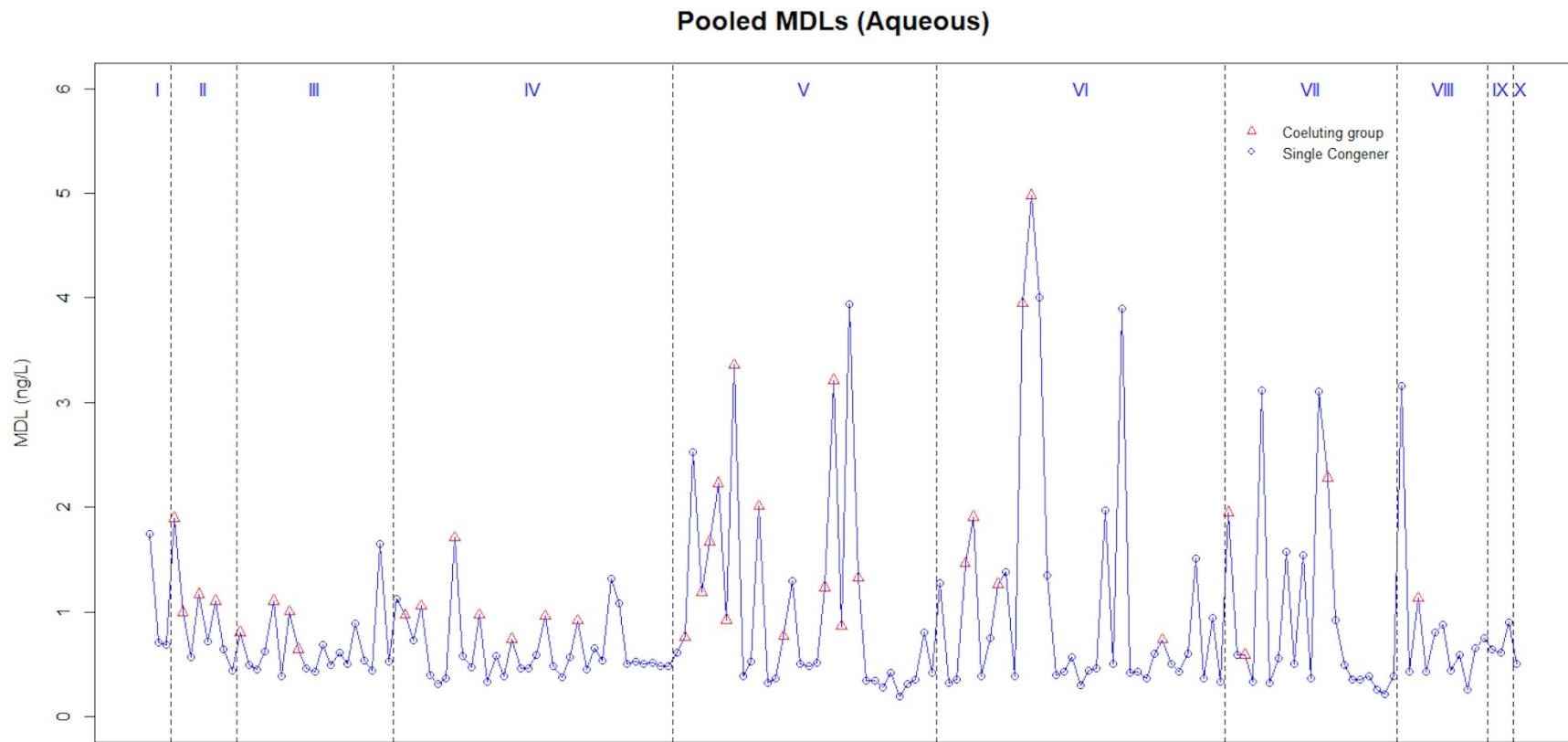


Figure 2. Pooled Aqueous MDLs Values in Elution Order

Dotted lines and Roman numerals delineate the levels of chlorination. The red triangle symbols denote a group of two or more coeluting congeners, while the blue diamond symbols denote single congeners.

Through these MDL data and the routine method blank results generated during the course of the validation study, EPA has demonstrated that background levels in typical laboratories are not a limiting factor in the application of this method, but that some laboratories do have much better control of background levels than others.

Soil/Sediment and Biosolids Sample MDL Determinations

The laboratories also determined the MDLs for the 209 PCB congeners in solid matrices, using Ottawa sand as the reference matrix. Separate MDLs were not determined for the soil/sediment and biosolids matrices in this study. Rather, the solid sample MDLs were applied to the biosolids samples for the purposes of the study. However, in practice, each laboratory performing biosolids analyses using this procedure would determine its own MDLs for the biosolids matrix.

CSRA provided all of the laboratories with instructions for preparing their solid MDL samples based on the MDL results from the single-laboratory validation study. The same 100 ng/mL spiking solution used for the aqueous MDL was used for the solid MDL determination. Laboratories were permitted to increase that concentration if their initial attempts to determine the MDL did not yield detectable results for each of the congeners.

CSRA determined the pooled MDLs for solid samples using the data from six laboratories. The results are summarized in Table 23, below. The analytes listed in the shaded rows in Table 23 are the congeners and coeluting congeners that represent the analytes that have direct calibration data for this method.

Table 23. Solid Sample Pooled MDL and ML Results (ng/g)

Analyte	# Labs	Pooled MDL _s	Max. MDL _s	# MDL _b	Max. MDL _b	Pooled ML	Max. ML
PCB-1	6	0.63	1.72		0.00	2	5
PCB-2	6	0.06	0.13		0.00	0.2	0.5
PCB-3	6	0.10	0.22		0.00	0.2	0.5
PCB-4+10	6	0.15	0.34		0.00	0.5	1
PCB-8+5	6	0.22	0.62		0.00	0.5	2
PCB-6	6	0.09	0.23		0.00	0.2	0.5
PCB-7+9	6	0.24	0.65		0.00	1	2
PCB-11	6	0.42	1.27	1	1.46	1	5
PCB-12+13	6	0.21	0.59		0.00	0.5	2
PCB-14	6	0.11	0.30		0.00	0.2	1
PCB-15	6	0.09	0.23		0.00	0.2	0.5
PCB-16+32	6	0.14	0.32		0.00	0.5	1
PCB-17	6	0.07	0.18		0.00	0.2	0.5
PCB-18	6	0.07	0.13	1	0.05	0.2	0.5
PCB-19	6	0.08	0.21		0.00	0.2	0.5
PCB-33+20+21	6	0.30	0.75		0.00	1	2
PCB-22	6	0.08	0.19		0.00	0.2	0.5
PCB-34+23	6	0.11	0.27		0.00	0.2	1
PCB-24+27	6	0.09	0.21		0.00	0.2	0.5
PCB-25	6	0.08	0.19		0.00	0.2	0.5
PCB-26	6	0.09	0.24		0.00	0.2	1
PCB-28	6	0.15	0.41		0.00	0.5	1
PCB-29	6	0.06	0.16		0.00	0.2	0.5
PCB-30	6	0.08	0.21		0.00	0.2	0.5
PCB-31	6	0.07	0.16		0.00	0.2	0.5
PCB-35	6	0.21	0.41		0.00	0.5	1
PCB-36	6	0.17	0.46		0.00	0.5	1
PCB-37	6	0.18	0.50		0.00	0.5	2
PCB-38	6	0.14	0.33		0.00	0.5	1

Table 23. Solid Sample Pooled MDL and ML Results (ng/g)

Analyte	# Labs	Pooled MDL _s	Max. MDL _s	# MDL _b	Max. MDL _b	Pooled ML	Max. ML
PCB-39	6	0.10	0.23		0.00	0.2	0.5
PCB-40	6	0.16	0.38		0.00	0.5	1
PCB-41+64	6	0.17	0.48		0.00	0.5	2
PCB-42	6	0.10	0.22		0.00	0.2	0.5
PCB-49+43	6	0.24	0.65		0.00	1	2
PCB-44	6	0.11	0.29		0.00	0.5	1
PCB-45	6	0.09	0.24		0.00	0.2	1
PCB-46	6	0.06	0.16		0.00	0.2	0.5
PCB-47+48+75	6	0.24	0.60	1	0.02	1	2
PCB-50	6	0.07	0.20		0.00	0.2	0.5
PCB-51	6	0.06	0.17		0.00	0.2	0.5
PCB-52+73	6	0.17	0.43		0.00	0.5	1
PCB-53	6	0.05	0.13		0.00	0.2	0.5
PCB-54	6	0.06	0.16		0.00	0.2	0.5
PCB-55	6	0.08	0.21		0.00	0.2	0.5
PCB-56+60	6	0.13	0.26		0.00	0.5	1
PCB-57	6	0.10	0.29		0.00	0.2	1
PCB-58	6	0.11	0.33		0.00	0.5	1
PCB-59	6	0.07	0.18		0.00	0.2	0.5
PCB-74+61	6	0.14	0.36		0.00	0.5	1
PCB-62	6	0.11	0.32		0.00	0.5	1
PCB-63	6	0.08	0.21		0.00	0.2	0.5
PCB-65	6	0.10	0.29		0.00	0.2	1
PCB-66+80	6	0.19	0.50		0.00	0.5	2
PCB-67	6	0.11	0.31		0.00	0.2	1
PCB-68	6	0.16	0.45		0.00	0.5	1
PCB-69	6	0.12	0.32		0.00	0.5	1
PCB-70	6	0.08	0.14	1	0.06	0.2	0.5
PCB-71	6	0.14	0.39		0.00	0.5	1
PCB-72	6	0.10	0.24		0.00	0.2	1
PCB-76	6	0.11	0.30		0.00	0.2	1
PCB-77	6	0.07	0.16		0.00	0.2	0.5
PCB-78	6	0.10	0.23		0.00	0.2	0.5
PCB-79	6	0.08	0.17		0.00	0.2	0.5
PCB-81	6	0.09	0.22		0.00	0.2	0.5
PCB-82	6	0.06	0.13		0.00	0.2	0.5
PCB-83+109	6	0.14	0.28		0.00	0.5	1
PCB-84	6	0.07	0.16		0.00	0.2	0.5
PCB-85+120	6	0.15	0.31		0.00	0.5	1
PCB-97+86	6	0.11	0.18		0.00	0.2	0.5
PCB-87+115+116	6	0.37	0.82	1	0.09	1	2
PCB-88+121	6	0.12	0.29		0.00	0.5	1
PCB-90+101+89	6	0.24	0.49	2	0.15	1	2
PCB-91	6	0.05	0.13		0.00	0.2	0.5
PCB-92	6	0.06	0.10		0.00	0.2	0.2
PCB-95+93	6	0.12	0.29	1	0.06	0.5	1
PCB-94	6	0.06	0.18		0.00	0.2	0.5
PCB-96	6	0.06	0.14		0.00	0.2	0.5
PCB-98+102	6	0.12	0.29		0.00	0.5	1
PCB-99	6	0.10	0.20		0.00	0.2	0.5
PCB-100	6	0.17	0.51		0.00	0.5	2
PCB-103	6	0.15	0.44		0.00	0.5	1
PCB-104	6	0.05	0.12		0.00	0.2	0.5

Table 23. Solid Sample Pooled MDL and ML Results (ng/g)

Analyte	# Labs	Pooled MDL _s	Max. MDL _s	# MDL _b	Max. MDL _b	Pooled ML	Max. ML
PCB-105+127	6	0.19	0.38	1	0.06	0.5	1
PCB-118+106	6	0.39	0.91	1	0.15	1	2
PCB-107+108	6	0.16	0.39		0.00	0.5	1
PCB-110	6	0.31	0.72	1	0.18	1	2
PCB-111+117	6	0.21	0.51		0.00	0.5	2
PCB-112	6	0.09	0.25		0.00	0.2	1
PCB-113	6	0.08	0.22		0.00	0.2	0.5
PCB-114	6	0.06	0.14		0.00	0.2	0.5
PCB-119	6	0.08	0.22		0.00	0.2	0.5
PCB-122	6	0.07	0.17		0.00	0.2	0.5
PCB-123	6	0.09	0.24		0.00	0.2	1
PCB-124	6	0.08	0.19		0.00	0.2	0.5
PCB-125	6	0.07	0.15		0.00	0.2	0.5
PCB-126	6	0.07	0.17		0.00	0.2	0.5
PCB-128	6	0.08	0.14		0.00	0.2	0.5
PCB-129	6	0.07	0.13		0.00	0.2	0.5
PCB-130	6	0.07	0.12		0.00	0.2	0.5
PCB-131+142	6	0.10	0.26		0.00	0.2	1
PCB-132+168	6	0.18	0.35	1	0.05	0.5	1
PCB-133	6	0.07	0.18		0.00	0.2	0.5
PCB-134	6	0.08	0.17		0.00	0.2	0.5
PCB-144+135	6	0.19	0.52		0.00	0.5	2
PCB-136	6	0.06	0.16		0.00	0.2	0.5
PCB-137	6	0.08	0.17		0.00	0.2	0.5
PCB-138+163+164	6	0.34	0.72	2	0.12	1	2
PCB-149+139	6	0.20	0.42	2	0.10	0.5	1
PCB-140	6	0.06	0.13		0.00	0.2	0.5
PCB-141	6	0.09	0.16		0.00	0.2	0.5
PCB-143	6	0.07	0.15		0.00	0.2	0.5
PCB-145	6	0.08	0.22		0.00	0.2	0.5
PCB-146	6	0.07	0.15		0.00	0.2	0.5
PCB-147	6	0.08	0.20		0.00	0.2	0.5
PCB-148	6	0.07	0.19		0.00	0.2	0.5
PCB-150	6	0.07	0.19		0.00	0.2	0.5
PCB-151	6	0.08	0.19		0.00	0.2	0.5
PCB-152	6	0.07	0.17		0.00	0.2	0.5
PCB-153	6	0.20	0.48	5	0.15	0.5	2
PCB-154	6	0.08	0.19		0.00	0.2	0.5
PCB-155	6	0.05	0.10		0.00	0.1	0.2
PCB-156	6	0.06	0.10		0.00	0.2	0.2
PCB-157	6	0.07	0.12		0.00	0.2	0.5
PCB-158+160	6	0.12	0.20		0.00	0.5	0.5
PCB-159	6	0.06	0.13		0.00	0.2	0.5
PCB-161	6	0.07	0.14		0.00	0.2	0.5
PCB-162	6	0.06	0.12		0.00	0.2	0.5
PCB-165	6	0.07	0.15		0.00	0.2	0.5
PCB-166	6	0.08	0.15		0.00	0.2	0.5
PCB-167	6	0.06	0.10		0.00	0.2	0.2
PCB-169	6	0.10	0.26		0.00	0.2	1
PCB-170+190	6	0.14	0.24		0.00	0.5	1
PCB-171	6	0.07	0.13		0.00	0.2	0.5
PCB-172+192	6	0.13	0.26		0.00	0.5	1
PCB-173	6	0.07	0.17		0.00	0.2	0.5

Table 23. Solid Sample Pooled MDL and ML Results (ng/g)

Analyte	# Labs	Pooled MDL _s	Max. MDL _s	# MDL _b	Max. MDL _b	Pooled ML	Max. ML
PCB-174	6	0.09	0.22		0.00	0.2	0.5
PCB-175	6	0.07	0.16		0.00	0.2	0.5
PCB-176	6	0.06	0.12		0.00	0.2	0.5
PCB-177	6	0.07	0.16		0.00	0.2	0.5
PCB-178	6	0.09	0.22		0.00	0.2	0.5
PCB-179	6	0.05	0.10		0.00	0.2	0.2
PCB-180	6	0.07	0.14	2	0.05	0.2	0.5
PCB-181	6	0.07	0.16		0.00	0.2	0.5
PCB-187+182	6	0.15	0.35		0.00	0.5	1
PCB-183	6	0.08	0.17		0.00	0.2	0.5
PCB-184	6	0.05	0.11		0.00	0.2	0.2
PCB-185	6	0.07	0.15		0.00	0.2	0.5
PCB-186	6	0.07	0.16		0.00	0.2	0.5
PCB-188	6	0.06	0.12		0.00	0.2	0.5
PCB-189	6	0.06	0.11		0.00	0.2	0.2
PCB-191	6	0.07	0.12		0.00	0.2	0.5
PCB-193	6	0.07	0.12		0.00	0.2	0.5
PCB-194	6	0.18	0.32		0.00	0.5	1
PCB-195	6	0.93	3.16		0.00	2	10
PCB-195 (w/o Lab 8)	5	0.07	0.12		0.00	0.2	0.5
PCB-196+203	6	0.15	0.35	1	0.15	0.5	1
PCB-197	6	0.06	0.10		0.00	0.2	0.2
PCB-198	6	0.10	0.19		0.00	0.2	0.5
PCB-199	6	0.08	0.19	1	0.17	0.2	0.5
PCB-200	6	0.06	0.13		0.00	0.2	0.5
PCB-201	6	0.06	0.11		0.00	0.2	0.5
PCB-202	6	0.05	0.08		0.00	0.2	0.2
PCB-204	6	0.07	0.12		0.00	0.2	0.5
PCB-205	6	0.06	0.12		0.00	0.2	0.5
PCB-206	6	0.06	0.10	1	0.28	0.2	0.2
PCB-207	6	0.06	0.10		0.00	0.2	0.2
PCB-208	6	0.05	0.09		0.00	0.2	0.2
PCB-209	6	0.26	0.87	3	0.41	1	2

The analytes listed in the shaded rows are the congeners and coeluting congeners that represent the analytes that have direct calibration data for this method.

Overall, the pooled MDL_s values ranged from 0.05 ng/g to 0.93 ng/g. However, the pooled MDL_s of 0.93 ng/g for PCB-195 was dramatically influenced by the results from Lab 8, at 3.16 ng/g. Review of that MDL_s value at CSRA and the laboratory did not uncover any calculation errors, but their MDL_s was over 25 times the next highest MDL_s for that congener in any of the other laboratories (0.12 ng/g). Therefore, EPA decided to report the MDL values in Table 23 both with and without the PCB-195 result from Lab 8. Without the MDL_s from Lab 8, the pooled MDL_s for PCB-195 is only 0.07 ng/g.

In total, 109 of the pooled MDL_s values in Table 23 are less than or equal to 0.1 ng/g, 42 more are between 0.1 and 0.2 ng/g, and 16 pooled MDL_s values are greater than 0.2 ng/g. The pooled MDL_s values for all 167 analytes are presented graphically in Figure 3. The x-axis is in the same order as the analytes are listed in Table 23.

The pooled ML for each of the 167 analytes was calculated as a multiplier of 3.18 times the pooled MDL value, and then rounded to the nearest multiple of 1, 2, or 5, in order to facilitate future preparation of calibration standards at the ML.

The 167 pooled ML values ranged from 0.5 ng/g to 2.0 ng/g. In total, 1 pooled ML value is at 0.1 ng/g, 113 pooled ML values are at 0.2 ng/g, 41 pooled ML values are at 0.5 ng/g, 11 pooled ML values are at 1.0 ng/g, and 1 value is at 2.0 (for PCB-1).

Table 23 includes a column that provides the number of times that any one of the six laboratories in the solids portion of the study reported a non-zero MDL_b value. EPA evaluated those MDL_b values and the frequencies at which they were reported in a similar manner as for the aqueous MDL values. In total, solid MDL_b values were reported by one or more laboratories for 19 of the 167 analytes. Table 24 includes a summary of the frequencies at which the six laboratories reported solid MDL_b values.

Table 24. Frequency of Solid MDL_b values by Lab

Lab #	3	4	6	7	8	9
# MDL _b values	0	1	7	11	9	1

As with the aqueous MDLs, the need to employ an MDL_b was lab-specific. However, the patterns of occurrences within each laboratory often differed between the aqueous and solid phases of the study. For example, of the 9 solid MDL_b values reported by Laboratory 8, only three of them overlapped with the four MDL_b values that they reported in the aqueous phase (PCB-149+139, PCB-153, and PCB-209).

Conversely, in Laboratory 7, 10 of the 11 solid MDL_b values were for congeners that also had aqueous MDL_b values in that laboratory. This may be an indication that there is a common source in Laboratory 7 for those 10 congeners that affected both the aqueous and solid portion of the study, while a separate source may be responsible for the other 14 congeners with MDL_b values in the aqueous portion of the study.

PCB-153 was the one congener for which five of the six laboratories in the solid phase of the study reported an MDL_b value. Those five MDL_b values ranged from about 0.047 to 0.153 ng/g. The pooled MDL_s calculated from the study results for PCB-153 was 0.200 ng/g.

Overall, these MDL data demonstrate that background levels in typical laboratories are not a limiting factor in the application of this method, but that some laboratories do have much better control of background levels than others.

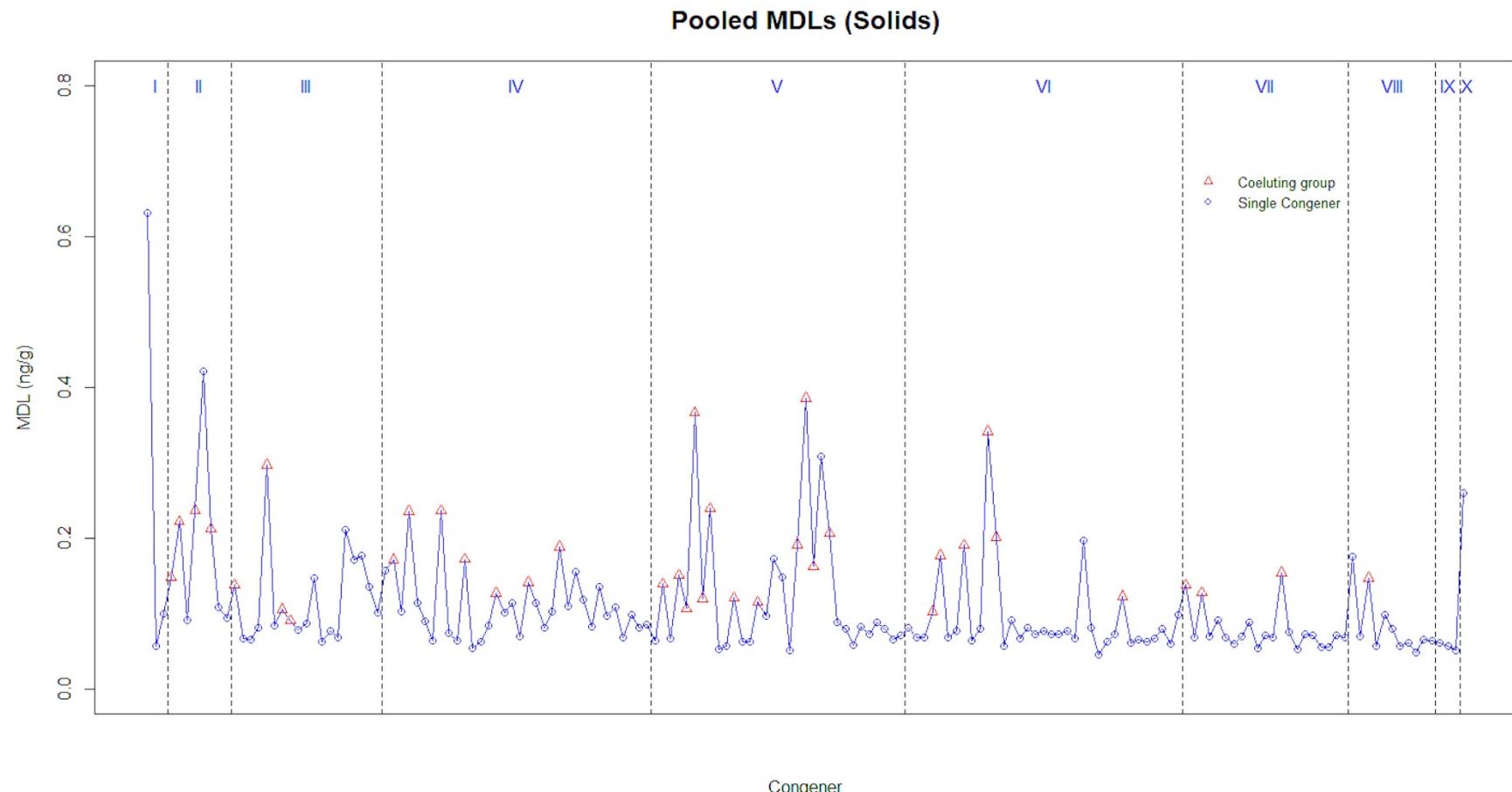


Figure 3. Pooled Solid MDL_s Values in Elution Order

Dotted lines and Roman numerals delineate the levels of chlorination. The red triangle symbols denote a group of two or more coeluting congeners, while the blue diamond symbols denote single congeners.

Tissue Sample MDL Determinations

The laboratories also determined the MDLs for the 209 PCB congeners in tissue matrices, using the 90:10 mixture of Ottawa sand and canola oil as the reference matrix.

CSRA provided all of the laboratories with instructions for preparing their tissue MDL samples based on the MDL results from the single-laboratory validation study. The same 100 ng/mL spiking solution used for the aqueous MDL was used for the aqueous and solid MDL determinations. Laboratories were permitted to increase that concentration if their initial attempts to determine the MDL did not yield detectable results for each of the congeners.

CSRA determined the pooled MDLs for tissue samples using the data from four laboratories. The results are summarized in Table 25, below. The analytes listed in the shaded rows are the congeners and coeluting congeners that represent the analytes that have direct calibration data for this method.

Table 25. Tissue Sample Pooled MDL and ML Results (ng/g)

Analyte	# Labs	Pooled MDL	Max. MDL _s	# MDL _b	Max. MDL _b	Pooled ML	Max. ML
PCB-1	4	0.11	0.18		0.00	0.2	0.5
PCB-2	4	0.13	0.29		0.00	0.5	1.0
PCB-3	4	0.11	0.23		0.00	0.2	0.5
PCB-4+10	3	0.23	0.43		0.00	0.5	1.0
PCB-8+5	4	0.18	0.28		0.00	0.5	1.0
PCB-6	4	0.10	0.18		0.00	0.2	0.5
PCB-7+9	3	0.22	0.38		0.00	0.5	1.0
PCB-11	4	0.06	0.09		0.00	0.2	0.2
PCB-12+13	4	0.13	0.24		0.00	0.5	1.0
PCB-14	4	0.07	0.14		0.00	0.2	0.5
PCB-15	4	0.06	0.11		0.00	0.2	0.2
PCB-16+32	4	0.18	0.32		0.00	0.5	1.0
PCB-17	4	0.08	0.13		0.00	0.2	0.5
PCB-18	4	0.09	0.15		0.00	0.2	0.5
PCB-19	4	0.07	0.10		0.00	0.2	0.2
PCB-33+20+21	4	0.20	0.34		0.00	0.5	1.0
PCB-22	4	0.10	0.22		0.00	0.2	0.5
PCB-34+23	4	0.13	0.25		0.00	0.5	1.0
PCB-24+27	4	0.11	0.18		0.00	0.5	0.5
PCB-25	4	0.08	0.16		0.00	0.2	0.5
PCB-26	4	0.07	0.13		0.00	0.2	0.5
PCB-28	4	0.14	0.33		0.00	0.5	1.0
PCB-29	4	0.08	0.14		0.00	0.2	0.5
PCB-30	4	0.08	0.12		0.00	0.2	0.5
PCB-31	4	0.09	0.18		0.00	0.2	0.5
PCB-35	4	0.14	0.32		0.00	0.5	1.0
PCB-36	4	0.10	0.22		0.00	0.2	0.5
PCB-37	4	0.12	0.24		0.00	0.5	1.0
PCB-38	4	0.13	0.31		0.00	0.5	1.0
PCB-39	4	0.06	0.10		0.00	0.2	0.2
PCB-40	4	0.13	0.28		0.00	0.5	1.0
PCB-41+64	4	0.15	0.25		0.00	0.5	1.0
PCB-42	4	0.09	0.18		0.00	0.2	0.5
PCB-49+43	4	0.22	0.53		0.00	0.5	2.0
PCB-44	4	0.09	0.18		0.00	0.2	0.5
PCB-45	4	0.07	0.13		0.00	0.2	0.5
PCB-46	4	0.07	0.14		0.00	0.2	0.5
PCB-47+48+75	4	0.23	0.51		0.00	0.5	2.0
PCB-50	4	0.07	0.13		0.00	0.2	0.5
PCB-51	4	0.07	0.13		0.00	0.2	0.5

Table 25. Tissue Sample Pooled MDL and ML Results (ng/g)

Analyte	# Labs	Pooled MDL	Max. MDL _s	# MDL _b	Max. MDL _b	Pooled ML	Max. ML
PCB-52+73	4	0.24	0.48		0.00	1.0	2.0
PCB-53	4	0.05	0.08		0.00	0.2	0.2
PCB-54	4	0.06	0.09		0.00	0.2	0.2
PCB-55	4	0.10	0.24		0.00	0.2	1.0
PCB-56+60	4	0.09	0.16	1	0.03	0.2	0.5
PCB-57	4	0.07	0.15		0.00	0.2	0.5
PCB-58	4	0.09	0.22		0.00	0.2	0.5
PCB-59	4	0.08	0.13		0.00	0.2	0.5
PCB-74+61	4	0.12	0.25		0.00	0.5	1.0
PCB-62	4	0.06	0.09		0.00	0.2	0.2
PCB-63	4	0.08	0.19		0.00	0.2	0.5
PCB-65	4	0.07	0.12		0.00	0.2	0.5
PCB-66+80	4	0.16	0.34		0.00	0.5	1.0
PCB-67	4	0.07	0.14		0.00	0.2	0.5
PCB-68	4	0.10	0.22		0.00	0.2	0.5
PCB-69	4	0.06	0.12		0.00	0.2	0.5
PCB-70	4	0.09	0.20		0.00	0.2	0.5
PCB-71	4	0.07	0.11		0.00	0.2	0.5
PCB-72	4	0.11	0.26		0.00	0.2	1.0
PCB-76	4	0.08	0.16		0.00	0.2	0.5
PCB-77	4	0.09	0.22		0.00	0.2	0.5
PCB-78	4	0.11	0.26		0.00	0.5	1.0
PCB-79	4	0.06	0.10	1	0.02	0.2	0.2
PCB-81	4	0.07	0.14		0.00	0.2	0.5
PCB-82	4	0.08	0.19		0.00	0.2	0.5
PCB-83+109	4	0.10	0.18		0.00	0.2	0.5
PCB-84	4	0.06	0.14		0.00	0.2	0.5
PCB-85+120	4	0.17	0.37		0.00	0.5	1.0
PCB-97+86	4	0.07	0.13		0.00	0.2	0.5
PCB-87+115+116	4	0.22	0.39		0.00	0.5	1.0
PCB-88+121	4	0.13	0.23		0.00	0.5	0.5
PCB-90+101+89	4	0.10	0.20		0.00	0.2	0.5
PCB-91	4	0.05	0.08		0.00	0.2	0.2
PCB-92	4	0.05	0.11		0.00	0.2	0.2
PCB-95+93	4	0.10	0.18	1	0.02	0.2	0.5
PCB-94	4	0.03	0.06		0.00	0.1	0.2
PCB-96	4	0.05	0.08		0.00	0.1	0.2
PCB-98+102	4	0.12	0.20		0.00	0.5	0.5
PCB-99	4	0.06	0.11		0.00	0.2	0.2
PCB-100	4	0.06	0.10		0.00	0.2	0.2
PCB-103	4	0.06	0.10		0.00	0.2	0.2
PCB-104	4	0.05	0.08		0.00	0.2	0.2
PCB-105+127	4	0.14	0.32		0.00	0.5	1.0
PCB-118+106	4	0.12	0.24		0.00	0.5	1.0
PCB-107+108	4	0.13	0.28		0.00	0.5	1.0
PCB-110	4	0.06	0.11		0.00	0.2	0.5
PCB-111+117	4	0.16	0.32		0.00	0.5	1.0
PCB-112	4	0.06	0.11		0.00	0.2	0.5
PCB-113	4	0.04	0.08		0.00	0.1	0.2
PCB-114	4	0.07	0.16		0.00	0.2	0.5
PCB-119	4	0.09	0.18		0.00	0.2	0.5
PCB-122	4	0.05	0.11		0.00	0.2	0.2
PCB-123	4	0.06	0.12		0.00	0.2	0.5
PCB-124	4	0.06	0.15		0.00	0.2	0.5
PCB-125	4	0.05	0.11		0.00	0.2	0.5
PCB-126	4	0.10	0.19		0.00	0.2	0.5

Table 25. Tissue Sample Pooled MDL and ML Results (ng/g)

Analyte	# Labs	Pooled MDL	Max. MDL _s	# MDL _b	Max. MDL _b	Pooled ML	Max. ML
PCB-128	4	0.08	0.16	1	0.02	0.2	0.5
PCB-129	4	0.08	0.18		0.00	0.2	0.5
PCB-130	4	0.06	0.11		0.00	0.2	0.5
PCB-131+142	4	0.19	0.44		0.00	0.5	1.0
PCB-132+168	4	0.14	0.26		0.00	0.5	1.0
PCB-133	4	0.07	0.15		0.00	0.2	0.5
PCB-134	4	0.06	0.11		0.00	0.2	0.2
PCB-144+135	4	0.11	0.21		0.00	0.5	0.5
PCB-136	4	0.05	0.08		0.00	0.1	0.2
PCB-137	4	0.06	0.12		0.00	0.2	0.5
PCB-138+163+164	4	0.17	0.32	1	0.02	0.5	1.0
PCB-149+139	4	0.12	0.25		0.00	0.5	1.0
PCB-140	4	0.06	0.10		0.00	0.2	0.2
PCB-141	4	0.07	0.14		0.00	0.2	0.5
PCB-143	4	0.07	0.15		0.00	0.2	0.5
PCB-145	4	0.06	0.10	1	0.04	0.2	0.2
PCB-146	4	0.04	0.07		0.00	0.1	0.2
PCB-147	4	0.07	0.13		0.00	0.2	0.5
PCB-148	4	0.06	0.10		0.00	0.2	0.2
PCB-150	4	0.11	0.23		0.00	0.2	0.5
PCB-151	4	0.05	0.08		0.00	0.2	0.2
PCB-152	4	0.06	0.09		0.00	0.2	0.2
PCB-153	4	0.09	0.15	3	0.07	0.2	0.5
PCB-154	4	0.06	0.09		0.00	0.2	0.2
PCB-155	4	0.05	0.11		0.00	0.2	0.5
PCB-156	4	0.07	0.13	1	0.04	0.2	0.5
PCB-157	4	0.08	0.14	1	0.01	0.2	0.5
PCB-158+160	4	0.13	0.23		0.00	0.5	0.5
PCB-159	4	0.06	0.10		0.00	0.2	0.2
PCB-161	4	0.07	0.13		0.00	0.2	0.5
PCB-162	4	0.05	0.09	1	0.01	0.2	0.2
PCB-165	4	0.04	0.08	1	0.01	0.1	0.2
PCB-166	4	0.09	0.21		0.00	0.2	0.5
PCB-167	4	0.06	0.10		0.01	0.2	0.2
PCB-169	4	0.06	0.13		0.00	0.2	0.5
PCB-170+190	3	0.15	0.28		0.00	0.5	1.0
PCB-171	4	0.13	0.32		0.00	0.5	1.0
PCB-172+192	4	0.12	0.20		0.00	0.5	0.5
PCB-173	4	0.21	0.52		0.00	0.5	2.0
PCB-174	4	0.08	0.13		0.00	0.2	0.5
PCB-175	4	0.08	0.17		0.00	0.2	0.5
PCB-176	4	0.07	0.14		0.00	0.2	0.5
PCB-177	4	0.06	0.12		0.00	0.2	0.5
PCB-178	4	0.09	0.18		0.00	0.2	0.5
PCB-179	4	0.05	0.09		0.00	0.2	0.2
PCB-180	4	0.19	0.45	1	0.03	0.5	1.0
PCB-181	4	0.08	0.17		0.00	0.2	0.5
PCB-187+182	4	0.13	0.24		0.00	0.5	1.0
PCB-183	4	0.07	0.15		0.00	0.2	0.5
PCB-184	4	0.06	0.10		0.00	0.2	0.2
PCB-185	4	0.06	0.10		0.00	0.2	0.2
PCB-186	4	0.05	0.08		0.00	0.2	0.2
PCB-188	4	0.05	0.09		0.00	0.2	0.2
PCB-189	4	0.07	0.12		0.00	0.2	0.5
PCB-191	4	0.05	0.10		0.00	0.2	0.2
PCB-193	4	0.06	0.11		0.00	0.2	0.2

Table 25. Tissue Sample Pooled MDL and ML Results (ng/g)

Analyte	# Labs	Pooled MDL	Max. MDL _s	# MDL _b	Max. MDL _b	Pooled ML	Max. ML
PCB-194	3	0.10	0.17		0.00	0.2	0.5
PCB-195	4	0.08	0.15		0.00	0.2	0.5
PCB-196+203	4	0.17	0.32		0.00	0.5	1.0
PCB-197	4	0.04	0.08		0.00	0.1	0.2
PCB-198	3	0.06	0.09		0.00	0.2	0.2
PCB-199	4	0.10	0.20		0.00	0.2	0.5
PCB-200	4	0.05	0.10		0.00	0.2	0.2
PCB-201	4	0.14	0.33		0.00	0.5	1.0
PCB-202	4	0.05	0.09		0.00	0.2	0.2
PCB-204	4	0.09	0.20		0.00	0.2	0.5
PCB-205	4	0.11	0.27		0.00	0.5	1.0
PCB-206	4	0.06	0.10		0.00	0.2	0.2
PCB-207	4	0.06	0.10		0.00	0.2	0.2
PCB-208	4	0.05	0.10		0.00	0.2	0.2
PCB-209	4	0.09	0.20		0.00	0.2	0.5

The analytes listed in the shaded rows are the congeners and coeluting congeners that represent the analytes that have direct calibration data for this method.

Initial examination of the MDL_s values from all four laboratories showed good consistency for the majority of the congeners, with pooled MDL_s values ranging from about 0.035 to 0.60 for 162 of the 167 analytes. The initial pooled MDL_s values for PCB-4+10, PCB-7+9, PCB-170+190, PCB-194 and PCB-198 were significantly higher than those for all the other congeners. Closer examination revealed that those higher values were driven by the MDL_s values from Lab 6, which ranged from 1.55 to 10.8 ng/g for those five analytes. All of those results were reviewed in detail, and while no obvious issues were identified, the project team opted to recalculate the pooled MDL_s values for those five analytes without the results from Lab 6. Those recalculated values are the ones presented in Table 25. The pooled MDL_s values ranged from about 0.035 to 0.23 ng/g for all 167 analytes. The pooled MDL_s values for all 167 analytes are presented graphically in Figure 4. The x-axis is in the same order as the analytes are listed in Table 25.

The pooled ML for each of the 167 analytes was calculated as a multiplier of 3.18 times the pooled MDL value, and then rounded to the nearest multiple of 1, 2, or 5, in order to facilitate future preparation of calibration standards at the ML.

