United States Environmental Protection Agency Region 10 Laboratory

7411 Beach Drive East, Port Orchard, WA 98366 (360) 871-8700 Fax: (360) 871-8747

EPA LABORATORY RECORD FOR FISH CONTAMINANT STUDY COLUMBIA RIVER BASIN

EPA SAMPLE ALIQUOT RECORD

Aliquot Samples Prepared by (name): _____

Date: _____ Time: _____

Comments:

_____
_____
_____
_____
_____

NUMBER OF ALIQUOTS PREPARED, LABELED AND SHIPPED

Assigned EPA Group Composite Sample Number	Composite Homogenate Weight (g)	Number of 2 Ounce Bottles					
		EPA Analyses		Contract Analyses		Archival	
		Need	Filled	Need	Filled	Need	Filled
EPA		6		4		16	
EPA		6		4		16	
EPA		6		4		16	
EPA		6		4		16	
EPA		6		4		16	
EPA		6		4		16	
EPA		6		4		16	
EPA		6		4		16	
EPA		6		4		16	
EPA		6		4		16	

EPA Sample Aliquots and Archival Aliquots
Should be sent to address listed below:

US EPA
Region 10 Laboratory
7411 Beach Dr. East
Port Orchard, Washington 98366
(360) 871-8700 contact:
Fax (360) 871-8747

STATEMENT OF WORK

A. EPA Region/Client: Region 10

B. Authorized By: _____

C. Prepared By: ROBERT MELTON

D. Date: _____

E. Site Name: _____

1. General description of analytical service requested:

Note: This SOW is written for the measurement of marine tissue samples. The SOW is customized to meet the Data Quality Objectives (DQOs) of the applicable Quality Assurance Project Plan (QAPP) for the Assessment of Chemical Contaminants In Fish Consumed By Four American Indian Tribes In The Columbia River Basin, Revision 5.0., 06/03/96 Therefore, the SOW requires special procedures in the extraction, clean-up, analysis, and reporting of data in order to meet the DQOs of the project. The use of the letters, xxxx, in the following text requires input from the user of the SOW in order to comply with the specifications of the QAPP.

This SOW requires the high resolution capillary column gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) analyses for Polychlorinated Dibenzo-p-Dioxins (PCDDs) and Polychlorinated Dibenzo-p-Furans (PCDFs) such as 2,3,7,8-tetrachlorodibenzo-p-dioxin, 2,3,7,8-tetrachlorodibenzo-p-furan, tetra through octa polychlorinated dibenzodioxin homologues, and tetra through octa polychlorinated dibenzofuran homologues listed in EPA Method 1613B in xxxx fish tissue samples plus one Performance Evaluation (PE) fish tissue sample using EPA Method 1613B: Tetra- through Octa- Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS. All the performance specifications of Method 1613B shall be used and achieved by the laboratory.

Percent lipid determination for all marine tissue samples as per Method 1613B is required. Confirmation of 2,3,7,8-Tetrachloro-p-dibenzofuran is required using a dissimilar capillary GC column such as DB-225, SP-2330, or equivalent. Measurement results for 2,3,7,8-TCDF must be made from the confirmation column. This requires full calibration (both

initial and continuing calibration) of the confirmation column in order to report 2,3,7,8-TCDF results. The laboratory must provide written documentation that all quality control (QC) requirements of Method 1613B have been met.

2. Definition and number of work units involved (specify whether whole samples or fractions; whether organics or inorganics; whether aqueous or marine tissue; and whether low, medium or high concentration):

xxxx low-level fish tissue samples plus one Performance Evaluation (PE) sample will be submitted for PCDD/PCDF measurements. In addition, the laboratory will purchase and analyze two samples each (total of six PE samples) of fish matrix reference material PE samples EDF-2524, EDF-2525, and EDF-2526 in the same manner and at the same time that project samples are measured. Accuracy requirements of acceptable recovery ranges for these PE samples have been documented by Cambridge Isotope Laboratories. These acceptable accuracy recovery ranges will be required by the laboratory which measures PCDDs/PCDFs. The estimated cost of procuring two alliquotes each of PE samples EDF-2524, EDF-2525, and EDF-2526 is approximately $2100. This cost should be added to the laboratory's bid price for completion of this SOW.

The subcontract laboratory which is responsible for measuring PCDDs/PCDFs will measure one sample each of PE samples EDF-2524, EDF-2525, and EDF-2526 when the first Sample Delivery Group is measured using Methods 1613B. The Contractor will designate a second SDG during the latter phase of the project for the subcontract laboratory to measure one sample each of PE samples EDF-2524, EDF-2525, and EDF-2526.

3. Purpose of analysis (specify whether Superfund (enforcement or remedial action), RCRA, NPDES, etc.):

xxxx -- The samples will be collected by EPA to assess chemical contaminant exposure from consumption of Columbia River fish by four Native American Tribes. The first phase of this study was completed in October of 1994 by the Columbia River Inter-Tribal Fish Commission (CRITFC).

4. Estimated date(s) of collection:

The samples will be collected between xxxx and xxxx.

5. Estimated date(s) and method of shipment:

The samples will be shipped via Federal Express during the week(s) listed in item 4. Tissue samples are to be kept at minus (-) 20 degrees C until analysis. Marine tissue samples are to be maintained at -20 °C for six months by the lab. All tissue sample amounts that remain after extraction is completed and extract solutions shall be retained by the lab at -20 °C for a period of one year from date of sample arrival. EPA has the right to request these remaining sample amounts and extracts for a period of one year from the time of sample arrival. Two samples each of PE samples EDF-2524, EDF-2525, and EDF-2526 will be procured directly by the laboratory for this project.

6. Number of days analysis and data required after laboratory receipt of samples:

The complete data package is required within 35 days of Validated Time of Sample Receipt of the last sample in each Sample Delivery Group (SDG). Tissue samples shall be extracted within 30 days of sample collection. All sample extracts shall be injected within 40 days from date of extraction.

7. Analytical instrumentation and protocols required:

Project samples and PE samples are to be prepared, analyzed, confirmed, documented and reported as specified in EPA Method 1613B: Tetra- through Octa- Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, except as is specified in this SOW for laboratory services. The use of other analytical methods is not be acceptable for this work. Only labs with experience using this method shall perform this work. Labs bidding on this SOW are required to submit a Lab Quality Assurance Plan (Lab QAP) and Standard Operating Procedures (SOPs) for the measurement of project samples and the PE sample using Method 1613B and this SOW. Labs bidding on this SOW are required to own and use two high resolution GC/MS instruments for this work because second GC column TCDF confirmation and calibration is required by this SOW.

8. Special technical instructions (if outside protocol requirements, specify compound names, CAS numbers, detection limits, etc.):

The compounds to be reported are listed in Table 1 of Method 1613B.

The laboratory which measures project samples will use the following procedure prior to removing a ground sample from a sample bottle for analysis of target compounds:

- Place sample container containing ground fish tissue/eggs in a 34°C to 40°C refrigerator 24 hours prior to removing sample.

- Remove sample bottle from the refrigerator and place on the lab bench at room temperature until all ice crystals in the sample bottle have melted.

- Hand stir the thawed tissue vigorously with a 1/4 inch solid glass rod for 3 minutes.

- Immediately remove sample containing tissue and liquid from sample bottle for weighing and laboratory analysis.

- Fill out a Corrective Action Form (see Attachment 18 to the QAPP) if any sample bottles contain contain either chunks of fish tissue or pieces of fish skin. A copy of this Corrective Action Form must be sent to the Contractor Project Manager.

  * The Laboratory shall achieve a Minimum (Quantitation) Limit (ML) of 0.2 ng/Kg (wet weight) for isomers 2,3,7,8-TCDD and 2,3,7,8-TCDF. This lower ML shall be achieved by the use of a low initial calibration point of 0.1 ng/ml and an ultra-low sensitivity HRMS system.

  * Final volume of sample extracts is 20 uL or lower.

  * This Initial Calibration of the instrument system for both the primary and secondary confirmation GC column must be determined within 30 days of the time that the first sample in each SDG is measured on the GC/MS system. All labeled and native standards used to measure initial calibration standards, method blanks, verification standards, calibration verification standards, and sample extracts must be from the same lot number and preparation date.

  * Fortify project samples and the PE sample with isotopically labeled $^{13}C_{12}$-PCDD and $^{13}C_{12}$-PCDF internal calibration standards as is specified in Method 1613B.

* Measure specified PCDDs and PCDFs in sample extracts using the cleanup procedure and isotopically labeled recovery standards specified in Method 1613B. The lowest level of the initial calibration standards shall be at or below the required Minimum Quantitation Limits (MQLs) specified in this SOW.

* All instructions in Method 1613B shall be followed for all aspects of sample analyses, including but not limited to:

1) Preparation, storage and analysis of all standards.

2) Preparation and storage of all project samples and the PE sample.

3) Cleanup, storage and analysis of all sample extracts.

4) Instrument calibration.

5) Quality Assurance/Quality Control.

6) The option in the method for reporting the analytical results using a 2,3,7,8-TCDD Toxicity Equivalency Factor (TEF) must be used.

7) Second GC column confirmation and measurement of 2,3,7,8-TCDF on the secondary GC column which is calibrated for TCDF measurements is required when 2,3,7,8-TCDF is detected on the primary column. This requires full calibration (both initial and continuing calibration) of the confirmation column in order to report 2,3,7,8-TCDF results.

8) If polychlorinated diphenyl ether (PCDPE) interferences to the measurement of PCDF isomers are present after initial cleanup and analysis procedures are used, then the laboratory must remove these PCDPE interferences prior to final analysis of the extracts using the PCDPE cleanup procedure described in Method 1613B. If the PCDPE interferences are still present after additional PCDPE cleanup steps, then the laboratory must contact the Contractor for instructions.

9) The laboratory shall trace and report the accuracy of the initial calibration curve and of calibration verification standards by measuring a Quality Control

(QC) Check Sample which originates from a source which is different from the source of standards used for the initial calibration curve and calibration verification standards.

9. Analytical results required (if known, specify format for data sheets, QA/QC reports, Chain-of-Custody documentation, etc.) If not completed, format of results will be left to program discretion.

The data package shall include all original documentation generated in support of this Statement of Work and Method 1613B. This includes, but is not limited to: sample tags, custody records, shipping information, standards and sample preparation records, instrument printouts such as chromatograms, extracted ion current profiles (EICPs), quality control requirements, precision and accuracy requirements, etc. When information and documentation required by this SOW or Method 1613B is recorded in permanently bound notebooks and in computer files, copies of the appropriate information shall be submitted to EPA as part of data deliverables

The following additional deliverables are required. Note that the following requirements are specified in order to emphasize general documentation requirements and are not intended to supersede or change requirements of Method 1613B:

* The lab must submit a copy of the analytical contract, the original sample packing list, chain-of-custody records, sample log-in records, and a Case Narrative describing the analyses and discussing any and all problems experienced during the analyses. The Case Narrative shall include a discussion of the presence of any interferences, the steps used to remove PCDPE interferences from extracts, the criteria used to qualitatively identify target isomers, and the failure of the lab to meet any of the requirements of the SOW or Method 1613B. The lab shall also submit Sample tags, custody seals, chain-of-custody records, and laboratory log in records with the data package. In addition, the data package shall contain the following records and data:

* Analyst bench records describing dilutions, weighing of project samples and the PE sample, sample size, final extract volumes, amount injected, and example calculations such that an independent data reviewer may recreate the calculations from the raw data which is submitted with the

data package.

* Detailed explanation of the quantitation and identification procedure used for each of the homologous series and for isomer specific analysis.

* Example calculations of response ratios (RRFs), sample results and detection limits.

* Tabulated recoveries of spiked labeled PCDDs and PCDFs, Internal Standards, Cleanup Standards, and Surrogates used to measure each sample.

* Standard curve RFs, RRFs and %RSDs for initial and calibration verification.

* Simultaneous offset display of single ion chromatograms (EICPs) for each GC column for analyte peaks and for polychlorinated diphenyl ether (PCDPE) peaks in order to check for PCDPE interferences which may co-elute with native target compounds. The hard copy of the EICP of PCDPE interference peak to 2,3,7,8-TCDF must be expanded on both GC columns to between 50 to 100% full scale in order to visually inspect for PCDPE interference to the measurement of 2,3,7,8-TCDF.

* Tabulated sample detection limits for analytes which are not measured in each sample.

* Deliverables to the Region shall be in the form of a purge file - i.e. **paginated original documents**, not copies of original documents. If an original document cannot be provided in each SDG, then the exact location of the original shall be stamped or recorded in ink on the copy.

After delivery of analytical results and data to EPA, the laboratory shall respond within seven days to written requests from EPA for additional information or explanations that result from the Government's inspection activities. Submissions of re-calculated data, missing deliverables, etc. shall be paginated for easy inclusion into the purge file. For example, if a Form I was left out of the purge file and should have followed page 5555, the submission of the missing page should have page 5556 or page 5555a recorded depending upon whether page 5556 has already been assigned to another page in the purge file.

* All Sample Tracking Reports (i.e. the signed chain-of-custody forms and the signed packing lists).

* DC-1 (Sample Log-In Form)

* DC-2 (Inventory Sheet) - This provides a Table of Contents for data sections in the Case File Purge.

* All of the Sample tags.

* The custody seals.

* A **copy** (not the original) of the SOW.

* Any telephone logs referring to the project samples and the PE sample.

* A Case Narrative signed by the laboratory manager or his/her designee certifying the accuracy and validity of all data reported and describing any changes to requirements in Method 1613B and in the SOW and problems encountered during the analyses along with documenting their resolution(s). **In addition:** any pre-award conditions/specifications accepted by a regional representative shall be documented in or attached to each case narrative. In the Case Narrative, the laboratory manager shall acknowledge that these measurement results are submitted in support of US EPA regulations and/or programs.

* Tabulated sample results, with units, percent solids, and sample weights or volumes clearly specified.

* Blank data with tabulated results. Specify which samples go with which blank.

* Submit all GPC cleanup calibration and extract run information.

* Submit all additional sample cleanup records and data.

* Sample data including:

  - Tabulated results.
  - All data system printouts.
  - Manual worksheets.

* Raw QC data including:

  - Blank data in chronological order:
  I) Tabulated results.
  ii) All blank data system printouts.

  - Initial Precision and Accuracy data as required

by Method 1613B.

- Calibration Verification data.

- Ongoing Precision and Accuracy data.

- Results from the measurement of the QC Check Sample and six PE samples (two each of EDF-2524, EDF-2525, and EDF-2526.

* Detailed explanation of the quantitation and identification procedure used for each of the homologous series and for isomer specific analysis.

* List of exact ion masses, response factors and retention times used for each isomer/class.

* Tabulated recoveries of Labeled Internal Standards and Clean-up Standards compared to the concentration used.

* Calibration curve(s) labeled with date and time of preparation.

* Standard curve RFs, RRFs and %RSDs for initial and calibration verification standards.

* EICPs of performance check mixtures showing first and last eluting compounds of each homologous series as well as the percent valley resolution for labeled TCDD and TCDF isomers as is required by Method 1613B.

* Complete documentation of initial and calibration verification and samples to include tabulated results of ion ratios and offset simultaneous displays of the single ion chromatograms of the two most abundant ions in the molecular ion region.

* Bench sheets for sample preparation indicating dates, times, methods of sample digestion/preparation and analysis, and volumes/amounts/concentrations of standard and reagents added, instrument run time/date, dilutions made, etc. Submit preparation/weight logs for percent moisture and percent lipid determinations. All bench sheets and logs will be labeled with the date and shall bear the analyst's signature.

* A formula (including definitions) showing how measurement results were calculated, with examples of actual calculations of response ratios (RRFs), sample results and detection limits.

Ship all regional deliverables to:

Laura Castrilli
USEPA Region 10 9th Floor
1200 Sixth Avenue MS/ES-095
Seattle, WA 98101

10. Other (use additional sheets or attach supplementary information as needed):

All hardcopy data reports and raw data shall be clear and legible. If discrepancies are found, the laboratory shall be required to resubmit non-compliant data or reports at no additional cost to the Government within 10 days after request by EPA.

11. Name of sampling/shipping contact:
Phone:

xxxx

12. Data Requirements

Minimum (Quantitation) Limits (MLs)

The following are required MLs:

Media, Units	Tetra CDDs/CDFs	Penta-Hepta CDDs/CDFs	Octa CCDs/CDFs
tissue ng/kg	0.2	5.0	10.0

13. Additional QC Requirements of the SOW

[1] The sum of the area counts for the two quantitation masses listed in Table 8 of Method 1613B for each of the two instrument recovery internal standards for samples, blanks, and standards (such as OPR standards and VER standards) must not vary by more than a factor of two (-50% to +100%) from the associated average areas of the five initial calibration standards.

14. Action Required if Limits are Exceeded

Laboratory:

If any QC limits specified in Method 1613B or in this SOW are exceeded, the laboratory must contact EPA for resolution.

EPA Region 10 SOP For the Validation of Polychlorinated Dibenzodioxin (PCDD) and Polychlorinated Dibenzofuran (PCDF) Data

EPA Region 10
Environmental Services Division
1200 Sixth Avenue
Seattle, WA 98101

Revision 2.0
January 31, 1996

APPROVAL:

Quality Assurance Manager:

_____ _____
Barry Towns Date:

Region 10 Chemists:

_____ _____
Peggy Knight Date:

_____ _____
Robert Melton Date:

TABLE OF CONTENTS

EPA Region 10 SOP For the Validation of Polychlorinated Dibenzodioxin (PCDD) and Polychlorinated Dibenzofuran (PCDF) Data

The Office of Quality Assurance (OQA) of EPA Region 10 has developed the following guidelines which should be used to access the quality of PCDD and PCDF data from samples originating from Region 10 sampling sites. This SOP is based upon the data validation principles specified in National Functional Guidelines For Organic Data Review, December, 1990, and the quality control (QC) requirements of EPA Method 1613B, October, 1994, and EPA Method 8290, Revision 0, 9/94.