The 167 pooled ML values ranged from 0.1 ng/g to 1.0 ng/g. In total, 7 pooled ML values are at 0.1 ng/g, 116 pooled ML values are at 0.2 ng/g, 43 pooled ML values are at 0.5 ng/g, and 1 pooled ML value is at 1.0 ng/g.

Table 25 includes a column that provides the number of times that any one of the four laboratories in the tissue portion of the study reported a non-zero MDL_b value. EPA evaluated those MDL_b values and the frequencies at which they were reported in a similar manner as for the other two matrices. In total, tissue MDL_b values were reported by one or more laboratories for 13 of the 167 analytes. Table 26 includes a summary of the frequencies at which the four laboratories reported tissue MDL_b values.

Table 26. Frequency of Tissue MDL_b values by Lab

Lab #	2	4	6	9
# MDL _b values	0	1	6	8

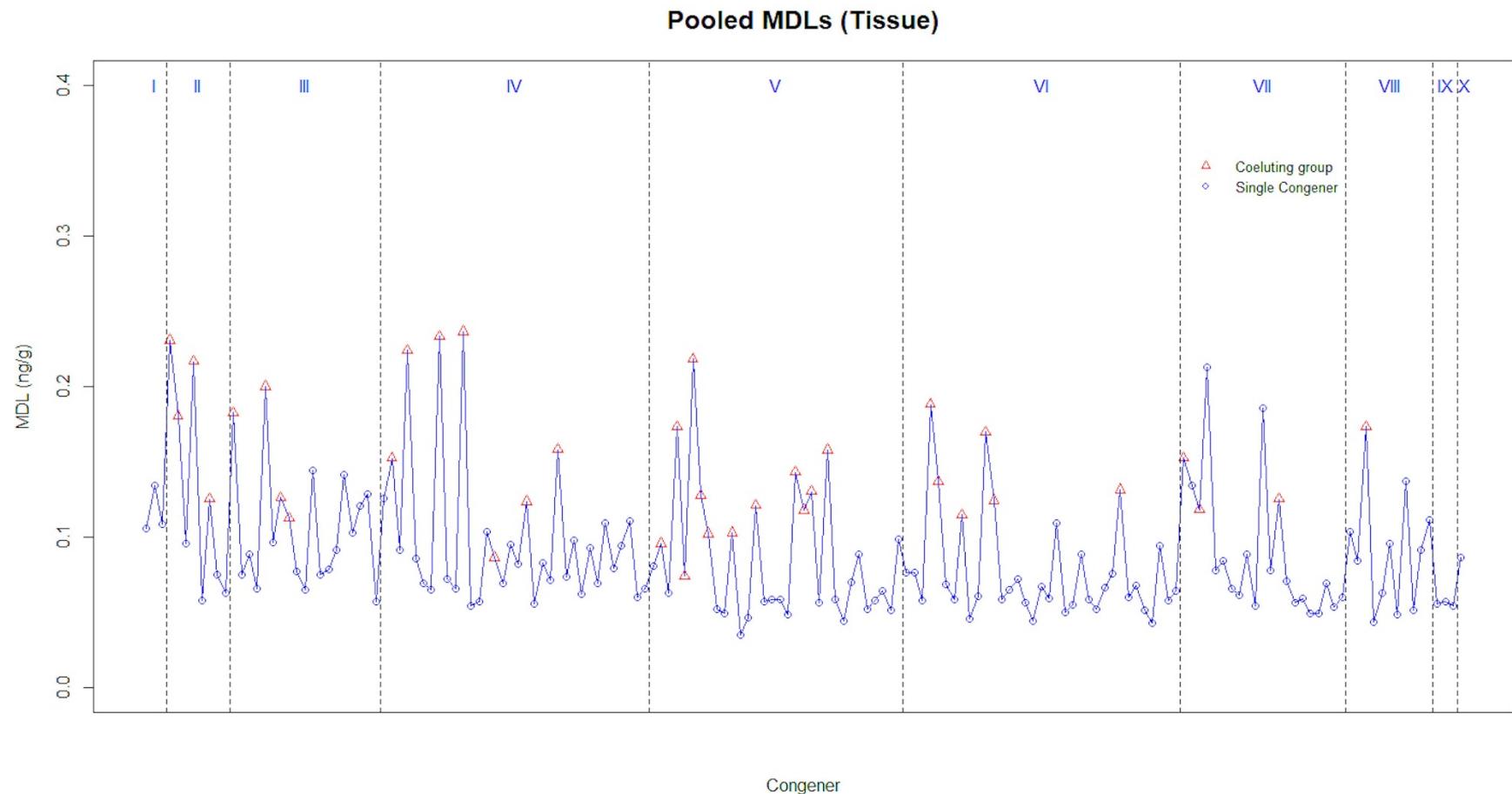


Figure 4. Pooled Tissue MDL_s Values in Elution Order

Dotted lines and Roman numerals delineate the levels of chlorination. The red triangle symbols denote a group of two or more coeluting congeners, while the blue diamond symbols denote single congeners.

The patterns of tissue MDL_b values differed across the four laboratories, as was found for the other two matrices. The patterns also differed between those matrices within some of the four laboratories in the tissue portion of the study. For example, Laboratory 9 only reported one MDL_b in the aqueous and solid portions of the study, for PCB-153 in both cases. They also reported a tissue MDL_b value for PCB-153, but they reported seven more MDL_b values in tissue, all of them occurring only at Laboratory 9.

In contrast, Laboratory 6 reported seven MDL_b values in the solid portion of the study and six in the tissue portion. Four of those six MDL_b values in tissue were unique to Laboratory 6, but other than PCB-153, none of them overlapped with the solid MDL_b values from that laboratory.

Once again, PCB-153 was the one congener in common across the majority of the laboratories in the study, with three of the four laboratories reporting an MDL_b value for this congener. Those three MDL_b values ranged from about 0.04 to 0.07 ng/g, and the pooled MDL_s values for PCB-153 in Table 25 is 0.09 ng/g.

As with the other two matrices, these MDL data demonstrate that background levels in typical laboratories are not a limiting factor in the application of this method, but that some laboratories have better control of background levels than others.

7. Unspiked Sample Analyses

The results for all of the unspiked sample analyses are summarized in the section below.

Wastewater Samples

Each of the seven laboratories that completed the wastewater portion of the study analyzed all nine of the wastewater samples as received, unspiked. As described in the draft procedure, all of the wastewater samples received the copper and Florisil cleanups. Wastewater #2 presented significant analytical challenges to all of the laboratories. Four of the laboratories applied additional cleanups, and two other laboratories diluted an aliquot of the final extract of wastewater #2 before analysis. The various cleanups applied by each laboratory as presented in Table 27.

Table 27. Summary of Wastewater Sample Cleanups

Sample Clean-up	Lab 1	Lab 3	Lab 4 ²	Lab 6	Lab 7	Lab 8	Lab 9	Recon
Acid - Sulfuric			X				X	
Alumina			X					X
Copper	X	X	X	X	X	X	X	X
Florisil®	X	X	X	X	X	X	X	X
GPC			X	X				X
Silica			X					
Dilution ¹					X	X		

¹ Dilution was only applied to Sample #2

² Full list of cleanups only applied to Sample #2

The results of the wastewater sample analyses are summarized in Table 28. Given the total number of analytes in the draft method and the fact that the purpose of the analyses of the unspiked samples was to allow the laboratories to calculate their matrix spike recoveries and not to characterize the unspiked samples otherwise, we have limited the results in the table to those congeners that were spiked into the matrix spike samples later (see Section 8 of this report). We also only included those detected congeners that met the identification criteria in the draft procedure and where the results were at least 5 times the results for the associated method blank. The results from the reconnaissance analyses are also included in Table 28.

Table 28. Unspiked Wastewater Sample Results in ng/L

Analyte	Lab 1	Lab 3	Lab 4	Lab 6	Lab 7	Lab 8	Lab 9	Recon
Wastewater #2								
PCB-4+10							2.80	2.43
PCB-8+5							3.64	
PCB-18	17.95			10.96			10.8	10.5
PCB-19	0.675			2.13			2.21	2.07
PCB-28		33.2		11.64			7.35	5.68
PCB-31		35.9					7.54	4.97
PCB-36		163						
PCB-41+64	23.59						3.34	2.45
PCB-44				5.53			7.14	5.12
PCB-52+73	12.81			9.31			9.99	7.73
PCB-70							2.10	
PCB-90+101+89	2.09			2.81			2.93	1.91
PCB-95+93	3.49			2.81			3.96	2.37
PCB-99				1.36			1.27	0.827
PCB-105+127	6.62				2.23		2.38	1.69
PCB-110								
PCB-132+168							1.07	0.419

Table 28. Unspiked Wastewater Sample Results in ng/L

Analyte	Lab 1	Lab 3	Lab 4	Lab 6	Lab 7	Lab 8	Lab 9	Recon
PCB-138+163+164							1.77	1.08
PCB-149+139	1.74 ^B			1.55			2.00	0.875
PCB-153	3.38 ^{B*}			2.04 ^{B*}	12.28		1.78 ^{B*}	0.909
PCB-177	0.319							
PCB-180				0.776			0.825	0.415
PCB-182+187								0.28
PCB-199				0.582			0.954	
PCB-206				0.388			0.495	
PCB-209								0.235
Wastewater #3 - no congeners found								
Wastewater #4								
PCB-28	1.24							
PCB-180						3.88		
Wastewater #5								
PCB-209					0.097			
Wastewater #6								
PCB-1	1.98 ^{B*}							
PCB-11	0.822 ^B						5.08	
PCB-70	1.26 ^B							
PCB-90+101+89			0.9				0.998	
PCB-99	0.358							
PCB-110	0.756		0.9	0.788			0.777	0.623
PCB-118+106				0.690			0.622	
PCB-138+163+164	0.954		1.18				0.959	
PCB-149+139	0.653 ^B		0.72					0.464
PCB-153	2.12 ^{B*}			1.18			1.02	0.535
PCB-180	0.464		0.51		0.459			0.413
PCB-182+187								0.215
Wastewater #7								
PCB-3				13.37				
PCB-31	3.32							
PCB-66+80	11.67 ^B							
PCB-110	0.412			1.58				
PCB-153	2.51 ^{B*}			2.67			0.863	
PCB-177				0.693				
PCB-180				1.29			0.719	
PCB-189							0.869	
PCB-199							0.908	
PCB-205							0.913	
PCB-206							0.931	
PCB-208							0.543	
PCB-209							0.820	
Wastewater #8 - no congeners found								
Wastewater #9								
PCB-153				0.882				
PCB-180						2.91		
Wastewater #10								
PCB-153							0.438	

^{B*} Congener found in the associated method blank. Sample result is between 5x and 10x the blank value.^B Congener found in the associated method blank. Sample result is >10x the blank value.

The results for the unspiked samples varied across both the samples and the laboratories. Two of the samples (#3 and #8) had no congeners detected by any laboratory, including the reconnaissance lab. Four

of the samples had only one or two congeners reported by any laboratory. Those results are not surprising, since PCBs are only slightly soluble in water, and are more likely to be associated with the suspended particulates in the wastewater (IARC, 2016). The aqueous samples distributed for this study were thoroughly mixed, but it is impossible to perfectly distribute the amount of particulates present in hundreds of 1-L samples. Samples #6 and #7 had 12 and 13 congeners detected by any of the laboratories. Sample #2 presented the greatest analytical challenge, but had the largest number congeners detected, 39 in all, including the coeluting congeners.

Labs 3, 7, and 8 found the smallest numbers of congeners across all nine samples. The performance of Labs 7 and 8 was most affected by their dilutions of Wastewater #2, which decreased their overall sensitivity to the point that many of the congeners present at low levels could not be detected. Lab 3 only detected three congeners in Wastewater #2, and none in any of the other eight samples. Lab 1 had more congeners with potential method blank issues than the other laboratories, however, even then, many of the concentration that they reported were similar to the results from the other labs that reported those same congeners. Labs 1, 6, and 9 reported results for many of these congeners that were quite similar to one another and similar to the results from the reconnaissance analyses by the laboratory that developed the draft procedure. In many of the cases where a minority of laboratories detected a congener, the congener was present at a concentration near the lowest detection limits of any of the laboratories and therefore laboratories with higher detection limits could not detect the congener. That said, the purpose of the study was not to assess the performance of the individual laboratories.

Sediment Samples

Each of the six laboratories that completed the sediment portion of the study analyzed the three sediment samples as received, unspiked. As described in the draft procedure, all of the sediment samples received the copper and Florisil cleanups. All of the laboratories applied additional cleanups, and four of the laboratories diluted an aliquot of the final extract of sediment #1 and #3 before analysis. The various cleanups applied by each laboratory are presented in Table 29.

Table 29. Summary of Sediment Sample Cleanups

Sample Clean-up	Lab 3 ¹	Lab 4 ²	Lab 6	Lab 7	Lab 8 ⁴	Lab 9	Recon ³
Acid - Sulfuric	X					X	
Alumina	X	X	X	X	X	X	X
Copper	X	X	X	X	X	X	X
Florisil®	X	X	X	X	X	X	X
GPC		X	X				X
Silica	X	X	X	X	X		X
Dilution		X		X	X		X

¹ Sample #1 only had alumina, silica and acid cleanup performed in addition to copper and Florisil

² Sample #1 had all procedures except acid cleanup

³ Sample #1 had GPC and sample #3 had dilution in addition to the other cleanup procedures

⁴ Dilution on samples #1 and #3

The results of the sediment sample analyses as summarized in Table 30. As with the wastewater sample results, we have limited the results in the table to those congeners that were spiked into the matrix spike samples later. We also only included those detected congeners that met the identification criteria in the draft procedure and where the results were at least 5 times the results for the associated method blank. The results from the reconnaissance analyses are also included in Table 30.

Note: For ease of comparison, as well as preparation of the table, all of the sediment sample results are reported to three decimal places. However, the results have at most three significant figures, so trailing zeroes and the figures past one decimal place in results larger than 10 are not significant.

Table 30. Unspiked Sediment Sample Results in ng/g (dw)

Analyte	Lab 3	Lab 4	Lab 6	Lab 7	Lab 8	Lab 9	Recon
Sediment #1							
PCB-1		4.216		5.978		8.030	29.300
PCB-3		3.018				2.430	5.920
PCB-4+10	55.164	24.752	16.085	46.241		32.900	82.200
PCB-8+5	12.096	8.367	4.357	11.994	0.292	11.100	61.600
PCB-11	7.148	1.139					8.630
PCB-15		7.214	4.320	17.615	0.544	12.300	25.600
PCB-18	16.350	17.023	4.688	20.374	0.813	12.400	21.900
PCB-19		7.497	2.904	8.857		6.780	14.300
PCB-28	30.527	14.550	6.526		4.221	14.600	13.700
PCB-31	23.602	26.389	6.507	30.437	5.789	20.000	32.300
PCB-37		3.371				3.820	
PCB-41+64	24.067		2.665		1.280	5.360	6.840
PCB-44	32.261	17.811	6.857	20.459	1.295	15.800	19.200
PCB-52+73	61.312		15.129	44.642	8.984	35.700	45.100
PCB-54			0.055			0.152	0.212
PCB-66+80		14.545	6.507	19.108	3.445	12.300	14.400
PCB-70	28.439	16.489	8.290	23.275	4.023	18.300	18.500
PCB-72	3.357			2.992		0.911	0.936
PCB-74+61		13.308	2.537		1.884	5.450	5.970
PCB-77		1.359					
PCB-85+120		6.470	2.794	6.533	1.464	6.080	6.410
PCB-90+101+89	62.736	40.945	17.665	47.169	12.772	38.300	40.300
PCB-95+93	41.459	31.133	12.390	34.713	6.975	28.200	31.700
PCB-96	3.923		0.129			0.281	0.342
PCB-98+102		1.623	0.460	1.544		1.350	1.500
PCB-99	32.689	21.236	8.934	24.619	5.981	18.800	20.300
PCB-104	5.320						
PCB-105+127		9.991	4.651	11.417	2.211	9.650	10.400
PCB-110	63.369	42.933	18.971	52.013	8.024	42.200	44.300
PCB-118+106		35.405	15.938	44.139	10.387	34.600	37.200
PCB-132+168	12.357	12.356	7.555		3.084	10.500	12.800
PCB-138+163+164	48.072	41.722	15.956	42.536	11.589	35.400	38.700
PCB-147	6.616	1.270	0.331	1.098		0.785	0.845
PCB-149+139	32.112	25.609	10.110	28.504	10.199	23.000	23.500
PCB-153	35.076	29.746	12.224	35.575		27.700	28.900
PCB-155	4.653						
PCB-156		4.194	1.544	4.714	1.582	3.640	4.290
PCB-169	11.519						
PCB-177	8.534	4.094	1.324	4.018		3.120	3.260
PCB-180	24.060	18.796	6.728	20.305	5.997	15.900	16.700
PCB-184	3.736						
PCB-187+182	23.063	14.771	5.221	16.835	5.449	12.000	12.200
PCB-188	5.296						
PCB-189	13.448						0.211
PCB-199	16.843	13.649	6.654	22.104		11.800	10.800
PCB-202		3.336	1.305	5.778	1.491	2.660	2.740
PCB-205			0.110			0.357	5.000
PCB-206	22.865	13.933	5.074	28.552	5.976	11.700	11.700
PCB-208		4.282	1.857	8.653		3.400	3.600
PCB-209	19.345	11.983	4.210	16.062	6.331	10.700	10.500

Table 30. Unspiked Sediment Sample Results in ng/g (dw)

Analyte	Lab 3	Lab 4	Lab 6	Lab 7	Lab 8	Lab 9	Recon
Sediment #2							
PCB-1	12.218	2.379				1.790	8.440
PCB-3	7.279	2.477	1.052	1.652	1.277	1.020	3.310
PCB-4+10	25.143	7.643	7.391	13.956	8.529	5.960	25.600
PCB-8+5	7.174	3.388		4.253	1.810	1.600	6.550
PCB-11		0.109					
PCB-15		3.124	3.001		2.533		9.260
PCB-18	4.394	3.530	2.538	3.553	1.910	1.460	7.840
PCB-19	3.725	2.256	1.416	3.099	1.503	1.140	5.720
PCB-28		2.644	2.258		1.527	1.540	4.970
PCB-31	6.041	5.291	4.306	8.195	3.591	2.350	11.900
PCB-37					0.192		0.691
PCB-41+64	3.800					0.410	1.930
PCB-44	2.365	2.738	1.725	3.068	1.528	1.140	4.780
PCB-52+73	6.984	4.934	3.717	6.286	3.841	2.620	11.300
PCB-54					0.046		0.131
PCB-66+80			0.898	1.316		0.593	2.300
PCB-70		1.197	0.729	1.188	0.777	0.716	2.050
PCB-74+61			0.309		0.334	0.336	1.270
PCB-90+101+89		1.696	0.827		0.901	1.130	2.410
PCB-95+93	2.266	1.928	1.052	2.328	1.110	1.200	3.640
PCB-96		0.068					0.117
PCB-98+102		0.194					0.354
PCB-99		0.951	0.393		0.413	0.514	1.170
PCB-105+127			0.309			0.357	0.726
PCB-107+108		1.556					
PCB-110		2.166	1.248	3.102		1.360	3.980
PCB-118+106		1.727		2.067			
PCB-126	17.378						
PCB-132+168		1.369			0.287	0.401	0.643
PCB-138+163+164	1.908	2.201	1.178	2.586	1.022	1.390	
PCB-147		1.888					
PCB-149+139	2.228	1.856	0.996	2.299		1.270	1.950
PCB-153	1.721	1.252	1.206	3.156	1.042	1.630	2.220
PCB-169	4.488						
PCB-177		0.546	1.290		0.210	0.448	0.575
PCB-180	2.795	3.104	1.851	3.448		2.850	2.820
PCB-187+182	2.645	2.568		2.937	1.270	2.430	2.440
PCB-199	1.892	2.859	1.487	3.615	1.652	3.000	2.110
PCB-202		0.622	0.365		0.372	0.718	
PCB-204			0.266				0.556
PCB-206		2.993	1.529	3.536		3.190	2.590
PCB-208		0.870	0.561			0.897	0.808
PCB-209	1.796	2.479	0.603	3.060		2.040	2.150
Sediment #3							
PCB-1		16.195	4.130		0.534	10.400	15.500
PCB-3	18.567	15.168	3.732	14.309	1.368	9.990	14.200
PCB-4+10	77.696	72.225	17.161	70.259	5.882	52.600	72.900
PCB-8+5	51.276	45.722	8.106	41.243	6.909	33.000	40.200
PCB-11		1.775		1.744		1.170	1.830
PCB-15	72.781	63.165	15.341	68.986	9.816	46.300	67.400
PCB-18	38.448	38.251	5.950	33.639	8.194	24.700	35.500
PCB-19	23.740	21.044	4.237	20.558	2.502	14.600	20.400

Table 30. Unspiked Sediment Sample Results in ng/g (dw)

Analyte	Lab 3	Lab 4	Lab 6	Lab 7	Lab 8	Lab 9	Recon
PCB-28	90.003	70.738	13.398	67.861	10.654	47.700	64.700
PCB-31	61.234	66.985	12.465	63.961	10.212	43.000	62.100
PCB-37		10.918	2.065	10.314	1.675	6.790	9.940
PCB-41+64	30.154	30.629		29.137	6.131	19.400	25.700
PCB-44	54.141	54.151	8.810	51.757	9.454	35.100	50.600
PCB-52+73	78.735	79.641	12.695	75.627	11.961	52.800	77.400
PCB-54	0.990	0.964	0.184	0.901	0.151	0.665	0.909
PCB-66+80	53.547	55.274	8.932	53.301	7.753	36.700	55.100
PCB-70	48.891	50.594	8.014	49.165	8.262	34.600	46.000
PCB-72		1.452	0.184			0.752	1.080
PCB-74+61	31.221	31.620	5.216	30.469	4.087	20.900	28.400
PCB-77	6.592	6.505	0.713	4.030	0.987	3.840	5.450
PCB-79		0.332					
PCB-85+120	8.019	7.212	1.101	6.901	1.161	4.890	6.700
PCB-90+101+89	24.290	22.425	3.258	21.691	3.848	15.300	20.300
PCB-95+93	20.054	19.563	2.432	18.310	5.178	13.400	18.300
PCB-96	0.558	0.643	0.092	0.601	0.197	0.437	0.578
PCB-98+102	2.971	2.635	0.352	2.368	0.561	15.300	2.500
PCB-99	17.003	15.582	2.340	14.895	2.287	10.500	13.900
PCB-104		0.118				0.085	
PCB-105+127	12.408	11.445	1.728	11.293	2.414	7.790	9.840
PCB-107+108		2.023	0.260	1.785			1.730
PCB-110	33.250	30.734	3.824	28.527		20.400	27.300
PCB-118+106	24.848	22.864	3.288	22.351	3.682	15.400	20.500
PCB-132+168	4.971	3.257	0.382	3.067	1.256	2.220	3.070
PCB-138+163+164	4.042	10.409	1.514	10.159	1.821	7.030	9.780
PCB-147	0.505	0.410	0.061			0.296	0.365
PCB-149+139	8.447	7.635	0.979	7.341	2.003	5.200	6.830
PCB-152		0.039					
PCB-153	7.376	7.866	1.269	8.167	1.528	5.630	7.150
PCB-156	1.091	1.133	0.153	1.065	0.361	0.727	0.952
PCB-169	0.639						
PCB-177	1.908	1.803	0.229	1.612		1.250	1.660
PCB-180	6.794	6.599	0.994	6.118	1.198	4.980	6.230
PCB-187+182	4.143	3.744	0.489	3.323	0.802	2.750	3.510
PCB-189		0.193	0.031			0.115	0.142
PCB-199	1.774	2.026	0.306	1.894		1.500	1.710
PCB-202	0.274	0.331	0.031	0.348		0.230	0.273
PCB-205		0.163				0.112	0.129
PCB-206		0.890		0.866		0.671	0.750
PCB-208		0.179				0.131	0.179
PCB-209		0.234				0.203	0.236

In general, all of the laboratories who completed the sediment portion of the study reported more congeners in these three sediment samples than they did in the wastewater samples, which would be expected since the sediments came from contaminated sites. For example, Laboratory 3, that only reported 3 congeners across all 8 wastewater samples, reported 90 results for the three sediment samples. The other five laboratories reported between 84 and 119 congeners across all three samples.

As with the unspiked wastewater sample results, there is some variability across the laboratories. Some of that variability may be due to the challenge of homogenizing a bulk sediment sample. In many of the cases where a minority of laboratories detected a congener, the congener was present at a concentration

near the lowest detection limits of any of the laboratories and therefore laboratories with higher detection limits could not detect the congener. However, Laboratory 6 and Laboratory 8 often reported lower values, and sometimes much lower values for some congeners than any other laboratory, suggesting that they have a low bias. For example, compare their results in Table 29 for PCB-18, PCB-31, and PCB-44 in Sediment #1. Similar comparisons for other congeners indicate that the differences between the results from Laboratory 6 and Laboratory 8 and the other four laboratories are not related to concentration (for example, see their results in Sediment #3 for PCB-4+10 and PCB-54). Despite a review of their results, CSRA did not identify any obvious or likely causes.

Biosolids Samples

Each of the four laboratories that completed the biosolids portion of the study analyzed the three biosolids samples as received, unspiked. As described in the draft procedure, all of the biosolids samples received the copper and Florisil cleanups. All of the laboratories applied additional cleanups, and two of the laboratories diluted an aliquot of the final extract before analysis. The various cleanups applied by each laboratory are presented in Table 31.

Table 31. Summary of Biosolids Sample Cleanups

Sample Clean-up	Lab 3	Lab 4 ¹	Lab 6	Lab 7 ²	Recon ³
Alumina		X	X	X	X
Copper	X	X	X	X	X
Florisil®	X	X	X	X	X
GPC	X	X			X
Silica		X	X	X	X
Dilution		X		X	

¹ Dilution and GPC was applied to Samples #1 and #3

² Dilution for all 3 biosolid samples

³ GPC was performed on Samples #1 and #3

As with the wastewater sample results, we have limited the results in Table 32 to those congeners that were spiked into the matrix spike samples later. We also only included those detected congeners that met the identification criteria in the draft procedure and where the results were at least 5 times the results for the associated method blank. The results from the reconnaissance analyses are also included in the table.

Table 32. Unspiked Biosolids Sample Results in ng/g (dw)

Analyte	Lab 3	Lab 4	Lab 6	Lab 7	Recon
Biosolids Sample #1					
PCB-1				2.690	1.300 # B
PCB-3	2.682 #			2.610	
PCB-11			3.465		2.180 #
PCB-15					0.383 #
PCB-18			1.475		0.848
PCB-28			3.023		1.270
PCB-31			2.138		1.270
PCB-41+64					1.040 #
PCB-44			4.350		2.110 #
PCB-52+73	3.347		6.857	9.490	3.540 #
PCB-54			0.074		
PCB-66+80			3.244		1.950 #
PCB-70			5.013		3.040 #
PCB-74+61			2.654		1.280 #
PCB-77					0.227
PCB-79		14.331			
PCB-85+120			1.180		0.958 #
PCB-90+101+89	6.293 #	7.602	9.216	13.840	6.140 #

Table 32. Unspiked Biosolids Sample Results in ng/g (dw)

Analyte	Lab 3	Lab 4	Lab 6	Lab 7	Recon
PCB-95+93	3.801	5.588	6.488	13.260	4.790 #
PCB-96			0.147		
PCB-98+102			0.295		0.149 #
PCB-99	2.300 #	3.187	3.686	6.550	2.380 #
PCB-104			0.147		
PCB-105+127			2.507		1.940 #
PCB-107+108			0.369 #		0.302 #
PCB-110	9.510 #	7.807	7.004	15.050	6.360 #
PCB-118+106	6.142 #	6.755	5.530	12.920	5.000 #
PCB-126					0.118 #
PCB-132+168		2.520	2.359	5.260	1.910 #
PCB-138+163+164	7.343	7.434	6.857	16.320	6.490 #
PCB-147			0.147		0.127 #
PCB-149+139	3.658	5.233	4.719	11.630	3.810 #
PCB-153	5.193 #	6.997	5.308 B	15.14	4.570 #
PCB-155			0.295		0.163 #
PCB-156			0.811		0.687 #
PCB-169	8.739 #				
PCB-177			0.516	2.450	0.464
PCB-180	1.926	2.375	1.991		1.970
PCB-184			0.442		0.330
PCB-187+182	0.699 #	1.555	1.327	5.410	1.190
PCB-188			0.074		
PCB-189			0.074		
PCB-199	0.361 #		0.590	2.820	0.626 #
PCB-202			0.147		0.150 #
PCB-206			0.295		0.321 #
PCB-208			0.147		
PCB-209			0.221		
Biosolids Sample #2					
PCB-1					1.790 # B
PCB-3	15.700 #				
PCB-11			2.077 #		
PCB-18			0.651		
PCB-28			0.855		
PCB-31			0.651		
PCB-44			1.344		
PCB-52+73			2.239		
PCB-54			0.081		
PCB-66+80			0.774		
PCB-70			1.140		
PCB-72			0.244 #		
PCB-74+61			0.651		
PCB-85+120			0.244		
PCB-90+101+89	0.719 #		1.995		0.971 #
PCB-95+93			1.425		0.733 #
PCB-98+102			0.0814 #		
PCB-99			0.774		0.383 #
PCB-104			0.081		
PCB-105+127			0.448		
PCB-107+108			0.081		
PCB-110	0.538		1.221		0.984 #
PCB-118+106	0.643 #		0.896		0.814 #

Table 32. Unspiked Biosolids Sample Results in ng/g (dw)

Analyte	Lab 3	Lab 4	Lab 6	Lab 7	Recon
PCB-132+168			0.407		
PCB-138+163+164	1.546 #		1.099		1.180 #
PCB-149+139	0.354		0.692		0.643 #
PCB-152			0.041		
PCB-153	0.258 #		0.977 B		0.698 #
PCB-155			0.122		
PCB-156			0.122		
PCB-180			0.285		0.301
PCB-184					
PCB-187+182			0.204		0.180 #
PCB-209			0.244		
Biosolids Sample #3					
PCB-1					2.34 # B
PCB-3	5.367				
PCB-4+10			1.282 #		0.998 #
PCB-11	3.609 #				2.890 #
PCB-15					0.759 #
PCB-18			2.172		2.280
PCB-19			0.677		0.594
PCB-28			3.811		3.110
PCB-31			2.885		2.990
PCB-36					0.670 # B*
PCB-41+64			2.244		1.770 #
PCB-44			4.843		4.250 #
PCB-52+73	11.133		9.473		8.760 #
PCB-54			0.783		0.710 #
PCB-66+80	16.010 #		4.879		3.830 #
PCB-70	8.728 #		6.588		5.810 #
PCB-72	2.665 #				0.080 #
PCB-74+61			2.350		2.100 #
PCB-77					0.319 #
PCB-85+120			2.101		2.040 #
PCB-90+101+89	16.097	14.623	15.135	6.750	14.000 #
PCB-95+93	10.841	9.490	10.292	7.840	9.900 #
PCB-96					0.099 #
PCB-98+102	10.760		0.677		0.570 #
PCB-99	6.584		6.268		5.600 #
PCB-104			0.214		0.205 #
PCB-105+127	7.555 #		4.701		4.410 #
PCB-107+108					0.812 #
PCB-110	21.134 #	13.481	13.925	5.680	13.200 #
PCB-118+106	12.278 #	11.998	11.503	4.210	11.100 #
PCB-132+168			6.054		4.760 #
PCB-138+163+164	18.776		17.450	7.300	16.70 #
PCB-147			0.783		0.648 #
PCB-149+139	12.522	11.422	11.396	5.13	9.830 #
PCB-152			0.071		0.047 #
PCB-153	17.201	15.971	13.141 B	8.100	12.300 #
PCB-155			0.178		0.175 #
PCB-156			1.709		1.730 #
PCB-166	1.608 #				0.058 #
PCB-169	9.737 #				
PCB-177	1.924		2.208		1.800

Table 32. Unspiked Biosolids Sample Results in ng/g (dw)

Analyte	Lab 3	Lab 4	Lab 6	Lab 7	Recon
PCB-180		7.839	8.583	2.290	7.470
PCB-184			0.321		0.269
PCB-187+182	4.927		5.306	1.570	4.330
PCB-188			0.036		
PCB-189					0.117
PCB-199	2.384	2.525	1.709		2.280 #
PCB-202			0.499		0.458 #
PCB-204	0.147		0.285		
PCB-205			0.071		0.697 #
PCB-206	1.229		1.318		1.200 #
PCB-208			0.427 #		0.391 #
PCB-209	0.344		0.997		0.520 #

= Analyte did not meet the ion abundance ratio criterion, but met all of the other identification criteria

B* = Analyte detected in the sample at a concentration between 5 and 10 times that in associated method blank

B = Analyte detected in the sample at a concentration above 10 that in associated method blank

The results for the unspiked biosolids samples are influenced by several factors. As part of the study design, EPA instructed the laboratories to apply their solid sample MDLs to the biosolids analyses, rather than developing separate MDLs for biosolids samples. Because the draft method calls for using a 5-g sample size for biosolids, as opposed to a 10-g sample for sediments. Therefore, adjusting the MDLs for the biosolids samples by a factor of 2 higher meant that the sensitivity would be less for biosolids.

In the case of Laboratory 3, their MDLs for the solid samples often were much higher than those for the other laboratories, and this means that their biosolids MDLs were also much higher than the adjusted MDLs from the other three laboratories. That issue was exacerbated by the fact that Laboratory 3 also deviated from the draft method and only extracted 1 to 2 g of biosolids (dry weight).

In contrast, Laboratory 6 had many of the lowest MDL values for solid samples, so they reported many more congeners in the unspiked samples than the other three laboratories. The results from Laboratories 4 and 7 do not follow an obvious pattern. Neither laboratory detected any congeners for Biosolids sample #2, despite having lower MDLs than Laboratory 3. It may be that their applications of the cleanup procedures were not as effective at reducing interferences in this sample as Laboratories 3 or 6.

Tissue Samples

Each of the four laboratories that completed the tissue portion of the study analyzed the three tissue samples as received, unspiked. All four laboratories performed the Florisil® and GPC cleanup described in the draft method; however, two laboratories performed additional cleanup of the sample extracts, including the use of copper, alumina, silica, and an acid wash. As with the wastewater sample results, we have limited the results in Table 33 to those congeners that were spiked into the matrix spike samples later. We also only included those detected congeners that met the identification criteria in the draft procedure and where the results were at least 5 times the results for the associated method blank. The results from the reconnaissance analyses are also included in Table 33.

Table 33. Unspiked Tissue Sample Results in ng/g

Analyte	Lab 3	Lab 4	Lab 6	Lab 9	Recon
Tissue sample #1					
PCB-28					0.030
PCB-31					0.024
PCB-41+64					0.032
PCB-44					0.034
PCB-52+73					0.040

Table 33. Unspiked Tissue Sample Results in ng/g

Analyte	Lab 3	Lab 4	Lab 6	Lab 9	Recon
PCB-70				0.088	
PCB-85+120		0.050			0.037
PCB-90+101+89		0.200	0.163		0.153
PCB-95+93		0.072			0.061
PCB-99		0.128	0.113	0.126	0.105
PCB-107+108					0.019
PCB-105+127		0.074			0.048
PCB-110		0.164	0.138	0.172	0.137
PCB-118+106		0.214	0.163	0.201	0.174
PCB-132+168					0.038
PCB-138+163+164		0.344	0.300	0.336	0.296
PCB-149+139		0.144	0.125		0.107
PCB-153		0.393	0.350		0.312
PCB-156					0.021
PCB-177		0.039			0.028
PCB-180		0.171		0.166	0.135
PCB-187+182		0.120	0.138		0.094
PCB-199		0.066			
PCB-206		0.058			0.054
PCB-208					0.017
PCB-209		0.030			0.032
Tissue Sample #2					
PCB-28					0.046
PCB-31					0.027
PCB-41+64					0.044
PCB-44				0.086	0.068
PCB-52+73		0.119		0.227	0.179
PCB-66+80		0.122	0.170	0.234	0.185
PCB-70		0.132	0.150	0.208	0.153
PCB-72				0.128	
PCB-74+61		0.070			0.101
PCB-85+120		0.097	0.160	0.217	0.162
PCB-90+101+89	2.080	0.421	0.630	0.986	0.749
PCB-95+93		0.162	0.260	0.333	0.254
PCB-99	1.890	0.403	0.660	0.764	0.582
PCB-105+127		0.170	0.230	0.328	0.239
PCB-110		0.348	0.560	0.713	0.538
PCB-118+106	2.090	0.477	0.670	1.070	0.795
PCB-132+168			0.170	0.182	0.145
PCB-138+163+164	4.360	1.110	1.700	2.240	1.800
PCB-147					0.031
PCB-149+139	1.890	0.463	0.740	0.832	0.623
PCB-153	5.720	1.352	2.060	2.450	
PCB-156		0.086	0.130	0.151	0.117
PCB-177	0.358	0.078		0.138	0.099
PCB-180	2.270	0.545	0.830	0.973	0.762
PCB-187+182	1.340	0.451	0.720	0.748	0.585
PCB-189					0.012
PCB-199		0.158		0.292	0.230
PCB-202		0.056	0.100	0.090	0.078
PCB-206		0.132	0.180	0.250	0.225
PCB-208	0.136	0.068	0.090	0.145	0.130
PCB-209	0.955	0.165	0.290	0.417	0.350

Table 33. Unspiked Tissue Sample Results in ng/g

Analyte	Lab 3	Lab 4	Lab 6	Lab 9	Recon
Tissue Sample #3					
PCB-3		0.100			
PCB-4+10		0.072			
PCB-11			0.267		0.283
PCB-18		0.227	0.089	0.113	0.075
PCB-19		0.087			
PCB-28		0.450	0.389	0.493	0.404
PCB-31		0.306	0.200	0.261	0.230
PCB-37		0.090			0.057
PCB-41+64	0.611	0.371	0.500	0.518	0.456
PCB-44		0.684	0.611	0.712	0.636
PCB-52+73	2.760	1.333	1.178	1.490	1.440
PCB-54		0.057			
PCB-66+80	2.310		1.200	1.480	1.530
PCB-70			1.200	1.570	1.370
PCB-72					0.029
PCB-74+61	1.390	0.747	0.744	0.868	0.790
PCB-78			0.011		
PCB-85+120	1.480	1.025	0.911	1.170	1.010
PCB-90+101+89	9.110	5.399	4.900	6.280	5.570
PCB-95+93	2.700	1.838	1.600	2.090	
PCB-98+102					0.051
PCB-99	4.720	2.925	2.578	3.370	2.930
PCB-104		0.049			
PCB-105+127	2.600	1.584	1.444	1.760	1.550
PCB-107+108	0.795	0.548	0.444	4.580	0.497
PCB-110	6.800	3.847	3.567	6.210	3.940
PCB-118+106	8.800	5.142	4.556	1.380	5.310
PCB-132+168	2.430	1.241	1.111	13.100	1.340
PCB-138+163+164	16.000	10.800	9.622		11.300
PCB-147		0.242	0.156		0.162
PCB-149+139	6.590	4.514	3.944	4.830	4.000
PCB-152		0.047			
PCB-153	16.300	11.296	9.889	13.600	11.000
PCB-155		0.053			
PCB-156	0.953	0.917	0.767	0.904	0.836
PCB-166		0.209			0.042
PCB-177		0.987	1.022	1.100	0.961
PCB-180	6.870	4.611	4.756	5.880	4.960
PCB-184		0.047			
PCB-187+182	4.060	3.041	3.278	3.700	3.040
PCB-188		0.061			
PCB-189		0.126		0.083	0.063
PCB-199	1.470	1.390	0.900	1.560	1.150
PCB-202	0.196	0.275	0.211	0.262	0.208
PCB-204		0.046			
PCB-205		0.106			0.641
PCB-206	0.543	0.664	0.444	0.760	0.549
PCB-208		0.274	0.167	0.276	0.231
PCB-209	0.089	0.455	0.422	0.453	0.382

The unspiked tissue sample results varied across the three samples and the four laboratories. Laboratory 3 did not detect any congeners in Tissue Sample #1 at all, whereas the other three laboratories reported

between 6 and 16 congeners in that sample, and the reconnaissance laboratory reported 45 congeners. Those differences in detection were largely driven by the MDLs in each of those laboratories. For example, Laboratory 3 had the highest tissue MDLs for 127 of the 167 analytes, and the results reported by the other laboratories were often at concentrations below the detection limits in Laboratory 3. Laboratory 6 had the highest MDLs for 31 of the 167 analytes, which may explain their lower numbers of detects in Tissue Sample #1 as well. In contrast, the reconnaissance laboratory had many of the lowest MDLs, as a result of their experience with the method, and they detected far more congeners in Tissue Sample #1, often at very low levels that were below the MDLs of the other laboratories. In many of the cases where not all of the laboratories detected a congener, the congener was present at a concentration near the lowest detection limits of any of the laboratories and therefore laboratories with higher detection limits could not detect the congener.