The EPA Analytical Operations Branch (AOB) of the Hazardous Site Evaluation Division in EPA Headquarters recently prepared a draft SOP for the validation of dioxin/furan data using low resolution GC/MS and Contract Laboratory Program (CLP) protocol, DFLM01.1. The title of this AOB SOP is, National Functional Guidelines For Dioxin/Furan Data Validation, January, 1996. This draft SOP does not apply for the validation of high resolution GC/MS data from EPA Methods 1613B and 8290, because CLP protocol DFLM01.1 uses a different procedure to calibrate the GC/MS system, and because the quality control requirements of CLP protocol DFLM01.1 are very different from the QC approach in high resolution methods 1613B and 8290. Therefore, National Functional Guidelines For Dioxin/Furan Data Validation, January, 1996, will not be used as the basis for the validation of Method 1613B and Method 8290 high resolution GC/MS data in EPA Region 10.

The validator of PCDD and PCDF data should obtain a copy of the site-specific Quality Assurance Project Plan (QAPP) and use the Data Quality Objectives and QA requirements of the QAPP to assess the data. This SOP requires that the following criteria be evaluated when determining the quality of high resolution PCDD and PCDF data:

1.0 HOLDING TIME AND PRESERVATION OF SAMPLES

1.1 Objective. To determine the validity of the measurement results based upon EPA requirements for preservation and holding time of the samples from day of collection to day of extraction. EPA also has holding time requirements for extracts which is the time from extraction of the samples to injection of the sample extracts.

1.2 Criteria. Holding time and preservation requirements for the measurement of 2,3,7,8-TCDD in water samples under the CWA (40CFR Part 136), SDWA, and RCRA have been promulgated and codified under 40CFR. These regulations require that

water samples be preserved by neutralizing any chlorine residual with 0.008% sodium thiosulfate, and cooling to 4°C using a holding time of 7 days from day of collection to day of extraction of the sample. In addition, the maximum holding time of extracts is 40 days from day of extraction to day of injection of the extract.

The holding time and preservation requirements of 2,3,7,8-TCDD and of other measured PCDD and PCDF isomers in non-water matrixes have not been promulgated by EPA. Therefore, the data validator should use the holding time specified in the EPA approved site-specific Quality Assurance Project Plan (QAPP).

> Method 8290, Revision 9/94 specifies that all samples, except fish and adipose tissue samples, must be stored at 4°C in the dark, extracted within 30 days, and completely analyzed within 45 days of extraction. Fish and adipose samples must be stored at -20°C in the dark, extracted within 30 days, and completely analyzed within 45 days of collection (see Section 6.4 of Method 8290).
>
> Method 1613B does not set holding times for PCDD or PCDF isomers. The Method does state that water samples which contain a chlorine residual should be treated with 80-mg of sodium thiosulfate per liter of water, samples should be maintained at 4°C in the dark, and extracts should be analyzed within 40 days of extraction.
>
> Method 1613B, October, 1994, has recommended a holding time of one year for tissue samples which are frozen at < -10°C. Once frozen tissue samples are thawed, tissue samples must be extracted within 24 hours.

It should be noted that the above reference data validation SOP, National Functional Guidelines For Dioxin/Furan Data Validation, January, 1996, does not address either holding time or preservation requirements for environmental samples which are measured for PCDDs/PCDFs.

1.3 Action. If **40CFR Part 136** and the QAPP for the samples do not specify a holding time, then the holding time which is recommended by applicable EPA method -- Method 1613B or EPA Method 8290, Revision 9/94, should be used. Whenever samples or extracts are analyzed after holding time expiration date, the results should be considered to be minimum concentrations and must be qualified with a "J3". Samples which are not preserved correctly should be qualified with a "J" flag.

2.0 GC/MS PERFORMANCE CHECK

2.1 Objective. Gas chromatograph/mass spectrometer (GC/MS) instrument performance checks are performed to ensure mass resolution, identification, and to

some degree, sensitivity. These criteria are not sample specific. Conformance is determined using standard materials, therefore, these criteria should be met in all circumstances.

2.2 Criteria. For the PFK molecular leak, the resolution must be greater than or equal to 10,000. The deviation between the exact mass and the theoretical mass (Table 3 in 1613B) for each of the three to five ions monitored must be less than 5 ppm. If the mass spectrometer is adjusted the resolution must be tested again and the resolution documented. (1613B/10.1.2.2; 8290/7.6.2.2)

The mass spectrometer shall be operated in a mass-drift correction mode using PFK to provide lock-masses. Each lock-mass shall be monitored and shall not vary by more than +/-20% throughout each respective time window. (1613B/10.2.1.2)

Ion abundance ratios. All labeled and unlabeled PCDDs and PCDFs in the CS1 standard shall be within the QC limits in 1613B Table 3A or 8290 Table 8 for their respective ion abundance ratios. (1613B/10.2.2; 8290/7.7.2.3)

The HRGC/HRMS must meet the minimum levels in 1613B Table 2. All labeled and unlabeled analytes in the CS1 calibration standard must have signal to noise ratios greater than or equal to 10.0. (1613B/10.2.3)

The absolute retention time of $^{13}C_{12}$-1,2,3,4-TCDD shall exceed 25.0 minutes on the DB-5 column, and the retention time of $^{13}C_{12}$-1,2,3,4-TCDD shall exceed 15.0 minutes on the DB-225 column. (1613B/10.2.4)

The compound pairs in the window defining mixtures shall be determined and meet the elution requirements of Table 5. (1613B/10.3)

The height of the valley between the most closely eluted isomers and the 2,3,7,8-substituted isomers shall be less than 25% (1613B/10.4.2)

2.3 Action. Failure to meet either the resolution or the retention window criteria invalidates all calibration or sample data collected during the 12 hour time window.

3.0 INITIAL CALIBRATION

3.1 Objective. Compliance requirements for satisfactory instrument calibration are established to ensure that the instrument is capable of producing acceptable qualitative and quantitative data for PCDDs and PCDFs. Initial calibration demonstrates that the instrument is capable of producing a linear calibration curve.

3.2 Criteria. There shall be an initial calibration curve consisting of five points for each analyte. The initial calibration curve shall be determined less than 30 days from the time the first samples of a Sample Delivery Group (SDG) are measured by the lab. The lab shall use the same calibration standards with the same lot number, for all internal standards, and labeled standards used in measuring the initial calibration curve, verification standards, field samples, and method blanks on both the primary GC column and on the secondary confirmation GC column. If an analyte is calculated by the isotope dilution method, an averaged response factor may be used if the RSD is less than 20% For analytes calculated by the internal standard method, an averaged response factor may be used if the RSD is less than 35%. Otherwise, for either calculation method, the complete curve must be used (1613B/10.5.4). [There is a variance with 8290 which requires 20% and 30% respectively and also requires the use of the average RF.]

3.3 Action. If the Initial Calibration Curve is older that 30 days, or if internal standards or labeled standards used in measuring of the initial calibration curve, verification standards, field samples, and method blanks on both the primary GC column and on the secondary confirmation GC column or not from the same lot number, then all measurement data should be qualified with a "J" qualifier and non-detects qualified "UJ".

If the RSD exceeds 20% for those analytes analyzed by isotope dilution or 35% for those analytes analyzed by the internal standard method qualify positive results with "J", and non-detects qualified "UJ". At the reviewer's discretion, a more in-depth review may be conducted to minimize data qualification by examining the entire curve and the quantitation method used.

4.0 CALIBRATION VERIFICATION MEASUREMENTS

4.1 Objective. Compliance requirements for satisfactory instrument calibration are established to ensure that the instrument remains capable of producing acceptable qualitative and quantitative data.

4.2 Criteria. The individual analytes shall meet the acceptance criteria in Table 7 of 1613B. [Method 8290 requires that the RRFs of the unlabeled analytes to be within 20% and the labeled analytes to be within 30%.

It should be noted that CLP protocol DFLM01.1 require that the GC/MS system must be calibrated based upon a daily Calibration Check Standard, whereas, EPA Methods 1613B and 8290 required that the GC/MS system the criteria of a daily calibration verification standard must be met with each 12-hour batch of samples measured, and

that responsed factors for native target compounds are derived from the 5-point initial calibration."

4.3 Action. The reviewer should use professional judgement to determine if it is necessary to qualify the data. The following are guidelines:

> If the %D for an analyte is outside the acceptance window qualify positive results "J" and non-detected "UJ" for that analyte. If the ion abundance criteria are not met results qualify all results for that analyte "R".

5.0 SYSTEM PERFORMANCE

5.1 Objective. The performance of the method by the laboratory is examined by determination of their initial ability to perform the method (Initial Precision and Recovery (IPR) study) and demonstration of continuing ability to perform the analysis (PAR). See Section 8.2 of Method 1613B for requirements of IPR data.

As part of measuring system performance, Methods 1613B and 8290 require that samples and standards be measured within require QC limits. QC criteria such as required relative retention times of labeled and native isomers, theoretical ion abundance ratios, recovery limits for OPR and VER standards, and recovery limits for spiked labeled target compounds must be met in order to demonstrate that the measurement system is within control limits.

5.2 Criteria. Initial precision and accuracy. All cleanup steps used in processing samples shall be included in the IPR study. All analytes shall be within the IPR limits in Table 7 of 1613B. Note that Method 8290 does not require a IPR study.

Ongoing Precision and recovery (PAR). There will be one PAR sample for each sample set analyzed. All analytes must meet the PAR limits in Table 7. [There are no requirements for PAR samples in Method 8290.]

QC limits such as required relative retention times of labeled and native isomers, theoretical ion abundance ratios, recovery limits for OPR and VER standards, and recovery limits for spiked labeled target compounds must be within control limits of the method.

5.3 Action. Results for analytes which do not meet either IPR or PAR requirements should be qualified with either "J" or "UJ".
If an analyte is not recovered for an PAR sample, results must be qualified with an "R" for that analyte. Failure to meet QC limits of the method may result in measurement

values to be qualified with a "J" or "UJ". In specific cases where major QC limits are exceeded, the data validator may determine that the measurement system is out of control, which would require that measurement results be qualified with a "J", "UJ", or "R" flag.

6.0 METHOD BLANKS

6.1 Objective. To determine the existence and magnitude of contamination of samples resulting from laboratory activities. The criteria for evaluation of blanks will apply to any blank associated with the samples, including any method blanks, instrument blanks, field equipment blanks, transfer blanks, trip blanks, or solvent blanks.

6.2 Criteria.

1. The criteria for the frequency of extraction and analysis of method blanks as stated in section 8.5 of Method 1613B shall be followed and demonstrated in the documented data. The maximum amount of PCDD and PCDF isomer contamination in method blanks is stated in Table 2 of Method 1613B.

2. The method blank must be measured on each GC/MS system which is used to measure a group of samples. This requirement includes measuring method blanks on a second GC column if confirmatory analysis of sample extracts on a second column is required by the method or by the Lab SOW.

6.3 Action. If the maximum contamination requirements of specific TCDD and TCDF isomers stated in Table 2 of Method 1613B are not met, then all isomers in all samples associated with a method blanks shall be qualified with a "J1" flag. If the frequency of measuring method blanks is not met by the laboratory in the data submitted, then the results of all samples which do not meet the frequency of extraction and measurement of method blanks shall be qualified with a "R" flag. Any PCDD or PCDF measurement in a sample that is also measured in any associated blank, is qualified with a "U" flag if the sample concentration is less than 5 times the blank concentration.

7.0 RECOVERY OF C-13 LABELED ISOTOPE DILUTION INTERNAL STANDARDS

7.1 Objective. Labeled PCDDs and PCDFs are added to each sample and method blank prior to extraction. The role of these C-13 labeled spiked compounds is to be an internal standard for the quantitation of native PCDD and PCDF isomers, and to serve as surrogates for the assessment of method performance in the sample matrix.

7.2 Criteria. The recovery of each C-13 labeled PCDD and PCDF isomer using Method 1613B must be within 25-150%. The acceptable recovery limits for Method 8290 data must be between 40 and 135%.

7.3 Action. If any of the 15 labeled percent recoveries are outside the guideline windows for individual analytes, the individual isomer for that sample will be qualified with a "J" flag. For non-detected PCDD and PCDF compounds whose percent recoveries are outside the guideline windows for individual analytes, these will be qualified with a "UJ" flag.

8.0 INSTRUMENT RECOVERY INTERNAL STANDARDS

8.1 Objective. The purpose of adding the two instrument recovery internal standards ($^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD) prior to injecting sample extracts and standards into the GC/MS is to determine the recovery efficiency of the extraction and cleanup procedures, to determine if the GC/MS sensitivity and response are stable during every analytical run, and to determine if the same amount of extract was injected into the GC/MS.

8.2 Criteria. The sum of the area counts of two masses for each of the two instrument recovery internal standards for samples, blanks, and standards must not vary by more than a factor of four (-25% to +400%) from the sum of the associated average areas from the five initial calibration standards.

8.3 Action. The reviewer should use professional judgement to determine if it is necessary to qualify the data. The following are guidelines:

1. If the sum of the two quantitation area counts of either of the two instrument recovery standards in the samples or blanks are outside the -25% to +400% window, then positive results for compounds measured should be qualified with a "J".

2. If the sum of the two quantitation area counts is greater that 400%, then non-detected compounds should not be qualified.

3. If the sum of the two area counts is less than 25%, then non-detect compounds should be qualified with a "UJ".

4. If the sum of the area counts is less than 10%, then non-detect target compounds should be qualified with a "R".

9.0 PROJECT AND REGIONAL QUALITY ASSURANCE SAMPLES

9.1 Objective: The data validator should consider the data of samples which are identified as field duplicates, transfer blanks, trip blanks, blind spikes, blind blanks, and performance evaluation (PE) samples.

9.2 Criteria. If QA samples are included among the field samples for measurement by the laboratory, then the data validator should refer to the applicable QAPP for any QA requirements regarding QA samples. Results from the measurement of project and regional QA samples will reflect upon the ability of the laboratory to report analytical results of known and documented quality which meet the PARCC requirements of the QAPP.

9.3 Action. The data validator should recommend action in accordance with Regional specifications and the criteria for acceptable PE sample results. Poor performance by the laboratory on blind PE samples may indicate that the laboratory analytical system is out of control, or that the amount of PCDD and PCDF isomers reported by the laboratory is an estimated quantity. The data validator should use her/his professional judgement to assess if "J" or "R" qualifiers should be placed upon the data due to the measurement of QA or PE samples.

10.0 COMPOUND IDENTIFICATION

10.1 Objective. The qualitative criteria for target compound identification have been established by EPA Method 1613B and EPA Method 8290 to minimize the number of erroneous identifications. An erroneous identification can be either a false-positive (reporting a target compound when it is not present in the sample), or false-negative (not reporting a compound that is present in the sample). The addition of single or double blind PE samples among field samples provides ancillary data to support the laboratory's ability to meet QAPP objectives.

10.2 Criteria. EPA Method 1613B and EPA Method 8290 specify certain requirements and guidelines for the positive identification of certain PCDD and PCDF isomers. The most frequently encountered interfering compounds to the measurement of PCDDs and PCDFs are chlorinated substances such as polychlorinated diphenyl ethers (PCDPEs), polychlorinated biphenyls (PCBs), polychlorinated alkyldibenzofurans, and polychlorinated napthalenes that may be found at concentrations several orders of magnitude higher than the analytes of interest. Interferences are such a major problem to Methods 1613B and 8290, that each method requires that PCDPE interference ions be scanned at the same time that PCDD and PCDF mass ions are measured. Both methods require that certain PCDF isomers such as 2,3,7,8-TCDF be measured on a second dissimilar GC column before specific TCDF identifications can be made.

In this part of the SOP for the validation of PCDD and PCDF data, the following criteria must be met for a GC peak to be identified as a PCDD or PCDF (either unlabeled or labeled compound):

1. The signals for the two exact m/z's being monitored must be present, and must maximize within plus or minus 2 seconds of one another (see 1613B/Section 15.1; 8290/Section 7.8.4.1.4).

2. The signal-to-noise ratio (S/N) of each of the two exact m/z's must be greater than or equal to 2.5 for a sample extract, and greater than or equal to 10 for a calibration standard (see 1613B/Section 15.2; 8290/Section 7.8.4.3).

3. The ratio of the integrated ion currents (EICPs) of both the exact m/z's monitored must be within the limits of the method (see 1613B/Section 15.3; 8290/Section 7.8.4.2).

4. The relative retention time (RRT) of the peaks representing a unlabeled 2,3,7,8 substituted PCDD or PCDF must be within the limits given in the method. The retention time (RT) of peaks representing non-2,3,7,8-substituted PCDDs or PCDFs must be within the RT windows established in the method (see 1613B/Section 15.4; 8290/Section 7.8.4.1.1).

5. The measurement of 2,3,7,8-TCDF on the primary DB-5 GC column must be confirmed by analysis on a confirmatory column such as DB-225, SP-2330, DB-DIOXIN, or equivalent. All QC requirements of the method must be met on both the primary and secondary GC columns (see 1613B/Section 15.5; 8290/Section 3.4). If a PCDPE interference peak to the measurement of 2,3,7,8-TCDF is detected on the secondary GC column, then the laboratory should remove PCDPE interferences by additional cleanup procedures such as is described in one of the following references:

a) Method 1613B, October, 1994, Section 13.1.2 and Section 13.4 (Alumina column cleanup).

b) J. R. Ryan, R. Lizotte, and W. H. Newsome, J. of Chromatography,

303 (1984) 351-360 (Activated Florisil column cleanup.

6. If non-PCDPE interferences to the measurement of 2,3,7,8-TCDF on the secondary GC column are present, then the laboratory should measure 2,3,7,8-TCDF on a third dissimilar GC column in order to separate the 2,3,7,8-TCDF peak from the non-PCDPE interference peak. Measurement of 2,3,7,8-TCDF on a third dissimilar GC column requires full calibration (both initial and calibration verification) on the third GC column.

7. The identification of a GC peak (on either primary or confirmatory GC column) as a PCDF can only be made if no signal having a S/N $\geq$ 2.5 is detected at the same retention time (± 2 seconds) in the corresponding polychlorinated diphenyl ether (PCDPE) channel. This criteria requires that the laboratory document the EICP of all PCDPE m/z's which are scanned (see 8290/Section 7.8.4.4).

8. The retention times of target compounds must be verified using reference standards before identifications can be determined (see 8290/Section 3.3).

9. The valley height between 2,3,7,8-TCDD and the other TCDD isomers at m/z 319.8965, and between 2,3,7,8-TCDF and the other TCDF isomers at m/z 303.9016 shall not exceed 25% on their respective columns (see 1613B/Section 14.4.2.2; 8290/Section 7.9.7.1.1 and 7.9.7.1.2).

10.3 Action. The validator of the data must use his/her professional judgement in evaluating the data using the above identification criteria. Generally, if all of the above criteria for the identification of PCDD and PCDF isomers are not met, then each reported positive measurement of a PCDD or PCDF isomer should be considered a non-detect, and therefore flagged with a "R" flag. The "R" flag in this case is based upon the fact that the presence of the isomer in the sample can not be corroborated by the laboratory data.

11.0 LABORATORY CONTACTS

Provide and attached to the validation memo a copy of all telephone logs and correspondence with the laboratory concerning the quality of data submitted by the laboratory.

12.0 OVERALL ASSESSMENT OF THE QUALITY OF THE DATA

12.1 Objective. The overall assessment of a data package is a brief narrative in which the data reviewer expresses concerns and comments of the quality of the data. The overall assessment of the data should be made after the data validator considers the DQOs and other QA requirements of the site-specific QAPP. It should be noted that the data reviewer does not determine or report the useability of the data. This determination is made by the Site Manager and by the other users of the data.

12.2 Criteria. The criteria for overall assessment is the QA and DQO criteria of the QAPP and the criteria listed above in this data validation SOP.

12.3 Action. Use professional judgement to determine if there is a need to further qualify the data. Write a brief narrative to give the user an indication of any analytical limitations of the data. Note if there are any inconsistencies observed between the raw data and the laboratory reported sample results.

DATA QUALIFIER DEFINITIONS

U - The analyte was analyzed for, but was not detected above the sample quantitation limit. The associated numerical value indicates the approximate concentration necessary to detect the analyte in this sample.

If a decision requires quantitation of the analyte below the associated numerical level, reanalysis or alternative analytical methods should be considered.

J - The analyte was analyzed for and was positively identified, but the associated numerical value may not be consistent with the amount actually present in the environmental sample.

A subscript may be appended to the "J" that indicates which of the following quality control criteria were not met:

J1 Blank Contamination: indicates possible high bias and/or false positives.

J2 Calibration range exceeded: indicates possible low bias.

J3 Holding times not met: indicates low bias for most analytes with the exception of common laboratory contaminants and chlorinated ethenes (i.e.: trichloroethene, 1,1-dichloroethene, vinyl chloride).

J4 Other QC parameter outside control limits: bias not readily determined.

J5 Other QC parameter outside control limits. The reported results appear to be biased high. The actual value of target compound in the sample may be lower than the value reported by the laboratory.

J6 Other QC parameter outside control limits. The reported results appear to be biased low. The actual value of target compound in the sample may be higher than the value reported by the laboratory.

J7 2,3,7,8-TCDF is reported from the value measured on the primary GC Column, DB-5. The reported value on the primary GC column may be biased high because other TCDF isomers may elute at this same retention time. The actual value of 2,3,7,8-TCDF in the sample may be lower than the value reported by the laboratory due to possible co-elution of other TCDF isomers on the primary GC column.

J8 The measurement of 2,3,7,8-TCDF on the secondary GC column used by the Laboratory appears to have chemical interferences which co-elute with the native 2,3,7,8-TCDF GC peak. Therefore, the value of 2,3,7,8-TCDF on the secondary GC column is rejected and is qualified with a "R" flag. Consequently, the measured value of 2,3,7,8-TCDF on the primary GC column should be used as the measured value of 2,3,7,8-TCDF in the sample. The reported value of 2,3,7,8-TCDF on the primary GC column is qualified with a "J8", and may be biased high because other TCDF isomers may elute at this same retention time. The actual value of 2,3,7,8-TCDF on the primary GC column may be lower than the value reported by the laboratory due to possible co-elution of other TCDF isomers.

R - The sample results are rejected due to serious deficiencies in the ability to analyze the sample and meet critical quality control criteria. The presence or absence of the analyte cannot be verified.

Resampling and reanalysis are necessary to confirm or deny the presence of the analyte.

UJ - The analyte was analyzed for and was not detected above the reported quantitation limit. However, the reported quantitation limit is approximate and may or may not represent the actual limit of quantitation necessary to accurately and precisely measure the analyte in this sample.

If a decision requires quantitation of the analyte close to the associated numerical level, reanalysis or alternative analytical methods should be considered.

METHOD 1668

Toxic Polychlorinated Biphenyls by Isotope Dilution High Resolution Gas Chromatography/High Resolution Mass Spectrometry

March 1997

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

This method has been reviewed by the Engineering and Analysis Division, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Note: *This method is a draft based on preliminary validation in a single laboratory. In surveys of several laboratories using this method, EPA has found that it is normal for background levels of certain congeners that are found in high concentrations in PCBs to be present in the analytical systems in this method. Therefore, the concentrations of certain congeners in calibration and other solutions have been adjusted for these backgrounds. EPA welcomes constructive suggestions for improvement of this method.*

Introduction

Method 1668 was developed by the United States Environmental Protection Agency's Office of Science and Technology for congener-specific determination of the toxic co-planar and mono-ortho-substituted polychlorinated biphenyls (PCBs) in aqueous, solid, and tissue matrices by isotope dilution, high resolution capillary column gas chromatography (HRGC)/high resolution mass spectrometry (HRMS).

Questions and comments concerning this method or its application should be addressed to:

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Method 1668

Toxic Polychlorinated Biphenyls by Isotope Dilution High Resolution Gas Chromatography/High Resolution Mass Spectrometry

1.0 Scope and Application

1.1 This method is for determination of the toxic polychlorinated biphenyls (PCBs) in water, soil, sediment, sludge, tissue, and other sample matrices by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). The method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act. The method is based on a compilation of methods from the technical literature (References 1-3) and on EPA Method 1613.

1.2 The toxic PCBs listed in Table 1 (Reference 4) and other specific congeners may be determined by this method.

1.3 The detection limits and quantitation levels in this method are usually dependent on the level of interferences rather than instrumental limitations. The minimum levels (MLs) in Table 2 are the levels at which the PCBs can be determined with only common laboratory interferences present. The Method Detection Limit (MDL) for PCB #126 has been determined as 40 pg/L (picograms/Liter; parts-per-quadrillion) in water using this method.

1.4 The GC/MS portions of this method are for use only by analysts experienced with HRGC/HRMS or under the close supervision of such qualified persons. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 9.2.

1.5 This method is performance-based. The analyst is permitted to modify the method to overcome interferences or lower the cost of measurements, provided that all performance criteria in this method are met. The requirements for establishing method equivalency are given in Section 9.1.2.

1.6 Any modification of this method, beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR Parts 136.4 and 136.5.

2.0 Summary of Method

Flow charts that summarize procedures for sample preparation, extraction, and analysis are given in Figure 1 for aqueous and solid samples, Figure 2 for multi-phase samples, and Figure 3 for tissue samples.

2.1 Extraction.

2.1.1 Aqueous samples (samples containing less than 1% solids)—Stable isotopically labeled analogs of the toxic PCBs are spiked into a 1-L sample, and the sample is vacuum-filtered through a glass-fiber filter on top of a solid-phase extraction (SPE) disk. Sample components on the filter and disk are eluted with methylene chloride and the eluant is concentrated for cleanup.

2.1.2 Solid, semi-solid, and multi-phase samples (but not tissue)—The labeled compounds are spiked into a sample containing 10 g (dry weight) of solids. Samples containing multiple phases are pressure filtered and any aqueous liquid is discarded. Coarse solids are ground or homogenized. Any non-aqueous liquid from multi-phase samples is combined with the solids and extracted in an SDS extractor. The extract is concentrated for cleanup.

2.1.3 Fish and other tissue—A 20-g aliquot of sample is homogenized, and a 10-g aliquot is spiked with the labeled compounds. The sample is mixed with sodium sulfate, allowed to dry for 12-24 hours, and extracted for 18-24 hours using methylene chloride:n-hexane (1:1) in a Soxhlet extractor. The extract is evaporated to dryness, and the lipid content is determined.

2.2 After extraction, samples are cleaned up using back-extraction with sulfuric acid and/or base, and gel permeation, silica gel, Florisil and activated carbon chromatography. High-performance liquid chromatography (HPLC) can be used for further isolation of specific isomers or congeners. Prior to the cleanup procedures cited above, tissue extracts are cleaned up using an anthropogenic isolation column.

2.3 After cleanup, the extract is concentrated to near dryness. Immediately prior to injection, internal standards are added to each extract, and an aliquot of the extract is injected into the gas chromatograph. The analytes are separated by the GC and detected by a high-resolution ($\geq$10,000) mass spectrometer. Two exact m/z's are monitored for each analyte.

2.4 An individual PCB congener is identified by comparing the GC retention time and ion-abundance ratio of two exact m/z's with the corresponding retention time of an authentic standard and the theoretical or acquired ion-abundance ratio of the two exact m/z's. Isomer specificity for the toxic PCBs is achieved using GC columns that resolve these congeners from the other PCBs.

2.5 Quantitative analysis is performed using selected ion current profile (SICP) areas in one of two ways:

2.5.1 For PCBs with labeled analogs (see Table 1), the GC/MS system is calibrated, and the concentration of each compound is determined using the isotope dilution technique.

2.5.2 For PCBs without labeled compounds, the GC/MS system is calibrated, and the concentration of each compound is determined using the internal standard technique.

2.6 The quality of the analysis is assured through reproducible calibration and testing of the extraction, cleanup, and GC/MS systems.

3.0 Definitions

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

4.0 Contamination and Interferences

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Where possible, reagents are cleaned by extraction or solvent rinse. The non-coplanar PCB congeners 105, 114, 118, 123, 156, 157, 167, and 180 have been shown to be very difficult to completely eliminate from the laboratory at the MDLs in this method, and baking of glassware in a kiln or furnace at 450-500 °C may be necessary to remove these and other contaminants.

4.2 Proper cleaning of glassware is extremely important because glassware may not only contaminate the samples but may also remove the analytes of interest by adsorption on the glass surface.

4.2.1 Glassware should be rinsed with solvent and washed with a detergent solution as soon after use as is practical. Sonication of glassware containing a detergent solution for approximately 30 seconds may aid in cleaning. Glassware with removable parts, particularly separatory funnels with fluoropolymer stopcocks, must be disassembled prior to detergent washing.

4.2.2 After detergent washing, glassware should be rinsed immediately; first with methanol, then with hot tap water. The tap water rinse is followed by another methanol rinse, then acetone, and then methylene chloride.

4.2.3 Baking of glassware in kiln or other high temperature furnace (450-500 °C) may be warranted after particularly dirty samples are encountered. However, baking should be minimized, as repeated baking of glassware may cause active sites on the glass surface that may irreversibly adsorb PCBs.

4.2.4 Immediately prior to use, the Soxhlet apparatus should be pre-extracted with toluene for approximately 3 hours (see Sections 12.3.1-12.3.3). The solid-phase extraction apparatus (Section 6.4.1.5) should be rinsed with methylene chloride/toluene (80/20 mixture).

4.3 All materials used in the analysis shall be demonstrated to be free from interferences by running reference matrix method blanks (Section 9.5) initially and with each sample batch (samples started through the extraction process on a given 12-hour shift to a maximum of 20 samples).

4.3.1 The reference matrix must simulate as closely as possible the sample matrix under test. Ideally, the reference matrix should not contain the PCBs in detectable amounts, but should contain potential interferents in the concentrations expected to be found in the samples to be analyzed.

4.3.2 When a reference matrix that simulates the sample matrix under test is not available, reagent water (Section 7.6.1) can be used to simulate water samples; playground sand (Section 7.6.2) or white quartz sand (Section 7.3.2) can be used to simulate soils; filter paper (Section 7.6.3) can be used to simulate papers and similar materials; and corn oil (Section 7.6.4) can be used to simulate tissues.

4.4 Interferences coextracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled. Interfering compounds may be present at concentrations several orders of magnitude higher than the PCBs. The most frequently encountered interferences are chlorinated dioxins and dibenzofurans, methoxy biphenyls, hydroxydiphenyl ethers, benzylphenyl ethers, polynuclear aromatics, and pesticides. Because very low levels of PCBs are measured by this method, the elimination of interferences is essential. The cleanup steps given in Section 13 can be used to reduce or eliminate these interferences and thereby permit reliable determination of the PCBs at the levels shown in Table 2.

4.5 Each piece of reusable glassware should be numbered to associate that glassware with the processing of a particular sample. This will assist the laboratory in tracking possible sources of contamination for individual samples, identifying glassware associated with highly contaminated samples that may require extra cleaning, and determining when glassware should be discarded.

4.6 Cleanup of tissue—The natural lipid content of tissue can interfere in the analysis of tissue samples for the PCBs. The lipid contents of different species and portions of tissue can vary widely. Lipids are soluble to varying degrees in various organic solvents and may be present in sufficient quantity to overwhelm the column chromatographic cleanup procedures used for cleanup of sample extracts. Lipids must be removed by the lipid removal procedures in Section 13.6, followed by Florisil (Section 13.7), and carbon (Section 13.4) as minimum additional cleanup steps.

5.0 Safety

5.1 The toxicity or carcinogenicity of each chemical used in this method has not been precisely determined; however, each compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

5.1.1 The PCBs have been tentatively classified as known or suspected human or mammalian carcinogens. On the basis of the available toxicological and physical properties of the PCBs, pure standards should be handled only by highly trained personnel thoroughly familiar with handling and cautionary procedures and the associated risks.

5.1.2 It is recommended that the laboratory purchase dilute standard solutions of the analytes in this method. However, if primary solutions are prepared, they shall be prepared in a hood, and a NIOSH/MESA approved toxic gas respirator shall be worn when high concentrations are handled.

5.2 The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should also be made available to all personnel involved in these analyses. It is also suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this method and that the results of this monitoring be made available to the analyst. Additional information on laboratory safety can be found in References 5-8. The references and bibliography at the end of Reference 8 are particularly comprehensive in dealing with the general subject of laboratory safety.

5.3 The pure PCBs and samples suspected to contain these compounds are handled using essentially the same techniques employed in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from State Departments of Health or Labor, many of which have an industrial health service. Each laboratory must develop a strict safety program for handling these compounds. The practices in Reference 11 for handling chlorinated dibenzo-*p*-dioxins and dibenzofurans (CDDs/CDFs) are also recommended for handling the toxic PCBs.

5.3.1 Facility—When finely divided samples (dusts, soils, dry chemicals) are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leak tight or in a fume hood demonstrated to have adequate air flow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in the case of an accident.

5.3.2 Protective equipment—Disposable plastic gloves, apron or lab coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be used. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters. Eye protection equipment (preferably full face shields) must be worn while working with exposed samples or pure analytical standards. Latex gloves are commonly used to reduce exposure of the hands. When handling samples suspected or known to contain high concentrations of the PCBs, an additional set of gloves can also be worn beneath the latex gloves.

5.3.3 Training—Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

5.3.4 Personal hygiene—Hands and forearms should be washed thoroughly after each manipulation and before breaks (coffee, lunch, and shift).

5.3.5 Confinement—Isolated work areas posted with signs, segregated glassware and tools, and plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Effluent vapors—The effluents of sample splitters from the gas chromatograph (GC) and from roughing pumps on the mass spectrometer (MS) should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high-boiling alcohols to condense PCB vapors.

5.3.7 Waste Handling—Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel must be trained in the safe handling of waste.

5.3.8 Decontamination.

5.3.8.1 Decontamination of personnel—Use any mild soap with plenty of scrubbing action.

5.3.8.2 Glassware, tools, and surfaces—Chlorothene NU Solvent is a less toxic solvent that should be effective in removing PCBs. Satisfactory cleaning may be accomplished by rinsing with Chlorothene, then washing with any detergent and water. If glassware is first rinsed with solvent, then the dish water may be disposed of in the sewer. Given the cost of disposal, it is prudent to minimize solvent wastes.

5.3.9 Laundry—Clothing known to be contaminated should be collected in plastic bags. Persons who convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. The clothing may be put into a washer without contact if the launderer knows of the potential problem. The washer should be run through a cycle before being used again for other clothing.