As noted in Table 4, Tissue Sample #1 was characterized as the low concentration sample in this study and as is shown in Table 33, most of the concentrations reported by the laboratories were fairly low. Setting aside the results from Laboratory 3, there were four analytes detected in Tissue Sample #1 by all three of the other laboratories and the reconnaissance laboratory. The results for those four congeners are remarkably similar across those four laboratories, as shown in Table 34.

Table 34. Results for Congeners Detected by Four Labs for Tissue Sample #1 in ng/g

Analyte	Lab 4	Lab 6	Lab 9	Recon
PCB-99	0.128	0.113	0.126	0.105
PCB-110	0.164	0.138	0.172	0.137
PCB-118+106	0.214	0.163	0.201	0.174
PCB-138+163+164	0.344	0.300	0.336	0.296

Tissue Sample #2 was designed to be a medium level sample. As a result of the higher concentrations in this sample compared to Tissue Sample #1, Laboratory 3 detected 11 analytes, even with their higher detection limits, and the other three laboratories in the study detected 20 to 25 analytes each. Of the 11 analytes detected by Laboratory 3, 10 also were detected in the other three laboratories in the study. For 9 of those 10 those analytes, Laboratory 3 reported markedly higher results than the other three laboratories, or the reconnaissance laboratory, and the results from the other three laboratories were generally quite similar, as shown in Table 35.

Table 35. Results for Congeners Detected by Five Labs for Tissue Sample #2 in ng/g

Analyte	Lab 3	Lab 4	Lab 6	Lab 9	Recon
PCB-90+101+89	2.080	0.421	0.630	0.986	0.749
PCB-99	1.890	0.403	0.660	0.764	0.582
PCB-118+106	2.090	0.477	0.670	1.070	0.795
PCB-138+163+164	4.360	1.110	1.700	2.240	1.800
PCB-149+139	1.890	0.463	0.740	0.832	0.623
PCB-153	5.720	1.352	2.060	2.450	
PCB-177	0.358	0.078		0.138	0.099
PCB-180	2.270	0.545	0.830	0.973	0.762
PCB-187+182	1.340	0.451	0.720	0.748	0.585
PCB-208	0.136	0.068	0.090	0.145	0.130
PCB-209	0.955	0.165	0.290	0.417	0.350

Tissue Sample #3 was designed to be a higher concentration sample and that characterization held true. Laboratory #3 detected 23 analytes, and the other three laboratories in the study detected 30 to 43 analytes each. As in Tissue Sample #2, Laboratory 3 reported higher concentrations than the other laboratories or the reconnaissance laboratory for many of the analytes, and the results from the other three laboratories were generally quite similar. Although CSRA examined the results in detail, as well as the associated

calibration data, no obvious reason for the higher results were immediately apparent. However, the potential bias does appear to be consistent for Laboratory 3. A comparison of these results is shown in Table 36.

Table 36. Results for Congeners Detected by Five Labs for Tissue Sample #3 in ng/g

Analyte	Lab 3	Lab 4	Lab 6	Lab 9	Recon
PCB-41+64	0.611	0.371	0.500	0.518	0.456
PCB-52+73	2.760	1.333	1.178	1.490	1.440
PCB-74+61	1.390	0.747	0.744	0.868	0.790
PCB-85+120	1.480	1.025	0.911	1.170	1.010
PCB-90+101+89	9.110	5.399	4.900	6.280	5.570
PCB-99	4.720	2.925	2.578	3.370	2.930
PCB-105+127	2.600	1.584	1.444	1.760	1.550
PCB-107+108	0.795	0.548	0.444	4.580	0.497
PCB-110	6.800	3.847	3.567	6.210	3.940
PCB-118+106	8.800	5.142	4.556	1.380	5.310
PCB-132+168	2.430	1.241	1.111	1.380	1.340
PCB-149+139	6.590	4.514	3.944	4.830	4.000
PCB-153	16.300	11.296	9.889	13.600	11.000
PCB-156	0.953	0.917	0.767	0.904	0.836
PCB-180	6.870	4.611	4.756	5.880	4.960
PCB-187+182	4.060	3.041	3.278	3.700	3.040
PCB-199	1.470	1.390	0.900	1.560	1.150
PCB-202	0.196	0.275	0.211	0.262	0.208
PCB-206	0.543	0.664	0.444	0.760	0.549
PCB-209	0.089	0.455	0.422	0.453	0.382

8. Matrix Spike Analyses

Isotope dilution methods generate recovery data for all of the labeled compounds spiked into every sample, so EPA has not included the use of matrix spike (MS) and matrix spike duplicate (MSD) samples as part of the routine per-sample-batch quality control operations in those methods. However, in order to demonstrate the performance of the draft procedure in real-world samples that contain the native analytes, EPA required each laboratory in the study to prepare an MS/MSD pair for each of the study samples that they agreed to analyze (e.g., all nine wastewaters, three sediments, three biosolids, and three fish tissues).

Generation of the most useful MS/MSD data requires knowledge of the background levels of the analytes in the unspiked samples so that appropriate spiking levels can be chosen (not so high as to be unrealistic in the context of actual sample concentrations, and not so low that the spiked amount is difficult to discern given the background concentration in the original sample). Rather than waiting for each participant laboratory to analyze all of their unspiked samples and then go back and develop a customized spiking scheme based on those results, EPA and CSRA used the reconnaissance analysis results described in Section 3 to develop sample-specific spiking instructions for all of the samples in the study.

The basic approach was to spike all 48 high-priority congeners, using the native compound spiking solution described in the draft procedure and provided to each laboratory in the study by EPA. The same spiking level of 16 ng per sample used for the IPR samples was used for the MS/MSD samples where practical.

However, in order to gather data on additional congeners, EPA and CSRA instructed the laboratory to prepare an additional spiking solution by diluting one of the retention time standards by a factor of 200, using acetonitrile to make the solution water miscible. Retention time Mix #7 (from the set of 9 standards provided by EPA) contains a total of 14 congeners. Of those, 13 are not among the 48 high-priority congeners in the native congener spiking solution. The one congener in common to both solutions is PCB-166. Both solutions contain some congeners that coelute with other congeners in the analysis.

EPA and CSRA instructed the laboratories to spike two aliquots of each study sample with 200 µL of the native compound spiking solution, and 200 µL of the diluted retention time Mix #7. The list of congeners spiked into the samples is shown in Table 37. Based on the volumes of the spiking solutions, each MS/MSD aliquot received 16 ng of each of the congeners, except PCB-166, for which the total mass spiked was 32 ng. The same spiking scheme was used for all four matrix types.

Table 37. Composition of Matrix Spiking Solutions

Analyte	Source Solution	Analyte	Source Solution
PCB-1	Native spiking solution	PCB 105	Native spiking solution
PCB-3	Native spiking solution	PCB-106	RT solution #7
PCB-4	Native spiking solution	PCB-108	RT solution #7
PCB-8	Native spiking solution	PCB 110	Native spiking solution
PCB-11	Native spiking solution	PCB 118	Native spiking solution
PCB-15	Native spiking solution	PCB 126	Native spiking solution
PCB-18	Native spiking solution	PCB 132	Native spiking solution
PCB-19	Native spiking solution	PCB 138	Native spiking solution
PCB-28	Native spiking solution	PCB 147	Native spiking solution
PCB-31	Native spiking solution	PCB 149	Native spiking solution
PCB-36	RT solution #7	PCB-152	RT solution #7
PCB-37	Native spiking solution	PCB 153	Native spiking solution
PCB-44	Native spiking solution	PCB 155	Native spiking solution
PCB-52	Native spiking solution	PCB 156	Native spiking solution
PCB-54	Native spiking solution	PCB 166	Both
PCB-64	Native spiking solution	PCB 169	Native spiking solution

Table 37. Composition of Matrix Spiking Solutions

Analyte	Source Solution	Analyte	Source Solution
PCB-66	Native spiking solution	PCB 177	Native spiking solution
PCB-70	Native spiking solution	PCB 180	Native spiking solution
PCB-72	RT solution #7	PCB-182	RT solution #7
PCB-74	Native spiking solution	PCB-184	RT solution #7
PCB-77	Native spiking solution	PCB 187	Native spiking solution
PCB-78	RT solution #7	PCB 188	Native spiking solution
PCB-79	RT solution #7	PCB 189	Native spiking solution
PCB-85	Native spiking solution	PCB 199	Native spiking solution
PCB-89	RT solution #7	PCB 202	Native spiking solution
PCB-95	Native spiking solution	PCB-204	RT solution #7
PCB-96	RT solution #7	PCB 205	Native spiking solution
PCB-98	RT solution #7	PCB 206	Native spiking solution
PCB-99	Native spiking solution	PCB 208	Native spiking solution
PCB-101	Native spiking solution	PCB 209	Native spiking solution
PCB-104	Native spiking solution		

Each laboratory reported their unspiked sample results, as well as the MS and MSD concentrations, recoveries, and relative percent differences (RPDs) for each matrix type that they analyzed. CSRA used those data to evaluate method performance, as described in the subsections that follow.

Aqueous Sample MS/MSD Results

Each of the seven laboratories that completed the wastewater portion of the study analyzed MS/MSD aliquots of all nine of the wastewater samples. Unfortunately, Lab 1 failed to follow the study instructions and did not spike the congeners in RT Mix #7 into the MS/MSD aliquots. However, they did spike the other analytes in the native spiking solution.

In addition, the analytical challenges evident in the analyses of the unspiked aliquots of Wastewater #2 were also present in the MS/MSD analyses. Although a few of the laboratories were able to generate useful MS/MSD results for that sample, other laboratories provided results with calculated recoveries that were negative numbers or well over 150%, and both those extremes are evidence of problems with the choice of spiking levels or with interferences. As a result, EPA decided not to evaluate the MS/MSD results for Wastewater #2 any further. That decision was based not only on the results from the study, but also after considering that the sample represented a landfill leachate, which is not a matrix type that is discharged to surface water without some level of treatment. Rather, the leachate in question is collected at the landfill and sent to a POTW for treatment, and the effluent from the POTW is what is subject to a discharge permit and NPDES monitoring requirements.

CSRA calculated the mean recovery of each spiked analyte by sample across all seven laboratories, along with the minimum and maximum observed values. Given the number of samples and analytes, those results are presented in Tables 38 and 39, for the MS/MSD analyses of wastewater samples #3 to #6 and samples #7 to #10, respectively. The mean MS/MSD recoveries are presented graphically in Figure 5, for all eight of the wastewater samples. The highlighted rows show the congeners that were quantified indirectly, using labeled standards of similar congeners in the same level of chlorination. Values in parentheses, next to zero values, represent the number of results which were reported as non-detects by the laboratories. Of the 8 wastewater samples summarized in Tables 38 and 39, four samples (Wastewater #3, #6, #7 and #10) had false negatives reported by the laboratories, with Wastewater #7 having the most reported false negatives by Labs 1, 6, 7 and 9. Although six wastewater samples had false negatives reported, the percentage was less than 0.2% of the 7128 total data points.

Table 38. Matrix Spike Recoveries for Wastewater Samples #3 to #6 (%)

Analyte	# Results	Wastewater #3			Wastewater #4			Wastewater #5			Wastewater #6		
		Mean Rec.	Min. Rec.	Max. Rec.	Mean Rec.	Min. Rec.	Max. Rec.	Mean Rec.	Min. Rec.	Max. Rec.	Mean Rec.	Min. Rec.	Max. Rec.
PCB-1	14	99.9	90	123	97.1	86	111	101.1	88	123	101.6	74	126
PCB-3	14	94.6	87	103	93.1	84	109	97.6	86	129	95.4	73	113
PCB-4+10	14	120.0	86	250	96.1	86	111	97.1	88	115	84.0	10	110
PCB-8+5	14	83.8	76	94	84.9	74	115	85.4	76	97	87.4	68	106
PCB-11	14	94.4	86	115	102.4	84	145	103.1	87	147	101.9	62	186
PCB-15	14	86.3	56	103	85.4	79	100	89.3	84	101	85.1	62	95
PCB-18	14	86.1	76	96	91.1	75	149	86.9	78	103	81.9	62	92
PCB-19	14	89.6	79	102	89.0	79	119	92.9	84	109	93.8	64	133
PCB-28	14	99.4	79	123	102.4	80	158	97.9	82	118	94.8	67	131
PCB-31	14	83.3	64	97	82.4	63	139	86.9	78	94	77.4	57	90
PCB-36	12	102.9	0 (3)	126	108.0	86	140	114.7	97	131	111.0	69	128
PCB-37	14	88.3	73	102	87.3	73	123	90.8	83	106	107.1	0 (2)	298
PCB-41+64	14	90.2	80	117	102.4	64	324	93.4	81	113	90.3	54	147
PCB-44	14	99.9	86	176	89.3	75	116	95.2	87	110	91.9	59	115
PCB-52+73	14	90.1	76	120	86.5	73	104	90.9	83	106	89.4	58	108
PCB-54	14	90.0	83	107	88.1	76	107	92.1	84	101	88.9	58	103
PCB-74+61	14	91.4	82	104	83.4	11	98	93.8	84	108	84.6	15	123
PCB-66+80	14	91.2	76	118	124.3	73	585	90.2	75	110	109.9	52	307
PCB-70	14	86.3	76	96	87.6	73	131	88.2	82	96	91.1	54	155
PCB-72	12	99.7	85	110	103.8	86	114	104.3	98	113	103.7	62	120
PCB-77	14	87.1	78	114	88.6	74	149	87.1	78	108	88.7	50	135
PCB-78	12	101.9	90	114	101.6	75	119	107.2	89	117	109.5	57	129
PCB-79	12	104.6	80	125	107.0	68	124	114.5	88	155	117.4	65	164
PCB-85+120	14	85.4	70	98	84.1	68	96	87.9	79	100	89.0	46	125
PCB-90+101+89	14	84.8	60	99	85.5	67	100	90.2	82	101	88.3	47	106
PCB-95+93	14	87.2	69	100	82.6	45	113	86.9	63	101	88.1	53	107
PCB-96	12	97.2	87	107	93.6	76	103	102.4	88	114	94.0	60	105
PCB-98+102	12	100.7	80	111	98.6	73	112	102.3	78	115	86.9	16	115
PCB-99	14	87.7	72	99	88.2	69	103	91.3	79	107	92.7	49	109
PCB-104	14	87.3	74	102	87.4	69	108	91.6	81	105	86.8	50	104
PCB-105+127	14	86.6	72	102	85.6	67	103	89.8	76	110	92.7	47	115
PCB-118+106	14	87.1	71	106	86.8	65	98	91.0	82	103	88.6	45	103
PCB-107+108	12	97.3	81	108	94.0	57	108	102.3	92	113	102.0	55	114
PCB-110	14	87.4	62	109	84.8	65	116	88.1	76	103	90.2	44	138

Table 38. Matrix Spike Recoveries for Wastewater Samples #3 to #6 (%)

Analyte	# Results	Wastewater #3			Wastewater #4			Wastewater #5			Wastewater #6		
		Mean Rec.	Min. Rec.	Max. Rec.	Mean Rec.	Min. Rec.	Max. Rec.	Mean Rec.	Min. Rec.	Max. Rec.	Mean Rec.	Min. Rec.	Max. Rec.
PCB-128	14	85.4	71	106	88.5	67	107	87.3	77	100	87.9	41	117
PCB-132+168	14	95.6	76	214	91.6	68	137	90.6	78	106	88.4	46	109
PCB-138+163+164	14	81.6	60	102	86.6	62	120	86.5	73	105	88.0	39	116
PCB-149+139	14	85.4	58	102	89.7	64	117	88.0	72	108	89.1	48	121
PCB-147	14	83.5	61	95	88.8	63	102	88.3	75	103	87.4	44	106
PCB-152	12	101.7	54	125	105.0	81	122	105.3	72	126	100.8	63	124
PCB-153	14	85.3	48	142	88.1	61	117	86.9	65	110	85.8	39	126
PCB-155	14	80.2	58	92	84.6	61	100	88.4	71	107	84.7	39	105
PCB-156	14	79.4	47	96	88.6	47	111	87.1	64	117	91.4	44	114
PCB-166	14	90.6	68	104	97.3	68	113	97.5	81	113	99.7	48	114
PCB-169	14	77.9	48	101	83.3	64	103	85.6	69	103	88.2	42	115
PCB-177	14	77.7	50	96	89.1	60	116	86.1	68	107	89.3	40	120
PCB-180	14	71.7	44	86	83.7	59	124	81.8	63	112	84.5	39	130
PCB-187+182	14	84.9	56	102	94.9	65	113	93.6	77	108	96.3	43	124
PCB-184	12	83.6	56	98	88.3	63	107	93.0	80	103	88.8	43	100
PCB-188	14	73.7	50	88	84.4	58	107	84.2	64	108	83.4	36	109
PCB-189	14	70.5	48	85	83.5	56	102	81.5	60	111	84.2	36	114
PCB-199	14	59.7	19	93	74.2	14	97	73.2	2	96	123.6	40	419
PCB-202	14	65.9	40	84	81.1	55	104	78.6	61	99	80.4	36	104
PCB-204	12	80.6	47	98	89.3	63	102	92.8	78	104	90.8	40	105
PCB-205	14	61	40	82	79.9	54	99	77.0	56	104	81.1	48	103
PCB-206	14	58.4	30	84	76.5	54	99	81.0	58	123	84.5	37	114
PCB-208	14	59.6	31	101	77.7	53	98	78.0	56	98	79.8	33	102
PCB-209	14	54.6	25	95	81.1	52	122	78.4	56	103	82.8	36	107

Table 39. Matrix Spike Recoveries for Wastewater Samples #7 to #10 (%)

Analyte	# Results	Wastewater #7			Wastewater #8			Wastewater #9			Wastewater #10		
		Mean Rec.	Min. Rec.	Max. Rec.	Mean Rec.	Min. Rec.	Max. Rec.	Mean Rec.	Min. Rec.	Max. Rec.	Mean Rec.	Min. Rec.	Max. Rec.
PCB-1	14	187.2	-33	453	105.2	93	143	106.5	96	163	94.5	0 (1)	114
PCB-3	14	98.7	58	158	95.4	88	107	97.2	88	117	96.9	89	113
PCB-4+10	14	96.6	38	164	106.4	85	266	85.4	14	139	95.4	13	147
PCB-8+5	14	93.1	59	165	83.0	70	99	81.6	56	101	81.6	31	114
PCB-11	14	97.7	33	157	94.1	88	121	93.2	86	104	114.4	85	263
PCB-15	14	90.4	60	116	87.1	74	93	85.7	68	92	86.7	82	92
PCB-18	14	90.4	41	145	76.9	55	85	78.7	53	86	79.7	34	101
PCB-19	14	93.6	47	166	89.6	83	98	88.8	79	98	90.5	84	103
PCB-28	14	85.4	43	122	97.9	76	122	96.9	76	127	98.5	77	123
PCB-31	14	76.6	35	118	82.2	71	96	84.4	66	104	83.6	74	94
PCB-36	12	97.4	0 (3)	174	114.5	74	138	109.8	72	138	116.8	98	144
PCB-37	14	74.5	0 (2)	236	89.0	82	97	90.4	85	100	87.9	82	92
PCB-41+64	14	118.2	26	255	79.2	65	94	80.9	63	96	82.3	64	89
PCB-44	14	82.6	31	109	91.6	71	110	89.0	66	100	94.2	88	102
PCB-52+73	14	87.0	32	171	87.1	66	104	84.9	64	92	89.1	84	105
PCB-54	14	86.7	34	108	89.1	74	100	86.1	65	97	91.5	85	104
PCB-74+61	14	90.4	29	114	87.8	62	110	93.1	61	143	88.9	67	97
PCB-66+80	14	116.6	29	293	89.0	63	120	84.6	60	92	86.9	68	91
PCB-70	14	80.7	29	114	88.6	62	156	81.2	60	90	83.9	68	90
PCB-72	12	116.7	30	193	100.4	69	119	97.2	67	115	104.5	75	123
PCB-77	14	73.2	24	92	84.1	62	96	81.4	59	101	80.3	44	97
PCB-78	12	95.5	19	151	105.6	67	128	100.8	59	122	103.5	77	115
PCB-79	12	111.5	12	220	111.3	73	141	106.0	67	139	108.7	82	128
PCB-85+120	14	76.0	26	95	86.0	57	122	81.1	56	93	86.1	52	112
PCB-90+101+89	14	75.7	22	94	93.2	57	203	83.1	54	95	86.1	58	95
PCB-95+93	14	77.9	26	101	84.9	60	153	81.5	59	97	81.6	58	92
PCB-96	12	85.6	22	106	99.3	69	121	92.8	64	115	100.6	93	112
PCB-98+102	12	91.7	22	120	97.2	67	112	96.6	64	116	99.4	64	110
PCB-99	14	77.2	26	98	91.5	57	163	84.0	56	98	86.3	57	93
PCB-104	14	76.3	26	95	86.6	60	104	81.9	59	91	87.2	76	94
PCB-105+127	14	74.2	23	129	90.6	55	161	83.4	53	97	82.7	45	92
PCB-118+106	14	76.4	20	96	94.8	56	209	83.6	55	99	85.9	48	95
PCB-107+108	12	91.9	20	113	100.3	59	132	94.8	60	114	97.6	55	109
PCB-110	14	81.7	25	133	93.9	56	280	81.7	57	104	81.5	47	110

Table 39. Matrix Spike Recoveries for Wastewater Samples #7 to #10 (%)

Analyte	# Results	Wastewater #7			Wastewater #8			Wastewater #9			Wastewater #10		
		Mean Rec.	Min. Rec.	Max. Rec.	Mean Rec.	Min. Rec.	Max. Rec.	Mean Rec.	Min. Rec.	Max. Rec.	Mean Rec.	Min. Rec.	Max. Rec.
PCB-126	14	82.4	22	228	83.1	54	92	82.6	54	99	84.1	41	103
PCB-132+168	14	58.1	0 (2)	103	88.9	53	135	83.6	54	101	84.6	42	97
PCB-138+163+164	14	84.2	24	141	91.7	50	197	82.9	49	97	82.6	38	96
PCB-149+139	14	82.2	23	112	89.8	50	179	84.0	51	100	83.5	39	97
PCB-147	14	77.8	21	103	84.1	49	95	82.3	49	102	83.7	37	99
PCB-152	12	98.6	18	130	99.5	67	121	99.9	68	130	100.3	51	124
PCB-153	14	93.1	23	159	91.2	53	180	82.6	49	109	83.3	38	99
PCB-155	14	67.9	22	91	85.6	50	99	78.6	49	89	83.6	46	91
PCB-156	14	73.1	23	139	88.1	53	113	82.4	50	100	84.4	41	100
PCB-166	14	86.7	20	142	97.4	54	112	91.9	53	108	94.5	44	109
PCB-169	14	75.5	0 (1)	138	84.6	48	97	81.4	47	101	80.8	32	97
PCB-177	14	68.8	25	101	85.5	46	102	82.4	46	121	81.1	31	102
PCB-180	14	60.4	15	96	84.4	44	97	73.7	44	95	78.1	31	92
PCB-187+182	14	73.1	19	132	92.6	45	105	88.4	45	130	88.1	33	105
PCB-184	12	66.4	11	89	91.8	45	106	81.9	46	104	89.0	40	103
PCB-188	14	61.6	24	87	83.9	44	94	77.3	45	90	80.9	35	93
PCB-189	14	61.4	20	103	82.2	44	96	75.6	42	98	77.6	29	92
PCB-199	14	85.6	14	188	84.4	50	106	80.7	47	151	79.0	38	98
PCB-202	14	57.8	24	107	79.6	43	93	73.0	41	91	75.7	29	87
PCB-204	12	73.0	12	150	92.4	41	111	85.6	42	116	92.5	38	122
PCB-205	14	60.8	0 (1)	135	74.3	40	92	74.0	42	96	75.5	29	96
PCB-206	14	57.5	16	100	80.1	38	118	73.7	37	94	76.7	29	111
PCB-208	14	47.8	15	76	76.9	38	91	71.2	37	93	77.9	27	118
PCB-209	14	79.9	13	356	77.9	36	92	77.1	36	122	79.1	29	99

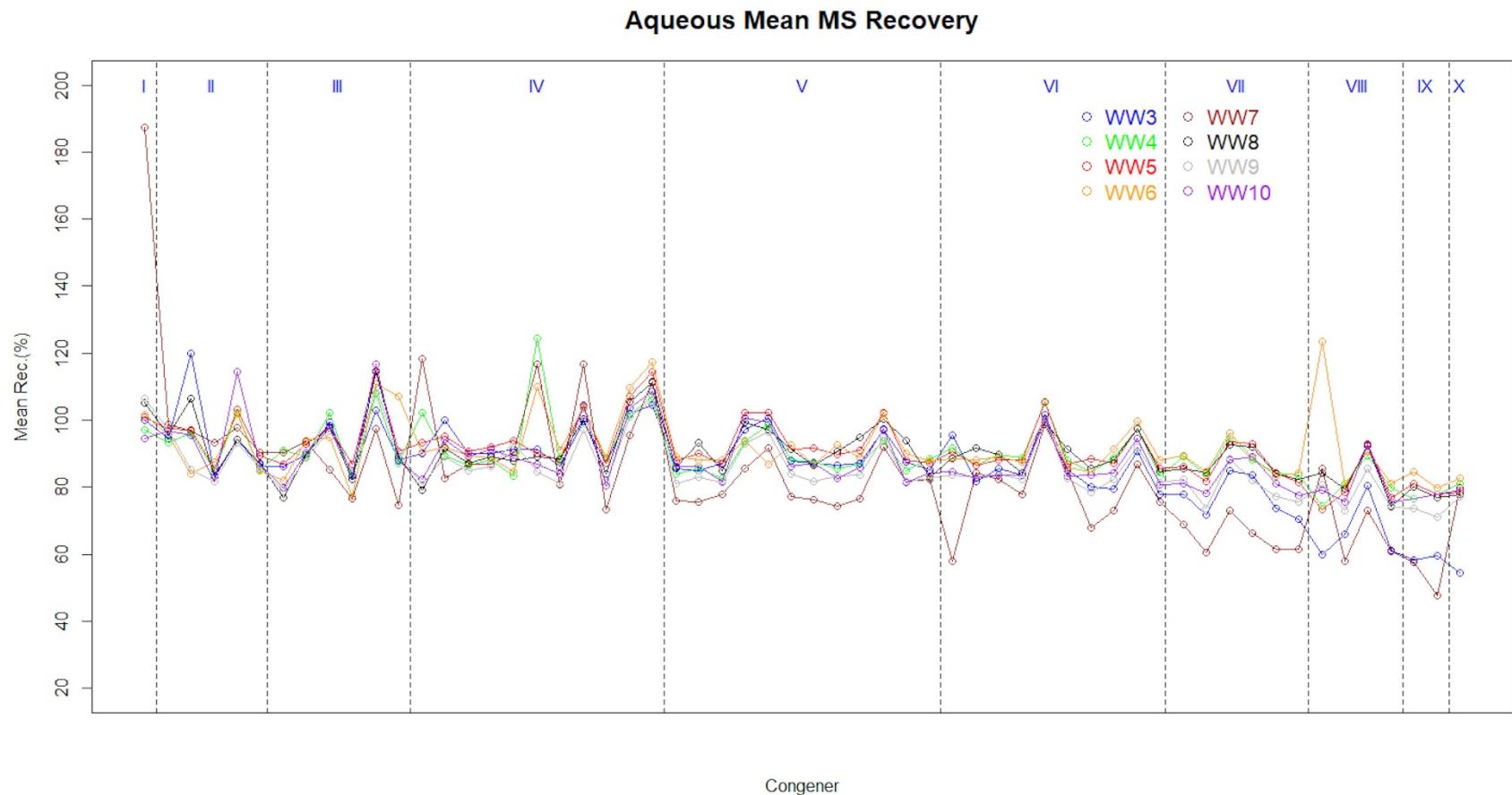


Figure 5. Mean Matrix Spike Wastewater Recoveries by Sample, in Elution Order (without Sample #2)

Dotted lines and Roman numerals delineate the levels of chlorination. The colored symbols and lines denote each of the eight wastewater samples that were spiked.

The majority of the mean matrix spike recoveries fall within the range of 60 to 120%. Of 464 mean recoveries, only 8 were below 60% and only 3 were over 120%. The mean recovery of 187% for PCB-1 in wastewater #7 was driven by the results from Lab 1, Lab 6, and Lab 8, with reported recoveries between approximately 200 and 500%. All of those results for PCB-1 also exhibited issues with the ion abundance ratio for this congener, indicating an interference was present. The mean recovery for PCB-199 in wastewater #6 was only slightly higher than 120% and was driven by the results from Lab 8, which were reported between approximately 300 and 420%. The mean recovery for PCB-66+80 in wastewater #4 was only slightly higher than 120% and was driven by the results from Lab 1, with a recovery in the MSD aliquot of 585%. That result was likely driven by the very low recovery of the labeled compound used to quantify this analyte in the MSD aliquot. In contrast, the MS recovery from Lab 1 was 94%.

Sediment Sample MS/MSD Results

Each of the six laboratories that completed the sediment portion of the study analyzed MS/MSD aliquots of the three sediment samples. CSRA calculated the mean recovery of each spiked analyte by sample across all six laboratories, along with the minimum and maximum observed values and presented those results in Table 40. Because the non-wastewater matrices in this study were a lower priority for EPA, and because more of the laboratories experienced issues with qualitative identification of the target analytes in these other matrices, if the results from a given laboratory did not meet the identification criteria for the analyte, then the recoveries from that laboratory were not included in the mean, and the number of results (#) for that sample in Table 40 will be less than 12. The highlighted rows show the congeners that were quantified indirectly, using labeled standards of similar congeners in the same level of chlorination. Values in parentheses, next to the zero values, represent the number of results which were reported as non-detects by the laboratories. Although there were some false negative results reported for Sediment #1, the percentage was less than 2.0% of the 2088 total data points. There were no false negatives for Sediments #2 and #3.

The mean MS/MSD recoveries are presented graphically in Figure 6, for all three of the sediment samples.

Table 40. Matrix Spike Recoveries for Sediment Samples

Analyte	#	Sediment #1			Sediment #2			Sediment #3		
		Mean Rec.	Min Rec.	Max Rec.	Mean Rec.	Min Rec.	Max Rec.	Mean Rec.	Min Rec.	Max Rec.
PCB-1	12	147	-63	380	125	36	260	176	-106	420
PCB-3	12	102	4	163	71	33	150	71	-86	170
PCB-4+10	12	107	-213	805	137	-67	971	121	-613	529
PCB-8+5	12	55	-29	145	89	18	368	24	-498	306
PCB-11	12	79	11	165	89	56	114	87	5	142
PCB-15	12	114	-158	334	157	-27	621	97	-456	542
PCB-18	12	142	-27	366	81	-35	389	34	-418	293
PCB-19	12	92	-5	239	89	16	322	74	-197	219
PCB-28	12	227	-112	1092	111	-2	279	87	-860	633
PCB-31	12	113	-154	392	88	-51	557	97	-744	573
PCB-36	12	59	0 (4)	148	108	71	167	117	14	156
PCB-37	12	56	0 (2)	117	88	0 (2)	296	110	-61	294
PCB-41+64	12	98	-14	284	86	53	153	83	-346	285
PCB-44	12	51	-256	220	75	-2	290	71	-675	442
PCB-52+73	12	461	-222	2761	102	-27	565	90	-1005	591
PCB-54	12	72	4	125	80	46	97	77	-5	102
PCB-66+80	12	155	-73	525	115	66	182	103	-642	504
PCB-70	12	50	-188	291	85	31	182	92	-572	451
PCB-72	12	73	0 (2)	121	109	83	143	122	20	258

Table 40. Matrix Spike Recoveries for Sediment Samples

Analyte	#	Sediment #1			Sediment #2			Sediment #3		
		Mean Rec.	Min Rec.	Max Rec.	Mean Rec.	Min Rec.	Max Rec.	Mean Rec.	Min Rec.	Max Rec.
PCB-74+61	12	117	-141	325	99	44	146	87	-335	319
PCB-77	12	70	0 (2)	134	92	75	110	78	-35	146
PCB-78	12	55	0 (4)	133	109	51	172	109	15	151
PCB-79	12	262	0 (2)	1129	118	62	166	104	15	160
PCB-85+120	12	69	-172	228	93	51	122	80	-14	139
PCB-90+101+89	12	57	-157	305	93	44	135	68	-103	168
PCB-95+93	12	100	-171	354	83	36	227	7	-500	174
PCB-96	12	62	-88	148	92	55	108	97	-8	217
PCB-98+102	12	146	-14	815	103	63	142	44	-783	448
PCB-99	12	50	-162	271	94	43	135	78	-126	190
PCB-104	12	45	-88	93	79	44	92	79	13	101
PCB-105+127	12	119	-44	283	96	61	122	87	-68	186
PCB-107+108	12	220	43	485	93	21	130	121	32	207
PCB-110	12	181	-183	472	99	41	231	107	-338	262
PCB-118+106	12	204	-118	638	101	63	174	83	-105	196
PCB-126	12	44	0 (4)	99	75	-26	107	81	10	104
PCB-132+168	12	55	-77	212	90	46	130	81	-11	119
PCB-138+163+164	12	109	-230	424	96	57	210	91	-74	189
PCB-147	12	59	-12	100	73	-20	101	82	19	106
PCB-149+139	12	99	-213	334	98	60	191	76	-7	127
PCB-152	12	102	15	139	103	60	131	98	29	123
PCB-153	12	197	-107	615	95	46	236	79	-15	136
PCB-155	12	50	-72	92	81	45	93	80	15	105
PCB-156	12	94	4	162	90	58	114	80	10	122
PCB-166	12	92	0 (1)	160	104	65	146	95	21	132
PCB-169	12	73	0 (2)	117	115	70	278	74	12	99
PCB-177	12	73	8	163	90	49	127	80	4	113
PCB-180	12	75	-85	316	112	43	275	79	-9	125
PCB-184	12	61	-65	110	93	54	110	90	20	121
PCB-187+182	12	55	-45	157	107	53	149	90	13	125
PCB-188	12	59	-6	93	83	46	101	81	19	111
PCB-189	12	54	-82	130	93	48	115	78	19	101
PCB-199	12	91	-30	232	94	-12	232	85	10	117
PCB-202	12	77	-16	159	91	43	120	83	22	123
PCB-204	12	77	0 (2)	141	95	47	128	93	6	130
PCB-205	12	74	0 (2)	152	85	38	106	78	7	105
PCB-206	12	34	-117	224	130	-3	411	82	25	108
PCB-208	12	92	18	173	101	64	123	86	30	120
PCB-209	12	114	32	244	95	40	140	88	34	105

As can be seen in Table 40, there are many congeners with minimum recoveries that are negative numbers. Although all of the laboratories reported some negative recoveries, four of the laboratories reported 26 to 63 negative recoveries across all the 348 results for the three sediment samples, while the other two laboratories reported only 3 and 4 negative recoveries. Although negative recoveries have no “physical” meaning, in that the analytes are not in fact removed from the sample by the analytical procedures, they can be indicative of issues with interferences of either the spiked sample analysis, or the unspiked sample analysis that bias the results of one or both of those analyses. CSRA examined the patterns of negative recoveries across the laboratories. Although Laboratory 8 had 63 negative recoveries, most of those occurred in Sediment #1 and Sediment #3, and usually only in one of the two spiked aliquots (i.e., the MS or the MSD, but not both). That pattern suggests an interference with one, but not both of the spiked samples for the affected congeners. In contrast, Laboratory 3 had all of its

negative recoveries in Sediments #1 and #2, and almost always for the same congeners in both aliquots. That pattern suggests that the negative values may be due to issues with the results for the unspiked aliquots, where a positive bias in the unspiked sample results leads to negative recoveries. The results for the unspiked sample analyses in Table 30 indicate that Laboratory 3 often reported higher results for the affected congeners than the other five laboratories. The patterns of negative recoveries from Laboratory 6 are similar, with all 26 of the negative recoveries occurring in Sediments #1 and #2, and almost always for the same congeners in both aliquots. In contrast, Laboratory 4 reported 47 negative recoveries, 35 from Sediment #3, but with 28 of those in the MSD aliquot, and only 7 in the MS aliquot. That pattern suggests a mix of both issue with the unspiked sample results and interferences in the MSD aliquot.

In addition to the negative recoveries, there were a number of very high positive recoveries reported, a few over 2,000%. As with the negative recoveries, the high recoveries were distributed across all six laboratories, but unevenly among the laboratories, as well as among the three sediment samples. In some laboratories, the higher than expected recoveries were reported in both the MS and MSD aliquots for the same congeners, suggesting that the issue may have been due to a low bias in the unspiked sample analyses. In other cases, the high recoveries were only in one of the spiked aliquots, indicative of aliquot-specific interferences.

Another potential source of the unusual recoveries is sample homogeneity, or lack thereof. Although the vendor took extensive measures to thoroughly blend and homogenize the bulk sediments used to create these study samples and divide them into aliquots for each laboratory, there still may be some inhomogeneity from aliquot to aliquot. This is because PCBs are sorbed onto the sediment particles, and it may not be possible to evenly distribute any more highly contaminated particles across the entire bulk sample, despite careful preparation. Therefore, one of the aliquots of a given sample distributed to each laboratory may have had more PCBs than the other two aliquots used for the unspiked and spiked sample analyses. Such differences will affect the assumptions in the analyte recovery calculations.