5.3.10 Wipe tests—A useful method of determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper. Extraction and analysis by GC with an electron capture detector (ECD) can achieve a limit of detection of 0.1 g per wipe; analysis using this method can achieve an even lower detection limit. Less than 0.1 µg per wipe indicates acceptable

cleanliness; anything higher warrants further cleaning. More than 10 µg on a wipe constitutes an acute hazard and requires prompt cleaning before further use of the equipment or work space, and indicates that unacceptable work practices have been employed.

6.0 Apparatus, Equipment and Supplies

Note: *Brand names, suppliers, and part numbers are for illustration purposes only and no endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here. Meeting the performance requirements of this method is the responsibility of the laboratory.*

6.1 Sampling equipment for discrete or composite sampling.

6.1.1 Sample bottles and caps.

6.1.1.1 Liquid samples (waters, sludges and similar materials containing 5% solids or less)—Sample bottle, amber glass, 1.1-L minimum, with screw cap.

6.1.1.2 Solid samples (soils, sediments, sludges, paper pulps, filter cake, compost, and similar materials that contain more than 5% solids)—Sample bottle, wide mouth, amber glass, 500-mL minimum.

6.1.1.3 If amber bottles are not available, samples shall be protected from light.

6.1.1.4 Bottle caps—Threaded to fit sample bottles. Caps shall be lined with fluoropolymer.

6.1.1.5 Cleaning.

6.1.1.5.1 Bottles are detergent water washed, then solvent rinsed before use.

6.1.1.5.2 Liners are detergent water washed and rinsed with reagent water (Section 7.6.1).

6.1.2 Compositing equipment—Automatic or manual compositing system incorporating glass containers cleaned per bottle cleaning procedure above. Only glass or fluoropolymer tubing shall be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing shall be thoroughly rinsed with methanol, followed by repeated rinsing with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

6.2 Equipment for glassware cleaning—Laboratory sink with overhead fume hood.

6.3 Equipment for sample preparation.

6.3.1 Laboratory fume hood of sufficient size to contain the sample preparation equipment listed below.

6.3.2 Glove box (optional).

6.3.3 Tissue homogenizer—VirTis Model 45 Macro homogenizer (American Scientific Products H-3515, or equivalent) with stainless steel Macro-shaft and Turbo-shear blade.

6.3.4 Meat grinder—Hobart, or equivalent, with 3- to 5-mm holes in inner plate.

6.3.5 Equipment for determining percent moisture.

6.3.5.1 Oven—Capable of maintaining a temperature of 110 ±5 °C.

6.3.5.2 Desiccator.

6.3.6 Balances.

6.3.6.1 Analytical—Capable of weighing 0.1 mg.

6.3.6.2 Top loading—Capable of weighing 10 mg.

6.4 Extraction apparatus.

6.4.1 Water samples.

6.4.1.1 pH meter, with combination glass electrode.

6.4.1.2 pH paper, wide range (Hydrion Papers, or equivalent).

6.4.1.3 Graduated cylinder, 1-L capacity.

6.4.1.4 Liquid/liquid extraction—Separatory funnels, 250-, 500-, and 2000-mL, with fluoropolymer stopcocks.

6.4.1.5 Solid-phase extraction.

6.4.1.5.1 1-L filtration apparatus, including glass funnel, frit support, clamp, adapter, stopper, filtration flask, and vacuum tubing (Figure 4). For wastewater samples, the apparatus should accept 90 or 144 mm disks. For drinking water or other samples containing low solids, smaller disks may be used.

6.4.1.5.2 Vacuum source capable of maintaining 25 in. Hg, equipped with shutoff valve and vacuum gauge.

6.4.1.5.3 Glass-fiber filter—Whatman GMF 150 (or equivalent), 1 micron pore size, to fit filtration apparatus in Section 6.4.1.5.1.

6.4.1.5.4 Solid-phase extraction disk containing octadecyl (C_{18}) bonded silica uniformly enmeshed in an inert matrix—Fisher Scientific 14-378F (or equivalent), to fit filtration apparatus in Section 6.4.1.5.1.

6.4.2 Soxhlet/Dean-Stark (SDS) extractor (Figure 5 and Reference 12) for filters and solid/sludge samples.

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

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

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

6.4.2.4 Heating mantle—Hemispherical, to fit 500-mL round-bottom flask (Cal-Glass LG-8801-112, or equivalent).

6.4.2.5 Variable transformer—Powerstat (or equivalent), 110-volt, 10-amp.

6.4.3 Beakers—400- to 500-mL.

6.4.4 Spatulas—Stainless steel.

6.5 Filtration apparatus.

6.5.1 Pyrex glass wool—Solvent-extracted by SDS for 3 hours minimum.

6.5.2 Glass funnel—125- to 250-mL.

6.5.3 Glass-fiber filter paper—Whatman GF/D (or equivalent), to fit glass funnel in Section 6.5.2.

6.5.4 Drying column—15- to 20-mm ID Pyrex chromatographic column equipped with coarse-glass frit or glass-wool plug.

6.5.5 Buchner funnel—15-cm.

6.5.6 Glass-fiber filter paper for Buchner funnel above.

6.5.7 Filtration flasks—1.5- to 2.0-L, with side arm.

6.5.8 Pressure filtration apparatus—Millipore YT30 142 HW, or equivalent.

6.6 Centrifuge apparatus.

6.6.1 Centrifuge—Capable of rotating 500-mL centrifuge bottles or 15-mL centrifuge tubes at 5,000 rpm minimum.

6.6.2 Centrifuge bottles—500-mL, with screw-caps, to fit centrifuge.

6.6.3 Centrifuge tubes—12- to 15-mL, with screw-caps, to fit centrifuge.

6.7 Cleanup apparatus.

6.7.1 Automated gel permeation chromatograph (Analytical Biochemical Labs, Inc, Columbia, MO, Model GPC Autoprep 1002, or equivalent).

6.7.1.1 Column—600-700 mm long × 25 mm ID, packed with 70 g of SX-3 Bio-beads (Bio-Rad Laboratories, Richmond, CA, or equivalent).

6.7.1.2 Syringe—10-mL, with Luer fitting.

6.7.1.3 Syringe filter holder—stainless steel, and glass- fiber or fluoropolymer filters (Gelman 4310, or equivalent).

6.7.1.4 UV detectors—254-nm, preparative or semi-preparative flow cell (Isco, Inc., Type 6; Schmadzu, 5-mm path length; Beckman-Altex 152W, 8-µL micro-prep flow cell, 2-mm path; Pharmacia UV-1, 3-mm flow cell; LDC Milton-Roy UV-3, monitor #1203; or equivalent).

6.7.2 Reverse-phase high-performance liquid chromatograph.

6.7.2.1 Column oven and detector—Perkin-Elmer Model LC-65T (or equivalent) operated at 0.02 AUFS at 235 nm.

6.7.2.2 Injector—Rheodyne 7120 (or equivalent) with 50-µL sample loop.

6.7.2.3 Column—Two 6.2 mm × 250 mm Zorbax-ODS columns in series (DuPont Instruments Division, Wilmington, DE, or equivalent), operated at 30°C and 2.0 mL/min with gradient from TBD percent methanol:acetonitrile to 100 percent acetonitrile in TBD minutes.

6.7.2.4 Pump—Altex 110A (or equivalent).

6.7.3 Pipets.

6.7.3.1 Disposable, Pasteur, 150-mm long × 5-mm ID (Fisher Scientific 13-678-6A, or equivalent).

6.7.3.2 Disposable, serological, 50-mL (8- to 10- mm ID).

6.7.4 Glass chromatographic columns.

6.7.4.1 150-mm long × 8-mm ID, (Kontes K-420155, or equivalent) with coarse-glass frit or glass-wool plug and 250-mL reservoir.

6.7.4.2 200-mm long × 15-mm ID, with coarse-glass frit or glass-wool plug and 250-mL reservoir.

6.7.4.3 300-mm long x 22-mm ID, with coarse-glass frit, 300-mL reservoir, and glass or fluoropolymer stopcock.

6.7.5 Stirring apparatus for batch silica cleanup of tissue extracts.

6.7.5.1 Mechanical stirrer—Corning Model 320, or equivalent.

6.7.5.2 Bottle—500- to 600-mL wide-mouth clear glass.

6.7.6 Oven—For baking and storage of adsorbents, capable of maintaining a constant temperature (±5°C) in the range of 105-250°C.

6.8 Concentration apparatus.

6.8.1 Rotary evaporator—Buchi/Brinkman-American Scientific No. E5045-10 or equivalent, equipped with a variable temperature water bath.

6.8.1.1 Vacuum source for rotary evaporator equipped with shutoff valve at the evaporator and vacuum gauge.

6.8.1.2 A recirculating water pump and chiller are recommended, as use of tap water for cooling the evaporator wastes large volumes of water and can lead to inconsistent performance as water temperatures and pressures vary.

6.8.1.3 Round-bottom flask—100-mL and 500-mL or larger, with ground-glass fitting compatible with the rotary evaporator.

6.8.2 Kuderna-Danish (K-D) concentrator.

6.8.2.1 Concentrator tube—10-mL, graduated (Kontes K-570050-1025, or equivalent) with calibration verified. Ground-glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

6.8.2.2 Evaporation flask—500-mL (Kontes K-570001-0500, or equivalent), attached to concentrator tube with springs (Kontes K-662750-0012 or equivalent).

6.8.2.3 Snyder column—Three-ball macro (Kontes K-503000-0232, or equivalent).

6.8.2.4 Boiling chips.

6.8.2.4.1 Glass or silicon carbide—Approximately 10/40 mesh, extracted with methylene chloride and baked at 450°C for 1 hour minimum.

6.8.2.4.2 Fluoropolymer (optional)—Extracted with methylene chloride.

6.8.2.5 Water bath—Heated, with concentric ring cover, capable of maintaining a temperature within ±2°C, installed in a fume hood.

6.8.3 Nitrogen blowdown apparatus—Equipped with water bath controlled in the range of 30 - 60°C (N-Evap, Organomation Associates, Inc., South Berlin, MA, or equivalent), installed in a fume hood.

6.8.4 Sample vials.

6.8.4.1 Amber glass, 2- to 5-mL with fluoropolymer-lined screw-cap.

6.8.4.2 Glass, 0.3-mL, conical, with fluoropolymer-lined screw or crimp cap.

6.9 Gas chromatograph—Shall have splitless or on-column injection port for capillary column, temperature program with isothermal hold, and shall meet all of the performance specifications in Section 10.

6.9.1 GC columns—The pair of GC columns listed below are capable of resolving all 209 PCB congeners. Other GC columns may be used so long as PCBs 126 and 169 are each resolved from their respective most closely eluted leading and trailing congeners. The valley height between PCB 126 or 169 and its respective most closely eluted leading and trailing congeners must be less than 10 percent of the height of the shorter of the pair.

6.9.2 Column #1—30±5-m long × 0.25±0.02-mm ID; 0.25-µm film SPB-Octyl (Supelco 2-4218, or equivalent).

6.9.3 Column #2—30±5-m long x 0.25±0.02-mm ID; 0.25-µm film DB-1 (J&W, or equivalent).

6.10 Mass spectrometer—28- to 40-eV electron impact ionization, shall be capable of repetitively selectively monitoring 12 exact m/z's minimum at high resolution (≥10,000) during a period less than 1.5 seconds, and shall meet all of the performance specifications in Section 10.

6.11 GC/MS interface—The mass spectrometer (MS) shall be interfaced to the GC such that the end of the capillary column terminates within 1 cm of the ion source but does not intercept the electron or ion beams.

6.12 Data system—Capable of collecting, recording, and storing MS data.

7.0 Reagents and Standards

7.1 pH adjustment and back-extraction.

7.1.1 Potassium hydroxide—Dissolve 20 g reagent grade KOH in 100 mL reagent water.

7.1.2 Sulfuric acid—Reagent grade (specific gravity 1.84).

7.1.3 Hydrochloric acid—Reagent grade, 6N.

7.1.4 Sodium chloride—Reagent grade, prepare at 5% (w/v) solution in reagent water.

7.2 Solution drying and evaporation.

7.2.1 Solution drying—Sodium sulfate, reagent grade, granular, anhydrous (Baker 3375, or equivalent), rinsed with methylene chloride (20 mL/g), baked at 400°C for 1 hour minimum, cooled in a desiccator, and stored in a pre-cleaned glass bottle with screw-cap that prevents moisture from entering. If, after heating, the sodium sulfate develops a noticeable grayish cast (due to the presence of carbon in the crystal matrix), that batch of reagent is not suitable for use and should be discarded. Extraction with methylene chloride (as opposed to simple rinsing) and baking at a lower temperature may produce sodium sulfate that is suitable for use.

7.2.2 Tissue drying—Sodium sulfate, reagent grade, powdered, treated and stored as above.

7.2.3 Prepurified nitrogen.

7.3 Extraction.

7.3.1 Solvents—Acetone, toluene, n-hexane, methanol, methylene chloride, and nonane; distilled in glass, pesticide quality, lot-certified to be free of interferences.

7.3.2 White quartz sand, 60/70 mesh—For Soxhlet/Dean-Stark extraction (Aldrich Chemical, Cat. No. 27-437-9, or equivalent). Bake at 450°C for 4 hours minimum.

7.4 GPC calibration solution—Prepare a solution containing 300 mg/mL corn oil, TBD mg/mL PCB 209, 1.4 mg/mL pentachlorophenol, 0.1 mg/mL perylene, and 0.5 mg/mL sulfur. [To be modified if necessary.]

7.5 Adsorbents for sample cleanup.

7.5.1 Silica gel.

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

7.5.1.2 Acid silica gel (30% w/w)—Thoroughly mix 44.0 g of concentrated sulfuric acid with 100.0 g of activated silica gel in a clean container. Break up aggregates with a stirring rod until a uniform mixture is obtained. Store in a screw-capped bottle with fluoropolymer-lined cap.

7.5.1.3 Basic silica gel—Thoroughly mix 30 g of 1N sodium hydroxide with 100 g of activated silica gel in a clean container. Break up aggregates with a stirring rod until a uniform mixture is obtained. Store in a screw-capped bottle with fluoropolymer-lined cap.

7.5.1.4 Potassium silicate.

7.5.1.4.1 Dissolve 56 g of high purity potassium hydroxide (Aldrich, or equivalent) in 300 mL of methanol in a 750- to 1000-mL flat-bottom flask.

7.5.1.4.2 Add 100 g of activated silica gel (Section 7.5.1.1) and a stirring bar, and stir on a hot plate at 60-70°C for 1-2 hours.

7.5.1.4.3 Decant the liquid and rinse the potassium silicate twice with 100-mL portions of methanol, followed by a single rinse with 100 mL of methylene chloride.

7.5.1.4.4 Spread the potassium silicate on solvent-rinsed aluminum foil and dry for 2-4 hours in a hood.

7.5.1.4.5 Activate overnight at 200-250°C.

7.5.2 Carbon.

7.5.2.1 Carbopak C—(Supelco 1-0258, or equivalent).

7.5.2.2 Celite 545—(Supelco 2-0199, or equivalent).

7.5.2.3 Thoroughly mix 18.0 g Carbopak C and 18.0 g Celite 545 to produce a 50% w/w mixture. Activate the mixture at 130°C for a minimum of 6 hours. Store in a desiccator.

7.5.3 Anthropogenic isolation column—Pack the column in Section 6.7.4.3 from bottom to top with the following:

7.5.3.1 2 g activated silica gel (Section 7.5.1.1).

7.5.3.2 2 g potassium silicate (Section 7.5.1.4).

7.5.3.3 2 g granular anhydrous sodium sulfate (Section 7.2.1).

7.5.3.4 10 g acid silica gel (Section 7.5.1.2).

7.5.3.5 2 g granular anhydrous sodium sulfate.

7.5.4 Florisil column.

7.5.4.1 Florisil—PR grade, 60-100 mesh (U.S. Silica Corp, Berkeley Springs, WV, or equivalent). Fill a clean 1- to 2-L bottle 1/2 to 2/3 full with Florisil and place in an oven at 130-150 °C for a minimum of three days.

7.5.4.2 Immediately prior to use, dry pack a 300-mm x 22-mm ID glass column (Section 6.7.4.3) bottom to top with 0.5-1.0 cm of anhydrous sodium sulfate (Section 7.2.1), 10-10.5 cm of warm to hot activated Florisil (Section 7.5.4.1), and 1-2 cm of warm to hot anhydrous sodium sulfate. Allow the column to cool and wet immediately with 100 mL of n-hexane to prevent water from entering.

7.5.4.3 Using the procedure in Section 13.7, establish the elution pattern for each carton of Florisil received.

7.6 Reference matrices—Matrices in which the PCBs and interfering compounds are not detected by this method.

7.6.1 Reagent water—Bottled water purchased locally or prepared by passage through activated carbon.

7.6.2 High-solids reference matrix—Playground sand or similar material. Prepared by extraction with methylene chloride and/or baking at 450°C for a minimum of 4 hours.

7.6.3 Paper reference matrix—Glass-fiber filter, Gelman type A, or equivalent. Cut paper to simulate the surface area of the paper sample being tested.

7.6.4 Tissue reference matrix—Corn or other vegetable oil. May be prepared by extraction with methylene chloride.

7.6.5 Other matrices—This method may be verified on any reference matrix by performing the tests given in Section 9.2. Ideally, the matrix should be free of the PCBs, but in no case shall the background level of the PCBs in the reference matrix exceed three times the minimum levels in Table 2. If low background levels of the PCBs are present in the reference matrix, the spike level of the analytes used in Section 9.2 should be increased to provide a spike-to-background ratio in the range of 1:1 to 5:1 (Reference 11).

7.7 Standard solutions—Purchased as solutions or mixtures with certification to their purity, concentration, and authenticity, or prepared from materials of known purity and composition. If the chemical purity is 98% or greater, the weight may be used without correction to compute the concentration of the standard. When not being used, standards are stored in the dark at room temperature in screw-capped vials with fluoropolymer-lined caps. A mark is placed on the vial at the level of the solution so that solvent loss by evaporation can be detected. If solvent loss has occurred, the solution should be replaced.

7.8 Stock solutions.

7.8.1 Preparation—Prepare in nonane per the steps below or purchase as dilute solutions (Cambridge Isotope Laboratories (CIL), Woburn, MA, or equivalent). Observe the safety precautions in Section 5 and the recommendation in Section 5.1.2.

7.8.2 Dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 1 to 2 mg of PCB 126 to three significant figures in a 10-mL ground-glass-stoppered volumetric flask and fill to the mark with nonane. After the PCB is completely dissolved, transfer the solution to a clean 15-mL vial with fluoropolymer-lined cap.

7.8.3 Stock standard solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards. Reference standards that can be used to determine the accuracy of calibration standards are available from several vendors.

7.9 PAR stock solution.

7.9.1 All PCBs—Using the solutions in Section 7.8, prepare the PAR stock solution to contain the PCBs of interest at the concentrations shown in Table 3. When diluted, the solution will become the PAR (Section 7.14).

7.9.2 If the toxic, non-ortho, co-planar PCBs (PCBs 77, 126, and 169) only are to be determined, prepare the PAR stock solution to contain these compounds only.

7.10 Labeled-compound spiking solution.

7.10.1 All toxic PCBs—From stock solutions, or from purchased mixtures, prepare this solution to contain the labeled compounds in nonane at the concentrations shown in Table 3. This solution is diluted with acetone prior to use (Section 7.10.3).

7.10.2 If PCBs 77, 126, and 169 only are to be determined, prepare the labeled-compound solution to contain these compounds only. This solution is diluted with acetone prior to use (Section 7.10.3).

7.10.3 Dilute a sufficient volume of the labeled compound solution (Section 7.10.1 or 7.10.2) by a factor of 500 with acetone to prepare a diluted spiking solution. Each sample requires 1.0 mL of the diluted solution, but no more solution should be prepared than can be used in one day.

7.11 Cleanup standard—Prepare PCBs 81 and 111 in nonane at the concentration shown in Table 3. The cleanup standard is added to all extracts prior to cleanup to measure the efficiency of the cleanup process.

7.12 Internal standard(s).

7.12.1 All toxic PCBs—Prepare the internal standard solution to contain labeled PCBs 52, 101, 138, and 178 in nonane at the concentration shown in Table 3.

7.12.2 If PCBs 77, 126, and 169 only are to be determined, the internal standard solution may be prepared to contain PCBs 52, 101, and 138 only.

7.13 Calibration standards (CS1 through CS5)—Combine the solutions in Sections 7.9-7.12 to produce the five calibration solutions shown in Table 4 in nonane. These solutions permit the relative response (labeled to native) and response factor to be measured as a function of concentration. The CS3 standard is used for calibration verification (VER). If the PCBs 77, 126, and 169 only are to be determined, combine the solutions appropriate to these compounds.

7.14 Precision and recovery (PAR) standard—Used for determination of initial (Section 9.2) and ongoing (Section 15.5) precision and recovery (See Table 3). Dilute 200 µL of the PAR stock solution

(Section 7.9.1 or 7.9.2) to 10 mL with acetone for each sample matrix for each sample batch. One mL of each are required for the blank and OPR with each matrix in each batch.

7.15 GC retention time window defining solution and isomer specificity test standard—Used to define the beginning and ending retention times for the PCB congeners and to demonstrate isomer specificity of the GC columns employed for determination of PCB 126. The standard must contain the compounds listed in Table 5 (CIL , or equivalent), at a minimum. It is not necessary to monitor all of the window-defining compounds if PCBs 77, 126, and 169 only are to be determined. In this case, a congener-specificity test standard containing the most closely eluted isomers listed in Table 5
(CIL , or equivalent) may be used.

7.16 QC Check Sample—A QC Check Sample should be obtained from a source independent of the calibration standards. Ideally, this check sample would be a certified standard reference material (SRM) containing the PCBs in known concentrations in a sample matrix similar to the matrix being analyzed. The National Institute of Standards and Technology (NIST) in Gaithersburg, Maryland has SRMs for several individual PCB congeners, and as Aroclors in transformer and motor oil, in combination with pesticides in cod liver oil, and in combination with 2,3,7,8-TCDD in human serum.

7.17 Stability of solutions—Standard solutions used for quantitative purposes (Sections 7.9 through 7.15) should be analyzed periodically, and should be assayed against reference standards before further use.

8.0 Sample Collection, Preservation, Storage, and Holding Times.

8.1 Collect samples in amber glass containers following conventional sampling practices (Reference 12).

8.2 Aqueous samples.

8.2.1 Samples that flow freely are collected as grab samples or in refrigerated bottles using automatic sampling equipment.

8.2.2 If residual chlorine is present, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine (Reference 13).

8.2.3 Adjust sample pH 2-3 with sulfuric acid.

8.2.4 Maintain aqueous samples in the dark at 0-4°C from the time of collection until receipt at the laboratory. Store in the dark at 0-4°C.

8.3 Solid samples.

8.3.4 Solid samples are collected as grab samples using wide-mouth jars.

8.3.4 Maintain solid, semi-solid, oily, and mixed-phase samples in the dark at <4°C from the time of collection until receipt at the laboratory. Store solid, semi-solid, oily, and mixed-phase samples in the dark at <-10°C.

8.4 Fish and tissue samples.

8.4.1 Fish may be cleaned, filleted, or processed in other ways in the field, such that the laboratory may expect to receive whole fish, fish fillets, or other tissues for analysis.

8.4.2 Fish collected in the field should be wrapped in aluminum foil and must be maintained at a temperature less than 4°C from the time of collection until receipt at the laboratory.

8.4.3 Samples must be frozen upon receipt at the laboratory and maintained in the dark at <-10°C until prepared. Maintain unused sample in the dark at <-10°C.

8.5 Holding times.

8.5.1 There are no demonstrated maximum holding times associated with the PCBs in aqueous, solid, semi-solid, tissues, or other sample matrices. If stored in the dark at 0-4°C and preserved as given above (if required), aqueous samples may be stored for up to one year. Similarly, if stored in the dark at <-10°C, solid, semi-solid, multi-phase, and tissue samples may be stored for up to one year.

8.5.2 Store sample extracts in the dark at <-10°C until analyzed. If stored in the dark at <-10°C, sample extracts may be stored for up to one year.

9.0 Quality Assurance/Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 14). The minimum requirements of this program consist of an initial demonstration of laboratory capability, analysis of samples spiked with labeled compounds to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

If the method is to be applied to a sample matrix other than water (e.g., soils, filter cake, compost, tissue) the most appropriate alternate matrix (Sections 7.6.2-7.6.5 and 7.16) is substituted for the reagent water matrix (Section 7.6.1) in all performance tests.

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

9.1.2 In recognition of advances that are occurring in analytical technology and to allow the analyst to overcome sample matrix interferences, the analyst is permitted certain options to improve separations or lower the costs of measurements. These options include alternate extraction, concentration, cleanup procedures, and changes in columns and detectors. Alternate determinative techniques, such as the substitution of spectroscopic or immuno-assay techniques, and changes that degrade method performance, are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or better than the specificity of the techniques in this method for the analytes of interest.

9.1.2.1 Each time a modification is made to this method, the analyst is required to repeat the procedure in Section 9.2. If the detection limit of the method will be affected by the change, the laboratory is required to demonstrate that the MDL (40 CFR Part 136, Appendix B) is lower than one-third the regulatory compliance level or one-third the ML in this method, whichever is higher. If calibration will be affected by the change, the analyst must recalibrate the instrument per Section 10.

9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the following at a minimum:

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modifications.

9.1.2.2.2 A listing of pollutant(s) measured, by name and CAS Registry number.

9.1.2.2.3 A narrative stating reason(s) for the modifications.

9.1.2.2.4 Results from all quality control (QC) tests comparing the modified method to this method. These results are to include the following:

a) Calibration (Section 10.5-10.7).
b) Calibration verification (Section 15.3).
c) Initial precision and recovery (Section 9.2).
d) Labeled compound recovery (Section 9.3).
e) Analysis of blanks (Section 9.5).
f) Accuracy assessment (Section 9.4).

9.1.2.2.5 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include the following:

a) Sample numbers and other identifiers.
b) Extraction dates.
c) Analysis dates and times.
d) Analysis sequence/run chronology.
e) Sample weight or volume (Section 11).
f) Extract volume prior to each cleanup step (Section 13).
g) Extract volume after each cleanup step (Section 13).
h) Final extract volume prior to injection (Section 14).
i) Injection volume (Section 14.3).
j) Dilution data, differentiating between dilution of a sample or extract (Section 17.5).
k) Instrument and operating conditions.
l) Column (dimensions, liquid phase, solid support, film thickness, etc).
m) Operating conditions (temperatures, temperature program, flow rates).
n) Detector (type, operating conditions, etc).
o) Chromatograms, printer tapes, and other recordings of raw data.
p) Quantitation reports, data system outputs, and other data to link the raw data to the results reported.

9.1.3 Analyses of method blanks are required to demonstrate freedom from contamination (Section 4.3). The procedures and criteria for analysis of a method blank are described in Sections 9.5 and 15.6.

9.1.4 The laboratory shall spike all samples with labeled compounds to monitor method performance. This test is described in Section 9.3. When results of these spikes indicate atypical method performance for samples, the samples are diluted to bring method performance within acceptable limits. Procedures for dilution are given in Section 17.5.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and the analysis of the ongoing precision and recovery aliquot that the analytical system is in control. These procedures are described in Sections 15.1 through 15.5.

9.1.6 The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Section 9.4.

9.2 Initial precision and recovery (IPR)—To establish the ability to generate acceptable precision and recovery, the analyst shall perform the following operations.

9.2.1 For low solids (aqueous, < 1% solids) samples, extract, concentrate, and analyze four 1-L aliquots of reagent water spiked with the diluted labeled compound spiking solution (Section 7.10.3) and the precision and recovery standard (Section 7.14) according to the procedures in Sections 11 through 18. For an alternative sample matrix, four aliquots of the alternative reference matrix (Section 7.6) are used. All sample processing steps that are to be used for processing samples, including preparation (Section 11), extraction (Section 12), and cleanup (Section 13), shall be included in this test.

9.2.2 Using results of the set of four analyses, compute the average concentration (X) of the extracts in ng/mL and the standard deviation of the concentration (s) in ng/mL for each compound, by isotope dilution for PCBs with a labeled analog, and by internal standard for the PCBs without a labeled analog, and the labeled compounds.

9.2.3 For each PCB and labeled compound, compare s and X with the corresponding limits for initial precision and recovery in Table 6. If PCBs 77, 126, and 169 only are to be determined, compare s and X with the corresponding limits for initial precision and recovery in Table 6a. If s and X for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any individual s exceeds the precision limit or any individual X falls outside the range for accuracy, system performance is unacceptable for that compound. Correct the problem and repeat the test (Section 9.2).

9.3 The laboratory shall spike all samples with the diluted labeled compound spiking solution (Section 7.10.3) to assess method performance on the sample matrix.

9.3.1 Analyze each sample according to the procedures in Sections 11 through 18.

9.3.2 Compute the percent recovery of the labeled compounds and the cleanup standard using the internal standard method (Section 17.2).

9.3.3 The recovery of each labeled compound must be within the limits in Table 7 when all of the toxic PCBs are determined, and within the limits in Table 7a when PCBs 77, 126, and 169 only are determined. If the recovery of any compound falls outside of these limits, method performance is unacceptable for that compound in that sample. Additional cleanup procedures must then be employed to attempt to bring the recovery within the normal range. If the recovery cannot be brought within the normal range after all cleanup procedures have been employed, water samples are diluted and smaller amounts of soils, sludges, sediments, and other matrices are analyzed per Section 18.4.

9.4 Recovery of labeled compounds from samples should be assessed and records should be maintained.

9.4.1 After the analysis of five samples of a given matrix type (water, soil, sludge, pulp, etc.) for which the labeled compounds pass the tests in Section 9.3, compute the average percent recovery (R) and the standard deviation of the percent recovery (S_R) for the labeled compounds only. Express the assessment as a percent recovery interval from $R - 2S_R$ to $R + 2S_R$ for each matrix. For example, if R = 90% and S_R = 10% for five analyses of pulp, the recovery interval is expressed as 70 to 110%.

9.4.2 Update the accuracy assessment for each labeled compound in each matrix on a regular basis (e.g., after each five to ten new measurements).

9.5 Method blanks—Reference matrix method blanks are analyzed to demonstrate freedom from contamination (Section 4.3).

9.5.1 Prepare, extract, clean up, and concentrate a method blank with each sample batch (samples of the same matrix started through the extraction process on the same 12-hour shift, to a maximum of 20 samples). The matrix for the method blank shall be similar to sample matrix for the batch; e.g., a 1-L reagent water blank (Section 7.6.1), high-solids reference matrix blank (Section 7.6.2), paper matrix blank (Section 7.6.3), tissue blank (Section 7.6.4), or alternative reference matrix blank (Section 7.6.5). Analyze the blank immediately after analysis of the OPR (Section 15.5) to demonstrate freedom from contamination.

9.5.2 If any PCB (Table 1) is found in the blank at greater than the minimum level (Table 2) or one-third the regulatory compliance level whichever is greater, or if any potentially interfering compound is found in the blank at the minimum level for each level of chlorination given in Table 2 (assuming a response factor of 1 relative to the internal standard at that level of chlorination for compounds not listed in Table 1), analysis of samples is halted until the blank associated with the sample batch shows no evidence of contamination at this level. All samples must be associated with an uncontaminated method blank before the results for those samples may be reported for regulatory compliance purposes.

9.6 QC Check Sample—Analyze the QC Check Sample (Section 7.16) periodically to assure the accuracy of calibration standards and the overall reliability of the analytical process. It is suggested that the QC Check Sample be analyzed at least quarterly.

9.7 The specifications contained in this method can be met if the apparatus used is calibrated properly and then maintained in a calibrated state. The standards used for calibration (Section 10), calibration verification (Section 15.3), and for initial (Section 9.2) and ongoing (Section 15.5) precision and recovery should be identical, so that the most precise results will be obtained. A GC/MS instrument will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of PCBs by this method.

9.8 Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and spiked samples may be required to determine the accuracy of the analysis when the internal standard method is used.

10.0 Calibration

10.1 Establish the operating conditions necessary to meet the minimum retention times for the internal standards in Section 10.2.4 and the relative retention times for the PCBs in Table 2.

10.1.1 Suggested GC operating conditions:

Injector temperature:	290°C
Interface temperature:	290°C
Initial temperature:	150°C
Initial time:	2 minutes
Temperature program:	150 to 200°C at 10°C/minute
	200 to 280°C at 2.0°C/minute

Note: *All portions of the column that connect the GC to the ion source shall remain at or above the interface temperature specified above during analysis to preclude condensation of less volatile compounds.*

The GC conditions may be optimized for compound separation and sensitivity. Once optimized, the same GC conditions must be used for the analysis of all standards, blanks, IPR and OPR aliquots, and samples.

10.1.2 Mass spectrometer (MS) resolution—Obtain a selected ion current profile (SICP) of each analyte in Table 3 at the two exact m/z's specified in Table 8 and at ≥10,000 resolving power by injecting an authentic standard of the PCBs either singly or as part of a mixture in which there is no interference between closely eluted components.

10.1.2.1 The analysis time for PCBs may exceed the long-term mass stability of the mass spectrometer. Because the instrument is operated in the high-resolution mode, mass drifts of a few ppm (e.g., 5 ppm in mass) can have serious adverse effects on instrument performance. Therefore, a mass-drift correction is mandatory and a lock-mass m/z from PFK is used for drift correction. The lock-mass m/z is dependent on the exact m/z's monitored within each descriptor, as shown in Table 8. The level of PFK metered into the HRMS during analyses should be adjusted so that the amplitude of the most intense selected lock-mass m/z signal (regardless of the descriptor number) does not exceed 10% of the full-scale deflection for a given set of detector parameters. Under those conditions, sensitivity changes that might occur during the analysis can be more effectively monitored.

Note: *Excessive PFK (or any other reference substance) may cause noise problems and contamination of the ion source necessitating increased frequency of source cleaning.*

10.1.2.2 If the HRMS has the capability to monitor resolution during the analysis, it is acceptable to terminate the analysis when the resolution falls below 10,000 to save reanalysis time.

10.1.2.3 Using a PFK molecular leak, tune the instrument to meet the minimum required resolving power of 10,000 (10% valley) at m/z 304.9824 or any other reference signal close to m/z 305 (from PeCB). For each descriptor (Table 8), monitor and record the resolution and exact m/z's of three to five reference peaks covering the mass range of the descriptor. The resolution must be greater than or equal to 10,000, and the deviation between the exact m/z and the theoretical m/z (Table 8) for each exact m/z monitored must be less than 5 ppm.

10.2 Ion abundance ratios, minimum levels, signal-to-noise ratios, and absolute retention times—Choose an injection volume of either 1- or 2-µL, consistent with the capability of the HRGC/HRMS instrument. Inject a 1 or 2 µL aliquot of the CS1 calibration solution (Table 4) using the GC conditions from Section 10.1.1. If PCBs 77, 126, and 169 only are to be determined, the operating conditions and specifications below apply to analysis of those compounds only.