As can be seen in Figure 6, the highest mean recoveries were reported from Sediment #1, and represent PCB-28, PCB-52+73, PCB-66+80, PCB-79, PCB-107+108, PCB-110, PCB-118+106, and PCB-153.

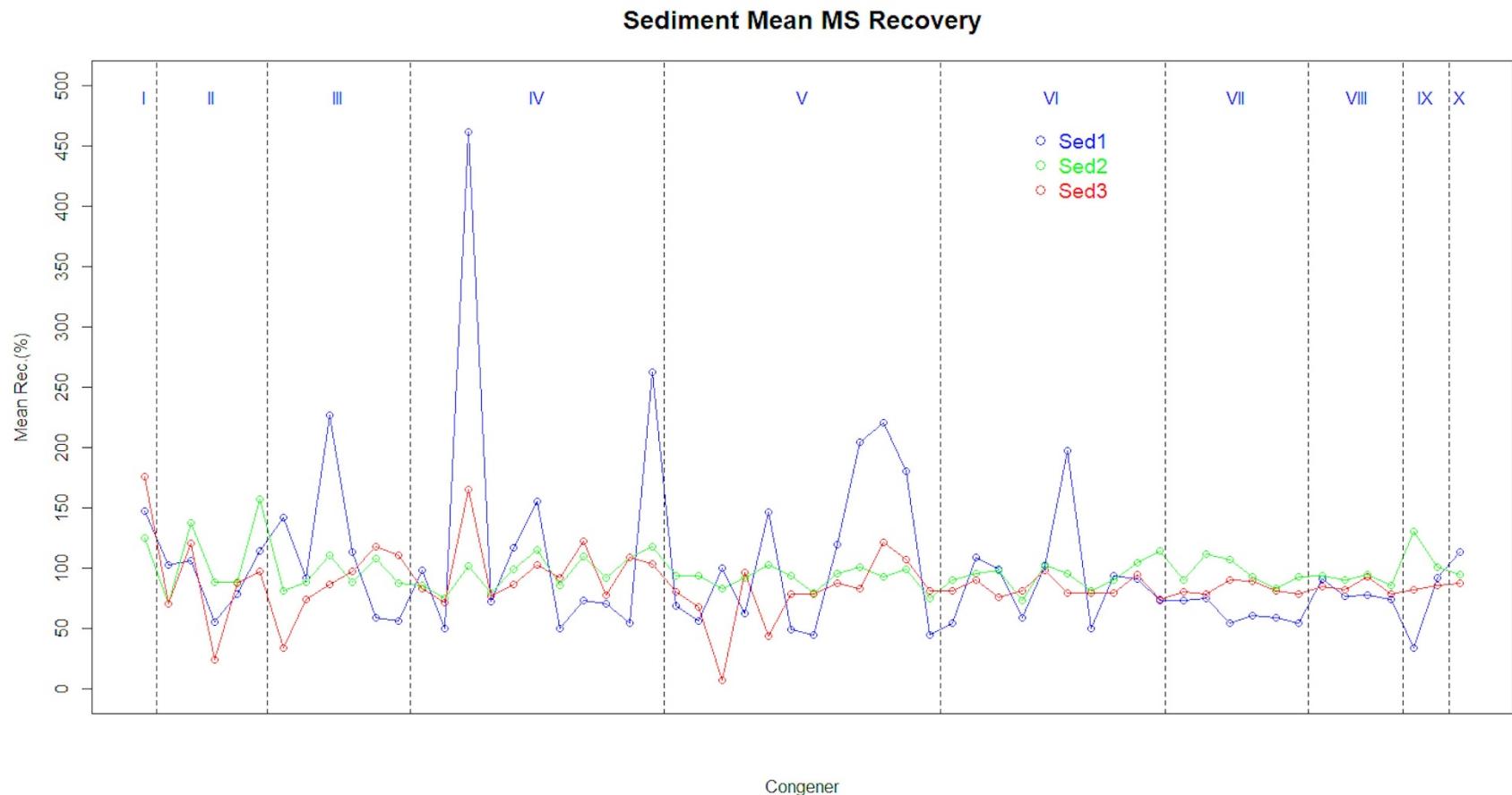


Figure 6. Mean Matrix Spike Sediment Recoveries by Sample, in Elution Order

Dotted lines and Roman numerals delineate the levels of chlorination. The colored symbols and lines denote each of the three sediment samples that were spiked.

Biosolids Sample MS/MSD Results

Each of the four laboratories that completed the biosolids portion of the study analyzed MS/MSD aliquots of the three biosolids samples. CSRA calculated the mean recovery of each spiked analyte by sample across all four laboratories, along with the minimum and maximum observed values and presented those results in Table 41. As noted in the discussion of the sediment sample results, because the non-wastewater matrices in this study were a lower priority for EPA, and because more of the laboratories experienced issues with qualitative identification of the target analytes in these other matrices, if the results from a given laboratory did not meet the identification criteria for the analyte, the recoveries from that laboratory were not included in the mean, and the number of results (#) for that sample in Table 41 will be less than 8. The highlighted rows show the congeners that were quantified indirectly, using labeled standards of similar congeners in the same level of chlorination.

Samples #1 and #3 had many false negatives with a percentage of 4.8% of the 928 combined data points for those two samples for all four labs; however, all the false negative results are from Laboratory 4. The laboratory performed GPC clean-up, plus an extra dilution, which may have caused loss of some target analytes. The congeners affected were from the monochlorinated up to the heptachlorinated congeners.

The mean MS/MSD recoveries are presented graphically in Figure 7, for all three of the biosolids samples.

Table 41. Matrix Spike Recoveries for Biosolids Samples

Analyte	# Results ¹	Biosolid #1			Biosolid #2			Biosolid #3		
		Mean Rec.	Min Rec.	Max Rec.	Mean Rec.	Min Rec.	Max Rec.	Mean Rec.	Min Rec.	Max Rec.
PCB-1	8	79	-86	151	113	88	146	135	73	257
PCB-3	8	106	56	194	177	41	478	224	83	543
PCB-4+10	8	94	24	130	186	33	354	83	63	104
PCB-8+5	8	78	49	108	71	28	117	101	64	144
PCB-11	8	100	18	130	91	33	169	178	71	427
PCB-15	8	71	32	94	72	26	121	84	74	96
PCB-18	8	95	14	165	74	13	131	82	54	110
PCB-19	8	78	21	128	70	25	104	89	44	130
PCB-28	8	103	28	149	90	14	133	107	73	147
PCB-31	8	95	29	164	79	18	105	104	44	144
PCB-36	8 (6)	86	0 (2)	154	101	44	141	84	0 (2)	126
PCB-37	8 (6)	59	0 (2)	109	89	39	128	92	52	124
PCB-41+64	8 (6)	72	0 (2)	108	78	37	120	68	0 (2)	105
PCB-44	8 (6)	70	0 (2)	116	80	21	100	80	0 (2)	129
PCB-52+73	8	93	25	129	71	4	100	94	54	152
PCB-54	8	83	24	118	72	20	91	85	54	110
PCB-66+80	8 (6)	86	0 (2)	160	99	46	154	94	0 (2)	194
PCB-70	8 (7)	111	0 (1)	242	83	36	120	90	0 (2)	149
PCB-72	8 (6)	80	0 (2)	132	104	42	144	94	0 (2)	149
PCB-74+61	8	185	62	463	88	39	116	84	0 (2)	154
PCB-77	8 (6)	52	0 (2)	86	80	61	97	101	0 (2)	281
PCB-78	8 (6)	84	0 (2)	145	101	84	119	112	0 (2)	233
PCB-79	8 (6)	90	0 (2)	157	120	79	189	101	0 (2)	150
PCB-85+120	8	100	80	138	82	61	90	99	76	122
PCB-90+101+89	8	77	60	91	85	42	104	75	40	150
PCB-95+93	8	93	50	137	86	26	126	97	55	159
PCB-96	8	85	47	95	81	35	98	91	76	100
PCB-98+102	8 (6)	74	0 (2)	115	95	49	117	40	-52	129
PCB-99	8	93	37	122	85	51	98	109	72	133

Table 41. Matrix Spike Recoveries for Biosolids Samples

Analyte	# Results ¹	Biosolid #1			Biosolid #2			Biosolid #3		
		Mean Rec.	Min Rec.	Max Rec.	Mean Rec.	Min Rec.	Max Rec.	Mean Rec.	Min Rec.	Max Rec.
PCB-104	8	73	36	85	70	23	86	76	59	91
PCB-105+127	8	121	97	189	90	81	100	81	0 (2)	132
PCB-107+108	8 (6)	89	0 (2)	147	105	82	129	89	0 (2)	130
PCB-110	8	101	74	150	92	58	113	121	67	228
PCB-118+106	8	90	63	117	96	72	107	108	68	155
PCB-126	8 (6)	67	0 (2)	97	89	80	98	78	0 (2)	136
PCB-132+168	8	87	66	115	92	73	108	116	59	181
PCB-138+163+164	8	86	68	100	102	82	123	105	59	166
PCB-147	8	95	81	120	85	68	92	94	88	104
PCB-149+139	8	92	78	123	93	69	109	91	61	151
PCB-152	8	103	69	133	95	50	116	108	98	118
PCB-153	8	100	73	142	99	77	116	93	59	137
PCB-155	8	79	57	92	76	39	87	80	70	89
PCB-156	8 (6)	70	0 (2)	102	90	68	107	74	0 (2)	122
PCB-166	8	105	71	144	103	98	108	98	73	114
PCB-169	8 (6)	74	0 (2)	136	94	74	132	58	0 (2)	108
PCB-177	8	93	87	99	89	81	96	98	90	109
PCB-180	8	151	78	361	86	83	91	118	70	194
PCB-184	8	106	87	128	95	83	101	99	83	119
PCB-187+182	8	95	80	109	96	87	103	93	71	117
PCB-188	8	88	79	102	83	67	92	85	77	92
PCB-189	8	89	60	108	91	86	94	96	83	119
PCB-199	8	89	61	112	90	75	100	83	48	111
PCB-202	8	93	86	110	87	82	90	91	86	98
PCB-204	8	102	95	108	101	92	108	101	88	117
PCB-205	8	84	63	105	90	77	110	80	58	106
PCB-206	8	115	84	199	88	79	105	96	73	110
PCB-208	8	92	79	120	94	82	109	95	82	121
PCB-209	8	98	82	112	91	73	117	99	88	119

¹ The value in parentheses is the number of results used to determine the mean recovery in biosolid sample #1

Overall, across all four laboratories and all three biosolids samples, the majority of the mean recoveries ranged from 70% to 185%, with six mean recoveries below 70%, four mean recoveries between 150% and 185%, and two mean recoveries above 185%.

Biosolids are well known as a challenging matrix for any analysis. In the case of PCB analyses, there are many potential organic components present that can affect the sample extraction processes that may require additional cleanup steps to remove from the extracts, which present instrumental interferences that affect the chromatographic separation, or that affect the identification of a peak as a target analyte. Despite such challenges, when compared to the sediment results, the biosolids results from this study demonstrate better recoveries across all four of the laboratories and all of the congeners. For example, there was only two instances of a congener with a minimum recovery less than 0%, and 22 congeners with maximum recoveries greater than 150%, out of 435 results in Table 41. In comparison, there were 81 instances of negative recoveries and 97 instances of recoveries greater than 150% out of the 2054 sediment results in Table 40. It is unclear if the apparent differences between the results for the two matrices are a function of issues in either of the two laboratories that performed sediment analyses but did not perform biosolids analyses, or if other factors may be involved. As can be seen in Figure 7, mean recoveries greater than 150% occurred in all three of the biosolids samples, but for different congeners in different samples.

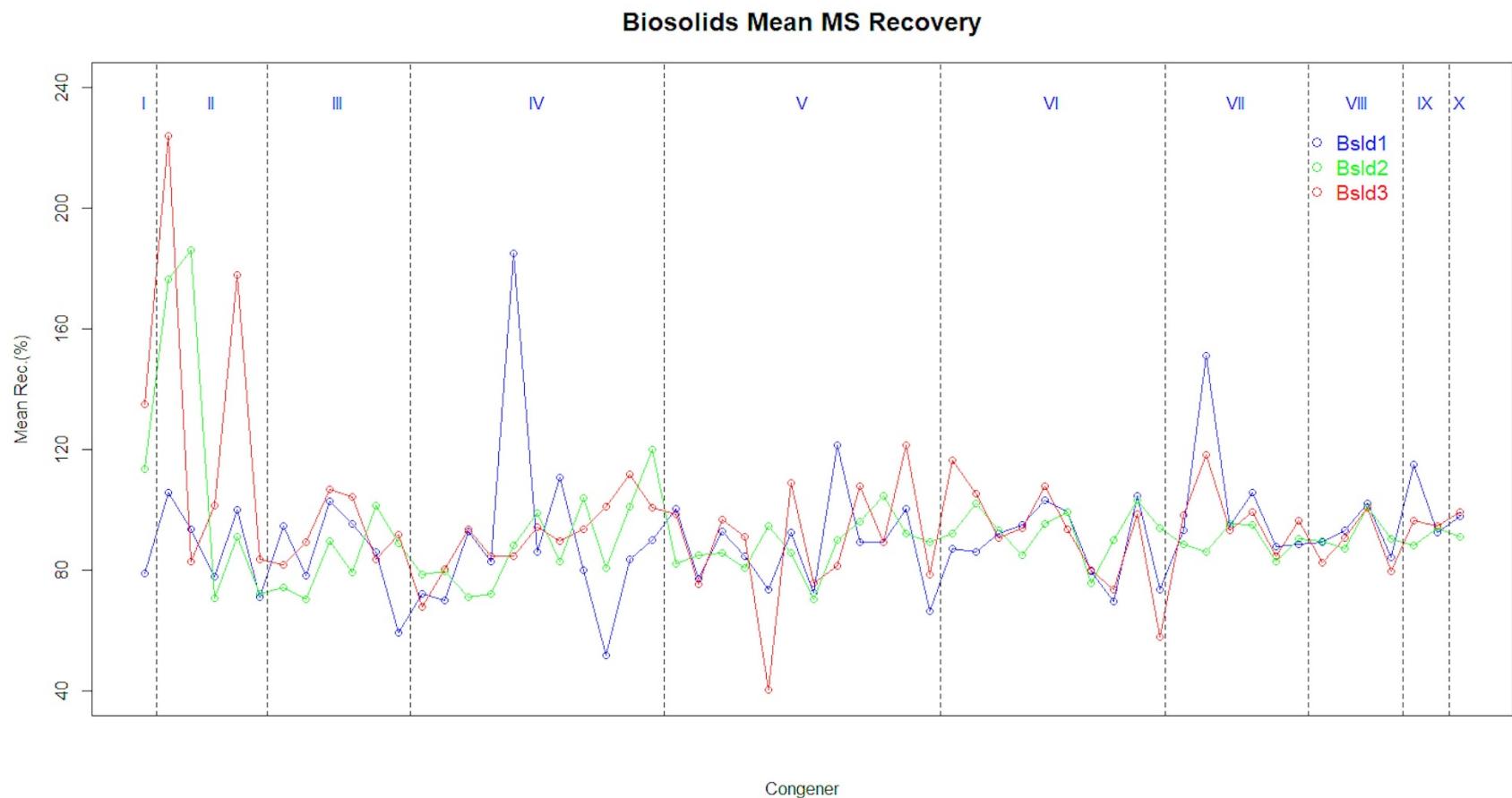


Figure 7. Mean Matrix Spike Biosolids Recoveries by Sample, in Elution Order

Dotted lines and Roman numerals delineate the levels of chlorination. The colored symbols and lines denote each of the three biosolids samples that were spiked.

Tissue Sample MS/MSD Results

Each of the four laboratories that completed the tissue portion of the study analyzed MS/MSD aliquots of the three tissue samples. CSRA calculated the mean recovery of each spiked analyte by sample across all six laboratories, along with the minimum and maximum observed values and presented those results in Table 42. The highlighted rows show the congeners that were quantified indirectly, using labeled standards of similar congeners in the same level of chlorination. Values in parentheses next to the zero values, represent the number of results which were reported at the same value of the unspiked samples by the laboratories. The percentage of false negatives for tissue samples was less than 0.1% of the 1392 total data points.

The mean MS/MSD recoveries are presented graphically in Figure 8, for all three of the tissue samples.

Table 42. Matrix Spike Recoveries for Tissue Samples

Analyte	# Results	Tissue #1			Tissue #2			Tissue #3		
		Mean Rec.	Min. Rec.	Max. Rec.	Mean Rec.	Min. Rec.	Max. Rec.	Mean Rec.	Min. Rec.	Max. Rec.
PCB-1	8	101.8	94	111	95.0	87	104	95.5	92	101
PCB-3	8	95.8	89	105	91.9	86	98	89.8	83	97
PCB-4+10	8	95.3	87	106	87.8	80	95	89.4	84	96
PCB-8+5	8	112.0	75	194	84.0	73	99	67.9	48	97
PCB-11	8	97.9	89	118	95.5	85	113	106.0	86	118
PCB-15	8	84.9	77	93	90.9	85	110	88.5	82	92
PCB-18	8	85.8	73	94	83.0	71	102	87.8	69	106
PCB-19	8	89.6	86	97	88.6	82	101	85.6	76	93
PCB-28	8	100.1	82	130	109.4	86	153	102.4	79	142
PCB-31	8	87.6	76	101	87.4	78	109	83.4	71	91
PCB-36	8	115.6	101	130	114.6	101	146	112.9	99	123
PCB-37	8	90.4	81	112	91.5	83	123	90.6	74	108
PCB-41+64	8	86.6	73	92	90.3	79	129	80.5	70	91
PCB-44	8	94.9	92	102	99.9	81	155	93.8	83	106
PCB-52+73	8	90.1	75	106	95.1	75	152	80.1	28	114
PCB-54	8	89.3	87	91	89.6	82	116	84.5	67	93
PCB-74+61	8	95.3	92	101	96.9	81	149	82.3	71	102
PCB-66+80	8	95.1	84	102	93.9	77	153	175.9	65	557
PCB-70	8	194.8	84	554	89.8	47	149	224.6	84	699
PCB-72	8	112.0	94	136	114.5	84	143	111.4	89	142
PCB-77	8	88.4	80	103	85.4	80	91	87.9	75	102
PCB-78	8	110.3	103	120	111.3	94	175	228.5	109	669
PCB-79	8	105.1	94	119	117.3	89	196	106.3	88	134
PCB-85+120	8	89.1	84	99	85.8	75	104	84.8	77	97
PCB-90+101+89	8	85.3	57	95	84.1	56	152	77.4	45	110
PCB-95+93	8	93.0	80	153	87.8	69	137	79.5	65	99
PCB-96	8	104.8	93	150	99.1	81	147	96.0	91	105
PCB-98+102	8	102.1	93	113	103.4	90	143	80.0	0 (2)	114
PCB-99	8	86.3	55	99	83.8	61	166	81.9	51	118
PCB-104	8	89.3	86	99	89.9	79	131	86.1	79	94
PCB-105+127	8	92.5	88	97	87.6	74	102	85.0	68	101
PCB-118+106	8	91.3	88	95	81.4	62	104	79.3	39	116
PCB-107+108	8	104.5	98	113	105.9	91	115	114.1	91	158
PCB-110	8	86.0	82	97	82.1	59	101	68.1	20	104
PCB-126	8	87.3	83	91	85.3	73	90	90.1	85	99
PCB-132+168	8	94.1	89	107	91.1	75	107	80.1	67	91
PCB-138+163+164	8	88.4	85	96	50.8	7	101	65.1	-12	143

Table 42. Matrix Spike Recoveries for Tissue Samples

Analyte	# Results	Tissue #1			Tissue #2			Tissue #3		
		Mean Rec.	Min. Rec.	Max. Rec.	Mean Rec.	Min. Rec.	Max. Rec.	Mean Rec.	Min. Rec.	Max. Rec.
PCB-149+139	8	92.0	86	97	71.6	54	94	77.1	38	108
PCB-147	8	89.5	82	95	87.5	82	92	87.5	84	94
PCB-152	8	108.0	94	121	102.4	88	120	105.9	95	117
PCB-153	8	95.8	84	112	43.3	6	96	65.0	-11	150
PCB-155	8	87.6	85	90	90.8	82	129	85.8	79	94
PCB-156	8	97.3	91	105	92.1	80	99	89.1	62	108
PCB-166	8	103.5	97	112	99.5	90	107	103	99	108
PCB-169	8	92.9	86	106	82.3	71	88	90.3	82	104
PCB-177	8	92.8	90	100	81.1	75	92	90.1	81	99
PCB-180	8	91.8	85	101	62.9	49	87	79.4	50	118
PCB-187+182	8	102.9	99	113	81.5	67	93	93.0	86	103
PCB-184	8	95.8	87	106	92.6	83	101	95.4	89	101
PCB-188	8	87.8	83	91	86.3	81	92	86.3	81	90
PCB-189	8	82.5	61	94	82.4	61	92	79.4	61	98
PCB-199	8	90.5	71	98	88.5	76	101	81.1	55	97
PCB-202	8	88.5	85	92	80.8	67	87	86.3	81	92
PCB-204	8	99.6	90	104	94.3	78	104	104.8	97	112
PCB-205	8	80.3	61	91	78.6	61	86	78.9	63	91
PCB-206	8	85.4	69	96	80.4	68	88	80.3	65	89
PCB-208	8	83.9	68	93	78.9	60	87	81.6	70	91
PCB-209	8	91.5	87	101	75.9	65	94	88.4	79	94

The matrix spike recoveries in the tissue samples were generally much more consistent than for the sediment samples. Across all four laboratories and all three tissue samples, the mean recoveries range from about 43% to 229%, with only 4 congeners with mean recoveries over 120% (PCB-66+80, PCB-70, and PCB-78) in Tissue Samples #1 and #3. Those four recoveries over 120% were driven by the very high reported recoveries from Laboratory 6 in those two samples. In contrast, Laboratory 6 reported recoveries of those three congeners in Tissue Sample #2 that ranged from about 84% to 104%, which suggests that whatever the cause of the very high recoveries in the other samples, it may have been sample-specific, and not an issue of laboratory bias or other error.

Laboratory 6 reported no recoveries for PCB-98+102 in Tissue Sample #3 in either the MS or MSD aliquots. Laboratory 3 also reported negative recoveries in Tissue Sample #3 for PCB-138+163+164 and PCB-153 in both the MS and MSD aliquots. Beyond those analytes, no other negative recoveries were reported.

As can be seen in Figure 8, the highest mean recoveries were reported from Tissue #1 and Tissue #3, and represent PCB-66+80, PCB-70, and PCB-78.

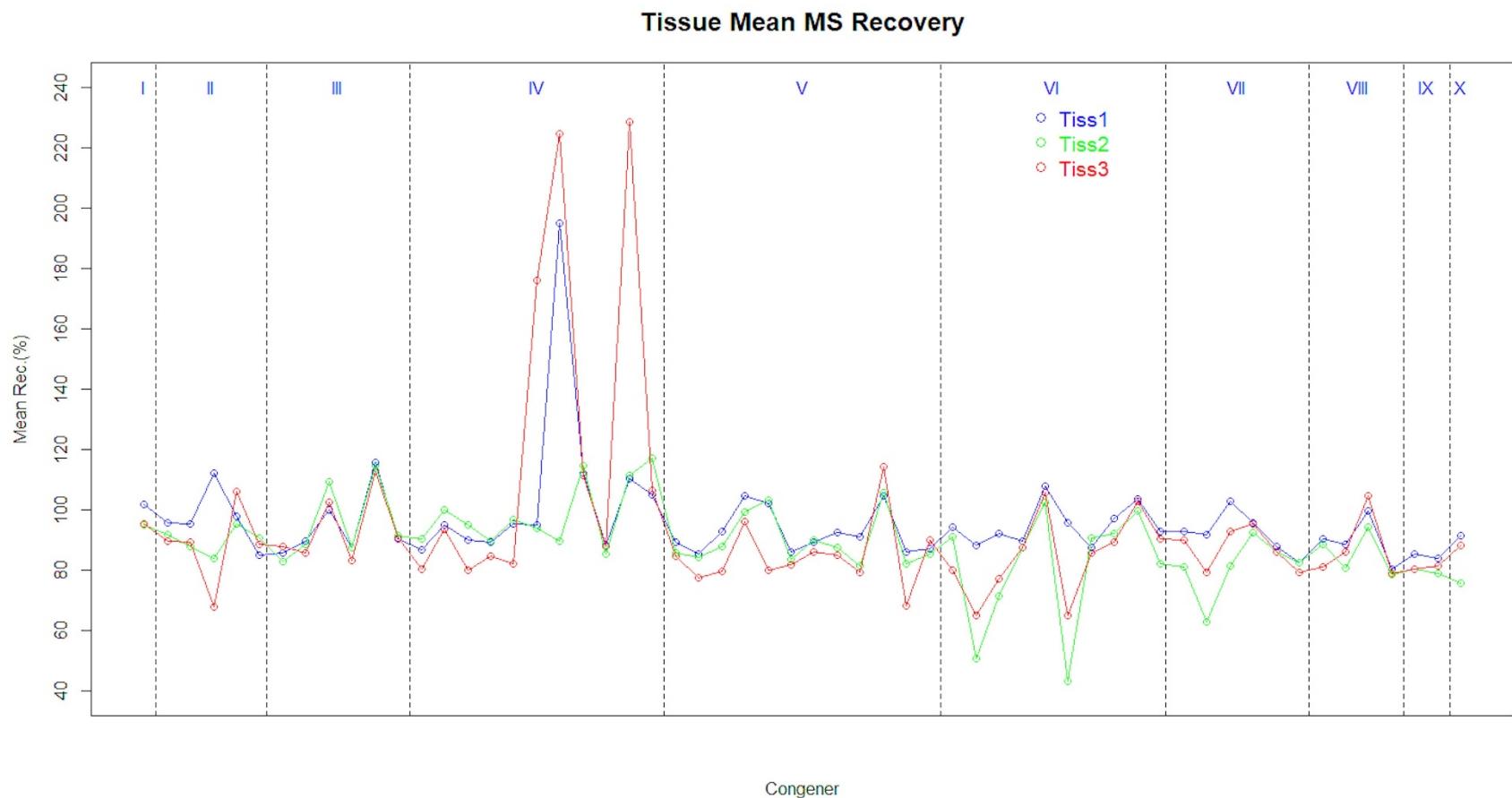


Figure 8. Mean Matrix Spike Tissue Recoveries by Sample, in Elution Order

Dotted lines and Roman numerals delineate the levels of chlorination. The colored symbols and lines denote each of the three tissue samples that were spiked.

9. Labeled Compound Results

One of the most important aspects of the draft PCB method study is its use of isotope dilution quantitation to determine the concentrations of the targeted analytes. As described in Section 4 of this report, each sample to be analyzed is spiked with a suite of 29 $^{13}\text{C}_{12}$ -labeled analogs of the target PCBs that are used as quantitation reference standards for both true isotope dilution quantitation and a modified form of isotope dilution for other target congeners in the same level of chlorination as the labeled compound.

This process results in an inherent correction of the target analyte concentration for the loss (or apparent gain) of the labeled compound throughout the entire analytical process, including the extraction steps as well as the many extract cleanup steps that are often necessary. Relative to the more commonly employed internal standards that are injected into the final sample extract shortly before the instrumental analysis, isotope dilution quantitation yields data that are both more accurate (less bias) and more precise.

Methods that rely on the analysis of MS/MSD samples to estimate accuracy and precision as a QC measure typically limit those MS/MSD analyses to a small subset of all the samples prepared together, with the typical frequency of 5%, or 1 in every 20 field samples. Whatever accuracy and precision information is generated is often assumed to apply to the entire sample batch, even when samples from different sources or locations are prepared and analyzed together.

In contrast, the labeled isotope dilution standards are added to every sample in the batch, so the analysis generates sample-specific accuracy data, in the form of the measured recovery of each of the labeled compounds in each sample.

CSRA and EPA compiled the labeled compound recovery data from all of analyses of spiked and unspiked study samples of wastewaters, sediments, biosolids, and fish tissues. Those results are discussed in the sections below, by matrix type.

Aqueous Sample Labeled Compound Results

The labeled compound recoveries from the seven laboratories that completed the aqueous sample portion of the study are summarized in Table 43 below. These data represent the analyses of the unspiked, MS, and MSD aliquots of all eight wastewater samples, for a total of 168 observations for each labeled compound (4,872 observations in all). The table contains the observed mean, minimum, and maximum recoveries from those 168 observations for each labeled compound, across all of the seven laboratories. Values in parentheses, next to zero values, represent the number of results which were reported as non-detects by the laboratories. The table also contains the QC acceptance criteria that CSRA calculated from those results.

Table 43. Observed Aqueous Labeled Compound Recoveries and Calculated Acceptance Criteria

Congener	# Labs	# Results	Observed Recoveries			Calculated Acceptance Criteria	
			Mean	Min.	Max.	Lower Limit	Upper Limit
$^{13}\text{C}_{12}$ -PCB-1	7	168	41.8	0.0 (1)	142.6	-31	115
$^{13}\text{C}_{12}$ -PCB-3	7	168	46.0	0.0 (1)	110.1	-21	114
$^{13}\text{C}_{12}$ -PCB-4	7	168	46.5	1.4	102.4	-20	113
$^{13}\text{C}_{12}$ -PCB-11	7	168	52.0	2.2	88.8	-11	115
$^{13}\text{C}_{12}$ -PCB-15	7	168	56.6	3.1	104.1	-10	123
$^{13}\text{C}_{12}$ -PCB-19	7	168	48.7	2.1	82.4	-13	111
$^{13}\text{C}_{12}$ -PCB-28	7	168	57.0	9.0	92.5	-8	122
$^{13}\text{C}_{12}$ -PCB-37	7	168	61.6	8.9	104.4	-11	134
$^{13}\text{C}_{12}$ -PCB-52	7	168	40.9	1.5	78.8	-18	99
$^{13}\text{C}_{12}$ -PCB-54	7	168	46.0	6.9	85.5	-16	108
$^{13}\text{C}_{12}$ -PCB-70	7	168	50.2	3.4	91.0	-20	120

Table 43. Observed Aqueous Labeled Compound Recoveries and Calculated Acceptance Criteria

Congener	# Labs	# Results	Observed Recoveries			Calculated Acceptance Criteria	
			Mean	Min.	Max.	Lower Limit	Upper Limit
¹³ C ₁₂ -PCB-77	7	168	56.6	6.0	117.7	-15	128
¹³ C ₁₂ -PCB-85	7	168	53.5	5.5	95.0	-12	119
¹³ C ₁₂ -PCB-101	7	168	52.7	5.6	95.0	-13	118
¹³ C ₁₂ -PCB-104	7	168	45.8	5.6	86.5	-12	104
¹³ C ₁₂ -PCB-118	7	168	55.3	5.5	95.5	-14	125
¹³ C ₁₂ -PCB-126	7	168	56.2	5.3	94.5	-17	130
¹³ C ₁₂ -PCB-138	7	168	54.5	4.9	96.5	-21	130
¹³ C ₁₂ -PCB-153	7	168	48.7	4.7	93.8	-14	111
¹³ C ₁₂ -PCB-155	7	168	53.5	5.0	97.3	-20	127
¹³ C ₁₂ -PCB-169	7	168	52.3	4.5	106.5	-31	135
¹³ C ₁₂ -PCB-180	7	168	53.5	4.2	97.8	-31	138
¹³ C ₁₂ -PCB-188	7	168	50.4	4.3	95.3	-24	125
¹³ C ₁₂ -PCB-189	7	168	52.0	3.3	123.5	-46	150
¹³ C ₁₂ -PCB-202	7	168	50.4	3.6	93.0	-34	135
¹³ C ₁₂ -PCB-205	7	168	51.5	1.1	143.5	-64	167
¹³ C ₁₂ -PCB-206	7	168	50.3	1.1	140.3	-63	164
¹³ C ₁₂ -PCB-208	7	168	51.0	0.6	150.3	-69	171
¹³ C ₁₂ -PCB-209	7	168	49.2	0.3	166.8	-69	168

Overall, the observed labeled compound recoveries are typical of what one would expect from a method with multiple cleanup procedures. The minimum recoveries ranged from 0% to 9% across the 29 labeled compounds, while the maximum recoveries ranged from about 79% to 167%. The mean recoveries across all seven laboratories, and all eight wastewater matrix types, both unspiked and spiked, ranged from about 41% to 62%.

Since the development of EPA's first isotope dilution methods for wastewater and other matrices in the late 1970s, the Office of Water has used two approaches to establishing QC acceptance limits for labeled compound recovery. Early efforts derived limits from the results of an interlaboratory method validation study, using the same statistical procedures used to derive the acceptance limits for the target analytes in IPR and OPR analyses. The second approach has been to set the acceptance limits as simpler whole number ranges and then evaluate those limits using the results of an interlaboratory method validation study.

The calculated acceptance criteria in Table 43 represent the first of those two approaches. As can be seen in Table 43, the calculated lower recovery limits for all 29 labeled congeners are negative numbers. As discussed previously in this report, negative recovery values have no physical meaning for either labeled compounds, or for spiked target analytes. Rather, the calculated acceptance criteria are a function of the variability in the observed recovery data across all of the laboratories and samples in the study.

The calculated upper recovery limits for all 29 labeled congeners ranged from 115% to 171%, with the upper limits for 25 of the 29 labeled compounds between 115% and 150%. The four labeled compounds with the highest upper limits are the four labeled compounds with the highest levels of chlorination, e.g., the labeled analogs of PCBs 205 to 209.

Both the lower and upper limits are driven by the variability within each laboratory and across all laboratories and all samples. Thus, if one or more of the laboratories have highly variable recoveries, the width of the acceptance limits for the labeled compounds can be very wide, even if no actual recoveries approach those limits.

Clearly, the calculated negative recovery values in Table 43 are not useful as lower limits. Therefore, CSRA examined the observed recovery data and compared them to two potential consensus-based acceptance limits to determine the frequencies at which the results from the study would fail to meet those potential acceptance limits. The draft method used acceptance limits of 15 - 130% for the labeled compounds, so those limits were used as a potential set of limits for the final method. However, EPA also evaluated the study results using two additional lower limits of 10% and 25%, and one additional upper limit of 150%. Table 44 contains the rates at which the results from the study failed to meet those potential lower and upper acceptance limits.

Table 44. Observed Aqueous Labeled Compound Recovery Failure Rates for Potential Acceptance Criteria

Congener	Total # Results	Observed Failure Rate (%)				
		If Lower Limit = 10%	If Lower Limit = 15%	If Lower Limit = 25%	If Upper Limit = 130%	If Upper Limit = 150%
¹³ C ₁₂ -PCB-1	168	6.5	10.7	19.0	1.2	0.0
¹³ C ₁₂ -PCB-3	168	5.4	8.3	14.9	0.0	0.0
¹³ C ₁₂ -PCB-4	168	3.0	7.1	14.3	0.0	0.0
¹³ C ₁₂ -PCB-11	168	0.6	3.0	8.3	0.0	0.0
¹³ C ₁₂ -PCB-15	168	0.6	3.0	6.5	0.0	0.0
¹³ C ₁₂ -PCB-19	168	1.8	6.0	9.5	0.0	0.0
¹³ C ₁₂ -PCB-28	168	1.2	3.0	6.5	0.0	0.0
¹³ C ₁₂ -PCB-37	168	0.6	2.4	5.4	0.0	0.0
¹³ C ₁₂ -PCB-52	168	4.8	6.5	13.7	0.0	0.0
¹³ C ₁₂ -PCB-54	168	1.8	3.6	8.9	0.0	0.0
¹³ C ₁₂ -PCB-70	168	4.2	6.0	8.9	0.0	0.0
¹³ C ₁₂ -PCB-77	168	1.8	3.6	7.7	0.0	0.0
¹³ C ₁₂ -PCB-85	168	1.8	3.6	7.7	0.0	0.0
¹³ C ₁₂ -PCB-101	168	1.8	3.6	7.7	0.0	0.0
¹³ C ₁₂ -PCB-104	168	2.4	3.6	9.5	0.0	0.0
¹³ C ₁₂ -PCB-118	168	1.8	3.6	7.7	0.0	0.0
¹³ C ₁₂ -PCB-126	168	1.8	3.6	7.1	0.0	0.0
¹³ C ₁₂ -PCB-138	168	2.4	3.6	8.3	0.0	0.0
¹³ C ₁₂ -PCB-153	168	3.0	3.6	8.9	0.0	0.0
¹³ C ₁₂ -PCB-155	168	2.4	3.6	8.9	0.0	0.0
¹³ C ₁₂ -PCB-169	168	1.8	6.0	12.5	0.0	0.0
¹³ C ₁₂ -PCB-180	168	2.4	6.0	9.5	0.0	0.0
¹³ C ₁₂ -PCB-188	168	3.6	4.2	11.3	0.0	0.0
¹³ C ₁₂ -PCB-189	168	4.2	8.9	15.5	0.0	0.0
¹³ C ₁₂ -PCB-202	168	3.6	7.1	13.1	0.0	0.0
¹³ C ₁₂ -PCB-205	168	8.3	11.9	16.1	3.0	0.0
¹³ C ₁₂ -PCB-206	168	8.3	11.9	17.9	3.0	0.0
¹³ C ₁₂ -PCB-208	168	11.9	13.7	16.7	1.2	0.0
¹³ C ₁₂ -PCB-209	168	11.9	13.1	18.5	1.8	0.6

The rate at which the study's aqueous results failed a 25% lower limit ranged from 5.4% to 19%, with the higher rates occurring for the lightest and heaviest of the labeled congeners. Using the 15% lower recovery limit, the failure rates ranged from 2.4% to 13.7%. The majority of those failures were concentrated in two of the laboratories (Labs 1 and 7 accounted for 232 of 293 failures at 15%, about 80% of the failures came from 2 of the 7 laboratories). Using a 10% lower recovery limit further reduced the failure rates to 0.6% to 11.9%, which were still concentrated in Labs 1 and 7.

The rate at which the study results failed a 130% upper recovery limit was trivial in comparison. Only 5 of the 29 labeled compounds exhibited any failures of the upper limit, and those failure rates ranged from

1.2% to 3.0%. The evaluation of the study result against the 150% upper recovery limit led to 0% failures.

EPA has previously shown that isotope dilution quantitation functions well even when the observed recovery of the labeled compound drops as low as 5%. Therefore, based on the observed recoveries in this study, EPA recommends retaining the 15 - 130% labeled compound recovery limits for aqueous samples in the final method. Given that there are 29 labeled compounds that are being tested simultaneously, a laboratory will be allowed to have up to three labeled compounds in a sample that do not meet the acceptance criterion, provided that those compounds have at least 5% recovery. EPA will also add language in the final version of the method to advise laboratories how to develop and utilize in-house limits for the recoveries of labeled compounds in all matrices (see Appendix B of this report).