10.2.1 Measure the SICP areas for each analyte, and compute the ion abundance ratios at the exact m/z's specified in Table 8. Compare the computed ratio to the theoretical ratio given in Table 9.

10.2.1.1 The exact m/z's to be monitored in each descriptor are shown in Table 8. Each group or descriptor shall be monitored in succession as a function of GC retention time to ensure that all of the toxic PCBs are detected. Additional m/z's may be monitored in each descriptor, and the m/z's may be divided among more than the descriptors listed in Table 8, provided that the laboratory is able to monitor the m/z's of all the PCBs that may elute from the GC in a given retention-time window. If PCBs 77, 126, and 169 only are to be determined, the descriptors may be modified to include only the exact m/z's for the tetra-, penta-, and hexa-, congeners, and the lock m/z's.

10.2.1.2 The mass spectrometer shall be operated in a mass-drift correction mode, using PFK to provide lock m/z's. The lock mass for each group of m/z's is shown in Table 8. Each lock mass shall be monitored and shall not vary by more than ±20% throughout its respective retention time window. Variations of the lock mass by more than 20% indicate the presence of coeluting interferences that may significantly reduce the sensitivity of the mass spectrometer. Reinjection of another aliquot of the sample extract will not resolve the problem. Additional cleanup of the extract may be required to remove the interferences.

10.2.2 All PCBs and labeled compounds in the CS1 standard shall be within the QC limits in Table 9 for their respective ion abundance ratios; otherwise, the mass spectrometer shall be adjusted and this test repeated until the m/z ratios fall within the limits specified. If the adjustment alters the resolution of the mass spectrometer, resolution shall be verified (Section 10.1.2) prior to repeat of the test.

10.2.3 Verify that the HRGC/HRMS instrument meets the minimum levels in Table 2. The peaks representing the PCBs and labeled compounds in the CS1 calibration standard must have signal-to-noise ratios (S/N) greater than or equal to 10.0. Otherwise, the mass spectrometer shall be adjusted and this test repeated until the minimum levels in Table 2 are met.

10.2.4 The absolute retention time of PCB 169 (Section 7.12) shall exceed 20 minutes on the SPB-Octyl column, and the retention time of PCB 157 shall exceed 25 minutes on the DB-1 column; otherwise, the GC temperature program shall be adjusted and this test repeated until the above-stated minimum retention time criteria are met.

10.3 Retention-time windows—Analyze the window defining mixtures (Section 7.15) using the optimized temperature program in Section 10.1. Table 5 gives the elution order (first/last) of the window-defining compounds. If PCBs 77, 126, and 169 only are to be determined, the window-defining tetra-, penta-, and hepta-PCBs are the only compounds that need to be tested.

10.4 Isomer specificity.

10.4.1 Analyze the isomer specificity test standards (Section 7.15) using the procedure in Section 14 and the optimized conditions for sample analysis (Section 10.1.1).

10.4.2 Compute the percent valley between the GC peaks that elute most closely to PCB 126 and 169 on the SPB-Octyl column and to PCB 156/157 on the DB-1 column, per Figures 6 and 7.

10.4.3 Verify that the height of the valley between the most closely eluted isomers and the PCBs given in Section 10.4.2 is less than 25% (computed as 100 x/y in Figures 6 and 7). If the valley exceeds 25%, adjust the analytical conditions and repeat the test or replace the GC column and recalibrate (Sections 10.1.2 through 10.7).

10.5 Calibration by isotope dilution—Isotope dilution calibration is used for the native PCBs for which labeled compounds are added to samples prior to extraction. The reference compound for each native compound is shown in Table 2.

10.5.1 A calibration curve encompassing the concentration range is prepared for each compound to be determined. The relative response (RR) (labeled to native) vs. concentration in standard solutions is plotted or computed using a linear regression. Relative response is determined according to the procedures described below. Five calibration points are employed.

10.5.2 The response of each native PCB relative to its labeled analog is determined using the area responses of both the primary and secondary exact m/z's specified in Table 8, for each calibration standard as follows:

$$RR = \frac{(A1_n + A2_n)\ C_l}{(A1_l + A2_l)\ C_n}$$

Where:

$A1_n$ *and* $A2_n$ = *The areas of the primary and secondary m/z's for the PCB.*

$A1_l$ *and* $A2_l$ = *The areas of the primary and secondary m/z's for the labeled compound.*

C_l = *The concentration of the labeled compound in the calibration standard* (*Table* 4).

C_n = *The concentration of the native compound in the calibration standard* (*Table* 4).

10.5.3 To calibrate the analytical system by isotope dilution, inject a volume of calibration standards CS1 through CS5 (Section 7.13 and Table 4) identical to the volume chosen in Section 10.2, using the procedure in Section 14 and the conditions in Section 10.1.1 and Table 2. Compute the relative response (RR) at each concentration.

10.5.4 Linearity—If the relative response for any compound is constant (less than 20% coefficient of variation) over the five-point calibration range, an averaged relative response may be used for that compound; otherwise, the complete calibration curve for that compound shall be used over the five-point calibration range.

10.6 Calibration by internal standard—The internal standard method is applied to determination of the native PCBs for which a labeled compound is not available and to the determination of labeled compounds for intralaboratory statistics (Sections 9.4 and 15.5.4).

10.6.1 Response factors—Calibration requires the determination of response factors (RF) defined by the following equation:

$$RF = \frac{(A1_s + A2_s)\ C_{is}}{(A1_{is} + A2_{is})\ C_s}$$

Where:

$A1_s$ *and* $A2_s$ = *The areas of the primary and secondary m/z's for the PCB.*

$A1_{is}$ *and* $A2_{is}$ = *The areas of the primary and secondary m/z's for the internal standard.*

C_{is} = *The concentration of the internal standard* (*Table* 4).

C_s = *The concentration of the compound in the calibration standard* (*Table* 4).

Note: *There is only one m/z for PCBs 81 and 111 (see Table 8).*

10.6.2 To calibrate the analytical system by internal standard, inject 1.0 or 2.0 µL of calibration standards CS1 through CS5 (Section 7.13 and Table 4) using the procedure in Section 14 and the conditions in Section 10.1.1 and Table 2. Compute the response factor (RF) at each concentration.

10.6.3 Linearity—If the response factor (RF) for any compound is constant (less than 35% coefficient of variation) over the five-point calibration range, an averaged response factor may be used for that compound; otherwise, the complete calibration curve for that compound shall be used over the five-point range.

10.7 Combined calibration—By using calibration solutions (Section 7.13 and Table 4) containing the native PCBs, labeled compounds, and the internal standards, a single set of analyses can be used to produce calibration curves for the isotope dilution and internal standard methods. These curves are verified (Section 15.3) each shift by analyzing the calibration verification standard (VER, Table 4). Recalibration is required if any of the calibration verification criteria (Section 15.3) cannot be met.

10.8 Data storage—MS data shall be collected, recorded, and stored.

10.8.1 Data acquisition—The signal at each exact m/z shall be collected repetitively throughout the monitoring period and stored on a mass storage device.

10.8.2 Response factors and multipoint calibrations—The data system shall be used to record and maintain lists of response factors (response ratios for isotope dilution) and multipoint calibration curves. Computations of relative standard deviation (coefficient of variation) shall be used to test calibration linearity. Statistics on initial performance (Section 9.2) and ongoing performance (Section 15.5) should be computed and maintained, either on the instrument data system or on a separate computer system.

11.0 Sample Preparation

11.1 Sample preparation involves modifying the physical form of the sample so that the PCBs can be extracted efficiently. In general, the samples must be in a liquid form or in the form of finely divided solids in order for efficient extraction to take place. Table 10 lists the phases and suggested quantities for extraction of various sample matrices.

For samples known or expected to contain high levels of the PCBs, the smallest sample size representative of the entire sample should be used (see Section 17.5).

For all samples, the blank and IPR/OPR aliquots must be processed through the same steps as the sample to check for contamination and losses in the preparation processes.

11.1.1 For samples that contain particles, percent solids and particle size are determined using the procedures in Sections 11.2 and 11.3, respectively.

11.1.2 Aqueous samples—Because PCBs may be bound to suspended particles, the preparation of aqueous samples is dependent on the solids content of the sample.

11.1.2.1 Aqueous samples containing 1% solids or less are prepared per Section 11.4 and extracted directly using the SPE technique in 12.2.

11.1.2.2 For aqueous samples containing greater than 1% solids, a sample aliquot sufficient to provide 10 g of dry solids is used as described in Section 11.5.

11.1.3 Solid samples are prepared using the procedure described in Section 11.5 followed by extraction via the SDS procedure in Section 12.3.

11.1.4 Multiphase samples—The phase(s) containing the PCBs is separated from the non-PCB phase using pressure filtration and centrifugation as described in Section 11.6. The PCBs will be in the organic phase in a multiphase sample in which an organic phase exists.

11.1.5 Procedures for grinding, homogenization, and blending of various sample phases are given in Section 11.7.

11.1.6 Tissue samples—Preparation procedures for fish and other tissues are given in Section 11.8.

11.2 Determination of percent suspended solids.

Note: *This aliquot is used for determining the solids content of the sample, not for determination of PCBs.*

11.2.1 Aqueous liquids and multi-phase samples consisting of mainly an aqueous phase.

11.2.1.1 Desiccate and weigh a GF/D filter (Section 6.5.3) to three significant figures.

11.2.1.2 Filter 10.0 ± 0.02 mL of well-mixed sample through the filter.

11.2.1.3 Dry the filter a minimum of 12 hours at 110 ± 5°C and cool in a desiccator.

11.2.1.4 Calculate percent solids as follows:

$$\%\ solids = \frac{weight\ of\ sample\ aliquot\ plus\ filter\ after\ drying\ (g) - weight\ of\ filter\ (g)}{10\ g} \times 100$$

11.2.2 Non-aqueous liquids, solids, semi-solid samples, and multi-phase samples in which the main phase is not aqueous, but not tissues.

11.2.2.1 Weigh 5 to 10 g of sample to three significant figures in a tared beaker.

11.2.2.2 Dry a minimum of 12 hours at 110 ± 5°C, and cool in a desiccator.

11.2.2.3 Calculate percent solids as follows:

$$\%\ solids = \frac{weight\ of\ sample\ aliquot\ after\ drying}{weight\ of\ sample\ aliquot\ before\ drying} \times 100$$

11.3 Determination of particle size.

11.3.1 Spread the dried sample from Section 11.2.2.2 on a piece of filter paper or aluminum foil in a fume hood or glove box.

11.3.2 Estimate the size of the particles in the sample. If the size of the largest particles is greater than 1 mm, the particle size must be reduced to 1 mm or less prior to extraction using the procedures in Section 11.7.

11.4 Preparation of aqueous samples containing 1% suspended solids or less.

11.4.1 Aqueous samples containing 1% suspended solids or less are prepared using the procedure below and extracted using the SPE technique in Section 12.2.

11.4.2 Preparation of sample and QC aliquots.

11.4.2.1 Mark the original level of the sample on the sample bottle for reference. Weigh the sample plus bottle to ±1 g.

11.4.2.2 Spike 1.0 mL of the diluted labeled-compound spiking solution (Section 7.10.3) into the sample bottle. Cap the bottle and mix the sample by careful shaking. Allow the sample to equilibrate for 1 to 2 hours, with occasional shaking.

11.4.2.3 For each sample or sample batch (to a maximum of 20 samples) to be extracted during the same 12-hour shift, place two 1.0-L aliquots of reagent water in clean sample bottles or flasks.

11.4.2.4 Spike 1.0 mL of the diluted labeled-compound spiking solution (Section 7.10.3) into both reagent water aliquots. One of these aliquots will serve as the method blank.

11.4.2.5 Spike 1.0 mL of the PAR standard (Section 7.14) into the remaining reagent water aliquot. This aliquot will serve as the OPR (Section 15.5).

11.4.2.6 Add 5 mL of methanol to the sample and QC aliquots. Cap and shake the sample and QC aliquots to mix thoroughly and proceed to Section 12.2 for extraction.

11.5 Preparation of samples containing greater than 1% solids.

11.5.1 Weigh a well-mixed aliquot of each sample (of the same matrix type) sufficient to provide 10 g of dry solids (based on the solids determination in Section 11.2) into a clean beaker or glass jar.

11.5.2 Spike 1.0 mL of the diluted labeled compound spiking solution (Section 7.10.3) into the sample.

11.5.3 For each sample or sample batch (to a maximum of 20 samples) to be extracted during the same 12 hour shift, weigh two 10-g aliquots of the appropriate reference matrix (Section 7.6) into clean beakers or glass jars.

11.5.4 Spike 1.0 mL of the diluted labeled compound spiking solution (Section 7.10.3) into each reference matrix aliquot. One aliquot will serve as the method blank. Spike 1.0 mL of the PAR standard (Section 7.14) into the other reference matrix aliquot. This aliquot will serve as the OPR (Section 15.5).

11.5.5 Stir or tumble and equilibrate the aliquots for 1 to 2 hours.

11.5.6 Decant excess water. If necessary to remove water, filter the sample through a glass-fiber filter (Section 6.5.6) and discard the aqueous liquid.

11.5.7 If particles >1 mm are present in the sample (as determined in Section 11.3.2), spread the sample on clean aluminum foil in a hood. After the sample is dry, grind to reduce the particle size (Section 11.7).

11.5.8 Extract the sample and QC aliquots using the SDS procedure in Section 12.3.

11.6 Multiphase samples.

11.6.1 Using the percent solids determined in Section 11.2.1 or 11.2.2, determine the volume of sample that will provide 10 g of solids, up to 1 L of sample.

11.6.2 Pressure filter the amount of sample determined in Section 11.6.1 through Whatman GF/D glass-fiber filter paper (Section 6.5.3). Pressure filter the blank and OPR aliquots through GF/D papers also. If necessary to separate the phases and/or settle the solids, centrifuge these aliquots prior to filtration.

11.6.3 Discard any aqueous phase (if present). Remove any non-aqueous liquid present and reserve the maximum amount filtered from the sample (Section 11.6.1) or 10 g, whichever is less, for combination with the solid phase (Section 12.3.5).

11.6.4 If particles >1 mm are present in the sample (as determined in Section 11.3.2) and the sample is capable of being dried, spread the sample and QC aliquots on clean aluminum foil in a hood. After the aliquots are dry, or if the sample cannot be dried, reduce the particle size using the procedures in Section 11.7 and extract the reduced particles using the SDS procedure in Section 12.3. If particles >1 mm are not present, extract the particles and filter in the sample and QC aliquots directly using the SDS procedure in Section 12.3.

11.7 Sample grinding, homogenization, or blending—Samples with particle sizes greater than 1 mm (as determined in Section 11.3.2) are subjected to grinding, homogenization, or blending. The method of reducing particle size to less than 1 mm is matrix-dependent. In general, hard particles can be reduced by grinding with a mortar and pestle. Softer particles can be reduced by grinding in a Wiley mill or meat grinder, by homogenization, or in a blender.

11.7.1 Each size-reducing preparation procedure on each matrix shall be verified by running the tests in Section 9.2 before the procedure is employed routinely.

11.7.2 The grinding, homogenization, or blending procedures shall be carried out in a glove box or fume hood to prevent particles from contaminating the work environment.

11.7.3 Grinding—Certain papers and pulps, slurries, and amorphous solids can be ground in a Wiley mill or heavy duty meat grinder. In some cases, reducing the temperature of the sample to freezing or to dry ice or liquid nitrogen temperatures can aid in the grinding process. Grind the sample aliquots from Sections 11.5.7 or 11.6.4 in a clean grinder. Do not allow the sample temperature to exceed 50°C. Grind the blank and reference matrix aliquots using a clean grinder.

11.7.4 Homogenization or blending—Particles that are not ground effectively, or particles greater than 1 mm in size after grinding, can often be reduced in size by high speed homogenization or blending. Homogenize and/or blend the particles or filter from Sections 11.5.7 or 11.6.4 for the sample, blank, and OPR aliquots.

11.7.5 Extract the aliquots using the SDS procedure in Section 12.3.

11.8 Fish and other tissues—Prior to processing tissue samples, the laboratory must determine the exact tissue to be analyzed. Common requests for analysis of fish tissue include whole fish–skin on, whole fish–skin removed, edible fish fillets (filleted in the field or by the laboratory), specific organs, and other portions. Once the appropriate tissue has been determined, the sample must be homogenized.

11.8.1 Homogenization.

11.8.1.1 Samples are homogenized while still frozen, where practical. If the laboratory must dissect the whole fish to obtain the appropriate tissue for analysis, the unused tissues may be rapidly refrozen and stored in a clean glass jar for subsequent use.

11.8.1.2 Each analysis requires 10 g of tissue (wet weight). Therefore, the laboratory should homogenize at least 20 g of tissue to allow for re-extraction of a second aliquot of the same homogenized sample, if re-analysis is required. When whole fish analysis is necessary, the entire fish is homogenized.

11.8.1.3 Homogenize the sample in a tissue homogenizer (Section 6.3.3) or grind in a meat grinder (Section 6.3.4). Cut tissue too large to feed into the grinder into smaller pieces. To assure homogeneity, grind three times.

11.8.1.4 Transfer approximately 10 g (wet weight) of homogenized tissue to a clean, tared, 400- to 500-mL beaker.

11.8.1.5 Transfer the remaining homogenized tissue to a clean jar with a fluoropolymer-lined lid. Seal the jar and store the tissue at <-10°C. Return any tissue that was not homogenized to its original container and store at <-10°C.

11.8.2 QC aliquots.

11.8.2.1 Prepare a method blank by adding approximately 10 g of the oily liquid reference matrix (Section 7.6.4) to a 400- to 500-mL beaker.

11.8.2.2 Prepare a precision and recovery aliquot by adding approximately 10 g of the oily liquid reference matrix (Section 7.6.4) to a separate 400- to 500-mL beaker. Record the weight to the nearest 10 mg. If the initial precision and recovery test is to be performed, use four aliquots; if the ongoing precision and recovery test is to be performed, use a single aliquot.

11.8.3 Spiking.

11.8.3.1 Spike 1.0 mL of the labeled compound spiking solution (Section 7.10.3) into the sample, blank, and OPR aliquot.

11.8.3.2 Spike 1.0 mL of the PAR standard (Section 7.14) into the OPR aliquot.

11.8.4 Extract the aliquots using the procedures in Section 12.4.

12.0 Extraction and Concentration

12.1 Extraction procedures include solid phase (Section 12.2) for aqueous liquids; Soxhlet/Dean-Stark (Section 12.3) for solids and filters; and Soxhlet extraction (Section 12.4) for tissues. Acid/base back-extraction (Section 12.5) is used for initial cleanup of extracts.

Macro-concentration procedures include rotary evaporation (Section 12.6.1), heating mantle (Section 12.6.2), and Kuderna-Danish (K-D) evaporation (Section 12.6.3). Micro-concentration uses nitrogen blowdown (Section 12.7).

12.2 SPE of samples containing less than 1% solids.

12.2.1 Disk preparation.

12.2.1.1 Remove the test tube from the suction flask (Figure 4). Place an SPE disk on the base of the filter holder and wet with methylene chloride. While holding a GMF 150 filter above the SPE disk with tweezers, wet the filter with methylene chloride and lay the filter on the SPE disk, making sure that air is not trapped between the filter and disk. Clamp the filter and SPE disk between the 1-L glass reservoir and the vacuum filtration flask.

12.2.1.2 Rinse the sides of the reservoir with approximately 15 mL of methylene chloride using a squeeze bottle or pipet. Apply vacuum momentarily until a few drops appear at the drip tip. Release the vacuum and allow the filter/disk to soak for approximately one minute. Apply vacuum and draw all of the methylene chloride through the filter/disk. Repeat the wash step with approximately 15 mL of acetone and allow the filter/disk to air dry.

12.2.2 Sample extraction.

12.2.2.1 Pre-wet the disk by adding approximately 20 mL of methanol to the reservoir. Pull most of the methanol through the filter/disk, retaining a layer of methanol approximately 2 mm thick on the filter. Do not allow the filter/disk to go dry from this point until the extraction is completed.

12.2.2.2 Add approximately 20 mL of reagent water to the reservoir and pull most through, leaving a layer approximately 2 mm thick on the filter/disk.

12.2.2.3 Allow the sample (Section 11.4.2.2) to stand for 1-2 hours, if necessary, to settle the suspended particles. Decant the clear layer of the sample, the blank (Section 11.4.2.4), or IPR/OPR aliquot (Section 11.4.2.5) into the reservoir and turn on the vacuum to begin the extraction. Adjust the vacuum to complete the extraction in no less than 10 minutes. For samples containing a high concentration of particles (suspended solids), the extraction time may be one hour or longer.

12.2.2.4 Before all of the sample has been pulled through the filter/disk, add approximately 50 mL of reagent water to the sample bottle, swirl to suspend the solids (if present), and pour into the reservoir. Pull through the filter/disk. Use additional reagent water rinses until all solids are removed.

12.2.2.5 Before all of the sample and rinses have been pulled through the filter/disk, rinse the sides of the reservoir with small portions of reagent water.

12.2.2.6 Partially dry the filter/disk under vacuum for approximately 3 minutes.

12.2.3 Elution of the filter/disk.

12.2.3.1 Release the vacuum, remove the entire filter/disk/reservoir assembly from the vacuum flask, and empty the flask. Insert a test tube for eluant collection into the flask. The test tube should have sufficient capacity to contain the total volume of the elution solvent (approximately 50 mL) and should fit around the drip tip. The drip tip should protrude into the test tube to preclude loss of sample from spattering when vacuum is applied. Reassemble the filter/disk/reservoir assembly on the vacuum flask.

12.2.3.2 Wet the filter/disk with 4-5 mL of acetone. Allow the acetone to spread evenly across the disk and soak for 15-20 seconds. Pull the acetone through the disk, releasing the vacuum when approximately 1 mm thickness remains on the filter.

12.2.3.3 Rinse the sample bottle with approximately 20 mL of methylene chloride and transfer to the reservoir. Pull approximately half of the solvent through the filter/disk and release the vacuum. Allow the filter/disk to soak for approximately 1 minute. Pull all of the solvent through the disk. Repeat the bottle rinsing and elution step with another 20 mL of methylene chloride. Pull all of the solvent through the disk.

12.2.3.4 Release the vacuum, remove the filter/disk/reservoir assembly, and remove the test tube containing the sample solution. Quantitatively transfer the solution to a 250-mL separatory funnel and proceed to Section 12.5 for back-extraction.

12.3 SDS extraction of samples containing particles.

12.3.1 Charge a clean extraction thimble (Section 6.4.2.2) with 5.0 g of 100/200 mesh silica (Section 7.5.1.1) topped with 100 g of quartz sand (Section 7.3.2).

Note: *Do not disturb the silica layer throughout the extraction process.*

12.3.2 Place the thimble in a clean extractor. Place 30 to 40 mL of toluene in the receiver and 200 to 250 mL of toluene in the flask.

12.3.3 Pre-extract the glassware by heating the flask until the toluene is boiling. When properly adjusted, 1 to 2 drops of toluene will fall per second from the condenser tip into the receiver. Extract the apparatus for a minimum of 3 hours.

12.3.4 After pre-extraction, cool and disassemble the apparatus. Rinse the thimble with toluene and allow to air dry.

12.3.5 Load the wet sample and/or filter from Sections 11.5.8, 11.6.4, 11.7.3, or 11.7.4 and any non-aqueous liquid from Section 11.6.3 into the thimble and manually mix into the sand layer with a clean metal spatula, carefully breaking up any large lumps of sample.

12.3.6 Reassemble the pre-extracted SDS apparatus, and add a fresh charge of toluene to the receiver and reflux flask. Apply power to the heating mantle to begin refluxing. Adjust the reflux rate to match the rate of percolation through the sand and silica beds until water removal lessens the restriction to toluene flow. Frequently check the apparatus for foaming during the first 2 hours of extraction. If foaming occurs, reduce the reflux rate until foaming subsides.

12.3.7 Drain the water from the receiver at 1 to 2 hours and 8 to 9 hours, or sooner if the receiver fills with water. Reflux the sample for a total of 16 to 24 hours. Cool and disassemble the apparatus. Record the total volume of water collected.

12.3.8 Remove the distilling flask. Drain the water from the Dean-Stark receiver and add any toluene in the receiver to the extract in the flask.

12.3.9 Concentrate the extracts from particles (Sections 11.5-11.7) to approximately 10 mL using the rotary evaporator or heating mantle (Section 12.6.1 or 12.6.2), transfer to a 250-mL separatory funnel, and proceed with back-extraction (Section 12.5).

12.4 Extraction of tissue.

12.4.1 Add 30 to 40 g of powdered anhydrous sodium sulfate to each of the beakers (Section 11.8.1.4, 11.8.2.1, and 11.8.2.2) and mix thoroughly. Cover the beakers with aluminum foil and allow to equilibrate for 12-24 hours. Remix prior to extraction to prevent clumping.

12.4.2 Assemble and pre-extract the Soxhlet apparatus per Sections 12.3.1-12.3.4, except use the methylene chloride:n-hexane (1:1) mixture for the pre-extraction and rinsing and omit the quartz sand. The Dean-Stark moisture trap may also be omitted, if desired.

12.4.3 Reassemble the pre-extracted Soxhlet apparatus and add a fresh charge of methylene chloride:n-hexane to the reflux flask.

12.4.4 Transfer the sample/sodium sulfate mixture (Section 12.4.1) to the Soxhlet thimble, and install the thimble in the Soxhlet apparatus.

12.4.5 Rinse the beaker with several portions of solvent mixture and add to the thimble. Fill the thimble/receiver with solvent. Extract for 18 to 24 hours.

12.4.6 After extraction, cool and disassemble the apparatus.

12.4.7 Quantitatively transfer the extract to a macro-concentration device (Section 12.6), and concentrate to near dryness. Set aside the concentration apparatus for re-use.

12.4.8 Complete the removal of the solvent using the nitrogen blowdown procedure (Section 12.7) and a water bath temperature of 60°C. Weigh the receiver, record the weight, and return the receiver to the blowdown apparatus, concentrating the residue until a constant weight is obtained.

12.4.9 Percent lipid determination—The lipid content is determined by extraction of tissue with the same solvent system (methylene chloride:n-hexane) that was used in EPA's National Dioxin Study (Reference 15) so that lipid contents are consistent with that study.

12.4.9.1 Redissolve the residue in the receiver in n-hexane and spike 1.0 mL of the cleanup standard (Section 7.11) into the solution.

12.4.9.2 Transfer the residue/n-hexane to the anthropogenic isolation column (Section 13.6.1), retaining the boiling chips in the concentration apparatus. Use several rinses to assure that all material is transferred. If necessary, sonicate or heat the receiver slightly to assure that all material is re-dissolved. Allow the receiver to dry. Weigh the receiver and boiling chips.

12.4.9.3 Calculate the lipid content to the nearest three significant figures as follows:

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

12.4.9.4 It is not necessary to determine the lipid content of the blank, IPR, or OPR aliquots.

12.5 Back-extraction with base and acid.

12.5.1 Spike 1.0 mL of the cleanup standard (Section 7.11) into the separatory funnels containing the sample and QC extracts from Section 12.2.3.4 or 12.3.9.

12.5.2 Partition the extract against 50 mL of potassium hydroxide solution (Section 7.1.1). Shake for 2 minutes with periodic venting into a hood. Remove and discard the aqueous layer. Repeat the base washing until no color is visible in the aqueous layer to a maximum of four washings. Minimize contact time between the extract and the base to prevent degradation of the PCBs. Stronger potassium hydroxide solutions may be employed for back-extraction, provided that the laboratory meets the specifications for labeled compound recovery and demonstrates acceptable performance using the procedure in Section 9.2.

12.5.3 Partition the extract against 50 mL of sodium chloride solution (Section 7.1.4) in the same way as with base. Discard the aqueous layer.

12.5.4 Partition the extract against 50 mL of sulfuric acid (Section 7.1.2) in the same way as with base. Repeat the acid washing until no color is visible in the aqueous layer to a maximum of four washings.

12.5.5 Repeat the partitioning against sodium chloride solution and discard the aqueous layer.

12.5.6 Pour each extract through a drying column containing 7 to 10 cm of granular anhydrous sodium sulfate (Section 7.2.1). Rinse the separatory funnel with 30 to 50 mL of solvent, and pour through the drying column. Collect each extract in a round-bottom flask. Re-concentrate the sample and QC aliquots per Sections 12.6-12.7, and clean up the samples and QC aliquots per Section 13.

12.6 Macro-concentration—Extracts in toluene are concentrated using a rotary evaporator or a heating mantle; extracts in methylene chloride or n-hexane are concentrated using a rotary evaporator, heating mantle, or Kuderna-Danish apparatus.

12.6.1 Rotary evaporation—Concentrate the extracts in separate round-bottom flasks.

12.6.1.1 Assemble the rotary evaporator according to manufacturer's instructions, and warm the water bath to 45°C. On a daily basis, preclean the rotary evaporator by concentrating 100 mL of clean extraction solvent through the system. Archive both the concentrated solvent and the solvent in the catch flask for a contamination check if necessary. Between samples, three 2- to 3-mL aliquots of solvent should be rinsed down the feed tube into a waste beaker.

12.6.1.2 Attach the round-bottom flask containing the sample extract to the rotary evaporator. Slowly apply vacuum to the system, and begin rotating the sample flask.

12.6.1.3 Lower the flask into the water bath, and adjust the speed of rotation and the temperature as required to complete concentration in 15 to 20 minutes. At the proper rate of concentration, the flow of solvent into the receiving flask will be steady, but no bumping or visible boiling of the extract will occur.

Note: *If the rate of concentration is too fast, analyte loss may occur.*

12.6.1.4 When the liquid in the concentration flask has reached an apparent volume of approximately 2 mL, remove the flask from the water bath and stop the rotation. Slowly and carefully admit air into the system. Be sure not to open the valve so quickly that the sample is blown out of the flask. Rinse the feed tube with approximately 2 mL of solvent.

12.6.1.5 Proceed to Section 12.6.4 for preparation for back-extraction or micro-concentration and solvent exchange.

12.6.2 Heating mantle—Concentrate the extracts in separate round-bottom flasks.

12.6.2.1 Add one or two clean boiling chips to the round-bottom flask, and attach a three-ball macro-Snyder column. Pre-wet the column by adding approximately 1 mL of solvent through the top. Place the round-bottom flask in a heating mantle, and apply heat as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood.

12.6.2.2 When the liquid has reached an apparent volume of approximately 10 mL, remove the round-bottom flask from the heating mantle and allow the solvent to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the glass joint into the receiver with small portions of solvent.

12.6.2.3 Proceed to Section 12.6.4 for preparation for back-extraction or micro-concentration and solvent exchange.

12.6.3 Kuderna-Danish (K-D)—Concentrate the extracts in separate 500-mL K-D flasks equipped with 10-mL concentrator tubes. The K-D technique is used for solvents such as methylene chloride and n-hexane. Toluene is difficult to concentrate using the K-D technique unless a water bath fed by a steam generator is used.

12.6.3.1 Add 1 to 2 clean boiling chips to the receiver. Attach a three-ball macro-Snyder column. Pre-wet the column by adding approximately 1 mL of solvent through the top. Place the K-D apparatus in a hot water bath so that the entire lower rounded surface of the flask is bathed with steam.

12.6.3.2 Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

12.6.3.3 When the liquid has reached an apparent volume of 1 mL, remove the K-D apparatus from the bath and allow the solvent to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of solvent. A 5-mL syringe is recommended for this operation.

12.6.3.4 Remove the three-ball Snyder column, add a fresh boiling chip, and attach a two-ball micro-Snyder column to the concentrator tube. Pre-wet the column by adding approximately 0.5 mL of solvent through the top. Place the apparatus in the hot water bath.

12.6.3.5 Adjust the vertical position and the water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

12.6.3.6 When the liquid reaches an apparent volume of 0.5 mL, remove the apparatus from the water bath and allow to drain and cool for at least 10 minutes.

12.6.3.7 Proceed to 12.6.4 for preparation for back-extraction or micro-concentration and solvent exchange.

12.6.4 Preparation for back-extraction or micro-concentration and solvent exchange.

12.6.4.1 For back-extraction (Section 12.5), transfer the extract to a 250-mL separatory funnel. Rinse the concentration vessel with small portions of n-hexane, adjust the n-hexane volume in the separatory funnel to 10 to 20 mL, and proceed to back-extraction (Section 12.5).

12.6.4.2 For determination of the weight of residue in the extract or for clean-up procedures other than back-extraction, transfer the extract to a blowdown vial using 2-3 rinses of solvent. Proceed with micro-concentration and solvent exchange (Section 12.7).

12.7 Micro-concentration and solvent exchange.

12.7.1 Extracts to be subjected to GPC or HPLC cleanup are exchanged into methylene chloride. Extracts to be cleaned up using silica gel, carbon, and/or Florisil are exchanged into n-hexane.

12.7.2 Transfer the vial containing the sample extract to a nitrogen blowdown device. Adjust the flow of nitrogen so that the surface of the solvent is just visibly disturbed.

Note: *A large vortex in the solvent may cause analyte loss.*

12.7.3 Lower the vial into a 45°C water bath and continue concentrating.

12.7.3.1 If the extract is to be concentrated to dryness for weight determination (Sections 12.4.8 and 13.6.4), blow dry until a constant weight is obtained.

12.7.3.2 If the extract is to be concentrated for injection into the GC/MS or the solvent is to be exchanged for extract cleanup, proceed as follows:

12.7.4 When the volume of the liquid is approximately 100 µL, add 2 to 3 mL of the desired solvent (methylene chloride for GPC and HPLC, or n-hexane for the other cleanups) and continue concentration to approximately 100 µL. Repeat the addition of solvent and concentrate once more.

12.7.5 If the extract is to be cleaned up by GPC, adjust the volume of the extract to 5.0 mL with methylene chloride. If the extract is to be cleaned up by HPLC, further concentrate the extract to 30 µL. Proceed with GPC or HPLC cleanup (Section 13.2 or 13.5, respectively).

12.7.6 If the extract is to be cleaned up by column chromatography (silica gel, Carbopak/Celite, or Florisil), bring the final volume to 1.0 mL with n-hexane. Proceed with column cleanups (Sections 13.3 - 13.4 and 13.7).

12.7.7 If the extract is to be concentrated for injection into the GC/MS (Section 14), quantitatively transfer the extract to a 0.3-mL conical vial for final concentration, rinsing the larger vial with n-hexane and adding the rinse to the conical vial. Reduce the volume to approximately 100 µL. Add 10 µL of nonane to the vial, and evaporate the solvent to the level of the nonane. Seal the vial and label with the sample number. Store in the dark at room temperature until ready for GC/MS analysis. If GC/MS analysis will not be performed on the same day, store the vial at <-10°C.