Sediment Sample Labeled Compound Results

The labeled compound recoveries from the six laboratories that completed the sediment sample portion of the study are summarized in Table 45 below. These data represent the analyses of the unspiked, MS, and MSD aliquots of all three sediment samples, for a total of 54 observations for each labeled compound.

Table 45. Observed Sediment Labeled Compound Recoveries and Calculated Acceptance Criteria

Congener	# Labs	# Results	Observed Recoveries			Calculated Acceptance Criteria	
			Mean	Min.	Max.	Lower Limit	Upper Limit
¹³ C ₁₂ -PCB-1	6	54	48.4	1.7	122.4	-99	196
¹³ C ₁₂ -PCB-3	6	54	55.0	2.8	128.7	-112	222
¹³ C ₁₂ -PCB-4	6	54	50.6	2.5	103.4	-84	186
¹³ C ₁₂ -PCB-11	6	54	69.3	5.3	190.2	-121	260
¹³ C ₁₂ -PCB-15	6	54	65.1	5.9	144.3	-94	224
¹³ C ₁₂ -PCB-19	6	54	56.0	3.9	118.2	-101	213
¹³ C ₁₂ -PCB-28	6	54	65.0	6.8	149.9	-97	227
¹³ C ₁₂ -PCB-37	6	54	74.7	10.8	185.9	-97	247
¹³ C ₁₂ -PCB-52	6	54	56.6	4.4	128.8	-156	270
¹³ C ₁₂ -PCB-54	6	54	58.4	6.5	140.2	-92	208
¹³ C ₁₂ -PCB-70	6	54	59.7	8.2	146.4	-82	201
¹³ C ₁₂ -PCB-77	6	54	63.8	10.3	142.2	-75	202
¹³ C ₁₂ -PCB-85	6	54	66.9	8.8	161.0	-114	248
¹³ C ₁₂ -PCB-101	6	54	63.8	8.2	156.0	-105	233
¹³ C ₁₂ -PCB-104	6	54	60.7	5.6	146.3	-127	248
¹³ C ₁₂ -PCB-118	6	54	65.9	9.5	160.9	-102	234
¹³ C ₁₂ -PCB-126	6	54	66.6	10.6	144.6	-108	241
¹³ C ₁₂ -PCB-138	6	54	66.2	9.7	157.3	-101	233
¹³ C ₁₂ -PCB-153	6	54	59.4	7.2	154.6	-96	215
¹³ C ₁₂ -PCB-155	6	54	64.1	9.4	159.7	-96	224
¹³ C ₁₂ -PCB-169	6	54	61.8	0.0	140.0	-92	216
¹³ C ₁₂ -PCB-180	6	54	62.0	10.4	158.8	-89	213
¹³ C ₁₂ -PCB-188	6	54	61.1	8.2	154.6	-95	217
¹³ C ₁₂ -PCB-189	6	54	55.8	9.1	131.6	-104	215
¹³ C ₁₂ -PCB-202	6	54	60.1	8.7	152.2	-92	213
¹³ C ₁₂ -PCB-205	6	54	53.4	9.5	125.5	-92	199
¹³ C ₁₂ -PCB-206	6	54	55.4	9.1	172.3	-97	208
¹³ C ₁₂ -PCB-208	6	54	50.7	10.3	118.1	-97	199
¹³ C ₁₂ -PCB-209	6	54	55.0	10.2	178.0	-120	230

Overall, the observed labeled compound recoveries are typical of what one would expect from a method with multiple cleanup procedures. The minimum recoveries ranged from 1.7% to 10.8% across the 29

labeled compounds, while the maximum recoveries ranged from about 103% to 190%. The mean recoveries across all 6 laboratories, 3 samples, both unspiked and spiked, ranged from about 48% to 75%.

The calculated acceptance criteria in Table 45 represent the same approach described for the aqueous sample results. As can be seen in Table 45, the calculated lower recovery limits for all 29 labeled congeners are negative numbers, and are more negative than for the calculated aqueous limits.

The calculated upper recovery limits for all 29 labeled congeners ranged from 186% to 270%, much greater than the upper limits for the aqueous samples.

However, EPA also evaluated the study results using two additional lower limits of 10% and 25%, and one additional upper limit of 150%. Table 46 contains the rates at which the results from the study failed to meet those potential lower and upper acceptance limits.

Table 46. Observed Sediment Labeled Compound Recovery Failure Rates for Potential Acceptance Criteria

Congener	Total # Results	Observed Failure Rate (%)				
		If Lower Limit = 10%	If Lower Limit = 15%	If Lower Limit = 25%	If Upper Limit = 130%	If Upper Limit = 150%
¹³ C ₁₂ -PCB-1	54	16.7	22.2	29.6	0.0	0.0
¹³ C ₁₂ -PCB-3	54	11.1	14.8	27.8	0.0	0.0
¹³ C ₁₂ -PCB-4	54	9.3	14.8	25.9	0.0	0.0
¹³ C ₁₂ -PCB-11	54	5.6	13.0	22.2	7.4	7.4
¹³ C ₁₂ -PCB-15	54	5.6	13.0	22.2	5.6	0.0
¹³ C ₁₂ -PCB-19	54	7.4	16.7	25.9	0.0	0.0
¹³ C ₁₂ -PCB-28	54	5.6	13.0	22.2	5.6	0.0
¹³ C ₁₂ -PCB-37	54	0.0	11.1	18.5	5.6	5.6
¹³ C ₁₂ -PCB-52	54	5.6	16.7	25.9	0.0	0.0
¹³ C ₁₂ -PCB-54	54	5.6	13.0	22.2	1.9	0.0
¹³ C ₁₂ -PCB-70	54	1.9	9.3	22.2	5.6	0.0
¹³ C ₁₂ -PCB-77	54	0.0	7.4	16.7	3.7	0.0
¹³ C ₁₂ -PCB-85	54	1.9	11.1	24.1	5.6	3.7
¹³ C ₁₂ -PCB-101	54	1.9	13.0	24.1	5.6	1.9
¹³ C ₁₂ -PCB-104	54	5.6	16.7	25.9	5.6	0.0
¹³ C ₁₂ -PCB-118	54	1.9	9.3	22.2	5.6	3.7
¹³ C ₁₂ -PCB-126	54	0.0	5.6	18.5	3.7	0.0
¹³ C ₁₂ -PCB-138	54	0.0	7.4	20.4	5.6	1.9
¹³ C ₁₂ -PCB-153	54	3.7	14.8	24.1	5.6	1.9
¹³ C ₁₂ -PCB-155	54	1.9	9.3	24.1	5.6	3.7
¹³ C ₁₂ -PCB-169	54	7.4	9.3	22.2	1.9	0.0
¹³ C ₁₂ -PCB-180	54	0.0	7.4	20.4	5.6	3.7
¹³ C ₁₂ -PCB-188	54	1.9	11.1	24.1	5.6	1.9
¹³ C ₁₂ -PCB-189	54	1.9	11.1	24.1	1.9	0.0
¹³ C ₁₂ -PCB-202	54	1.9	11.1	24.1	5.6	1.9
¹³ C ₁₂ -PCB-205	54	0.0	11.1	22.2	0.0	0.0
¹³ C ₁₂ -PCB-206	54	1.9	13.0	29.6	3.7	3.7
¹³ C ₁₂ -PCB-208	54	0.0	7.4	25.9	0.0	0.0
¹³ C ₁₂ -PCB-209	54	0.0	14.8	31.5	1.9	1.9

The rate at which the study's sediment results failed a 25% lower recovery limit ranged from 16.7% to 31.5% with rates above 20% spread across most of the labeled congeners. The results failed a 15% lower recovery limit ranged from 5.6% to 22.2%, with the higher rates occurring for the lightest and heaviest of the labeled congeners. The failure rates for the sediments are almost double that for the aqueous samples. However, the vast majority of those failures were concentrated in two of the laboratories (Labs 4 and 6).

accounted for 196 of 198 failures, and both those laboratories had many fewer failures for the aqueous samples in the study). The rate at which the study results failed a 10% lower recovery limit ranged from 0.0% to 16.7%, with 27 of the 29 labeled congeners having failure rates below 10% at that 10% recovery limit.

The rate at which the study results failed a 130% upper recovery limit ranged from 0% to 7.4%. Of the 29 labeled compounds, 15 compounds exhibited more than a 5% failure rate at the upper recovery limit, although most of those 15 congeners had a rate of 5.6%.

Given the very high numbers of failures observed for Labs 4 and 6 but moderately better performance of the other four laboratories that completed the sediment portion of the study, EPA recommends retaining the 15 - 130% labeled compound recovery limits for sediment samples in the final method. Given that there are 29 labeled compounds that are being tested simultaneously, a laboratory will be allowed to have up to three labeled compounds in a sample that do not meet the acceptance criterion, provided that those compounds have at least 5% recovery. As noted for the aqueous sample labeled compound recoveries, EPA will also add language in the final version of the method to advise laboratories how to develop and utilize in-house limits for the recoveries of labeled compounds in all matrices (see Appendix B of this report).

Biosolids Sample Labeled Compound Results

The labeled compound recoveries from the four laboratories that completed the biosolids sample portion of the study are summarized in Table 47 below. These data represent the analyses of the unspiked, MS, and MSD aliquots of all three biosolids samples, for a total of 36 observations for each labeled compound.

Table 47. Observed Biosolids Labeled Compound Recoveries and Calculated Acceptance Criteria

Congener	# Labs	# Results	Observed Recoveries			Calculated Acceptance Criteria	
			Mean	Min.	Max.	Lower Limit	Upper Limit
¹³ C ₁₂ -PCB-1	4	36	39.4	10.0	67.0	-17	96
¹³ C ₁₂ -PCB-3	4	36	46.8	11.0	75.0	-21	114
¹³ C ₁₂ -PCB-4	4	36	58.9	14.0	117.4	-35	153
¹³ C ₁₂ -PCB-11	4	36	70.3	21.5	104.5	-6	146
¹³ C ₁₂ -PCB-15	4	36	76.4	22.5	126.5	-27	180
¹³ C ₁₂ -PCB-19	4	36	57.2	18.0	90.0	-15	129
¹³ C ₁₂ -PCB-28	4	36	69.9	17.9	113.5	-53	193
¹³ C ₁₂ -PCB-37	4	36	88.8	22.4	201.5	-141	318
¹³ C ₁₂ -PCB-52	4	36	52.2	17.0	85.0	-21	125
¹³ C ₁₂ -PCB-54	4	36	61.2	22.7	99.5	-20	142
¹³ C ₁₂ -PCB-70	4	36	69.6	25.8	113.6	-10	149
¹³ C ₁₂ -PCB-77	4	36	69.7	0.0	117.9	-100	239
¹³ C ₁₂ -PCB-85	4	36	73.6	21.3	118.0	-22	170
¹³ C ₁₂ -PCB-101	4	36	67.3	18.8	112.0	-24	159
¹³ C ₁₂ -PCB-104	4	36	63.4	22.5	108.0	-22	148
¹³ C ₁₂ -PCB-118	4	36	72.7	18.3	119.0	-51	197
¹³ C ₁₂ -PCB-126	4	36	73.4	16.8	122.5	-126	272
¹³ C ₁₂ -PCB-138	4	36	75.8	22.5	119.0	-62	214
¹³ C ₁₂ -PCB-153	4	36	67.2	22.2	113.5	-19	153
¹³ C ₁₂ -PCB-155	4	36	71.9	19.4	118.5	-57	201
¹³ C ₁₂ -PCB-169	4	36	62.7	0.0	122.5	-129	254
¹³ C ₁₂ -PCB-180	4	36	75.2	19.9	117.0	-56	207
¹³ C ₁₂ -PCB-188	4	36	68.7	17.9	113.0	-42	180
¹³ C ₁₂ -PCB-189	4	36	71.6	16.2	113.7	-59	202
¹³ C ₁₂ -PCB-202	4	36	70.0	17.1	105.5	-53	193

Table 47. Observed Biosolids Labeled Compound Recoveries and Calculated Acceptance Criteria

Congener	# Labs	# Results	Observed Recoveries			Calculated Acceptance Criteria	
			Mean	Min.	Max.	Lower Limit	Upper Limit
¹³ C ₁₂ -PCB-205	4	36	66.6	18.2	105.5	-46	180
¹³ C ₁₂ -PCB-206	4	36	63.2	17.4	101.6	-30	156
¹³ C ₁₂ -PCB-208	4	36	65.3	14.3	103.5	-40	171
¹³ C ₁₂ -PCB-209	4	36	62.6	14.9	104.9	-43	168

Overall, the observed labeled compound recoveries are typical of what one would expect from a method with multiple cleanup procedures. The minimum recoveries ranged from 0% to 25.8% across the 29 labeled compounds, while the maximum recoveries ranged from about 67% to 202%. The mean recoveries across all 4 laboratories, 3 samples, both unspiked and spiked, ranged from about 39% to 89%.

The calculated acceptance criteria in Table 47 represent the same approach described for the aqueous sample results. As can be seen in Table 47, the calculated lower recovery limits for all 29 labeled congeners are negative numbers. Somewhat surprisingly, given the known analytical challenges of biosolids, the calculated lower recovery limits are not as extreme as those for the sediment samples (e.g., in Table 45).

The calculated upper recovery limits for all 29 labeled congeners ranged from 96% to 318%, much greater than the upper limits for the aqueous samples, but often lower than the corresponding upper limits for sediments.

However, EPA also evaluated the study results using two additional lower limits of 10% and 25%, and one additional upper limit of 150%. Table 48 contains the rates at which the results from the study failed to meet those potential lower and upper acceptance limits.

Table 48. Observed Biosolids Labeled Compound Recovery Failure Rates for Potential Acceptance Criteria

Congener	Total # Results	Observed Failure Rate (%)				
		If Lower Limit = 10%	If Lower Limit = 15%	If Lower Limit = 25%	If Upper Limit = 130%	If Upper Limit = 150%
¹³ C ₁₂ -PCB-1	36	0.0	2.8	16.7	0.0	0.0
¹³ C ₁₂ -PCB-3	36	0.0	2.8	13.9	0.0	0.0
¹³ C ₁₂ -PCB-4	36	0.0	2.8	2.8	0.0	0.0
¹³ C ₁₂ -PCB-11	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-15	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-19	36	0.0	0.0	5.6	0.0	0.0
¹³ C ₁₂ -PCB-28	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-37	36	0.0	0.0	2.8	11.1	8.3
¹³ C ₁₂ -PCB-52	36	0.0	0.0	8.3	0.0	0.0
¹³ C ₁₂ -PCB-54	36	0.0	0.0	5.6	0.0	0.0
¹³ C ₁₂ -PCB-70	36	0.0	0.0	0.0	0.0	0.0
¹³ C ₁₂ -PCB-77	36	16.7	16.7	16.7	0.0	0.0
¹³ C ₁₂ -PCB-85	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-101	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-104	36	0.0	0.0	5.6	0.0	0.0
¹³ C ₁₂ -PCB-118	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-126	36	0.0	0.0	8.3	0.0	0.0
¹³ C ₁₂ -PCB-138	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-153	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-155	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-169	36	16.7	16.7	25.0	0.0	0.0
¹³ C ₁₂ -PCB-180	36	0.0	0.0	2.8	0.0	0.0

Table 48. Observed Biosolids Labeled Compound Recovery Failure Rates for Potential Acceptance Criteria

Congener	Total # Results	Observed Failure Rate (%)				
		If Lower Limit = 10%	If Lower Limit = 15%	If Lower Limit = 25%	If Upper Limit = 130%	If Upper Limit = 150%
¹³ C ₁₂ -PCB-188	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-189	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-202	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-205	36	0.0	0.0	8.3	0.0	0.0
¹³ C ₁₂ -PCB-206	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-208	36	0.0	2.8	11.1	0.0	0.0
¹³ C ₁₂ -PCB-209	36	0.0	0.0	8.3	0.0	0.0

The rate at which the study's biosolids results failed a 25% lower recovery limit ranged from 0.0% to 25.0% with 17 of the 29 labeled compounds exhibiting failure rates of less than 5%. For a 15% lower recovery limit, the failure rates ranged from 0% to 16.7% (for labeled congeners 77 and 169). At the 10% lower recovery limit, only two labeled congeners failed at all, 77 and 169, both at 16.7%. The failure rates for the biosolids are surprisingly low and the majority of those failures were concentrated in one of the laboratories (Lab 4 accounted for 13 of 16 failures).

Only the labeled analog of PCB-37 failed the 130% upper recovery limit, at a rate of 11.1% (4 of 36 samples) and no labeled congeners failed the 150% upper recovery limit.

Given the data from this portion of the study, EPA recommends retaining the 15 - 130% labeled compound recovery limits for biosolids in the final method. Given that there are 29 labeled compounds that are being tested simultaneously, a laboratory will be allowed to have up to three labeled compounds in a sample that do not meet the acceptance criterion, provided that those compounds have at least 5% recovery. As noted for the aqueous and sediment sample labeled compound recoveries, EPA will also add language in the final version of the method to advise laboratories how to develop and utilize in-house limits for the recoveries of labeled compounds in all matrices (see Appendix B of this report).

Tissue Sample Labeled Compound Results

The labeled compound recoveries from the four laboratories that completed the tissue sample portion of the study are summarized in Table 49 below. These data represent the analyses of the unspiked, MS, and MSD aliquots of all three fish tissue samples, for a total of 36 observations for each labeled compound.

Table 49. Observed Tissue Labeled Compound Recoveries and Calculated Acceptance Criteria

Congener	# Labs	# Results	Observed Recoveries			Calculated Acceptance Criteria	
			Mean	Min.	Max.	Lower Limit	Upper Limit
¹³ C ₁₂ -PCB-1	4	36	50.5	13.5	103.7	-93	194
¹³ C ₁₂ -PCB-3	4	36	54.7	17.5	110.0	-89	198
¹³ C ₁₂ -PCB-4	4	36	54.9	17.8	113.0	-96	206
¹³ C ₁₂ -PCB-11	4	36	63.2	23.3	118.8	-89	215
¹³ C ₁₂ -PCB-15	4	36	63.5	20.6	120.6	-97	224
¹³ C ₁₂ -PCB-19	4	36	58.3	21.0	113.7	-86	202
¹³ C ₁₂ -PCB-28	4	36	66.8	24.0	116.8	-77	211
¹³ C ₁₂ -PCB-37	4	36	71.7	23.3	127.2	-55	199
¹³ C ₁₂ -PCB-52	4	36	49.6	19.0	81.0	-69	169
¹³ C ₁₂ -PCB-54	4	36	53.7	20.8	73.7	-48	155
¹³ C ₁₂ -PCB-70	4	36	59.8	24.1	103.3	-26	145
¹³ C ₁₂ -PCB-77	4	36	59.8	21.7	79.4	1	118
¹³ C ₁₂ -PCB-85	4	36	59.3	20.7	79.1	-16	134
¹³ C ₁₂ -PCB-101	4	36	57.6	20.3	77.4	-27	142

Table 49. Observed Tissue Labeled Compound Recoveries and Calculated Acceptance Criteria

Congener	# Labs	# Results	Observed Recoveries			Calculated Acceptance Criteria	
			Mean	Min.	Max.	Lower Limit	Upper Limit
¹³ C ₁₂ -PCB-104	4	36	52.6	18.7	74.7	-62	168
¹³ C ₁₂ -PCB-118	4	36	61.2	21.2	81.3	-6	128
¹³ C ₁₂ -PCB-126	4	36	59.7	15.9	84.1	-18	138
¹³ C ₁₂ -PCB-138	4	36	63.3	22.5	83.4	3	124
¹³ C ₁₂ -PCB-153	4	36	56.2	19.8	75.8	-40	152
¹³ C ₁₂ -PCB-155	4	36	61.7	22.4	79.5	-6	129
¹³ C ₁₂ -PCB-169	4	36	56.7	10.3	95.7	-113	226
¹³ C ₁₂ -PCB-180	4	36	65.1	24.4	92.6	-8	138
¹³ C ₁₂ -PCB-188	4	36	60.2	21.6	79.2	-15	136
¹³ C ₁₂ -PCB-189	4	36	66.7	23.5	92.0	-7	140
¹³ C ₁₂ -PCB-202	4	36	61.8	23.1	81.0	-13	137
¹³ C ₁₂ -PCB-205	4	36	65.6	24.2	88.3	-27	158
¹³ C ₁₂ -PCB-206	4	36	63.1	23.9	89.5	-68	195
¹³ C ₁₂ -PCB-208	4	36	64.4	21.6	89.3	-31	160
¹³ C ₁₂ -PCB-209	4	36	60.9	20.5	86.2	-93	215

Overall, the observed labeled compound recoveries in fish tissue were quite good, especially for a method with multiple cleanup procedures. The minimum recoveries ranged from 10.3% to 24.4% across the 29 labeled compounds, while the maximum recoveries ranged from about 73% to 127%. The mean recoveries across all 4 laboratories, 3 samples, both unspiked and spiked, ranged from about 50% to 72%.

The calculated acceptance criteria in Table 49 represent the same approach described for the aqueous sample results. As can be seen in Table 49, all but one of the calculated lower recovery limits are negative numbers, and the one positive value is at 1%. The calculated upper recovery limits for all 29 labeled congeners ranged from 118% to 226%.

However, EPA also evaluated the study results using two additional lower limits of 15% and 25%, and one additional upper limit of 150%. Table 50 contains the rates at which the results from the study failed to meet those potential lower and upper acceptance limits.

Table 50. Observed Tissue Labeled Compound Recovery Failure Rates for Potential Acceptance Criteria

Congener	Total # Results	Observed Failure Rate (%)				
		If Lower Limit = 10%	If Lower Limit = 15%	If Lower Limit = 25%	If Upper Limit = 130%	If Upper Limit = 150%
¹³ C ₁₂ -PCB-1	36	0.0	2.8	30.6	0.0	0.0
¹³ C ₁₂ -PCB-3	36	0.0	0.0	16.7	0.0	0.0
¹³ C ₁₂ -PCB-4	36	0.0	0.0	16.7	0.0	0.0
¹³ C ₁₂ -PCB-11	36	0.0	0.0	5.6	0.0	0.0
¹³ C ₁₂ -PCB-15	36	0.0	0.0	5.6	0.0	0.0
¹³ C ₁₂ -PCB-19	36	0.0	0.0	11.1	0.0	0.0
¹³ C ₁₂ -PCB-28	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-37	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-52	36	0.0	0.0	11.1	0.0	0.0
¹³ C ₁₂ -PCB-54	36	0.0	0.0	5.6	0.0	0.0
¹³ C ₁₂ -PCB-70	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-77	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-85	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-101	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-104	36	0.0	0.0	5.6	0.0	0.0
¹³ C ₁₂ -PCB-118	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-126	36	0.0	0.0	2.8	0.0	0.0

Table 50. Observed Tissue Labeled Compound Recovery Failure Rates for Potential Acceptance Criteria

Congener	Total # Results	Observed Failure Rate (%)				
		If Lower Limit = 10%	If Lower Limit = 15%	If Lower Limit = 25%	If Upper Limit = 130%	If Upper Limit = 150%
¹³ C ₁₂ -PCB-138	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-153	36	0.0	0.0	5.6	0.0	0.0
¹³ C ₁₂ -PCB-155	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-169	36	0.0	2.8	5.6	0.0	0.0
¹³ C ₁₂ -PCB-180	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-188	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-189	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-202	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-205	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-206	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-208	36	0.0	0.0	2.8	0.0	0.0
¹³ C ₁₂ -PCB-209	36	0.0	0.0	5.6	0.0	0.0

The rate at which the study's tissue results failed a 25% lower recovery limit ranged from 2.8% to 30.6%, with 24 of the 29 labeled congeners having failure rates below 10%. The 5 labeled compounds that failed the 25% lower recovery limit were concentrated in two of the laboratories (Labs 3 and 6 accounted for 62 of the 65 failures).

For the 15% lower recovery limit, there were only two congeners that failed at all, at 2.8% for the labeled analogs of PCB-1 and PCB-169 (i.e., 1 out of 36 samples). Those two failures occurred in two different laboratories. No labeled congeners failed the 10% lower recovery limit. No labeled congeners failed the 130% upper recovery limit or the 150% upper recovery limit.

EPA recommends retaining the 15 - 130% labeled compound recovery limits for tissue in the final method. Given that there are 29 labeled compounds that are being tested simultaneously, a laboratory will be allowed to have up to three labeled compounds in a sample that do not meet the acceptance criterion, provided that those compounds have at least 5% recovery. As noted for the aqueous, sediment, and biosolid sample labeled compound recoveries, EPA will also add language in the final version of the method to advise laboratories how to develop and utilize in-house limits for the recoveries of label compounds in all matrices (see Appendix B of this report).

10. Data Review and Validation

The results for all of the analyses in this study were submitted as electronic data deliverables (EDDs) in Excel format, and supported by raw data and reporting forms provided in PDF format equivalent to a hardcopy data package. Both the electronic data deliverables and the supporting raw data were reviewed for completeness and for data quality. Data were evaluated based on the preliminary method performance criteria described in the draft procedure, but because one purpose of the study is to develop formal acceptance criteria, all of the results were retained for further consideration. The formal method performance criteria will be established as a result of this multi-laboratory validation study. The data review process was patterned after that used for PCB results from various other Office of Water studies.

Completeness check – The supporting data provided in the “hardcopy package” were compared to the results in the EDD. The data report narratives in the hardcopy package were reviewed and any quality control or performance related issues were noted. The data was verified to be consistent with the narrative and appropriate validation qualifiers were applied. Electronic data deliverable (EDD) elements and results were checked for completeness and consistency with the hardcopy data. Elements checked included sample number and laboratory sample number identifiers, analysis date and time, Chemical Abstract Service Registry Number (CASRN), congener number identifier, coeluting congeners, laboratory qualifiers, found concentrations, detection limits, ion abundance ratios, relative retention times, sample sizes (volume or weight), dilution factors, spiked amounts, percent recoveries, extraction date, sample receipt date, and concentration units.

Hardcopy and EDD data were checked to ensure that no data were missing or inconsistent for all samples and blanks. Hardcopy data were checked to ensure that all chromatograms and quantitation reports were available for all analyses and that all samples were reported on the sample pretreatment, sample preparation, extraction worksheet records.

Instrument Sensitivity - Results between the MDL and the laboratory’s quantitation or nominal reporting limit were checked to confirm they were flagged by the laboratory. Any flagged results were checked to confirm the concentration warranted the qualifier (e.g., was between the MDL and the quantitation limit). All reported non-detects were checked to confirm no signal was present, or that they were detects below the MDL value.

Sample Dilutions – Results were checked to ensure they were within the range of the calibration curve. If results were above the curve, then a diluted reanalysis should have been performed and results were to be reported from the dilution.

Ion abundance ratios – Ion abundance ratios (IARs) were checked for all calibration standards, samples and blanks to determine if they were within the preliminary method control limits. Deviations were flagged, but the data retained.

Blank Contamination – Found concentrations in the method blank were compared to the associated samples. If a congener concentration in a sample was greater than 10 times the same congener concentration in the method blank, then the sample result was considered to be unaffected and the following validation flag was applied, “B, RNAF” to indicate that the blank contamination was at a low enough level to not significantly affect sample results.

IPR and OPR Recovery – The percent recoveries for native congeners in the OPR were checked to determine if they were within preliminary control limits (i.e., 60 – 130% for mono- through tri-chlorinated congeners in tissues and all other matrices, respectively; 70 – 130% for tetra- through deca-chlorinated congeners in all matrices). Results outside of those preliminary criteria were noted, but retained because one purpose of the study is to develop IPR and OPR acceptance criteria that are based on

actual study data. Percent recovery calculations for a few congeners were independently performed to ensure they were within 1% of the reported values.

Labeled Compound Recoveries – Labeled compound recoveries were checked to determine if they were within the preliminary control limits in all samples and blanks (i.e., 15 – 130% for ¹³C-PCB-1 through ¹³C-PCB-19 and 40 – 130% for the remaining labeled congeners in all matrices). Results outside of those preliminary criteria were noted, but retained because one purpose of the study is to develop labeled compound acceptance criteria that are based on actual study data.

Quantification Check - Concentration result calculations for several native and labeled congeners in each sample were independently performed by CSRA using equations provided in the draft method to ensure they were within 1% percent of the reported values.

Resolution of questions and issues - Any issues of missing data, calculation errors, or questions about specific results were communicated to the laboratory by email or telephone. The final resolution of the issues may have involved resubmission of the EDD or the raw data, or missing portions thereof. Correction of simpler issues, such as transposition errors in the EDD could be corrected by CSRA after receiving concurrence from the laboratory by email, in which case a copy of the email was retained in the project files.

11. Conclusions

The Office of Water's Engineering and Analysis Division completed a multi-laboratory validation study of a method for PCB congeners in wastewater, biosolids, sediments, and fish tissue. The multi-laboratory validation study achieved all its intended goals, as outlined below.

Study Goals

1. Obtain data from matrices that are representative of the method's intended use

As described in Section 3, the wastewater matrices were a diverse selection of wastewaters from multiple parts of the country with different physical parameters, as demonstrated in Table 3. The matrices chosen are typical of what might be analyzed by a laboratory performing NPDES compliance monitoring and included some pretreatment samples that would be more challenging than a typical NPDES compliance final effluent. The three biosolid, sediment, and fish tissue samples were all reasonably typical samples that might be analyzed for data gathering and monitoring in support of the Clean Water Act.

2. Obtain data from laboratories that are representative of those likely to use the approved method, but that were not directly involved in its development

The laboratories that participated in this method validation study were mostly commercial laboratories that routinely perform NPDES compliance monitoring analyses. One state and several EPA laboratories also participated. Commercial laboratories are those most likely to use this method for NPDES compliance monitoring, so the laboratories in this study are representative of the laboratories most likely to use the method.

3. Obtain feedback from laboratory users on the specifics of the draft method (e.g., is it clear and easy to understand, or are changes to the method text needed?)

Participant laboratories were all encouraged to provide feedback on the method, and most did. EPA has revised the method in response to such feedback.

4. Use study data to characterize performance of the method

All of the data collected during this study were reviewed and evaluated to characterize the performance of this method, as summarized in detail in Sections 4-9. This includes data on calibration, initial precision and recovery, method detection limits, performance in real-world matrices, and labeled compound recoveries.

5. Develop statistically derived QC acceptance criteria that will reflect method performance capabilities in real-world situations

Sections 4, 5, 6, and 9 contain statistically derived QC limits that were calculated from the data collected during this study. The laboratories that participated are representative of the real-world laboratories that would potentially run this method, and the matrices are typical of matrices that a laboratory using this method would analyze.

Method Performance

Method performance is summarized below for each matrix type tested.

Wastewater

Since Method 608.3 is the most commonly used EPA method approved at 40 CFR Part 136 for PCBs in wastewater, it was used as the basis of comparison for PCB congener Method 1628. The criteria used for developing Method 1628, which could replace Method 608.3, included: ability to identify and quantify individual PCB congeners instead of Aroclor mixtures, a higher sensitivity without being adversely affected by typical laboratory background contamination, and implementation at a typical mid-sized full-service environmental laboratory.

Before comparing Method 608.3 and Method 1628, it is important to state that Method 608 (subsequently revised as Method 608.3) was the best available technology in the late 1970s when it was validated. The dedicated laboratories, analysts, EPA employees, and contractors that developed Method 608 were using the best tools available to them at the time. Their efforts pioneered one of the first validated EPA analytical methods that is the foundation upon which Method 1628 and every other EPA method is built. Analytical technology, laboratory information management systems, and EPA method quality control monitoring have all improved significantly over the last 40 years, and these improvements make Method 1628 far superior to Method 608. Table 51 provides a side-by-side comparison of the two methods, which illuminates this point.

Table 51. Comparison of Method 608.3 and Method 1628 for Aqueous Samples

Method 608.3	Method 1628
Calibration	
3 to 5 calibration points for 2 Aroclors, one calibration point for the other 5	6-point calibration with 48 congeners that are representative of the 209 congeners.
Quantitation: Surrogates vs. Internal Standards	
One surrogate is required, no specific surrogates are specified, no criteria attached to the performance of the surrogate(s), nor has any testing been performed by EPA to validate any surrogates.	29 ^{13}C -labeled isotope dilution standards, representing each homolog and including the labeled analogs of the most commonly detected congeners in the environment. 3 ^{13}C -labeled non-extracted internal standards are used to calculate the recovery of the 29 isotope dilution standards. The performance of these standards was tested in a variety of wastewaters at seven laboratories to generate statistically derived performance criteria.
Initial Precision and Recovery	
Between 28-197% among the 7 Aroclor mixtures	Mostly between 50-130% recovery for the 48 calibrated congeners, with some outliers
Method Detection Limits	
Aroclor 1242 – 65 ng/L, no other Aroclor mixtures were tested.	MDL _s values ranged from 0.19 ng/L to 4.98 ng/L among the 209 congeners. None of the MDL _b values were higher than the pooled MDL _s values, so blank contamination was not a significant issue.
Ongoing Precision and Recovery	
Aroclor-specific recovery criteria vary from as narrow as 50-114%, to as wide as 10-215%	Congener-specific criteria for the 48 calibrated congeners vary from as narrow as about 70-120%, to as wide as 14-193%.
Wastewater Matrix Performance	
Only MS/MSD reproducibility is stated in the method. No recovery data are presented.	Mostly between 60-120% recovery for the 60 spiked congeners, with a few outliers. False negatives in less than 0.2% of the 7,128 total data points.

Calibration and Quantitation

Method 1628 is superior to Method 608.3 in its calibration and quantitation approach. Method 608.3 uses a multi-point calibration for two of the seven Aroclors, while the other Aroclor mixtures are quantified using a 1-point calibration. Method 608.3 also mentions that at least one surrogate should be used to represent all the pesticides and Aroclors in the method, yet it does not provide any criteria for this surrogate. On the other hand, Method 1628 has a 6-point calibration containing 48 congeners, representing every homolog of the 209 congeners, plus 29 isotope dilution standards and 3 non-extracted internal standards to track the measurement quality in every sample.

Method Detection Limits

When it comes to comparing Aroclor MDLs to individual congener MDLs, the process is not straightforward. Based on the data from Frame *et al.* (1996), the main constituents of Aroclor 1242 are PCB-8 (7.05%), PCB-18 (8.53%), PCB-28 (6.86%), PCB-31 (7.34%), and PCB-33 (5.01%). The pooled aqueous MDL calculated in this study for PCB-18, the largest component of Aroclor 1242, was 0.46 ng/L. Assuming that all of the PCB-18 came from unweathered Aroclor 1242, detecting 0.46 ng/L in an aqueous sample would equate to a concentration of 5.39 ng/L of Aroclor 1242. Similar estimates derived for the other major components of Aroclor 1242 are shown in Table 52.

Table 52. Estimated Aroclor 1242 Concentrations Using 5 Most Prevalent Congeners in Aqueous Matrices

Congener	% Contribution to Aroclor-1242	Pooled MDL in this study (ng/L)	Estimated Concentration of Aroclor 1242 at the Pooled MDL (ng/L)
8	7.05%	1.00	14.18
18	8.53%	0.46	5.39
28	6.86%	0.69	10.06
31	7.34%	0.50	6.81
33	5.01%	1.11	22.16

The published sensitivity data for Method 608.3 is only for Aroclor 1242, with an MDL of 65 ng/L in aqueous samples. The highest estimate of 22.16 ng/L in Table 52, derived from the pooled MDL for PCB-33, is roughly three times below the published MDL for Aroclor 1242. In fact, even the highest reported MDL_s value for these five congeners from any of the laboratories in the study, 3.15 ng/L for the coeluting congeners PCB-33+20+21 (see Table 21), would yield an estimated Aroclor 1242 concentration of 62.87 ng/L, which is still below the published MDL in Method 608.3.

Admittedly, the published MDL data for Aroclor 1242 date to the original version of Method 608. However, the analyses for Aroclors performed as part of this study reported no Aroclors in the original nine wastewater matrices. The MDL_s values used by that laboratory ranged from 2.8 to 9.5 ng/L for the seven common Aroclor mixtures, and were similar to MDL values provided by other laboratories solicited for the effort. The MDL_s for Aroclor 1242 was 9.5 ng/L, which is comparable to the Aroclor 1242 concentrations that were estimated from the congener results shown in Table 52. As shown in Table 4, while two of the wastewater matrices contained measurable concentrations of 5 and 40 of the congeners in this method, no Aroclors were reported, even with more recent GC/ECD instrumentation. This indicates that method sensitivity did not limit the ability of the laboratory to determine Aroclor 1242 using Method 608.3, but rather, it was the result of the lack of a recognizable Aroclor pattern, and supports the position that analysis of congeners is superior to analysis of Aroclors because it provides a direct measurement of the PCB contamination.

Performance in Wastewater

For wastewater analyses, Method 1628 is a more advanced method than the currently approved Method 608.3 by virtually any manner of comparison. In the original interlaboratory validation report for Method

608 (published in June 1984), recoveries of <20% for Aroclors were very common among the six test matrices, causing a significant quantity of data for the Aroclors to be rejected (e.g., over 15% of the data for Aroclor 1254). Also, the matrices used in that validation study were significantly less challenging (reagent water, a drinking water, a surface water, and three final effluents).

The validation study for Method 1628 used several pretreatment matrices that have not undergone any treatment (refer to Table 2). Pretreatment matrices are generally more challenging than treated final effluents. Ninety-eight percent of the mean recoveries for the matrix spike samples fell between 60% and 120%, with a false negative rate below 0.2%, demonstrating the ruggedness of the method across a range of wastewaters.

Method 1628 uses a mass spectrometer, which is less prone to interferences than the electron capture detector used in Method 608.3. The use of isotope dilution standards in Method 1628 corrects the target analyte concentration in every sample for the recovery of the labeled standards in that sample, thus accounting for matrix effects, and improving the accuracy and precision of the results, which is especially important in challenging matrices.

Most importantly, Method 608.3 does not actually measure PCBs, but instead it measures seven Aroclor mixture patterns. This is an indirect measurement that is prone to false negatives and low bias.

Manufacturing of PCBs has been banned in the U.S. for over 40 years. While Aroclor contamination is an important legacy source of PCB contamination in the environment, much of the PCB contamination in the environment is now so weathered that it no longer matches the original Aroclor mixture when analyzed. Method 1628 addresses this issue by directly measuring the 209 PCB congeners. Measurement of individual congeners has an added advantage because PCB contamination rarely involves just one congener. A particularly difficult matrix may cause an interference that invalidates a low-level detect for one congener, but it is unlikely that the sample will cause the same of interference for all of the PCB congeners in the sample.

Sediments and Biosolids

The reference matrix used for solid samples was Ottawa sand. Since the reference matrix represented solid samples as a category, the same reference matrix performance (IPRs, OPRs, and MDLs) was applied for sediments and biosolids. The observed mean IPR recoveries for the 48 spiked congeners ranged from about 86 to 114%. The calculated IPR ranges, while wider than the ranges for the same congeners in the aqueous IPR samples, are generally reasonable for solid samples. Almost all the calculated OPR criteria were between 25-160% recovery. The pooled MDL_s values ranged from about 0.05 to 0.93 ng/g. None of the MDL_b values were higher than the pooled MDL_s values; therefore, blank contamination was not a significant issue.

For real-world samples, the sediments and biosolids analyses differed in the weight of sample used. For sediments, the laboratories used 10 g, while 5 g was used for biosolids. Solids are well known for being an overall challenging matrix, with many potential interfering organic components, as well as being a difficult matrix to homogenize. Since PCBs are sorbed onto particles, it may not be possible to evenly distribute more highly contaminated particles across the entire bulk sample, despite careful preparation. Therefore, some aliquots of a given sample may have had more PCBs than other aliquots for the unspiked and spiked sample analyses. Such differences affected the assumptions in the analyte recovery calculations. Considering all the difficulties presented by the matrix, the method performed well overall for the sediment and biosolid matrices that were analyzed.

Mean recoveries among the sediment matrices mostly fell between 30% and 200%, with one laboratory reporting up to 79% of the congeners for two samples with negative values for either the matrix spike (MS) or the matrix spike duplicate (MSD), suggesting interference or homogeneity issues with one but

not both of the spiked samples. Less than 2% false detects were observed, with most of them coming from two of the six laboratories.

Mean recoveries ranged between 70% to 185% for 54 of the 60 spiked congeners in real-world biosolid samples. The other six congeners ranged between 40% and 230%, which may have been caused by interferences that were not completely removed with the sample clean-ups utilized, or by a lack of sample homogeneity. The false negative percentage was 4.8% and was only observed in two biosolids samples. All the observed false negatives among the 928 results were from one laboratory that used GPC cleanup, which was an optional cleanup, and an additional sample dilution for those two samples. While GPC is a very robust clean-up procedure, it is recommended that the laboratories become familiar with the procedure before implementing it for use in biosolids. Overall, laboratory performance was typical for organic analytes in a challenging matrix like biosolid.

The Clean Water Act does not approve methods for either sediment or biosolids; therefore, EPA used fewer laboratories and matrices than for wastewater. The performance criteria that will be listed in the method will be noted as advisory.

Tissue

The reference matrix used for tissue was a 90:10 mixture of Ottawa sand and canola oil to mimic a lipid level of 10% in tissue samples. The IPR and OPR calculated limits for most of the congeners were unrealistically wide and did not resemble the range seen among the laboratories. A recovery limit of 25 - 150% for IPR and OPR was adopted for most of the congeners, giving a low failure rate among the data collected. The pooled MDL_s values ranged from about 0.035 to 0.23 ng/g. None of the MDL_b values were higher than the pooled MDL_s values, so blank contamination was not a significant issue.

Among the four laboratories that analyzed three real-world fish tissue sample types, mean matrix spike recoveries ranged from about 43% to 229%, with only 4 congeners with mean recoveries over 120%. The high recoveries were observed only for two samples in one laboratory, suggesting a sample-specific issue most likely due to lack of homogenization. The percentage of false negatives for tissue samples was less than 0.1% of the 1392 total data points.

The Clean Water Act does not approve methods for fish tissue; therefore, EPA used fewer laboratories and matrices than for wastewater. The performance criteria that will be listed in the method will be noted as advisory.

Summary

EPA has demonstrated that Method 1628 is effective in all the matrices tested, and is far superior to the currently approved EPA method for PCBs in wastewater, Method 608.3. This multi-laboratory validation study also demonstrated that this method can be implemented at a typical full-service environmental laboratory. Currently, EPA's only other PCB congener method uses high-resolution mass spectrometry instrumentation, which many full-service laboratories do not own. This method provides access to PCB congener analysis to any laboratory using a typical gas chromatograph/mass spectrometer (GC/MS) instrument that is used for many other EPA methods.

12. References

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Appendix A

Labeled PCB Congeners to be used as Quantitation Standards

Appendix A
Labeled PCB Congeners to be used as Quantitation Standards
March 2016 (Revised April 2021)

This document provides information about PCB congeners in order to help EPA select appropriate ¹³C-labeled PCB standards to be used for quantitation purposes in the low-resolution GC/MS PCB method. These recommendations are based on information from a number of sources. Compiled below is a table that lists all 209 congeners with information on:

- Congener number
- Level of chlorination (LOC)
- Retention times of the DB-5 GC column specified in the AXYS SOP
- 40 congeners that were most prevalent in database queries
- Risk Priority based on abundance in fish and human tissues and the availability of toxicological data
- Labels used by AXYS at present
- Labels available as individual standards from commercial vendors
- Comments on appropriate choices

The retention time data are from the AXYS SOP, which uses a DB-5 column (primarily made of diphenyl dimethyl polysiloxane), congeners are sorted by level of chlorination (LOC) and then retention time. Co-eluting congeners are listed individually, but are listed with the same retention time in that column. Please note that the first and last eluters for each LOC are also the first and last eluters for a DB-1 and SPB-octyl column (the two other most common columns used for PCBs). The ¹³C-labeled PCB standards have very similar retention times to their parent congeners, usually within a second or less. The first and last eluting congeners were selected as isotope dilution standards (e.g., used for quantitation). If ¹³C-labeled PCB standards are present for both the first and last eluting congener of each LOC, the analyst will know that the selected ion monitoring descriptors of the mass spectrometer are set appropriately.

The “Top 40” congener list is a summary of the congeners that are most present in the environment, from querying the following databases of PCB congener data 1) Wastewater data from the Delaware River Basin Commission (DRBC) (2005 - 2013), 2) EPA National Lake Fish Tissue Survey data (2000 - 2004), 3) EPA National Sewage Sludge Survey (NSSS) data (2001), 4) Upper Trenton Chanel sediment data from the Great Lakes National Program Office.

Priority risk congeners are from Geniece Lehmann (ORD), based upon congeners that are known to be present in human blood and fish tissue, and whether risk data are available for these congeners; lower numbers indicate higher priorities.

The challenge with any choices for labels is assigning each label to a native congener for quantitation purposes. In traditional EPA full-scan GC/MS methods, the assignments of internal standards and target analytes simply is based on retention times, with the internal standard always associated with target analytes that elute at the same retention time or later, not with analytes that elute significantly before the internal standard.

For these labels, associations are made by LOC, as well as retention time. Thus, using the label for the last eluting congener means “reaching back” chromatographically to make some of those associations. While that can be done, it adds some level of arbitrariness to the process. Once the selected standards are run, EPA will need to determine whether native congeners are assigned to individual isotope dilution standards with a relative response factor, or if the responses from multiple isotopes should be used and averaged for the congeners in a given LOC.

The comments section contains some information gained from researching supplier catalogs for the labeled congeners and some recommendations reflect the apparent availability of individual labels versus existing mixtures, and other relevant details.

The goal is to select the labeled analogs of the first and last eluter of each LOC, then select additional labeled standards that are commercially available, spread out over the retention times for each LOC, and represent congeners that are believed to be the most abundant in the environment. Too many labeled standards will be overly burdensome for the laboratory community, so the number of labeled standards will be limited to about 30. The labeled congeners believed to be most appropriate to act as isotope dilution standards are in ***bold italics text*** in the table below.

Congener	LOC	RT (DB-5)	Top 40?	Risk Priority	WHO TEF	Comments
<i>PCB 1</i>	1	13.63				The most abundant monochloro congener in Aroclor mixtures (~0.5% of 1016 and 1242). Usually has poor retention during analysis. Available as an individual C13 standard
PCB 2	1	14.91				
<i>PCB 3</i>	1	15.04				Last eluter. Available as C13 standard
<i>PCB 4</i>	2	15.86	Y	5		It is the first eluter, and was the most abundant congener (by mean % contribution) detected in WW discharged to the Delaware River. One concern may be that labeled PCB 4 will elute very close to both its native and PCB 10. Available as C13 standard.
PCB 10	2	15.86				
PCB 7	2	16.84				
PCB 9	2	16.84				
PCB 6	2	17.28				
PCB 5	2	17.52				
PCB 8	2	17.52				A good choice, since it's the most abundant dichloro congener in Aroclor mixtures, but PCB 11 is more abundant in the environmental databases reviewed, so it was chosen instead.
PCB 14	2	18.12				
<i>PCB 11</i>	2	18.98	Y			The most abundant congener in the NSSS, and 6 th most common congener detected in the Delaware River WW data. Not a component of Aroclor mixtures, found in some inks. Available as C13 standard.
PCB 12	2	19.25				
PCB 13	2	19.25				
<i>PCB 15</i>	2	19.50				Last eluter, also makes up about 2% of 1016 and 1242. Available as C13 standard
<i>PCB 19</i>	3	18.40	Y			First eluting tri-CB. Available as C13 standard.
PCB 30	3	18.77				
PCB 18	3	19.40				
PCB 17	3	19.50				
PCB 24	3	19.88				
PCB 27	3	19.88				
PCB 16	3	20.27				
PCB 32	3	20.27				
PCB 23	3	20.73				
PCB 34	3	20.73				
PCB 29	3	20.90				
PCB 26	3	21.13				
PCB 25	3	21.51				

Congener	LOC	RT (DB-5)	Top 40?	Risk Priority	WHO TEF	Comments
PCB 31	3	21.58	Y			
PCB 28	3	22.03	Y	5		Available as an individual C13 standard.
PCB 20	3	22.40	Y			
PCB 21	3	22.40				
PCB 33	3	22.40				
PCB 22	3	22.69				
PCB 36	3	23.12				
PCB 39	3	23.58				
PCB 38	3	24.08				
PCB 35	3	24.45				
PCB 37	3	24.50				Last eluter, and available as a standard. Present at ~1% in Aroclor 1016, 1242, and 1248. Available as an individual C13 standard
PCB 54	4	20.91		5		First eluter, toxicity data is available, and it is available as C13 standard.
PCB 50	4	21.53				
PCB 53	4	22.09				
PCB 51	4	22.32		5		
PCB 45	4	22.66				
PCB 46	4	23.05				
PCB 69	4	23.18		4		
PCB 52	4	23.29	Y	3		One of the most commonly occurring congeners in the environment. Present at ~4-7% in every Aroclor mixture but 1260. Available as C13 standard.
PCB 73	4	23.29				
PCB 43	4	23.49				
PCB 49	4	23.49		4		
PCB 47	4	23.65	Y	3		A good backup choice since it breaks up the tetras by RT. Commonly occurring congener, but not as common as PCB 52. Available as a C13 standard.
PCB 48	4	23.65				
PCB 75	4	23.65				
PCB 62	4	23.82				
PCB 65	4	23.82	Y	4		
PCB 44	4	24.30	Y	4		
PCB 42	4	24.40				
PCB 59	4	24.40				
PCB 72	4	24.73				
PCB 41	4	24.92				
PCB 64	4	24.92	Y			
PCB 68	4	24.92				
PCB 71	4	24.92				
PCB 40	4	25.31				
PCB 57	4	25.39				
PCB 67	4	25.62				
PCB 58	4	25.79				
PCB 63	4	25.91				
PCB 61	4	26.11	Y	4		
PCB 74	4	26.11	Y	4		

Congener	LOC	RT (DB-5)	Top 40?	Risk Priority	WHO TEF	Comments
PCB 70	4	26.28	Y	4		Commonly detected in the environment, and a major component (~4-7%) of 1242, 1248, and 1254. Available as a C13 standard
PCB 76	4	26.28	Y	4		A good choice, but cannot find a commercially available C13 standard.
PCB 66	4	26.45	Y	4		A good choice, but cannot find a commercially available C13 standard.
PCB 80	4	26.45		5		
PCB 55	4	26.90				
PCB 56	4	27.32				
PCB 60	4	27.32				
PCB 79	4	28.03				
PCB 78	4	28.55				
PCB 81	4	29.06			0.000 3	
PCB 77	4	29.56		5	0.000 1	Last eluter and a WHO toxic. Available as a C13 standard.
PCB 104	5	24.08		5		The first eluter for the pentachloro congeners, and available as a C13 standard.
PCB 96	5	25.14				
PCB 103	5	25.34				
PCB 100	5	25.62		4		Not present in the original Aroclor mixtures.
PCB 94	5	26.03				
PCB 102	5	26.39		4		Barely present in the original Aroclor mixtures (<0.2%).
PCB 98	5	26.39		4		Not present in the original Aroclor mixtures.
PCB 93	5	26.51		4		Barely present in the original Aroclor mixtures (<0.1%).
PCB 95	5	26.51		3		Present at a few percent in some of the Aroclor mixtures, but very close in retention time to congener 104.
PCB 121	5	26.66				
PCB 88	5	26.66				
PCB 91	5	26.83				
PCB 92	5	27.41				
PCB 84	5	27.58				
PCB 101	5	27.70	Y	3		Good choice for mid-RT penta-CBs. Detected regularly in the environment, and one of the main components of Aroclor 1248, 1254, and 1260). Available as a C13 standard.
PCB 89	5	27.70				
PCB 90	5	27.70	Y	4		
PCB 113	5	27.88	Y	4		
PCB 99	5	27.97	Y	2		A good choice, but elutes close to 101, and is not usually detected in as high quantities as 101. Also, it's not available as C13 standard.
PCB 119	5	28.30		4		
PCB 112	5	28.41				
PCB 83	5	28.52	Y			
PCB 108	5	28.54		4		
PCB 86	5	28.80		4		
PCB 97	5	28.80		4		
PCB 125	5	28.92		4		
PCB 111	5	29.00				

Congener	LOC	RT (DB-5)	Top 40?	Risk Priority	WHO TEF	Comments
PCB 117	5	29.00		2		
PCB 115	5	29.08	Y			
PCB 116	5	29.08		2		
PCB 87	5	29.08		4		
PCB 120	5	29.28				
PCB 85	5	29.28		2		Present at 1-2% in Aroclor 1248 and 1254, and known to be present in fish and human tissue. Available as a C13 standard.
PCB 110	5	29.58	Y	3		110 is an ideal candidate for an isotopically labeled standard. It is a major component of Aroclor 1248, 1254, and 1260, and is present regularly in the environment. Unfortunately, a commercial C13 standard is not currently available.
PCB 82	5	30.14				
PCB 124	5	30.49				
PCB 107	5	30.67				
PCB 109	5	30.67		4		
PCB 123	5	30.80			0.000 03	
PCB 106	5	30.92				
PCB 118	5	30.92	Y	1	0.000 03	It's a WHO toxic, and a major component of Aroclor 1248 and 1254. It is also one of the most common congeners in the environment, fish tissue, and human tissue. Available as a C13 standard.
PCB 114	5	31.50		5	0.000 03	
PCB 122	5	31.63				
PCB 105	5	32.30	Y	3	0.000 03	A good choice, but elutes close to 118, and is not usually detected in as high quantities as 118.
PCB 127	5	32.30				
PCB 126	5	33.99		5	0.1	The last eluter, and the most toxic congener according to WHO. It is rarely detected in the environment. Available as a C13 standard.
PCB 155	6	27.23				The first eluter for the hexa congeners. Available as a C13 standard.
PCB 150	6	28.33				
PCB 152	6	28.71				
PCB 145	6	29.04				
PCB 148	6	29.26				
PCB 136	6	29.41				
PCB 154	6	29.64		4		
PCB 151	6	30.22		4		
PCB 135	6	30.46		4		
PCB 144	6	30.46				
PCB 147	6	30.64	Y			
PCB 139	6	30.84				
PCB 149	6	30.84	Y	3		A good candidate, commonly detected in the environment, and is at a good retention time to distribute the hexa standards. Currently not available as a C13 standard.
PCB 140	6	30.99				
PCB 134	6	31.31				
PCB 143	6	31.31				

Congener	LOC	RT (DB-5)	Top 40?	Risk Priority	WHO TEF	Comments
PCB 133	6	31.50				
PCB 131	6	31.63				
PCB 142	6	31.63				
PCB 165	6	31.72				
PCB 146	6	31.82	Y	2		A good choice, but too close to the retention time of 153, which is usually detected in environmental standards at higher concentrations. Currently not available as a C13 standard.
PCB 161	6	31.91				
PCB 153	6	32.12	Y	1		One of the most abundant congeners in the environment and in the Aroclor mixtures. Available as a C13 standard.
PCB 132	6	32.25	Y	3		All good choices, but too close to the retention time of 153, which is usually detected in environmental standards at higher concentrations.
PCB 168	6	32.25	Y	2		
PCB 141	6	32.77	Y	4		
PCB 137	6	33.12				
PCB 130	6	33.26				
PCB 138	6	33.52	Y	1		One of the more abundant congeners in the environment and Aroclor 1254 and 1260. Present in human and fish tissue and toxicity data is available. Available as a C13 standard.
PCB 163	6	33.52	Y	2		
PCB 164	6	33.52		4		
PCB 158	6	33.66				
PCB 160	6	33.66	Y	2		
PCB 129	6	33.95	Y	2		
PCB 166	6	34.27		4		
PCB 159	6	34.44				
PCB 162	6	34.71				
PCB 128	6	34.93		3		
PCB 167	6	35.01			0.000 03	
PCB 156	6	36.17		6	0.000 03	It's a WHO toxic congener, and available as a C13 standard. It was not selected since the RT is so close to 169.
PCB 157	6	36.46			0.000 03	
PCB 169	6	37.91		5	0.03	The last eluter, and the second most toxic congener according to the WHO. It is rarely detected in the environment. Available as a C13 standard.
PCB 188	7	31.77				First eluter and available as an individual standard.
PCB 184	7	32.12				
PCB 179	7	32.85				
PCB 176	7	33.22				
PCB 186	7	33.67				
PCB 178	7	34.00				Available as a C13 standard.
PCB 175	7	34.29				
PCB 182	7	34.42				
PCB 187	7	34.42	Y	2		A good choice, but not available as a C13 standard.
PCB 183	7	34.68		4		Not available as a C13 standard.
PCB 185	7	35.23				
PCB 174	7	35.68	Y	4		Not available as a C13 standard.

Congener	LOC	RT (DB-5)	Top 40?	Risk Priority	WHO TEF	Comments
PCB 181	7	35.68				
PCB 177	7	35.89	Y	2		Not available as a C13 standard.
PCB 171	7	36.12				
PCB 173	7	36.42				
PCB 172	7	36.75				
PCB 192	7	36.75				
PCB 180	7	37.06	Y	1		The most abundant congener in the Aroclor 1260 mixture, and regularly detected in the environment. Available as a C13 standard.
PCB 193	7	37.19	Y			
PCB 191	7	37.39				
PCB 170	7	38.22	Y	1		A good choice, and available as a standard, but too close to the retention time of 180, which is usually detected in environmental standards at higher concentrations.
PCB 190	7	38.22				
PCB 189	7	38.98		5	0.00003	Last eluter, a WHO toxic congener, and available as a C13 standard.
PCB 202	8	36.08				First eluter, and available as a C13 standard.
PCB 201	8	36.48				
PCB 204	8	36.58				
PCB 197	8	36.86				
PCB 200	8	37.61				
PCB 198	8	38.35		4		Not available as a C13 standard.
PCB 199	8	38.45		4		Not available as a C13 standard.
PCB 196	8	38.60				Not available as a C13 standard.
PCB 203	8	38.60		2		Not available as a C13 standard.
PCB 195	8	39.37				
PCB 194	8	39.90		2		Good choice and available as a C13 standard, but too close in retention time to 205.
PCB 205	8	40.03				Last eluter, and available as an individual C13 standard.
PCB 208	9	39.35				First eluter, and available as a C13 standard.
PCB 207	9	39.56				Least abundant nona-congener in Aroclor mixtures.
PCB 206	9	40.82		2		Last eluter, and available as a C13 standard. Most abundant nona-congener in Aroclor mixtures.
PCB 209	10	41.51				Only choice. Not abundant in Aroclor mixtures, but has been detected in the environment. May be present from non-Aroclor sources. Available as a C13 standard.
Non-extracted Internal Standards (NIS)						
PCB 79	4	28.03				Available as a C13 standard.
PCB 162	6	34.71				Available as an individual C13 standard
Non-extracted internal standards are not being used for quantification of the target analytes, but to determine the recovery of the other labeled standards added to each sample prior to extraction. As such, they are sometimes called "recovery standards" in other methods. The C13 congeners that were selected are not commonly present in the environment, nor are they abundant in Aroclor mixtures.						

Summary of Isotope Dilution Standards Selected by EPA	
Level of Chlorination	Labeled Congeners
1	1, 3
2	4, 11, 15
3	19, 28, 37
4	54, 52, 70, 77
5	104, 101, 85, 118, 126
6	155, 153, 138, 169
7	188, 180, 189
8	202, 205
9	208, 206
10	209

Priority Congeners by Risk (1=highest)		
Priority	Native Congeners	Rationale
1	118, 138, 153, 170, 180	Abundant in fish and human tissues, and tox data available
2	85, 99, 116, 117, 129, 146, 160, 163, 168, 177, 187, 194, 203, 206	Abundant in fish and human tissues
3	47, 52, 95, 101, 105, 110, 128, 132, 149	Abundant in fish, and tox data available
4	44, 49, 61, 65, 66, 69, 70, 74, 76, 86, 87, 90, 93, 97, 98, 100, 102, 108, 109, 113, 119, 125, 135, 141, 151, 154, 166, 174, 183, 198, 199	Abundant in fish
5	4, 28, 51, 54, 77, 80, 104, 114, 126, 169, 189	Toxicity data available
6	156	Abundant in human tissues and tox data available

Appendix B

Interim Quality Control Acceptance Criteria Arising from the Method Validation Study

Interim Quality Control Acceptance Criteria Arising from the Method Validation Study

The tables below present the interim QC acceptance criteria that EPA anticipates including in the draft method. The derivations of these criteria are described in the body of this report.

Aqueous Matrix IPR and OPR QC Acceptance Criteria for Target Analytes

Congener	Interim Acceptance Criteria (%)		
	IPR Mean	Max RSD	OPR
PCB-1	78- 130	18	71 - 136
PCB-3	74- 117	14	71 - 120
PCB-4	77- 112	14	72 - 117
PCB-8	42- 120	18	43 - 119
PCB-11	62- 125	9	63 - 124
PCB-15	70- 111	10	69 - 111
PCB-18	60- 107	17	57 - 111
PCB-19	77- 107	12	73 - 111
PCB-28	18- 184	17	21 - 180
PCB-31	46- 129	19	46 - 129
PCB-37	67- 112	9	68 - 111
PCB-44	44- 131	13	46 - 130
PCB-52	61- 128	8	62 - 127
PCB-54	67- 112	8	68 - 111
PCB-64	74- 108	10	73 - 110
PCB-66	64- 118	8	65 - 117
PCB-70	55- 127	8	57 - 126
PCB-74	74- 102	8	73 - 103
PCB-77	58- 118	9	59 - 116
PCB-85	68- 106	7	69 - 105
PCB-95	63- 117	12	63 - 117
PCB-99	66- 107	10	66 - 107
PCB-101	64- 118	9	65 - 117
PCB-104	64- 117	8	65 - 116
PCB-105	64- 120	10	65 - 119
PCB-118	61- 119	10	62 - 118
PCB-110	63- 106	12	62 - 107
PCB-126	63- 113	7	64 - 112
PCB-132	51- 133	11	53 - 131
PCB-138	61- 117	11	61 - 116
PCB-147	61- 117	12	62 - 117
PCB-149	57- 120	11	58 - 119
PCB-153	46- 134	16	48 - 132
PCB-155	64- 116	10	65 - 115
PCB-156	46- 149	23	45 - 150
PCB-166	34- 157	9	36 - 156
PCB-169	50- 122	10	52 - 121
PCB-177	47- 130	10	49 - 128
PCB-180	52- 124	11	53 - 123
PCB-187	36- 138	17	38 - 136
PCB-188	57- 122	11	58 - 121
PCB-189	56- 119	11	58 - 118
PCB-199	42- 164	57	14 - 193
PCB-202	55- 121	12	56 - 120
PCB-205	52- 118	18	51 - 119
PCB-206	35- 135	17	37 - 133
PCB-208	44- 125	15	45 - 124
PCB-209	31- 130	27	30 - 131

Aqueous Matrix IPR and OPR QC Acceptance Criteria for Labeled Compounds

Congener	Interim Acceptance Criteria (%)		
	IPR (each aliquot)	Max RSD	OPR
¹³ C ₁₂ -PCB-1	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-3	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-4	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-11	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-15	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-19	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-28	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-37	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-52	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-54	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-70	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-77	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-85	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-101	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-104	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-118	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-126	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-138	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-153	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-155	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-169	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-180	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-188	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-189	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-202	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-205	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-206	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-208	15 - 130	40	15 - 130
¹³ C ₁₂ -PCB-209	15 - 130	40	15 - 130

Solid Matrix IPR and OPR QC Acceptance Criteria for Target Analytes

Congener	Interim Acceptance Criteria (%)		
	IPR Mean	Max RSD	OPR
PCB-1	61 - 154	59	19 - 196
PCB-3	40 - 156	42	29 - 167
PCB-4	48 - 144	61	13 - 179
PCB-8	35 - 150	40	25 - 160
PCB-11	35 - 150	40	25 - 160
PCB-15	36 - 150	44	25 - 162
PCB-18	20 - 148	40	18 - 149
PCB-19	26 - 157	32	28 - 156
PCB-28	25 - 150	35	30 - 150
PCB-31	38 - 147	37	32 - 153
PCB-37	38 - 147	38	31 - 155
PCB-44	23 - 153	34	24 - 151
PCB-52	57 - 138	42	36 - 159
PCB-54	56 - 132	56	21 - 167
PCB-64	29 - 153	29	31 - 150
PCB-66	50 - 138	25	49 - 140
PCB-70	43 - 144	27	42 - 144

Solid Matrix IPR and OPR QC Acceptance Criteria for Target Analytes

Congener	Interim Acceptance Criteria (%)		
	IPR Mean	Max RSD	OPR
PCB-74	41 - 135	30	38 - 138
PCB-77	42 - 134	40	30 - 145
PCB-85	57 - 121	27	50 - 128
PCB-95	55 - 125	29	47 - 133
PCB-99	33 - 140	34	30 - 143
PCB-101	57 - 125	26	51 - 132
PCB-104	52 - 128	44	32 - 148
PCB-105	65 - 122	17	63 - 124
PCB-118	48 - 133	19	50 - 131
PCB-110	31 - 142	20	36 - 137
PCB-126	48 - 129	14	52 - 124
PCB-132	42 - 146	18	47 - 141
PCB-138	60 - 123	19	58 - 125
PCB-147	58 - 126	25	53 - 132
PCB-149	51 - 129	28	46 - 134
PCB-153	76 - 109	25	61 - 124
PCB-155	60 - 122	37	41 - 140
PCB-156	76 - 119	25	62 - 133
PCB-166	71 - 122	21	64 - 128
PCB-169	56 - 130	55	23 - 164
PCB-177	71 - 114	29	55 - 130
PCB-180	72 - 112	25	58 - 125
PCB-187	64 - 114	23	56 - 122
PCB-188	61 - 118	27	52 - 128
PCB-189	67 - 117	24	58 - 126
PCB-199	62 - 126	22	58 - 130
PCB-202	51 - 127	24	49 - 129
PCB-205	54 - 116	31	44 - 126
PCB-206	52 - 129	49	27 - 154
PCB-208	45 - 131	21	47 - 129
PCB-209	67 - 111	19	62 - 117

Solid Matrix IPR and OPR QC Acceptance Criteria for Labeled Congeners

Congener	Interim Acceptance Criteria (%)		
	IPR (each aliquot)	Max RSD	OPR
¹³ C ₁₂ -PCB-1	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-3	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-4	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-11	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-15	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-19	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-28	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-37	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-52	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-54	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-70	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-77	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-85	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-101	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-104	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-118	15 - 130	60	15 -130

Solid Matrix IPR and OPR QC Acceptance Criteria for Labeled Congeners

Congener	Interim Acceptance Criteria (%)		
	IPR (each aliquot)	Max RSD	OPR
¹³ C ₁₂ -PCB-126	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-138	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-153	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-155	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-169	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-180	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-188	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-189	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-202	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-205	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-206	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-208	15 - 130	60	15 -130
¹³ C ₁₂ -PCB-209	15 - 130	60	15 -130

Tissue Matrix IPR and OPR QC Acceptance Criteria for Target Analytes

Congener	Interim Acceptance Criteria (%)		
	IPR Mean	Max RSD	OPR Range
PCB-1	25 - 150	25	25 - 150
PCB-3	25 - 150	25	25 - 150
PCB-4	25 - 150	25	25 - 150
PCB-8	25 - 150	25	25 - 150
PCB-11	25 - 150	25	25 - 150
PCB-15	25 - 150	25	25 - 150
PCB-18	25 - 150	25	25 - 150
PCB-19	25 - 150	25	25 - 150
PCB-28	25 - 150	25	25 - 150
PCB-31	25 - 150	25	25 - 150
PCB-37	25 - 150	25	25 - 150
PCB-44	25 - 150	25	25 - 150
PCB-52	25 - 150	25	25 - 150
PCB-54	25 - 150	25	25 - 150
PCB-64	25 - 150	25	25 - 150
PCB-66	25 - 150	25	25 - 150
PCB-70	25 - 150	25	25 - 150
PCB-74	25 - 150	25	25 - 150
PCB-77	25 - 150	25	25 - 150
PCB-85	25 - 150	25	25 - 150
PCB-95	25 - 150	25	25 - 150
PCB-99	25 - 150	25	25 - 150
PCB-101	25 - 150	25	25 - 150
PCB-104	25 - 150	25	25 - 150
PCB-105	25 - 150	25	25 - 150
PCB-118	25 - 150	25	25 - 150
PCB-110	25 - 150	25	38 - 138
PCB-126	25 - 150	25	38 - 139
PCB-132	32 - 156	25	48 - 140
PCB-138	27 - 150	25	43 - 134
PCB-147	25 - 150	25	25 - 150
PCB-149	25 - 150	25	25 - 150
PCB-153	33 - 142	25	47 - 127
PCB-155	25 - 150	25	25 - 150

Tissue Matrix IPR and OPR QC Acceptance Criteria for Target Analytes

Congener	Interim Acceptance Criteria (%)		
	IPR Mean	Max RSD	OPR Range
PCB-156	25 - 150	25	25 - 150
PCB-166	25 - 150	25	25 - 150
PCB-169	25 - 150	25	25 - 150
PCB-177	25 - 150	25	25 - 150
PCB-180	42 - 137	25	52 - 127
PCB-187	39 - 137	25	49 - 126
PCB-188	25 - 150	25	25 - 150
PCB-189	25 - 150	25	25 - 150
PCB-199	34 - 153	25	45 - 142
PCB-202	33 - 145	25	47 - 131
PCB-205	25 - 150	25	37 - 144
PCB-206	25 - 150	25	25 - 150
PCB-208	25 - 150	25	25 - 150
PCB-209	25 - 150	25	25 - 150

Tissue Matrix IPR and OPR QC Acceptance Criteria for Labeled Compounds

Congener	Interim Acceptance Criteria (%)		
	IPR (each aliquot)	Max RSD	OPR
¹³ C ₁₂ -PCB-1	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-3	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-4	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-11	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-15	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-19	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-28	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-37	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-52	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-54	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-70	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-77	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-85	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-101	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-104	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-118	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-126	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-138	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-153	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-155	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-169	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-180	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-188	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-189	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-202	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-205	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-206	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-208	15 - 130	60	15 - 130
¹³ C ₁₂ -PCB-209	15 - 130	60	15 - 130

QC Acceptance Criteria for Recovery of Labeled Compounds in Samples

Congener	Interim QC Acceptance Criteria (%)
¹³ C ₁₂ -PCB-1	15 - 130
¹³ C ₁₂ -PCB-3	15 - 130
¹³ C ₁₂ -PCB-4	15 - 130
¹³ C ₁₂ -PCB-11	15 - 130
¹³ C ₁₂ -PCB-15	15 - 130
¹³ C ₁₂ -PCB-19	15 - 130
¹³ C ₁₂ -PCB-28	15 - 130
¹³ C ₁₂ -PCB-37	15 - 130
¹³ C ₁₂ -PCB-52	15 - 130
¹³ C ₁₂ -PCB-54	15 - 130
¹³ C ₁₂ -PCB-70	15 - 130
¹³ C ₁₂ -PCB-77	15 - 130
¹³ C ₁₂ -PCB-85	15 - 130
¹³ C ₁₂ -PCB-101	15 - 130
¹³ C ₁₂ -PCB-104	15 - 130
¹³ C ₁₂ -PCB-118	15 - 130
¹³ C ₁₂ -PCB-126	15 - 130
¹³ C ₁₂ -PCB-138	15 - 130
¹³ C ₁₂ -PCB-153	15 - 130
¹³ C ₁₂ -PCB-155	15 - 130
¹³ C ₁₂ -PCB-169	15 - 130
¹³ C ₁₂ -PCB-180	15 - 130
¹³ C ₁₂ -PCB-188	15 - 130
¹³ C ₁₂ -PCB-189	15 - 130
¹³ C ₁₂ -PCB-202	15 - 130
¹³ C ₁₂ -PCB-205	15 - 130
¹³ C ₁₂ -PCB-206	15 - 130
¹³ C ₁₂ -PCB-208	15 - 130
¹³ C ₁₂ -PCB-209	15 - 130

Practical Application of These QC Acceptance Criteria

One of the challenges in developing statistically based QC acceptance criteria is that the limits are sometimes seen by users as overly wide, and thus not providing some preconceived level of “control” over laboratory performance. However, those concerns should be weighed against the risk and cost of rejecting results from samples and QC operations such as the IPR and OPR based on random variability.

Every laboratory performing analyses in support of Clean Water Act compliance monitoring must have an effective quality management system in place. Such systems must include assessment of all results against the various QC acceptance limits in a given analytical method, but also should include procedures for longer-term internal evaluations of laboratory performance. In fact, most EPA methods include discussions of the use of control charts and the development of in-house performance criteria. EPA expects that responsible laboratories will perform such evaluations and develop and apply in-house criteria, which by virtue of being from a single laboratory, will be narrower than the acceptance criteria listed in this appendix and incorporated in the final PCB method.

Appendix C

Study Plan for Multi-laboratory Validation of the EAD PCB Congener Method

This appendix contains the study plan developed by EPA for the multi-laboratory method validation study. The pagination, footers, and formatting remain the same as in the original study plan.

Study Plan for Multi-laboratory Validation of the EAD PCB Congener Method

Prepared for

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EPA Scientific and Technical Support Contract EP-C-17-024

January 17, 2018

Approvals



Brian D'Amico, EPA Branch Chief

2/20/18

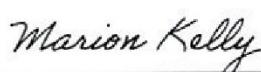
Date




Adrian Hanley, EPA Project Manager

1/18/2018

Date




Marion Kelly, EPA QA Coordinator

2/15/18

Date




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1/18/2018

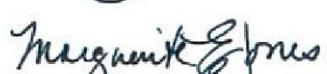
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1/18/2018

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DISCLAIMER

This document has been reviewed and approved by the Engineering and Analytical Support Branch of EAD. Mention of company names, trade names, or commercial products does not constitute endorsement or recommendation for use.

TABLE OF CONTENTS

	Page
Acknowledgments.....	i
Disclaimer	i
Distribution List.....	iii
Section 1. Background.....	1
Section 2. Study Objectives	2
Section 3. Study Management	3
Section 4. Technical Approach.....	4
4.1 Phase 1 - Soliciting Laboratories	4
4.2 Phase 2 - Procuring Standards and Study Samples.....	5
4.3 Phase 3 - Calibration and Demonstration of Capability	6
4.4 Phase 4 - Analyses of Study Samples	8
4.5 Phase 5 - Data Validation	9
4.6 Phase 6 - Development of QC Acceptance Criteria.....	10
Section 5. Quality Control Procedures.....	10
Section 6. Data Reporting	10
Section 7. Evaluation of Method Performance	12
Section 8. References.....	12

Appendix A	Standards Required for the Validation Study
Appendix B	Sample Types for the Validation Study
Appendix C	Procedures for Derivation of QC Acceptance Criteria from the Validation Study Results
Appendix D	Draft GC-LRMS-SIM Method

DISTRIBUTION LIST

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Study Plan for Multi-laboratory Validation of the EAD PCB Congener Method

SECTION 1. BACKGROUND

From the 1930s 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) gas chromatography (GC) with an electron capture detector (ECD). During this time period, EPA developed Method 608 for Aroclor determination in wastewater (Reference 8.1). The method detects seven of the most common Aroclor mixtures by comparing a peak pattern created by the congeners that make up each mixture. Method 608 does not target every known Aroclor mixture.

Aroclors are not the only source of PCB contamination, and the composition of Aroclors and other PCB sources within the environment changes over time due to variations in the stability, solubility, volatility, and other properties of the individual congeners. This weathering of PCB contamination often causes false nondetects and poor accuracy when measured as an Aroclor mixture. 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). Detecting the individual congeners instead of the peak pattern of Aroclor mixtures provides a more accurate result of how much PCB contamination is in the environmental sample. By the 1990’s PCB analysis research became focused on the development of high resolution GC (HRGC) high resolution MS (HRMS) methods.

In 1995, EPA developed Method 1668, which uses 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, California). 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. EPA subsequently drafted Revisions B and C of Method 1668, covering all 209 PCB congeners (Reference 8.2).

While Method 1668 is considered to be a highly sensitive and accurate method for the determination of individual PCB congeners, it is not without its limitations. HRGC/HRMS instrumentation is expensive and few environmental laboratories possess the capabilities to perform these analyses. In addition, sample analyses by Method 1668 are rather costly to perform and require highly technical personnel to interpret the data. The fact that Method 1668 is a highly sensitive method can also be problematic because PCBs are routinely detected below ambient background levels. For all these reasons, an EPA GC-LRMS method that quantifies individual congeners and is more sensitive than the GC-ECD procedure in Method 608, but more accessible to the laboratory community than Method 1668, would be highly useful for the determination of PCB congeners in environmental samples.

In 2016 and early 2017, the Engineering and Analysis Division (EAD) within the Office of Science and Technology, Office of Water conducted a single-laboratory study of a proprietary method from AXYS Analytical Services, Ltd (Reference 8.3). That single-laboratory study was successful in demonstrating the applicability of the GC-LRMS SIM method to monitoring PCB congeners in wastewater, soil/sediment, biosolids, and fish tissue samples relative to the needs of the Clean Water Act.

The next step is to conduct a multi-laboratory validation study of the draft EPA method that resulted from the single-laboratory study. This study plan presents EAD’s approach to that study.

SECTION 2. STUDY OBJECTIVES

The goals of the multi-laboratory validation study are to:

- Obtain data from matrices that are representative of the method's intended use
- Obtain data from laboratories that are representative of those likely to use the approved method, but that were not directly involved in its development
- Obtain feedback from laboratory users on the specifics of the draft method (e.g., is it clear and easy to understand, or are changes to the method text needed?)
- Use study data to characterize performance of the method
- Develop statistically derived QC acceptance criteria that will reflect method performance capabilities in real-world situations

In addition to the overall objective described above, EAD has two general quality objectives for this study:

1. Except where otherwise directed, all samples and data must be generated according to the analytical and quality assurance/quality control (QA/QC) procedures specified in this study plan and the GC-LRMS-SIM procedure. Alternatively, the data must be the result of pre-approved and documented changes to these procedures. This will allow EAD to collect data that accurately reflects the performance capabilities of the methodology and to use study results to identify the need for any further revisions to the procedure.
2. All data produced must be capable of being verified by an independent person reviewing the analytical data package.

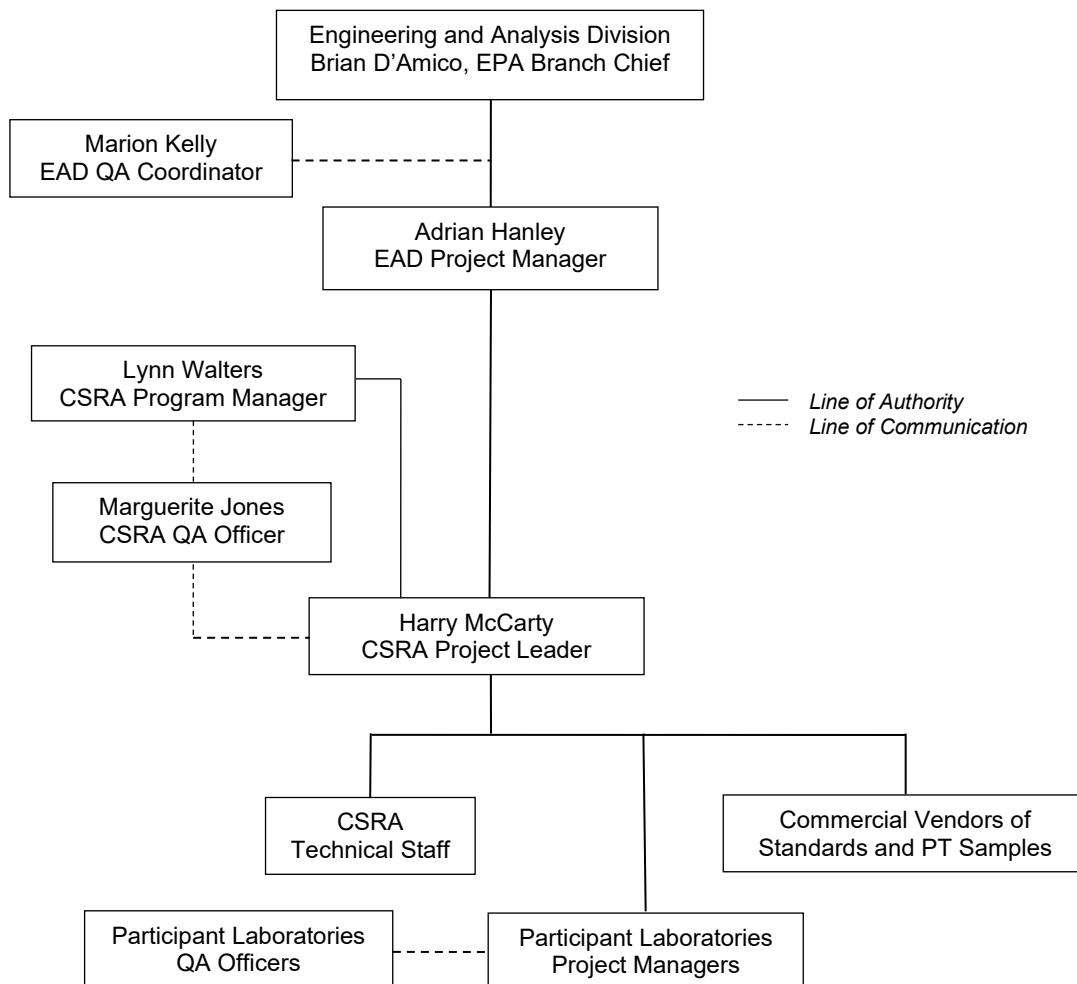
To meet these quality objectives, EPA and CSRA will employ the following QA/QC strategies:

- All CSRA activities will be performed in accordance with this study plan, which also serves as a Quality Assurance Project Plan (QAPP) for the study.
- The vendor responsible for preparing the study samples must have demonstrated experience in performing work of a similar nature and must have a comprehensive QA program in place and operating throughout their study operations.
- The vendor responsible for preparing the analytical standards must have demonstrated experience in performing work of a similar nature and must have a comprehensive QA program in place and operating throughout their study operations.
- Each participant laboratory (either contracted or volunteer) also must have demonstrated experience in GC-LRMS-SIM analyses of a similar nature and must have a comprehensive QA program in place and operating throughout their study operations.
- The study report and the final draft method will be reviewed by CSRA, EPA, and the EPA work group to ensure the QC requirements meet data quality objectives.

Cumulatively, these requirements are intended to ensure that the data produced in this study are of appropriate and documented quality.

SECTION 3. STUDY MANAGEMENT

The study will be managed by Adrian Hanley, the EAD Project Manager. Day-to-day management and coordination of study activities will be performed by CSRA Study Manager, Harry McCarty, under the supervision of the CSRA Program Manager, Lynn Walters and EAD oversight. Marion Kelly, the Quality Assurance Coordinator for EAD, will provide QA support for EPA. Marguerite Jones, the Quality Assurance Officer for CSRA, will provide QA oversight for CSRA. QA oversight of participant laboratory activities will be provided by each laboratory's QA Officer (or equivalent). Each of these QA positions is independent of the technical staff and managers who are responsible for the generation, analysis, and use of data in this study. The organization chart below illustrates the relationship of these parties.



A commercial vendor of performance testing (PT) samples will be used to prepare real-world and synthetic wastewater, soil/sediment, and tissue matrices for use in this study, with assistance from EAD and CSRA as needed. CSRA will be responsible for procuring and providing oversight of the vendor. EPA and CSRA will work together to obtain sufficient volumes of real-world wastewater, soil/sediment, and tissue matrices from various sources and delivering them to the selected PT vendor for homogenization, aliquoting, and shipment to the participant laboratories.

CSRA will be responsible for procuring the various PCB standards required to perform the method for each laboratory from one or more commercial vendors. A list of the required standards is provided in Appendix A.

CSRA will be responsible for procuring and providing oversight of commercial contract laboratories that will participate in the validation study. The number of contracted laboratories will be determined by EPA based on factors such as cost and EPA's ability to enlist other suitable laboratories as volunteer (unpaid) participants. In keeping with the approach described in ASTM Standard D2777 (Reference 8.4), EPA will solicit participation from a large number of laboratories, recognizing the possibility that some participants may drop out or otherwise fail to provide usable data. At this time, EPA is planning to include 20 laboratories in the study if possible, far in excess of the nine laboratories recommended in the ASTM Standard. Part of the rationale for the large number of participants is to gain additional support for promulgation of the final method from the commercial laboratory community.

In order to comply with recent EPA policies regarding laboratory competency, CSRA will request and evaluate information about each laboratory's certifications or accreditations relevant to the analysis of PCB congeners in environmental matrices during the solicitation process described in Section 4.1. CSRA recognizes that certifications or accreditations specific to analysis of individual PCB congeners may not be offered by all accrediting bodies, and CSRA will not use the lack of certifications or accreditations to exclude laboratories. CSRA will request similar information from any volunteer laboratories identified by EPA. Each laboratory supporting this study must have a comprehensive QA program in place and operating at all times during the study. CSRA will request copies of QA program documentation during the solicitation process, as part of an assessment of laboratory capabilities. Laboratories that cannot demonstrate competency in PCB analyses and that do not have an adequate QA program in place will not be included as participants in the study.

All analytical results will be submitted to CSRA. As described in Sections 4.5, 4.6 and 7, CSRA will review and evaluate all analytical data and assist EAD in drawing conclusions from the results. Depending on the availability of resources, CSRA will either prepare a draft study report that summarizes these results and conclusions for EAD review, or will provide data and technical assistance to aid EAD staff in preparing such a report. As appropriate, EAD will revise the draft GC-LRMS-SIM method to reflect study findings and add QC acceptance criteria developed from the study data.

SECTION 4. TECHNICAL APPROACH

The study will be performed in six phases. Phase 1 and 2 may occur simultaneously.

- Phase 1 (Section 4.1) involves soliciting laboratories (contracted and volunteer) to participate in the study
- Phase 2 (Section 4.2) involves procuring the standards required by each laboratory, as well as the study samples to be analyzed
- Phase 3 (Section 4.3) involves the initial steps (calibration, IDCs, and MDLs) demonstrating laboratory capability with standards and clean matrices using the draft method
- Phase 4 (Section 4.3) involves using the draft method to analyze the study samples by all of the participant laboratories
- Phase 5 (Section 4.5) involves validation of all of the study results by CSRA
- Phase 6 (Section 4.6) involves the development of QC acceptance criteria from the study data and production of the final version of the method document

4.1 Phase 1 - Soliciting Laboratories

Phase 1 of the study involves identifying and soliciting up to 20 laboratories to participate in the study. As noted earlier, some of those laboratories will be contracted by CSRA and others may participate as volunteers. While not a hard and fast differentiation, the contracted laboratories are likely to be commercial environmental laboratories, whereas the volunteer participants may be EPA Regional laboratories, utility laboratories, or other organizations that are unlikely to be able to accept payment for their participation.

CSRA and EPA will develop a broad list of likely participants and contact them in advance of a formal solicitation to determine their potential interest. Once the list of potential participants has been established, CSRA will competitively solicit bids using government-approved procurement procedures and an EAD-approved statement of work (SOW), or equivalent documentation that details the requirements for sample preparation, storage, shipment, analysis, and QA/QC. The SOW also will stipulate that the laboratory must have a comprehensive laboratory QA program in place and operating at all times during performance under the SOW and this program must be consistent with EPA guidance for quality systems (*Guidance for Developing Quality Systems for Environmental Programs*, EPA/240/R-02/008, November 2002) and the general laboratory procedures specified in the *Handbook for Analytical Quality Control in Water and Wastewater Laboratories* (EPA-600/4-79-019). CSRA will also work with EPA to develop suitable mechanisms to engage any volunteer laboratories identified by EPA. Such mechanisms may involve a memorandum of understanding (MOU) and/or a voluntary participation agreement form previously developed by EPA for similar studies.

Regardless of the nature of a laboratory's participation (contracted or volunteer), the same study requirements will apply and will be described in a study-specific statement of work and study-specific instructions.

4.2 Phase 2 - Procuring Standards and Study Samples

Phase 2 of the study involves procuring sufficient quantities of 1) the analytical standards needed to perform the method, and 2) the samples that will be analyzed in the study. Since the method is still in draft form, many of the standards are not available as ready-to-use commercial products. Having each laboratory prepare their own standards from neat materials or available stock solutions adds significant variability to the study results that is not likely to reflect routine laboratory practice when performing the method. Providing the same standards to each laboratory removes that aspect of variability and provides an incentive for both contracted and volunteer laboratories to participate.

CSRA and EPA previously identified the likely commercial sources of the needed standards. CSRA will use government-approved procurement procedures and an EPA-approved SOW (or equivalent) to obtain sufficient volumes of the needed standards and have them shipped directly to the participant laboratories. CSRA anticipates that these materials may need to be procured from multiple existing commercial sources, but is also investigating the possibility of finding a single vendor who can obtain materials from other vendors and prepare the entire suite of standards needed. The list of standards and quantities is provided in Appendix A.

Once the sources of the standards have been identified and CSRA purchase orders are in place, CSRA staff will work with the vendors to schedule and direct the shipments of materials to each participating laboratory. CSRA staff will notify each laboratory of impending shipments, track each shipment from the vendor to the laboratory, and confirm condition of the materials on receipt with each laboratory. CSRA will work with the vendors and laboratories to resolve any issues or discrepancies, and will communicate with EPA regularly.

The focus of the study is on analysis of real-world environmental matrices, including wastewaters, soil/sediment, biosolids, and fish tissue. A generalized list of sample types and quantities is provided in Appendix B. Given the breadth of the matrices and samples, EPA and CSRA anticipate procuring the services of an established commercial vendor of PT samples to prepare the study samples. EPA will work with municipal, state, and EPA Regional contacts to obtain sufficient volumes of several real-world wastewaters to be used in the study.

EPA plans to utilize nine wastewater matrices, submitting samples of each to all of the laboratories participating in the study. The wastewater samples will include effluents from a publicly owned treatment works (POTW), a substitute wastewater as specified in ASTM D 5905 - 98 (Reapproved 2013), Standard Specification for Substitute Wastewater (Reference 8.5), and wastewaters from specific

industrial discharges if they can be obtained in sufficient quantities. At least one of the wastewater matrix types should have one of the following characteristics, such that each criterion below is represented by at least one wastewater:

- Total suspended solids (TSS) greater than 40 mg/L
- Total dissolved solids (TDS) greater than 100 mg/L
- Oil and grease greater than 20 mg/L
- NaCl greater than 120 mg/L
- CaCO₃ greater than 140 mg/L

EPA and CSRA will work with other contacts to obtain sufficient masses of soils/sediments, biosolids, and fish tissues. All of these materials will be delivered to the selected PT vendor to be homogenized and aliquoted into study-specific sizes, and distributed to each laboratory in accordance with the EPA-approved PT vendor SOW. EPA plans to utilize three soil/sediment matrices, three biosolids matrices, and three fish tissue matrices, submitting samples of each to all of the laboratories participating in the study.

As with the standards, CSRA staff will work with the vendors to schedule and direct the shipments of materials to each participating laboratory. CSRA staff will notify each laboratory of impending shipments, track each shipment from the vendor to the laboratory, and confirm condition of the materials on receipt with each laboratory. CSRA will work with the vendors and laboratories to resolve any issues or discrepancies, and will communicate with EPA regularly.

4.3 Phase 3 - Calibration and Demonstration of Capability

Prior to analyzing any of the study samples, each laboratory will perform an initial multi-point calibration and conduct an initial demonstration of capability for each sample matrix, as described in the sections below.

4.3.1 Initial Calibration

Each laboratory will calibrate their instrumentation using the six standards provided by EPA for the study and as described in the draft method. The six calibration standards cover a concentration range from 10 ng/mL in the lowest standard to 2,000 ng/mL in the highest standard (equivalent to 1 ng/L to 200 ng/L in a one-liter aqueous sample). Each laboratory will report the relative responses (RRs) of the native congeners, using the calculations described in the method. They will also report the response factors (RFs) for each labeled congener, using the calculations described in the method. Each laboratory will report the calibration linearity metric that they use (e.g., the relative standard deviation) for each congener as well.

Twenty-three native (unlabeled) PCB congeners in the standards are calibrated by isotope dilution quantitation, by virtue of including the ¹³C₁₂-labeled analog of each of those congeners in the calibration standards. An additional 14 congeners are calibrated using modified isotope dilution, by virtue of coeluting with a congener that has a ¹³C₁₂-labeled analog in the calibration standards. Twenty-eight more congeners are calibrated using the response of a labeled congener in the same level of chlorination, via a process called extracted internal standard. The remaining 144 congeners are calibrated indirectly, using the response factor for a congener in the same level of chlorination. Each laboratory will report the response factors (RFs) of the native congeners, using the calculations described in the method. Each laboratory will report the calibration linearity metric that they use (e.g., the relative standard deviation) for each congener as well.

4.3.2 Initial Demonstration of Capability (IDC)

Each laboratory will perform an initial demonstration of capability (IDCs) for each of the three matrix types in the study (aqueous, soil/sediment/biosolid, and fish tissue) using a suitable spiked reference matrix. The spiked reference matrix is a clean matrix (void of target compounds at or above the MDL). The reference matrix for aqueous samples is 1 L of purified or reagent water. For soil/sediment/biosolid, the reference matrix will be clean sand, and for fish tissue, the reference matrix will be a 90/10 mixture of clean sand and vegetable oil, which simulates the lipid content of fish tissues.

Each IDC will include an initial precision and recovery (IPR) determination and a method detection limit (MDL) study. Although the method includes procedures for separatory funnel extraction and two forms of solid-phase extraction of aqueous samples, each laboratory participating in the study will use the separatory funnel extraction procedure for the IDC, because that procedure is readily available in all of the laboratories that are likely to employ the final promulgated method.

Data from the single-laboratory study indicate that solid-phase extraction can perform as well as separatory funnel extraction on real-world effluent samples. If a given laboratory participating in the study also has the necessary equipment for one of the two forms of solid-phase extraction, EPA may opt to have such laboratories also perform the IDC for aqueous using solid-phase extraction for comparative purposes. However, EPA anticipates developing a single set of QC acceptance criteria for the method for aqueous samples that are applicable to both types of extraction procedures.

4.3.3 IPR Determination

The IPR consists of four replicate samples of the reference matrix spiked with native congeners and labeled compounds and carried through the entire analytical process (sample preparation and analysis). The native congeners should be spiked around the midpoint of the calibration curve. Each laboratory will calculate the percent (%) recovery of each native congener using Equation 1:

Eq. 1

$$\text{Recovery} = \%R = \frac{C_s}{C_n} \times 100$$

where:

C_s = Measured concentration of the spiked sample aliquot

C_n = Nominal (theoretical) concentration of the spiked aliquot

The relative standard deviation (RSD) is calculated using the results of the four replicates for each native congener using Equation 2:

Eq. 2

$$RSD = \frac{SD}{C_{avg}} \times 100$$

where:

SD = Standard deviation of C_s for the four replicates

C_{avg} = Average measured concentration for the four replicates

Each laboratory will perform an IPR study for each matrix and/or extraction type and will report the results for the individual IPR samples, as well as the recoveries and RSDs for each analyte.

4.3.4 MDL studies

Each laboratory will perform an MDL study using the newly promulgated MDL procedure at 40 CFR Part 136 Appendix B, for each matrix type (Reference 8.6). Each MDL study will consist of seven replicate reference matrix samples spiked with native congeners (MDL_s) and seven replicate method blanks (MDL_b), all carried through the entire analytical process (sample preparation and analysis). The MDL study will be conducted for all 209 congeners. The native congeners will be spiked at a concentration near that of the lowest calibration standard in seven samples. Each laboratory will calculate and report the MDL_b and MDL_s, as well as the spiking levels and the individual MDL study results for all 14 aliquots in each matrix type. If an analyte is not detected, the spiking level will be raised, and the entire MDL study will be repeated for that analyte.

As with the IPR studies for aqueous samples, all laboratories will perform the MDL study for aqueous samples using the separatory funnel extraction procedures described in the method, and EPA may opt to have selected laboratories also determine MDLs using one of the solid-phase extraction procedures.

The draft method uses a 5-g sample aliquot for biosolids samples, and a 10-g aliquot for the other solid-phase samples. In order to reduce the effort at the laboratories, each laboratory will be instructed to use only 5 g for the MDL study for solids. CSRA and EPA anticipate that this may yield higher MDL values than for a 10-g sample size, but that the data will still be sufficient to characterize the general sensitivity of the method for solid matrices, since in practice, each laboratory using the final method will have to determine their own MDL values for compliance with method requirements.

4.4 Phase 4 - Analyses of Study Samples

The focus of Phase 4 is to evaluate the GC-LRMS-SIM procedure in various real-world matrices, including wastewaters, soils/sediments, biosolids, and fish tissues.

Wastewater Analyses

Each laboratory will receive three 1-L aliquots of each of nine wastewater samples (27 aliquots in total). One aliquot will be prepared and analyzed unspiked. The other two aliquots will be used by each laboratory to prepare a matrix spike/matrix spike duplicate (MS/MSD) pair. EPA and CSRA will provide the spiking levels of the native congeners to be used for each wastewater sample type to all of the laboratories, based on “reconnaissance” analyses conducted by SGS-AXYS Analytical, the laboratory that developed the draft method, using aliquots of the homogenized samples provided by the study sample vendor.

The draft method includes three extraction procedures for aqueous samples: the traditional separatory funnel extraction available in virtually any laboratory, and two forms of solid-phase extraction that rely on less common equipment from two or more vendors. EPA evaluated all three extraction procedures in side-by-side testing during the single-laboratory study and found them to yield similar results.

EPA anticipates having all laboratories use separatory funnel extraction for the wastewater samples. If a sufficient number of laboratories are identified who already possess the necessary equipment for one of the other of the solid-phase extraction procedures, EPA will provide additional sample aliquots to those laboratories and arrange for them to use solid-phase extraction *in addition* to the separatory funnel extraction procedure. (As noted in Section 4.3, EPA currently anticipates developing a single set of QC acceptance criteria for the method for aqueous samples that are applicable to both types of extraction procedures.)

Assuming that 20 laboratories participate in the study either under contract to CSRA or as volunteers, the study design will yield 180 results for unspiked wastewater samples and 360 matrix spike sample results for each of the native and labeled congeners. Even if fewer than 20 laboratories participate, or are able to

produce usable results, EPA will still have a significant body of performance data with which to judge the method's capabilities.

Soil/Sediment and Biosolids Analyses

Each laboratory will receive three 10-g aliquots of each of three soil/sediment samples (9 aliquots in total) and three 5-g aliquots of each of three biosolids samples (9 aliquots in total). As with the wastewater samples, one aliquot will be prepared and analyzed unspiked. The other two aliquots will be used by each laboratory to prepare a matrix spike/matrix spike duplicate (MS/MSD) pair. EPA and CSRA will provide the spiking levels of the native congeners to be used for each soil/sediment sample type and each biosolids sample type to all of the laboratories, based on "reconnaissance" analyses conducted by SGS-AXYS Analytical, the laboratory that developed the draft method, using aliquots of the homogenized samples provided by the study sample vendor.

Assuming that 20 laboratories participate in the study either under contract to CSRA or as volunteers, the study design will yield 60 results for unspiked soil/sediment samples, 60 results for unspiked biosolids samples, 120 soil/sediment matrix spike sample results, and 120 biosolids matrix spike sample results for each of the native and labeled congeners. Even if fewer than 20 laboratories participate, or are able to produce usable results, EPA will still have a significant body of performance data with which to judge the method's capabilities.

Fish Tissue Analyses

Each laboratory will receive three 10-g aliquots of each of three fish tissue samples (9 aliquots in total). As with the other matrices, one aliquot will be prepared and analyzed unspiked. The other two aliquots will be used by each laboratory to prepare a matrix spike/matrix spike duplicate (MS/MSD) pair. EPA and CSRA will provide the spiking levels of the native congeners to be used for each tissue sample type to all of the laboratories, based on "reconnaissance" analyses conducted by SGS-AXYS Analytical, the laboratory that developed the draft method, using aliquots of the homogenized samples provided by the study sample vendor.

Assuming that 20 laboratories participate in the study either under contract to CSRA or as volunteers, the study design will yield 60 results for unspiked tissue samples and 120 tissue matrix spike sample results for each of the native and labeled congeners. Even if fewer than 20 laboratories participate, or are able to produce usable results, EPA will still have a significant body of performance data with which to judge the method's capabilities.

4.5 Phase 5 - Data Verification and Validation

All of the results from all of the laboratories participating in the study will be reviewed and validated by CSRA relative to the study's goals. Every data submission will be checked for completeness (e.g., were all of the samples submitted to the laboratory analyzed and results submitted?) and to determine if the supporting documentation indicate that the laboratory followed the method and the study-specific instructions.

Each laboratory will be required to submit the raw data (e.g., instrument printouts and copies of bench records) that support the study results. CSRA will examine all of the raw data, perform spot checks of a percentage of the calculations from each laboratory, and ensure that the reported results can be traced back through all steps in the analytical process. If any issues are identified, CSRA will work with the laboratory to clarify the situation, obtain any missing information, and document the resolution. EPA will be advised of the status of the review efforts on a regular basis.

Because this is a method validation effort, there are no *a priori* quality control acceptance criteria, and data from the study will not be excluded from consideration simply because they appear to fail some

pre-conceived performance expectations. Every effort will be made to retain as many results as practical. CSRA will flag results from samples with obvious documented failures (e.g., extracts accidentally taken to dryness) for exclusion from use in developing method performance information and will document the rationale for such exclusions in the project files and/or the project database. However, in the absence of evidence of such failures, all of the results for the native and labeled congeners will be included in the initial data set. CSRA and EPA will use statistical tests in Phase 6 of the study to determine if results for specific laboratories, samples, or congeners may be outliers that should be removed from use in developing QC acceptance criteria. Suspected outliers will be examined in detail by CSRA and the laboratory before they are excluded from use in developing method performance summaries.

4.6 Phase 6 - Development of QC Acceptance Criteria

The last major phase of the study will be to develop statistically based QC acceptance criteria and summarize method performance in real-world samples. The overall procedures used for that process are described in Section 7 of this study plan.

SECTION 5. QUALITY CONTROL PROCEDURES

The GC-LRMS-SIM procedure includes many of the traditional quality control (QC) procedures found in EPA methods for the analysis of organic contaminants. The associated QC checks are summarized in Table 2 (following Section 8). Each laboratory is responsible for maintaining their instrumentation and ensuring that all study samples are analyzed on a properly calibrated instrument. Therefore, if the instrument calibrations or other instrument QC (i.e., mass spectrometer tune, mass calibration check, or qualitative identification criteria) are outside the normal criteria (see Table 2), the laboratory will take standard measures (e.g., cleaning the instrument, clipping column ends, or replacing column or other instrument parts) to correct the problem before any study samples are analyzed. The laboratory is also responsible for inspecting all study samples and standards to ensure they meet all study requirements. If standard measures do not correct identified problems or if study schedules will be impacted due to necessary repairs or replacement of study samples or standards, the laboratory will notify the CSRA Project Leader to indicate the impact on study schedules, the laboratory's plans to resolve the problem(s), and if any study samples will need to be reanalyzed.

Each laboratory will report the results from all procedure-specified QC operations, either in electronic format, or if necessary, in hard copy. CSRA will compile the QC results in a database specific to this project (See Section 6).

SECTION 6. DATA REPORTING AND DATA MANAGEMENT

6.1 Laboratory reporting requirements

Each laboratory participating in the study will be required to (1) report summary-level electronic data and supporting raw data, and (2) maintain their raw data for a period of five (5) years and provide them upon request (at additional cost negotiated as necessary). Raw data will include all calibration data, chromatograms, quantitation reports (including peak areas or heights), strip charts, spectra, bench sheets, and laboratory notebooks showing weights, volumes, manual calculations, and other data that will allow verification of the calculations performed and will allow the final results reported to be traced back to the raw data.

Each laboratory also will be instructed to adhere to the following rules when reporting data:

- All reports and documentation, including instrument printouts and other raw data, must be sequentially paginated, clearly labeled with the laboratory name, and labeled to provide sufficient identification for method blanks, calibration, interference checks, etc., necessary to link the raw data with associated summary reports.

- Results from *all* analyses must be reported, including calibration data and any dilutions or reanalysis performed. The laboratory also must include an explanation of any dilutions or reanalysis performed and identify which of the analyses the lab considers to be most appropriate for use.
- Results of all measurements must be reported to three significant figures in the appropriate reporting units (e.g., ng/L for water samples, ng/g for solid and tissue samples) to facilitate review and evaluation
- The terms “zero” and “trace” are not to be used; the term “not detected” (ND) is to be used for each measurement for which no signal is produced or if method-specified qualitative identification criteria are not met.
- If a signal is produced, the value must be reported, even if the value is negative. If the value is below the lowest calibration standard, a “J” flag must be applied to this value.
- Results must be reported for all study samples, including QC samples.

In addition, each laboratory will be required to submit a written “narrative report” with each data package. The narrative report will contain detailed descriptions of any difficulties encountered in the generation of the analytical results and QC data and any attempts to resolve the difficulties. It also will contain a detailed description of any modifications to the GC-LRMS-SIM procedure and the date that these modifications were pre-approved by CSRA.

Finally, each laboratory will be asked to provide comments on the draft method document, focusing on the clarity of the procedures, identifying any gaps in the descriptions of the analytical processes, inconsistencies, etc.

6.2 CSRA Data Management and Reporting

CSRA will store all submitted data (hard copy and electronic) in master files established for this study on CSRA’s secure local area network, which is backed up nightly, and/or in hardcopy files, depending on the source material. Cumulatively, these master files will include the following documents and records:

- This study plan (including all submitted draft versions, comments, and revisions)
- Documentation of the procedures used to assess the competency of laboratories participating in this study
- Documents and records associated with the solicitation and award of participant laboratories, including the SOWs or equivalent that describe participant laboratory requirements
- Documents and records associated with the procurement of standards and study samples, including SOWs or equivalent that describe the process used to collect and produce study samples
- The name, address, phone number and primary contact at the standards vendor and each participating laboratory
- Copies of all written correspondence (excluding emails) with laboratory staff, sampling personnel, and EPA staff regarding the study
- A log (or other record) that documents verbal communication with laboratory staff, sample coordinators, sampling personnel, and EPA staff regarding study status or problems
- Records concerning sample shipment and receipt
- All analytical data resulting from this study
- All laboratory comments on the method resulting from this study
- Records of all CSRA data review assessments and statistical analyses submitted to EPA
- All draft and final reports submitted to EPA pertaining to this study

CSRA and EPA will develop a schedule for routine communications during the course of the study, based on the specific activities underway at the time. For example, CSRA will communicate with the EPA Project Manager more frequently (e.g., daily) during those periods when samples are being shipped to the laboratories, versus less frequent communications during the periods when sample analyses are taking place.

SECTION 7. EVALUATION OF METHOD PERFORMANCE

EPA's overall goal is to develop method performance data for the GC-LRMS-SIM procedure. The results of the analyses in the first four phases of this study will be evaluated using common statistical procedures (References 8.4, 8.7, and 8.8). EPA and CSRA will use the results from the replicate samples to develop QC criteria for initial precision and recovery (IPR) tests, ongoing precision and recovery (OPR) tests, labeled compounds recoveries, duplicate precision, etc. A general description of the derivation of those QC acceptance criteria is provided in Appendix C and is based on EPA's existing new method evaluation protocol (Reference 8.9).

Finally, EPA and CSRA will develop tables of method performance data, including precision and accuracy, as a function of analyte concentration that will provide an indication of expected performance of the procedures under typical conditions. Such tables may be included in the revised procedure as further evidence of its overall capabilities or limitations.

Following completion of the method performance evaluation, CSRA and EPA will prepare a formal report on the results of the multi-laboratory validation study. EPA will submit that draft report to appropriate levels of management review within EPA and revise the report as needed. If the study is successful and EPA decides to move forward with a rulemaking to approve the new PCB method at 40 CFR Part 136 for use in nationwide compliance monitoring, the study report and records from the study will be placed into the rulemaking docket.

SECTION 8. REFERENCES

- 8.1 US Environmental Protection Agency. 1984. Method 608, Organochlorine Pesticides and PCBs. <http://www.epa.gov/cwa-methods/approved-cwa-test-methods-organic-compounds>
- 8.2 US Environmental Protection Agency. 2010. Method 1668C, Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS. Office of Water, Office of Science and Technology, Engineering and Analysis Division. EPA-820-R-10-005.
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- 8.4 ASTM 2013. Method D2777-13, Standard Practice for Determination of Precision and Bias of Applicable Methods of Committee D-19 on Water, ASTM International, West Conshohocken, PA. <http://www.astm.org>
- 8.5 ASTM 2013. Method D5095-13. Standard Practice for the Preparation of Substitute Wastewater, ASTM International, West Conshohocken, PA. <http://www.astm.org>
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- 8.7 SAS Institute Inc. 1994. SAS/STAT User's Guide, Volume 2, GLM-VARCOMP. Version 6, 4th Edition, June 1994.

- 8.8** Berry, D. A.; Lindgren, B. W. 1990. Statistics: Theory and Methods. pp. 286-290, 600-618. Brooks/Cole Publishing Company. Pacific Grove, California.
- 8.9** US Environmental Protection Agency. 2016. *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program*. U.S. Environmental Protection Agency. Office of Water, Engineering and Analysis Division. EPA 821-B-16-001.

Table 1. Names, Congener Numbers, and CAS Registry Numbers for Native Chlorinated Biphenyl (CB) Congeners

CB congener ¹	Congener number	CAS Number
2-MoCB	PCB 1	2051-60-7
3-MoCB	PCB 2	2051-61-8
4-MoCB	PCB 3	2051-62-9
2,2'-DiCB	PCB 4	13029-08-8
2,3-DiCB	PCB 5	16605-91-7
2,3'-DiCB	PCB 6	25569-80-6
2,4-DiCB	PCB 7	33284-50-3
2,4'-DiCB ²	PCB 8	34883-43-7
2,5-DiCB	PCB 9	34883-39-1
2,6-DiCB	PCB 10	33146-45-1
3,3'-DiCB	PCB 11	2050-67-1
3,4-DiCB	PCB 12	2974-92-7
3,4'-DiCB	PCB 13	2974-90-5
3,5-DiCB	PCB 14	34883-41-5
4,4'-DiCB	PCB 15	2050-68-2
2,2',3-TrCB	PCB 16	38444-78-9
2,2',4-TrCB	PCB 17	37680-66-3
2,2',5-TrCB ²	PCB 18	37680-65-2
2,2',6-TrCB	PCB 19	38444-73-4
2,3,3'-TrCB	PCB 20	38444-84-7
2,3,4-TrCB	PCB 21	55702-46-0
2,3,4'-TrCB	PCB 22	38444-85-8
2,3,5-TrCB	PCB 23	55720-44-0
2,3,6-TrCB	PCB 24	55702-45-9
2,3',4-TrCB	PCB 25	55712-37-3
2,3',5-TrCB	PCB 26	38444-81-4
2,3',6-TrCB	PCB 27	38444-76-7
2,4,4'-TrCB ²	PCB 28	7012-37-5
2,4,5-TrCB	PCB 29	15862-07-4
2,4,6-TrCB	PCB 30	35693-92-6
2,4',5-TrCB	PCB 31	16606-02-3
2,4',6-TrCB	PCB 32	38444-77-8
2',3,4-TrCB	PCB 33	38444-86-9
2',3,5-TrCB	PCB 34	37680-68-5
3,3',4-TrCB	PCB 35	37680-69-6
3,3',5-TrCB	PCB 36	38444-87-0
3,4,4'-TrCB	PCB 37	38444-90-5
3,4,5-TrCB	PCB 38	53555-66-1
3,4',5-TrCB	PCB 39	38444-88-1
2,2',3,3'-TeCB	PCB 40	38444-93-8
2,2',3,4-TrCB	PCB 41	52663-59-9
2,2',3,4'-TeCB	PCB 42	36559-22-5
2,2',3,5-TrCB	PCB 43	70362-46-8
2,2',3,5'-TeCB ³	PCB 44	41464-39-5
2,2',3,6-TrCB	PCB 45	70362-45-7
2,2',3,6'-TeCB	PCB 46	41464-47-5
2,2',4,4'-TeCB	PCB 47	2437-79-8

Table 1. Names, Congener Numbers, and CAS Registry Numbers for Native Chlorinated Biphenyl (CB) Congeners

CB congener ¹	Congener number	CAS Number
2,2',4,5-TeCB	PCB 48	70362-47-9
2,2',4,5'-TeCB	PCB 49	41464-40-8
2,2',4,6-TeCB	PCB 50	62796-65-0
2,2',4,6'-TeCB	PCB 51	68194-04-7
2,2',5,5'-TeCB ²	PCB 52	35693-99-3
2,2',5,6'-TeCB	PCB 53	41464-41-9
2,2',6,6'-TeCB	PCB 54	15968-05-5
2,3,3',4'-TeCB	PCB 55	74338-24-2
2,3,3',4'-TeCB	PCB 56	41464-43-1
2,3,3',5-TeCB	PCB 57	70424-67-8
2,3,3',5'-TeCB	PCB 58	41464-49-7
2,3,3',6-TeCB	PCB 59	74472-33-6
2,3,4,4'-TeCB	PCB 60	33025-41-1
2,3,4,5-TeCB	PCB 61	33284-53-6
2,3,4,6-TeCB	PCB 62	54230-22-7
2,3,4',5-TeCB	PCB 63	74472-34-7
2,3,4',6-TeCB	PCB 64	52663-58-8
2,3,5,6-TeCB	PCB 65	33284-54-7
2,3',4,4'-TeCB ²	PCB 66	32598-10-0
2,3',4,5-TeCB	PCB 67	73575-53-8
2,3',4,5'-TeCB	PCB 68	73575-52-7
2,3',4,6-TeCB	PCB 69	60233-24-1
2,3',4',5-TeCB	PCB 70	32598-11-1
2,3',4',6-TeCB	PCB 71	41464-46-4
2,3',5,5'-TeCB	PCB 72	41464-42-0
2,3',5',6-TeCB	PCB 73	74338-23-1
2,4,4',5-TeCB	PCB 74	32690-93-0
2,4,4',6-TeCB	PCB 75	32598-12-2
2',3,4,5-TeCB	PCB 76	70362-48-0
3,3',4,4'-TeCB ^{2,3}	PCB 77	32598-13-3
3,3',4,5-TeCB	PCB 78	70362-49-1
3,3',4,5'-TeCB	PCB 79	41464-48-6
3,3',5,5'-TeCB	PCB 80	33284-52-5
3,4,4',5-TeCB ⁶	PCB 81	70362-50-4
2,2',3,3',4-PeCB	PCB 82	52663-62-4
2,2',3,3',5-PeCB	PCB 83	60145-20-2
2,2',3,3',6-PeCB	PCB 84	52663-60-2
2,2',3,4,4'-PeCB	PCB 85	65510-45-4
2,2',3,4,5-PeCB	PCB 86	55312-69-1
2,2',3,4,5'-PeCB	PCB 87	38380-02-8
2,2',3,4,6-PeCB	PCB 88	55215-17-3
2,2',3,4,6'-PeCB	PCB 89	73575-57-2
2,2',3,4',5-PeCB	PCB 90	68194-07-0
2,2',3,4',6-PeCB	PCB 91	68194-05-8
2,2',3,5,5'-PeCB	PCB 92	52663-61-3
2,2',3,5,6-PeCB	PCB 93	73575-56-1
2,2',3,5,6'-PeCB	PCB 94	73575-55-0

Table 1. Names, Congener Numbers, and CAS Registry Numbers for Native Chlorinated Biphenyl (CB) Congeners

CB congener ¹	Congener number	CAS Number
2,2',3,5',6-PeCB	PCB 95	38379-99-6
2,2',3,6,6'-PeCB	PCB 96	73575-54-9
2,2',3',4,5-PeCB	PCB 97	41464-51-1
2,2',3',4,6-PeCB	PCB 98	60233-25-2
2,2',4,4',5-PeCB	PCB 99	38380-01-7
2,2',4,4',6-PeCB	PCB 100	39485-83-1
2,2',4,5,5'-PeCB ²	PCB 101	37680-73-2
2,2',4,5,6'-PeCB	PCB 102	68194-06-9
2,2',4,5,6'-PeCB	PCB 103	60145-21-3
2,2',4,6,6'-PeCB	PCB 104	56558-16-8
2,3,3',4,4'-PeCB ^{2,3}	PCB 105	32598-14-4
2,3,3',4,5-PeCB	PCB 106	70424-69-0
2,3,3',4',5-PeCB	PCB 107	70424-68-9
2,3,3',4,5'-PeCB	PCB 108	70362-41-3
2,3,3',4,6-PeCB	PCB 109	74472-35-8
2,3,3',4',6-PeCB	PCB 110	38380-03-9
2,3,3',5,5'-PeCB	PCB 111	39635-32-0
2,3,3',5,6-PeCB	PCB 112	74472-36-9
2,3,3',5',6-PeCB	PCB 113	68194-10-5
2,3,4,4',5-PeCB ⁶	PCB 114	74472-37-0
2,3,4,4',6-PeCB	PCB 115	74472-38-1
2,3,4,5,6-PeCB	PCB 116	18259-05-7
2,3,4',5,6-PeCB	PCB 117	68194-11-6
2,3',4,4',5-PeCB ^{2,3}	PCB 118	31508-00-6
2,3',4,4',6-PeCB	PCB 119	56558-17-9
2,3',4,5,5'-PeCB	PCB 120	68194-12-7
2,3',4,5,6'-PeCB	PCB 121	56558-18-0
2',3,3',4,5-PeCB	PCB 122	76842-07-4
2',3,4,4',5-PeCB ⁶	PCB 123	65510-44-3
2',3,4,5,5'-PeCB	PCB 124	70424-70-3
2',3,4,5,6'-PeCB	PCB 125	74472-39-2
3,3',4,4',5-PeCB ^{2,3}	PCB 126	57465-28-8
3,3',4,5,5'-PeCB	PCB 127	39635-33-1
2,2',3,3',4,4'-HxCB ³	PCB 128	38380-07-3
2,2',3,3',4,5-HxCB	PCB 129	55215-18-4
2,2',3,3',4,5'-HxCB	PCB 130	52663-66-8
2,2',3,3',4,6-HxCB	PCB 131	61798-70-7
2,2',3,3',4,6'-HxCB	PCB 132	38380-05-1
2,2',3,3',5,5'-HxCB	PCB 133	35694-04-3
2,2',3,3',5,6-HxCB	PCB 134	52704-70-8
2,2',3,3',5,6'-HxCB	PCB 135	52744-13-5
2,2',3,3',6,6'-HxCB	PCB 136	38411-22-2
2,2',3,4,4',5-HxCB	PCB 137	35694-06-5
2,2',3,4,4',5'-HxCB ²	PCB 138	35065-28-2
2,2',3,4,4',6-HxCB	PCB 139	56030-56-9
2,2',3,4,4',6'-HxCB	PCB 140	59291-64-4
2,2',3,4,5,5'-HxCB	PCB 141	52712-04-6

Table 1. Names, Congener Numbers, and CAS Registry Numbers for Native Chlorinated Biphenyl (CB) Congeners

CB congener ¹	Congener number	CAS Number
2,2',3,4,5,6-HxCB	PCB 142	41411-61-4
2,2',3,4,5,6'-HxCB	PCB 143	68194-15-0
2,2',3,4,5',6-HxCB	PCB 144	68194-14-9
2,2',3,4,6,6'-HxCB	PCB 145	74472-40-5
2,2',3,4',5,5'-HxCB	PCB 146	51908-16-8
2,2',3,4',5,6-HxCB	PCB 147	68194-13-8
2,2',3,4',5,6'-HxCB	PCB 148	74472-41-6
2,2',3,4',5',6-HxCB	PCB 149	38380-04-0
2,2',3,4',6,6'-HxCB	PCB 150	68194-08-1
2,2',3,5,5',6-HxCB	PCB 151	52663-63-5
2,2',3,5,6,6'-HxCB	PCB 152	68194-09-2
2,2',4,4',5,5'-HxCB ²	PCB 153	35065-27-1
2,2',4,4',5,6-HxCB	PCB 154	60145-22-4
2,2',4,4',6,6'-HxCB	PCB 155	33979-03-2
2,3,3',4,4',5-HxCB ³	PCB 156	38380-08-4
2,3,3',4,4',5'-HxCB ³	PCB 157	69782-90-7
2,3,3',4,4',6-HxCB	PCB 158	74472-42-7
2,3,3',4,5,5'-HxCB	PCB 159	39635-35-3
2,3,3',4,5,6-HxCB	PCB 160	41411-62-5
2,3,3',4,5',6-HxCB	PCB 161	74472-43-8
2,3,3',4',5,5'-HxCB	PCB 162	39635-34-2
2,3,3',4',5,6-HxCB	PCB 163	74472-44-9
2,3,3',4',5',6-HxCB	PCB 164	74472-45-0
2,3,3',5,5',6-HxCB	PCB 165	74472-46-1
2,3,4,4',5,6-HxCB	PCB 166	41411-63-6
2,3',4,4',5,5'-HxCB ³	PCB 167	52663-72-6
2,3',4,4',5,6-HxCB	PCB 168	59291-65-5
3,3',4,4',5,5'-HxCB ^{2,3}	PCB 169	32774-16-6
2,2',3,3',4,4',5-HpCB ²	PCB 170	35065-30-6
2,2',3,3',4,4',6-HpCB	PCB 171	52663-71-5
2,2',3,3',4,5,5'-HpCB	PCB 172	52663-74-8
2,2',3,3',4,5,6-HpCB	PCB 173	68194-16-1
2,2',3,3',4,5,6'-HpCB	PCB 174	38411-25-5
2,2',3,3',4,5',6-HpCB	PCB 175	40186-70-7
2,2',3,3',4,6,6'-HpCB	PCB 176	52663-65-7
2,2',3,3',4',5,6-HpCB	PCB 177	52663-70-4
2,2',3,3',5,5',6-HpCB	PCB 178	52663-67-9
2,2',3,3',5,6,6'-HpCB	PCB 179	52663-64-6
2,2',3,4,4',5,5'-HpCB ²	PCB 180	35065-29-3
2,2',3,4,4',5,6-HpCB	PCB 181	74472-47-2
2,2',3,4,4',5,6'-HpCB	PCB 182	60145-23-5
2,2',3,4,4',5',6-HpCB	PCB 183	52663-69-1
2,2',3,4,4',6,6'-HpCB	PCB 184	74472-48-3
2,2',3,4,5,5',6-HpCB	PCB 185	52712-05-7
2,2',3,4,5,6,6'-HpCB	PCB 186	74472-49-4
2,2',3,4',5,5',6-HpCB ²	PCB 187	52663-68-0
2,2',3,4',5,6,6'-HpCB	PCB 188	74487-85-7

Table 1. Names, Congener Numbers, and CAS Registry Numbers for Native Chlorinated Biphenyl (CB) Congeners

CB congener ¹	Congener number	CAS Number
2,3,3',4,4',5,5'-HpCB ³	PCB 189	39635-31-9
2,3,3',4,4',5,6-HpCB	PCB 190	41411-64-7
2,3,3',4,4',5,6'-HpCB	PCB 191	74472-50-7
2,3,3',4,5,5',6-HpCB	PCB 192	74472-51-8
2,3,3',4,5,5',6'-HpCB	PCB 193	69782-91-8
2,2',3,3',4,4',5,5'-OcCB	PCB 194	35694-08-7
2,2',3,3',4,4',5,6-OcCB ²	PCB 195	52663-78-2
2,2',3,3',4,4',5,6'-OcCB	PCB 196	42740-50-1
2,2',3,3',4,4',6,6'-OcCB	PCB 197	33091-17-7
2,2',3,3',4,5,5',6-OcCB	PCB 198	68194-17-2
2,2',3,3',4,5,5',6'-OcCB	PCB 199	52663-75-9
2,2',3,3',4,5,6,6'-OcCB	PCB 200	52663-73-7
2,2',3,3',4,5,6,6'-OcCB	PCB 201	40186-71-8
2,2',3,3',5,5',6,6'-OcCB	PCB 202	2136-99-4
2,2',3,4,4',5,5',6-OcCB	PCB 203	52663-76-0
2,2',3,4,4',5,6,6'-OcCB	PCB 204	74472-52-9
2,3,3',4,4',5,5',6-OcCB	PCB 205	74472-53-0
2,2',3,3',4,4',5,5',6-NoCB ²	PCB 206	40186-72-9
2,2',3,3',4,4',5,6,6'-NoCB	PCB 207	52663-79-3
2,2',3,3',4,5,5',6,6'-NoCB	PCB 208	52663-77-1
DeCB ²	PCB 209	2051-24-3

1. Abbreviations for chlorination levels (homologs)

MoCB	monochlorobiphenyl	HxCB	hexachlorobiphenyl
DiCB	dichlorobiphenyl	HpCB	heptachlorobiphenyl
TrCB	trichlorobiphenyl	OcCB	octachlorobiphenyl
TeCB	tetrachlorobiphenyl	NoCB	nonachlorobiphenyl
PeCB	pentachlorobiphenyl	DeCB	decachlorobiphenyl

2. National Oceanic and Atmospheric Administration (NOAA) congener of interest

3. World Health Organization (WHO) toxic congener

Table 2. Routine QC Checks

QC Check	Frequency	Acceptance Criterion	Study Requirements
Initial demonstration of capability	Once per matrix type and extraction technique	IPR %recovery and %RSD will be established after review of study data. Report all results as generated	Each laboratory will provide IPR data for each matrix type. Study data will be used to develop criteria
Initial calibration (ICAL), 6-point minimum	Once	%RSD of the RRFs should be \leq 20%. Report all results as generated.	ICAL data will be provided by each laboratory and compared to typical calibration criteria
Calibration verification (VER)	Initially and every 12 hrs. Initial Calibration can be used in place of initial VER if samples are analyzed within 12 hours of initial calibration.	VER RRFs should be within \pm 20% of the mean RRFs from the initial calibration. Report all results as generated.	VER data will be provided by each laboratory and compared to typical calibration criteria
Method blank	One per batch of 20 field samples or fewer	To be determined based on study data. Report all results as generated.	Perform as specified in the procedure
Laboratory control sample	One per preparation batch of 20 field samples or fewer	To be determined based on study data. Report all results as generated.	Perform as specified in procedure to demonstrate performance at the method-specified LCS concentration. Study data will be used to develop criteria
Matrix spike/ Matrix spike duplicate (MS/MSD)	One per preparation batch of 20 field samples or fewer	To be determined based on study data. Report all results as generated.	Not required for the method itself, since isotope dilution provides recovery data for every sample. For the study, the MS/MSD results will be used to demonstrate method performance. RPD between the MS and MSD analyses may be used to develop acceptance criteria for duplicate unspiked analyses.
Mass spectrometer tune (PFTBA)	Daily, at start up	To be determined based on study data. Report all results as generated.	Perform as specified in procedure
Mass calibration check (PFTBA)	Daily, at start up	<ul style="list-style-type: none"> • Peak drift for m/z 69, 219 and 502 $<$ 0.4 (or approx. 2 mm on the calibration printout). • Relative peak intensities (m/z 219 and 502 divided by m/z 69) between 50 – 150% 	Perform as specified in procedure
Qualitative Identification Criteria	All peaks in all analyses	<ul style="list-style-type: none"> • RRT within \pm 3 sec of VER calibration RRT • Ratio of 2 ions (Within 20% of theoretical) 	Perform as specified in procedure

Appendix A
Standards Required for the Validation Study

The standards in Tables A-1 to A-4 will be procured as custom mixtures from one or more commercial vendors to support this study. The standard mixes shown in Table A-5 are already commercially available.

Table A-1. Calibration Standard Solutions

Congener number	Calibration Standards (ng/mL)					
	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6
PCB-1	10	20	40	160	400	2,000
PCB-3	10	20	40	160	400	2,000
PCB-4	10	20	40	160	400	2,000
PCB-8	10	20	40	160	400	2,000
PCB-11	10	20	40	160	400	2,000
PCB-15	10	20	40	160	400	2,000
PCB-18	10	20	40	160	400	2,000
PCB-19	10	20	40	160	400	2,000
PCB-28	10	20	40	160	400	2,000
PCB-31	10	20	40	160	400	2,000
PCB-37	10	20	40	160	400	2,000
PCB-44	10	20	40	160	400	2,000
PCB-52	10	20	40	160	400	2,000
PCB-54	10	20	40	160	400	2,000
PCB-64	10	20	40	160	400	2,000
PCB-66	10	20	40	160	400	2,000
PCB-70	10	20	40	160	400	2,000
PCB-74	10	20	40	160	400	2,000
PCB-77	10	20	40	160	400	2,000
PCB-85	10	20	40	160	400	2,000
PCB-95	10	20	40	160	400	2,000
PCB-99	10	20	40	160	400	2,000
PCB-101	10	20	40	160	400	2,000
PCB-104	10	20	40	160	400	2,000
PCB-105	10	20	40	160	400	2,000
PCB-110	10	20	40	160	400	2,000
PCB-118	10	20	40	160	400	2,000
PCB-126	10	20	40	160	400	2,000
PCB-132	10	20	40	160	400	2,000
PCB-138	10	20	40	160	400	2,000
PCB-147	10	20	40	160	400	2,000
PCB-149	10	20	40	160	400	2,000
PCB-153	10	20	40	160	400	2,000
PCB-155	10	20	40	160	400	2,000
PCB-156	10	20	40	160	400	2,000
PCB-166	10	20	40	160	400	2,000
PCB-169	10	20	40	160	400	2,000
PCB-177	10	20	40	160	400	2,000
PCB-180	10	20	40	160	400	2,000
PCB-187	10	20	40	160	400	2,000
PCB-188	10	20	40	160	400	2,000
PCB-189	10	20	40	160	400	2,000
PCB-199	10	20	40	160	400	2,000

Table A-1. Calibration Standard Solutions

Congener number	Calibration Standards (ng/mL)					
	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6
PCB-202	10	20	40	160	400	2,000
PCB-205	10	20	40	160	400	2,000
PCB-206	10	20	40	160	400	2,000
PCB-208	10	20	40	160	400	2,000
PCB-209	10	20	40	160	400	2,000
¹³ C ₁₂ -PCB-1	400	400	400	400	400	400
¹³ C ₁₂ -PCB-3	400	400	400	400	400	400
¹³ C ₁₂ -PCB-4	400	400	400	400	400	400
¹³ C ₁₂ -PCB-11	400	400	400	400	400	400
¹³ C ₁₂ -PCB-15	400	400	400	400	400	400
¹³ C ₁₂ -PCB-19	400	400	400	400	400	400
¹³ C ₁₂ -PCB-28	400	400	400	400	400	400
¹³ C ₁₂ -PCB-37	400	400	400	400	400	400
¹³ C ₁₂ -PCB-52	400	400	400	400	400	400
¹³ C ₁₂ -PCB-54	400	400	400	400	400	400
¹³ C ₁₂ -PCB-70	400	400	400	400	400	400
¹³ C ₁₂ -PCB-77	400	400	400	400	400	400
¹³ C ₁₂ -PCB-85	400	400	400	400	400	400
¹³ C ₁₂ -PCB-101	400	400	400	400	400	400
¹³ C ₁₂ -PCB-104	400	400	400	400	400	400
¹³ C ₁₂ -PCB-118	400	400	400	400	400	400
¹³ C ₁₂ -PCB-126	400	400	400	400	400	400
¹³ C ₁₂ -PCB-138	400	400	400	400	400	400
¹³ C ₁₂ -PCB-153	400	400	400	400	400	400
¹³ C ₁₂ -PCB-155	400	400	400	400	400	400
¹³ C ₁₂ -PCB-169	400	400	400	400	400	400
¹³ C ₁₂ -PCB-180	400	400	400	400	400	400
¹³ C ₁₂ -PCB-188	400	400	400	400	400	400
¹³ C ₁₂ -PCB-189	400	400	400	400	400	400
¹³ C ₁₂ -PCB-202	400	400	400	400	400	400
¹³ C ₁₂ -PCB-205	400	400	400	400	400	400
¹³ C ₁₂ -PCB-206	400	400	400	400	400	400
¹³ C ₁₂ -PCB-208	400	400	400	400	400	400
¹³ C ₁₂ -PCB-209	400	400	400	400	400	400
¹³ C ₁₂ -PCB-8	400	400	400	400	400	400
¹³ C ₁₂ -PCB-79	400	400	400	400	400	400
¹³ C ₁₂ -PCB-162	400	400	400	400	400	400

Table A-2. Labeled Compound Standard Solution (1,250 ng/mL)

¹³ C ₁₂ -PCB-1	¹³ C ₁₂ -PCB-52	¹³ C ₁₂ -PCB-118	¹³ C ₁₂ -PCB-188
¹³ C ₁₂ -PCB-3	¹³ C ₁₂ -PCB-54	¹³ C ₁₂ -PCB-126	¹³ C ₁₂ -PCB-189
¹³ C ₁₂ -PCB-4	¹³ C ₁₂ -PCB-70	¹³ C ₁₂ -PCB-138	¹³ C ₁₂ -PCB-202
¹³ C ₁₂ -PCB-11	¹³ C ₁₂ -PCB-77	¹³ C ₁₂ -PCB-153	¹³ C ₁₂ -PCB-205
¹³ C ₁₂ -PCB-15	¹³ C ₁₂ -PCB-85	¹³ C ₁₂ -PCB-155	¹³ C ₁₂ -PCB-206
¹³ C ₁₂ -PCB-19	¹³ C ₁₂ -PCB-101	¹³ C ₁₂ -PCB-169	¹³ C ₁₂ -PCB-208
¹³ C ₁₂ -PCB-28	¹³ C ₁₂ -PCB-104	¹³ C ₁₂ -PCB-180	¹³ C ₁₂ -PCB-209
¹³ C ₁₂ -PCB-37			

Table A-3. Native Standard Spiking Solution (80 ng/mL)

PCB-1	PCB-52	PCB-105	PCB-169
PCB-3	PCB-54	PCB-110	PCB-177
PCB-4	PCB-64	PCB-118	PCB-180
PCB-8	PCB-66	PCB-126	PCB-187
PCB-11	PCB-70	PCB-132	PCB-188
PCB-15	PCB-74	PCB-138	PCB-189
PCB-18	PCB-77	PCB-147	PCB-199
PCB-19	PCB-85	PCB-149	PCB-202
PCB-28	PCB-95	PCB-153	PCB-205
PCB-31	PCB-99	PCB-155	PCB-206
PCB-37	PCB-101	PCB-156	PCB-208
PCB-44	PCB-104	PCB-166	PCB-209

Table A-4. Non-extracted Internal Standard Solution (1,000 ng/mL)

¹³ C ₁₂ -PCB-8	¹³ C ₁₂ -PCB-79	¹³ C ₁₂ -PCB-162
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Table A-5. **Retention Time Standards**

Recommended Mixtures								
#1	#2	#3	#4	#5	#6	#7	#8	#9
PCB-1	PCB-5	PCB-15	PCB-13	PCB-12	PCB-11	PCB-36	PCB-30	PCB-23
PCB-2	PCB-7	PCB-20	PCB-14	PCB-33	PCB-21	PCB-72	PCB-43	PCB-39
PCB-3	PCB-10	PCB-27	PCB-35	PCB-49	PCB-38	PCB-78	PCB-55	PCB-62
PCB-4	PCB-17	PCB-29	PCB-51	PCB-59	PCB-50	PCB-79	PCB-58	PCB-68
PCB-6	PCB-24	PCB-34	PCB-53	PCB-63	PCB-57	PCB-89	PCB-76	PCB-80
PCB-8	PCB-26	PCB-40	PCB-54	PCB-64	PCB-61	PCB-96	PCB-109	PCB-88
PCB-9	PCB-31	PCB-42	PCB-73	PCB-77	PCB-65	PCB-98	PCB-112	PCB-94
PCB-16	PCB-32	PCB-47	PCB-75	PCB-85	PCB-86	PCB-106	PCB-120	PCB-111
PCB-18	PCB-37	PCB-69	PCB-81	PCB-91	PCB-102	PCB-108	PCB-159	PCB-116
PCB-19	PCB-41	PCB-92	PCB-90	PCB-97	PCB-113	PCB-152	PCB-186	PCB-121
PCB-22	PCB-45	PCB-93	PCB-100	PCB-104	PCB-126	PCB-166	PCB-192	PCB-125
PCB-25	PCB-46	PCB-101	PCB-117	PCB-114	PCB-127	PCB-182	PCB-198	PCB-140
PCB-28	PCB-48	PCB-105	PCB-122	PCB-123	PCB-133	PCB-184		PCB-142
PCB-44	PCB-60	PCB-118	PCB-124	PCB-129	PCB-139	PCB-204		PCB-143
PCB-52	PCB-70	PCB-119	PCB-130	PCB-137	PCB-145			PCB-148
PCB-56	PCB-83	PCB-128	PCB-154	PCB-156	PCB-161			PCB-150
PCB-66	PCB-84	PCB-134	PCB-163	PCB-167	PCB-169			PCB-155
PCB-67	PCB-95	PCB-136	PCB-165	PCB-176	PCB-181			PCB-160
PCB-71	PCB-103	PCB-144	PCB-175	PCB-185				PCB-162
PCB-74	PCB-107	PCB-151	PCB-200	PCB-189				PCB-168
PCB-82	PCB-115	PCB-157	PCB-201					PCB-188
PCB-87	PCB-131	PCB-158	PCB-202					
PCB-99	PCB-132	PCB-190						
PCB-110	PCB-135	PCB-191						
PCB-138	PCB-141	PCB-207						
PCB-146	PCB-149	PCB-208						
PCB-147	PCB-164	PCB-209						
PCB-153	PCB-170							
PCB-173	PCB-171							
PCB-174	PCB-172							
PCB-177	PCB-178							
PCB-179	PCB-183							
PCB-180	PCB-193							
PCB-187	PCB-196							
PCB-194	PCB-197							
PCB-195	PCB-205							
PCB-199								
PCB-203								
PCB-206								

Appendix B

Sample Types for the Validation Study

Sample Matrix Types to be Obtained for the Study

Matrix Type	# Matrices	Approximate Amount	# Aliquots of each	Aliquot size
Wastewater	9	120 liters each	120*	1-liter
Soil/sediment	3	800 grams each	80	10-grams
Biosolids	3	400 grams each	80	5-grams
Fish tissue	3	800 grams each	80	10-grams
Total # Samples		1,800		

* The 120 wastewater aliquots will provide 80 aliquots for the separatory funnel extraction analyses (including 20 backup samples in the event of breakage or laboratory accidents) and an additional 40 aliquots to support possible solid-phase extraction analyses by a subset of the study participants.

Appendix C
Procedures for Derivation of QC Acceptance Criteria from the Validation Study Results

The information below has been excerpted from Reference 8.9 and all citations to section numbers and references apply to the original document, not this study plan. Not all of the calculations in the document may apply to this specific method study. The calculations used for this study will be adjusted for the actual number of laboratories involved.

Quality Control Acceptance Criteria Development for New Methods

Method Detection Limits and Minimum Levels

Each laboratory participating in the validation study must perform an MDL study as described in Section 3.1.1. The organization responsible for developing the new method must establish an MDL for the method, using a pooled MDL from the at least six laboratories. A pooled MDL is calculated from m individual laboratory MDLs by computing the square root of the mean of the squares of the individual MDLs and multiplying the result by a ratio of t -values to adjust for the increased degrees of freedom.

Note: The MDL values used in this calculation are those determined in each of the six or more laboratories. If one laboratory reports an MDL_s (from spiked samples), that value is used in conjunction with the MDL values from the other laboratories, including any values reported as MDL_b (from blanks).

$$MDL_{pooled} = \sqrt{\frac{d_1 \left(\frac{MDL_{Lab\ 1}}{t_{(0.99,d_1)}}\right)^2 + d_2 \left(\frac{MDL_{Lab\ 2}}{t_{(0.99,d_2)}}\right)^2 + \dots + d_m \left(\frac{MDL_{Lab\ m}}{t_{(0.99,d_m)}}\right)^2}{d_1 + d_2 + \dots + d_m}} \times t_{(0.99,[d_1+d_2+\dots+d_m])}$$

where:

m = The number of laboratories, and

d_i = The number of replicates used by Lab i to derive the MDL.

In the case of 9 laboratories with 7 replicates per laboratory, the equation simplifies to:

$$MDL_{pooled} = \sqrt{\frac{MDL_{Lab\ 1}^2 + MDL_{Lab\ 2}^2 + \dots + MDL_{Lab\ 9}^2}{9}} \times \frac{2.41}{3.14}$$

The organization responsible for developing the method must also use this MDL to develop an ML. Procedures for determining the ML are given in Section 3.1.1.

Calibration Linearity

The instrument or analytical system is then calibrated with six standards specified in the method to calculate an initial RSD for the response factor

The RSD and the RSD limit for the CF, RF, or RR is determined as follows:

1. Calculate the mean and standard deviation of the CFs, RFs, or RRs for each laboratory.

$$Mean\ Factor = \overline{Factor} = \frac{\sum_{i=1}^n Factor_i}{n}$$

$$s = \sqrt{\frac{\sum_{i=1}^n (\text{Factor}_i - \overline{\text{Factor}})^2}{n - 1}}$$

where:

Factor = The “Factor” terms are replaced by the CF, RF or RR terms, based on the quantitation approach described in the method in question, and
 n = The number of calibration points used in each laboratory.

2. Calculate the relative standard deviation of the CFs, RFs, or RRs of each laboratory and analyte as:

$$RSD_i = 100 \times \frac{s_i}{\overline{\text{Factor}}_i}$$

where s_i and $\overline{\text{Factor}}_i$ are the standard deviation and mean of the CFs, RFs, or RRs for laboratory i .

3. Calculate the pooled RSD of the CFs, RFs, or RRs for each analyte from *all* laboratories. The pooled RSD is calculated as the square root of the mean of the squares of the sample RSDs from each individual laboratory. For example, for nine laboratories, the pooled RSD is calculated as:

$$RSD_{pooled} = \sqrt{\frac{RSD_1^2 + RSD_2^2 + RSD_3^2}{3}}$$

4. Calculate RSD_{max} as the smaller of 35% and:

$$RSD_{max} = k(RSD_{pooled})$$

where:

k = The square root of the 95th percentile of an F distribution with $n - 1$ degrees of freedom in the numerator and $m(n - 1)$ degrees of freedom in the denominator,
 m = The number of laboratories, and
 n = The number of calibration points.

For nine laboratories using a five-point calibration ($m = 9$, $n = 5$), the value of k is 1.6. The maximum allowable specification for RSD_{max} is 35%.

Calibration Verification

As noted in Section 2.2., acceptance limits for calibration verifications can be determined in three different ways, each of which is described below.

The calibration verification criterion may be specified as a maximum relative distance between the mean CF, RF, or RR obtained by a future laboratory’s initial calibration ($\overline{\text{Factor}}$) and the CF, RF or RR obtained from its calibration verification standard (Factor_{VER}). The maximum allowable deviation is based on the pooled relative standard deviation (RSD_{pooled}) calculated in Section 3.2.2.

1. Determine k_{VER} by multiplying the 97.5th percentile of a Student’s t distribution with $(m[n-1])$ degrees of freedom times the square root of $(1+1/n)$, where there are n points in the calibration and m laboratories:

$$k_{VER} = t \sqrt{\left(1 + \frac{1}{n}\right)}$$

For a five-point calibration, the Student's *t* value is 2.0, resulting in combined multipliers of 2.4 for a three-point calibration, and 2.2 for a five-point calibration.

2. The calibration verification criterion for the new method would then be stated as the maximum percent difference as follows:

$$\text{Percent Difference} = 100 \times \left(\frac{\text{Factor}_{VER} - \overline{\text{Factor}}}{\overline{\text{Factor}}} \right) \leq k_{VER} \text{RSD}_{pooled}$$

where:

Factor = The "Factor" terms are replaced by the CF, RF or RR terms, based on the quantitation approach described in the method in question, and

For example, if the calibration verification criterion, calculated as $k_{VER} \text{RSD}_{pooled}$, equals 17%, then the difference between the $\overline{\text{Factor}}$ from the initial calibration and the Factor_{VER} from the calibration verification sample must be less than or equal to 17% of the $\overline{\text{Factor}}$.

When using either the concentration or the recovery approach, the calculations are very similar to those used for the "factor" limits shown above:

3. Calculate the upper and lower QC acceptance criteria for the known concentration of the analyte in the calibration verification standard, using the lower and upper percentages calculated in Step 2 above:

$$\text{Lower limit} = (\text{Lower Percentage in Step 2}) \times (\text{Known Concentration in Standard})$$

$$\text{Upper limit} = (\text{Upper Percentage in Step 2}) \times (\text{Known Concentration in Standard})$$

Alternatively, calibration verification criteria may be specified as the range of acceptable recoveries calculated for the analytes in the calibration verification standard, using the lower and upper percentages calculated in Step 2 above to create a window around 100% recovery.

Initial and Ongoing Precision and Recovery

For the IPR and OPR tests, QC acceptance criteria are calculated using the mean percent recovery and the standard deviation of recovery from the IPR tests of four aliquots of the reference matrix and the OPR test of one aliquot of the reference matrix (for a total of five samples) in nine laboratories. The QC acceptance criteria are developed using the following steps:

1. Calculate the mean percent recovery (\bar{X}) for each analyte, based on all data points from all laboratories, the between-laboratory standard deviation (s_b) of the mean results for each of the six or more laboratories (standard deviation of the nine laboratory means $\bar{X}_1 + \bar{X}_2 + \dots + \bar{X}_9$), and the pooled within-laboratory standard deviation (s_w). The value of s_w is calculated as the square root of the mean of all within-laboratory variances. For example, for nine laboratories:

$$s_b = \sqrt{\frac{\sum_{j=1}^m (\bar{X}_j - \bar{X})^2}{m - 1}}$$

where:

\bar{X}_j = The mean percent recovery for the *jth* laboratory

m = The number of laboratories, and

\bar{X} = The overall mean of the percent recoveries from all laboratories

$$s_w = \sqrt{\frac{s_1^2 + s_2^2 + \dots + s_9^2}{9}}$$

Note: CSRA will provide direction to the participating laboratories to ensure they are spiking IPR and OPR samples at the same concentration.

2. QC acceptance criteria for IPR recovery - Calculate the combined standard deviation for interlaboratory variability and estimation of the mean (s_c) as:

$$s_c = \sqrt{\left(1 + \frac{1}{m}\right) s_b^2 + \left(\frac{1}{4} - \frac{1}{n}\right) s_w^2}$$

where:

m = the number of laboratories, and

n = the number of data points per laboratory.

For 9 laboratories and 5 data points per laboratory, the calculation becomes:

$$s_c = \sqrt{\left(\frac{10}{9}\right) s_b^2 + \left(\frac{1}{20}\right) s_w^2}$$

3. Calculate the QC acceptance criteria for recovery in the IPR test by constructing a $\pm 2.3 s_c$ window around the mean percent recovery \bar{X} , where 2.3 is the 97.5th percentile Student's t value for 10 degrees of freedom (an estimated degrees of freedom based on the variance ratios observed with EPA Method 1625):

$$\text{Lower limit (\%)} = \bar{X} - 2.3s_c$$

$$\text{Upper limit (\%)} = \bar{X} + 2.3s_c$$

If more than 9 laboratories are used, the degrees of freedom for t will increase, but a complete calculation is beyond the scope of this document. An approximation of degrees of freedom equal to the number of laboratories will serve for most situations.

4. QC acceptance criterion for IPR precision - The maximum acceptable RSD for the four IPR aliquots is approximated by a 95% upper confidence limit around the observed RSD of the results from all of the laboratories. The RSD_{IPR} (computed as s_w divided by \bar{X}) is multiplied by the square root of a 95th percentile F value with 3 degrees of freedom in the numerator and $m(n - 1)$ degrees of freedom in the denominator, where m = the number of laboratories, and n is the number of data points per laboratory. For example, the resulting multiplier on the RSD for nine laboratories and five data points per laboratory will then be 1.7, and the QC acceptance criterion for precision in the IPR test is calculated as follows:

$$\text{Maximum } RSD_{IPR} = (1.7) \times RSD_{IPR}$$

5. QC acceptance criteria for OPR recovery - Calculate the combined standard deviation for interlaboratory variability and estimation of the mean (s_c) as:

$$s_c = \sqrt{\left(1 + \frac{1}{m}\right) s_b^2 + \left(1 - \frac{1}{n}\right) s_w^2}$$

where:

m = the number of laboratories, and
n = the number of data points per laboratory.

For 9 laboratories and 5 data points per laboratory,

$$s_c = \sqrt{\left(\frac{10}{9}\right)s_b^2 + \left(\frac{4}{5}\right)s_w^2}$$

6. Calculate the QC acceptance criteria for recovery in the OPR test by constructing a $\pm 2.1 s_c$ window around the mean percent recovery \bar{X} , where 2.1 is the 97.5th percentile Student's *t* value for 19 degrees of freedom (an estimated degrees of freedom based on the variance ratios observed with EPA Method 1625):

$$\text{Lower limit (\%)} = \bar{X} - 2.1s_c$$

$$\text{Upper limit (\%)} = \bar{X} + 2.1s_c$$

If more than 9 laboratories are used, the degrees of freedom for *t* will increase, but a complete calculation is beyond the scope of this document. An approximation of degrees of freedom equal to the number of laboratories will serve for most situations.

Matrix Spike and Matrix Spike Duplicate

Results of the MS/MSD analyses performed in the validation study are used to develop the MS/MSD QC acceptance criteria. Calculate the MS/MSD performance criteria as follows:

1. Calculate the mean and sample standard deviation of the recoveries of each MS/MSD pair, and then compute the overall mean recovery \bar{X} , the between-laboratory standard deviation (s_b) of the mean results for each of the nine laboratories, and the pooled within-laboratory standard deviation (s_w) for each target analyte using the MS and MSD analyses.

$$s_b = \sqrt{\frac{\sum_{j=1}^m (\bar{X}_j - \bar{X})^2}{m - 1}}$$

where:

\bar{X}_j = The mean percent recovery for the *jth* laboratory

m = The number of laboratories, and

\bar{X} = The overall mean of the percent recoveries from all laboratories

In order to allow for interlaboratory variability, calculate the combined standard deviation (s_c) for interlaboratory variability and estimation of the mean as:

$$s_c = \sqrt{\left(1 + \frac{1}{m}\right)s_b^2 + \frac{1}{2}s_w^2}$$

where:

m = the number of laboratories.

For nine labs, this becomes:

$$s_c = \sqrt{\left(\frac{10}{9}\right)s_b^2 + \left(\frac{1}{2}\right)s_w^2}$$

2. QC acceptance criteria for MS/MSD recovery - Calculate the QC acceptance criteria for recovery in the MS/MSD test by constructing a $\pm 2.2s_c$ window around the mean percent recovery (\bar{X}) using the combined standard deviation. This factor comes from a t value for an estimated 11 degrees of freedom (based on this experimental design and variance ratios observed in Method 1625):

$$Lower\ limit\ (\%) = \bar{X} - 2.2s_c$$

$$Upper\ limit\ (\%) = \bar{X} + 2.2s_c$$

If more than 9 laboratories are used, the degrees of freedom for t will increase, but a complete calculation is beyond the scope of this document. An approximation of degrees of freedom equal to the number of laboratories plus 2 will serve for most situations.

Note: For highly variable methods, it is possible that the lower limit for recovery for both the IPR and OPR analyses will be a negative number. In these instances, the data should either be log-transformed and the recovery window recalculated, or the lower limit established as “detected,” as was done with some of the methods in 40 CFR Part 136, Appendix A.

3. QC acceptance criteria for MS/MSD relative percent difference (RPD) - To evaluate a 95% upper confidence limit for precision, the RSD (computed using the pooled within-laboratory standard deviation s_w of the MS/MSD samples, divided by \bar{X} , is multiplied by the square root of the 95th percentile F value with 1 degrees of freedom in the numerator and m degrees of freedom in the denominator multiplied by the square root of 2 (i.e., $\sqrt{2}$), where m is the number laboratories. The resulting multiplier on the RSD for 3 laboratories will then be 3.2. The QC acceptance criterion for precision in the MS/MSD test (RPD_{max}) is calculated as follows:

$$RPD_{max} = 3.2 \text{ RSD}$$

Absolute and Relative Retention Time

Establishing QC acceptance criteria for RT and RRT precision is problematic when multiple laboratories are involved because laboratories have a tendency to establish the chromatographic conditions that suit their needs. Calculating mean RTs and RRTs based on different operating conditions will result in the establishment of erroneously wide windows. Therefore, it is advised that the organization developing the method specify to the participating laboratories the chromatographic conditions and columns to be used. Any future laboratories operating under different conditions will need to develop new acceptance criteria for RT and RRT precision.

Determine the mean retention time, \bar{RT} (and/or the mean relative retention time \bar{RRT}) and the standard deviation (s) of the RT and/or RRT for each analyte and standard. Determine the upper and lower retention time (or relative retention time) limits as follows:

$$Lower\ limit = \bar{RT} - \left(ts \sqrt{1 + \frac{1}{n}} \right)$$

$$Upper\ limit = \bar{RT} + \left(ts \sqrt{1 + \frac{1}{n}} \right)$$

where:

t = The 97.5th percentile of a t distribution with $n - 1$ degrees of freedom, and

n = The number of retention time or relative retention time values used.

The relative retention time upper and lower limits are determined by replacing \bar{RT} with \bar{RRT} in the equations above.

Blanks

Establish the QC acceptance criteria for blanks. The historical requirement has been that the concentration of an analyte in a blank must be below the ML or below one-third (1/3) the regulatory compliance level, whichever is higher. However, other limits (including those below the ML) may be used for a specific method. In instances where the level of the blank is close to the regulatory compliance level or the level at which measurements are to be made, it may be necessary to require multiple blank measurements and establish the QC acceptance criteria based on the mean of the blank measurements plus two standard deviations of the blank measurements.

Labeled Compound Recovery

The labeled compound recoveries from all of the samples analyzed in the validation study can be used to develop the labeled compound QC acceptance criteria. Calculate the labeled compound performance criteria as follows:

1. Calculate the mean and sample standard deviation of the recoveries of each labeled compound, and then compute the overall mean recovery \bar{X} , the between-laboratory standard deviation (s_b) of the mean results for each of the nine laboratories, and the pooled within-laboratory standard deviation (s_w) for each labeled compound.

$$s_b = \sqrt{\frac{\sum_{j=1}^m (\bar{X}_j - \bar{X})^2}{m - 1}}$$

where:

\bar{X}_j = The mean percent recovery for the j th laboratory

m = The number of laboratories, and

\bar{X} = The overall mean of the percent recoveries from all laboratories

In order to allow for interlaboratory variability, calculate the combined standard deviation (s_c) for interlaboratory variability and estimation of the mean as:

$$s_c = \sqrt{\left(1 + \frac{1}{m}\right)s_b^2 + \frac{1}{2}s_w^2}$$

where:

m = the number of laboratories.

For nine labs, this becomes:

$$s_c = \sqrt{\left(\frac{10}{9}\right)s_b^2 + \left(\frac{1}{2}\right)s_w^2}$$

2. QC acceptance criteria for labeled compound recovery - Calculate the QC acceptance criteria for recovery the labeled compounds by constructing a $\pm 2.2s_c$ window around the mean percent recovery (\bar{X}) using the combined standard deviation. This factor comes from a t value for an estimated 11 degrees of freedom (based on this experimental design and variance ratios observed in Method 1625):

$$\text{Lower limit (\%)} = \bar{X} - 2.2s_c$$

$$\text{Upper limit (\%)} = \bar{X} + 2.2s_c$$

If more than 9 laboratories are used, the degrees of freedom for t will increase, but a complete calculation is beyond the scope of this document. An approximation of degrees of freedom equal to the number of laboratories plus 2 will serve for most situations.