13.0 Extract Cleanup

13.1 Cleanup may not be necessary for relatively clean samples (e.g., treated effluents, groundwater, drinking water). If particular circumstances require the use of a cleanup procedure, the analyst may use any or all of the procedures below or any other appropriate procedure. Before using a cleanup procedure, the analyst must demonstrate that the requirements of Section 9.2 can be met using the cleanup procedure. If PCBs 77, 126, and 169 only are to be determined, the cleanup procedures may be optimized for isolation of these compounds.

13.1.1 Gel permeation chromatography (Section 13.2) removes high molecular weight interferences that cause GC column performance to degrade. It should be used for all soil and sediment extracts. It may be used for water extracts that are expected to contain high molecular weight organic compounds (e.g., polymeric materials, humic acids). It may also be used for tissue extracts after initial cleanup on the anthropogenic isolation column (Section 13.6).

13.1.2 Acid, neutral, and basic silica gel (Section 13.3) and Florisil (Section 13.7) are used to remove nonpolar and polar interferences.

13.1.3 Carbopak/Celite (Section 13.4) can be used to separate PCBs 77, 126, and 169 from the mono- and di- ortho-substituted PCBs, if desired.

13.1.4 HPLC (Section 13.5) is used to provide specificity for certain congeners and congener groups.

13.1.5 The anthropogenic isolation column (Section 13.6) is used for removal of lipids from tissue samples.

13.2 Gel permeation chromatography (GPC).

13.2.1 Column packing.

13.2.1.1 Place 70 to 75 g of SX-3 Bio-beads (Section 6.7.1.1) in a 400- to 500-mL beaker.

13.2.1.2 Cover the beads with methylene chloride and allow to swell overnight (a minimum of 12 hours).

13.2.1.3 Transfer the swelled beads to the column (Section 6.7.1.1) and pump solvent through the column, from bottom to top, at 4.5 to 5.5 mL/minute prior to connecting the column to the detector.

13.2.1.4 After purging the column with solvent for 1 to 2 hours, adjust the column head pressure to 7 to 10 psig and purge for 4 to 5 hours to remove air. Maintain a head pressure of 7 to 10 psig. Connect the column to the detector (Section 6.7.1.4).

13.2.2 Column calibration.

13.2.2.1 Load 5 mL of the calibration solution (Section 7.4) into the sample loop.

13.2.2.2 Inject the calibration solution and record the signal from the detector. The elution pattern will be corn oil, PCB 209, pentachlorophenol, perylene, and sulfur.

13.2.2.3 Set the "dump time" to allow >85% removal of the corn oil and >85% collection of PCB 209.

13.2.2.4 Set the "collect time" to the peak minimum between perylene and sulfur.

13.2.2.5 Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the pentachlorophenol is greater than 85%. If calibration is not verified, the system shall be recalibrated using the calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated GPC system.

13.2.3 Extract cleanup—GPC requires that the column not be overloaded. The column specified in this method is designed to handle a maximum of 0.5 g of high molecular weight material in a 5-mL extract. If the extract is known or expected to contain more than 0.5 g, the extract is split into aliquots for GPC, and the aliquots are combined after elution from the column. The residue content of the extract may be obtained gravimetrically by evaporating the solvent from a 50-µL aliquot.

13.2.3.1 Filter the extract or load through the filter holder (Section 6.7.1.3) to remove the particles. Load the 5.0-mL extract onto the column.

13.2.3.2 Elute the extract using the calibration data determined in Section 13.2.2. Collect the eluate in a clean 400- to 500-mL beaker.

13.2.3.3 Rinse the sample loading tube thoroughly with methylene chloride between extracts to prepare for the next sample.

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

13.2.3.5 Concentrate the eluate per Section 12.6 and Section 12.7 for further cleanup or injection into the GC/MS.

13.3 Silica gel cleanup.

13.3.1 Place a glass-wool plug in a 15-mm ID chromatography column (Section 6.7.4.2). Pack the column bottom to top with 1 g silica gel (Section 7.5.1.1), 4 g basic silica gel (Section 7.5.1.3),

1 g silica gel, 8 g acid silica gel (Section 7.5.1.2), 2 g silica gel, and 4 g granular anhydrous sodium sulfate (Section 7.2.1). Tap the column to settle the adsorbents.

13.3.2 Pre-elute the column with 50 to 100 mL of n-hexane. Close the stopcock when the n-hexane is within 1 mm of the sodium sulfate. Discard the eluate. Check the column for channeling. If channeling is present, discard the column and prepare another.

13.3.3 Apply the concentrated extract to the column. Open the stopcock until the extract is within 1 mm of the sodium sulfate.

13.3.4 Rinse the receiver twice with 1-mL portions of n-hexane, and apply separately to the column. Elute the PCBs with 25 mL of n-hexane and collect the eluate.

13.3.5 Concentrate the eluate per Section 12.6 and 12.7 for further cleanup or injection into the HPLC or GC/MS.

13.3.6 For extracts of samples known to contain large quantities of other organic compounds (such as paper mill effluents), it may be advisable to increase the capacity of the silica gel column. This may be accomplished by increasing the strengths of the acid and basic silica gels. The acid silica gel (Section 7.5.1.2) may be increased in strength to as much as 44% w/w (7.9 g sulfuric acid added to 10 g silica gel). The basic silica gel (Section 7.5.1.3) may be increased in strength to as much as 33% w/w (50 mL 1N NaOH added to 100 g silica gel), or the potassium silicate (Section 7.5.1.4) may be used.

Note: *The use of stronger acid silica gel (44% w/w) may lead to charring of organic compounds in some extracts. The charred material may retain some of the analytes and lead to lower recoveries of the PCBs. Increasing the strengths of the acid and basic silica gel may also require different volumes of n-hexane than those specified above to elute the analytes from the column. Therefore, the performance of the method after such modifications must be verified by the procedure in Section 9.2.*

13.4 Carbon column (Reference 16).

13.4.1 Cut both ends from a 50-mL disposable serological pipet (Section 6.7.3.2) to produce a 20-cm column. Fire-polish both ends and flare both ends if desired. Insert a glass-wool plug at one end, and pack the column with 3.6 g of Carbopak/Celite (Section 7.5.2.3) to form an adsorbent bed 20 cm long. Insert a glass-wool plug on top of the bed to hold the adsorbent in place.

13.4.2 Pre-elute the column with 20 mL each in succession of toluene, methylene chloride, and n-hexane.

13.4.3 When the solvent is within 1 mm of the column packing, apply the n-hexane sample extract to the column. Rinse the sample container twice with 1-mL portions of n-hexane and apply separately to the column. Apply 2 mL of n-hexane to complete the transfer.

13.4.4 Elute the column with 25 mL of n-hexane and collect the eluate. This fraction will contain the mono- and di-ortho PCBs. If carbon particles are present in the eluate, filter through glass-fiber filter paper.

13.4.5 Elute the column with 15 mL of methanol and discard the eluate. The fraction discarded will contain residual lipids and other potential interferents, if present.

13.4.6 Elute the column with 15 mL of toluene and collect the eluate. This fraction will contain PCBs 77, 126, and 169. If carbon particles are present in the eluate, filter through glass-fiber filter paper.

13.4.7 Concentrate the fractions per Section 12.6 and 12.7 for further cleanup or injection into the HPLC or GC/MS.

13.5 HPLC (Reference 17).

13.5.1 Column calibration.

13.5.1.1 Prepare a calibration standard containing the toxic congeners and other congeners of interest at a concentration of approximately TBD pg/µL in methylene chloride.

13.5.1.2 Inject 30 µL of the calibration solution into the HPLC and record the signal from the detector. Collect the eluant for reuse. The elution order will be the mono- through deca-congeners.

13.5.1.3 Establish the collection time for the congeners of interest. Following calibration, flush the injection system with copious quantities of methylene chloride, including a minimum of five 50-µL injections while the detector is monitored, to ensure that residual PCBs are removed from the system.

13.5.1.4 Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the PCBs from the calibration standard is 75 to 125% compared to the calibration (Section 13.5.1.1). If calibration is not verified, the system shall be recalibrated using the calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated system.

13.5.2 Extract cleanup—HPLC requires that the column not be overloaded. The column specified in this method is designed to handle a maximum of 30 µL of extract. If the extract cannot be concentrated to less than 30 µL, it is split into fractions and the fractions are combined after elution from the column.

13.5.2.1 Rinse the sides of the vial twice with 30 µL of methylene chloride and reduce to 30 µL with the evaporation apparatus (Section 6.8.3).

13.5.2.2 Inject the 30 µL extract into the HPLC.

13.5.2.3 Elute the extract using the calibration data determined in Section 13.5.1. Collect the fraction(s) in a clean 20-mL concentrator tube containing 5 mL of n-hexane:acetone (1:1 v/v).

13.5.2.4 If an extract containing greater than TBD ng/mL of total PCBs is encountered, a 30-µL methylene chloride blank shall be run through the system to check for carry-over.

13.5.2.5 Concentrate the eluate per Section 12.7 for injection into the GC/MS.

13.6 Anthropogenic isolation column (References 1-2)—Used for removal of lipids from tissue extracts.

13.6.1 Prepare the column as given in Section 7.5.3.

13.6.2 Pre-elute the column with 100 mL of n-hexane. Drain the n-hexane layer to the top of the column, but do not expose the sodium sulfate.

13.6.3 Load the sample and rinses (Section 12.4.9.2) onto the column by draining each portion to the top of the bed. Elute the PCBs from the column into the apparatus used for concentration (Section 12.4.7) using 200 mL of n-hexane.

13.6.4 Concentrate the cleaned up extract (Sections 12.6-12.7) to constant weight per Section 12.7.3.1. If more than 500 mg of material remains, repeat the cleanup using a fresh anthropogenic isolation column.

13.6.5 Redissolve the extract in a solvent suitable for the additional cleanups to be used (Section 13.2-13.5 and 13.7).

13.6.6 Spike 1.0 mL of the cleanup standard (Section 7.11) into the residue/solvent.

13.6.7 Clean up the extract using the procedures in Sections 13.2-13.5 and 13.7. Florisil (Section 13.7) and carbon (Section 13.4) are recommended as minimum additional cleanup steps.

13.6.8 Following cleanup, concentrate the extract to 10 µL as described in Section 12.7 and proceed with the analysis in Section 14.

13.7 Florisil cleanup (Reference 18).

13.7.1 Begin to drain the n-hexane from the column (Section 7.5.4). Adjust the flow rate of eluant to 4.5-5.0 mL/min.

13.7.2 When the n-hexane is within 1 mm of the sodium sulfate, apply the sample extract (in n-hexane) to the column. Rinse the sample container twice with 1-mL portions of n-hexane and apply to the column.

13.7.3 Elute the mono-ortho and di-ortho PCBs with approximately 165 mL of n-hexane and collect the eluate. Elute the non-ortho co-planar PCBs with approximately 100 mL of 6% ether:n-hexane and collect the eluate. The exact volumes of solvents will need to be determined for each batch of Florisil. If the mono/di-ortho PCBs are not to be separated from the non-ortho co-planar PCBs, elute all PCBs with 6% ether:n-hexane.

13.7.4 Concentrate the eluate(s) per Sections 12.6-12.7 for further cleanup or for injection into the HPLC or GC/MS.

14.0 HRGC/HRMS Analysis

14.1 Establish the operating conditions given in Section 10.1.

14.2 Add 10 µL of the appropriate internal standard solution (Section 7.12) to the sample extract immediately prior to injection to minimize the possibility of loss by evaporation, adsorption, or reaction. If an extract is to be reanalyzed and evaporation has occurred, do not add more instrument internal standard solution. Rather, bring the extract back to its previous volume (e.g., 19 µL) with pure nonane only (18 µL if 2 µL injections are used).

14.3 Inject 1.0 or 2.0 µL of the concentrated extract containing the internal standard solution, using on-column or splitless injection. The volume injected must be identical to the volume used for calibration (Section 10). Start the GC column initial isothermal hold upon injection. Start MS data collection after the solvent peak elutes. Stop data collection after the $^{13}C_{12}$-PCB 209 has eluted. If PCBs 77, 126, and 169 only are to be determined, stop data collection after $^{13}C_{12}$-PCB 169 has eluted. Return the column to the initial temperature for analysis of the next extract or standard.

15.0 System and Laboratory Performance

15.1 At the beginning of each 12-hour shift during which analyses are performed, GC/MS system performance and calibration are verified for all native PCBs and labeled compounds. For these tests,

analysis of the CS3 calibration verification (VER) standard (Section 7.13 and Table 4) and the congener specificity test standards (Section 7.15 and Table 5) shall be used to verify all performance criteria. Adjustment and/or recalibration (Section 10) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, IPRs, and OPRs be analyzed.

15.2 MS resolution—A static resolving power of at least 10,000 (10% valley definition) must be demonstrated at the appropriate m/z before any analysis is performed. Static resolving power checks must be performed at the beginning and at the end of each 12-hour shift according to procedures in Section 10.1.2. Corrective actions must be implemented whenever the resolving power does not meet the requirement.

15.3 Calibration verification.

15.3.1 Inject the VER standard using the procedure in Section 14.

15.3.2 The m/z abundance ratios for all PCBs shall be within the limits in Table 9; otherwise, the mass spectrometer shall be adjusted until the m/z abundance ratios fall within the limits specified, and the verification test shall be repeated. If the adjustment alters the resolution of the mass spectrometer, resolution shall be verified (Section 10.1.2) prior to repeat of the verification test.

15.3.3 The peaks representing each native PCB and labeled compound in the VER standard must be present with a S/N of at least 10; otherwise, the mass spectrometer shall be adjusted and the verification test repeated.

15.3.4 Compute the concentration of each native PCB compound by isotope dilution (Section 17.1) for those compounds that have labeled analogs (Table 1). Compute the concentration of each native compound that does not have a labeled analog and of each labeled compound by the internal standard method (Section 17.2). These concentrations are computed based on the calibration data in Section 10.

15.3.5 For each compound, compare the concentration with the calibration verification limit in Table 6. If PCBs 77, 126, and 169 only are to be determined, compare the concentration to the limit in Table 6a. If all compounds meet the acceptance criteria, calibration has been verified and analysis of standards and sample extracts may proceed. If, however, any compound fails its respective limit, the measurement system is not performing properly for that compound. In this event, prepare a fresh calibration standard or correct the problem causing the failure and repeat the resolution (Section 15.2) and verification (Section 15.3) tests, or recalibrate (Section 10).

15.4 Retention times and GC resolution.

15.4.1 Retention times.

15.4.1.1 Absolute—The absolute retention times of the GC/MS internal standards in the verification test (Section 15.3) shall be within ±15 seconds of the retention times obtained during calibration (Section 10.2.4).

15.4.1.2 Relative—The relative retention times of native PCBs and labeled compounds in the verification test (Section 15.3) shall be within 5 percent of the relative retention times given in Table 2.

15.4.2 GC resolution.

15.4.2.1 Inject the isomer specificity standards (Section 7.15) on their respective columns.

15.4.2.2 The valley height between PCBs 123 and 118 at m/z 325.8804 shall not exceed 10 percent on the SPB-Octyl column, and the valley height between PCBs 156 and 157 shall not exceed 10 percent at m/z 359.8415 on the DB-1 column (Figures 6 and 7).

15.4.3 If the absolute retention time of any compound is not within the limits specified or if the congeners are not resolved, the GC is not performing properly. In this event, adjust the GC and repeat the verification test (Section 15.3) or recalibrate (Section 10), or replace the GC column and either verify calibration or recalibrate.

15.5 Ongoing precision and recovery.

15.5.1 Analyze the extract of the ongoing precision and recovery (OPR) aliquot (Section 11.4.2.5, 11.5.4, 11.6.2, 11.7.4, or 11.8.3.2) prior to analysis of samples from the same batch.

15.5.2 Compute the concentration of each native PCB by isotope dilution for those compounds that have labeled analogs (Section 17.1). Compute the concentration of the native PCBs that have no labeled analog and each labeled compound by the internal standard method (Section 17.2).

15.5.3 For each PCB and labeled compound, compare the concentration to the OPR limits given in Table 6. If PCBs 77, 126, and 169 only are to be determined, compare the concentration to the limits in Table 6a. If all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may proceed. If, however, any individual concentration falls outside of the range given, the extraction/concentration processes are not being performed properly for that compound. In this event, correct the problem, re-prepare, extract, and clean up the sample batch and repeat the ongoing precision and recovery test (Section 15.5).

15.5.4 Add results that pass the specifications in Section 15.5.3 to initial and previous ongoing data for each compound in each matrix. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory accuracy for each congener in each matrix type by calculating the average percent recovery (R) and the standard deviation of percent recovery (S_R). Express the accuracy as a recovery interval from $R - 2S_R$ to $R + 2S_R$. For example, if R = 95% and S_R = 5%, the accuracy is 85 to 105%.

15.6 Blank—Analyze the method blank extracted with each sample batch immediately following analysis of the OPR aliquot to demonstrate freedom from contamination and freedom from carryover from the OPR analysis. The results of the analysis of the blank must meet the specifications in Section 9.5.2 before sample analyses may proceed.

16.0 Qualitative Determination

A PCB or labeled compound is identified in a standard, blank, or sample when all of the criteria in Sections 16.1 through 16.4 are met.

16.1 The signals for the two exact m/z's in Table 8 must be present and must maximize within the same two seconds.

16.2 The signal-to-noise ratio (S/N) for the GC peak at each exact m/z must be greater than or equal to 2.5 for each PCB detected in a sample extract, and greater than or equal to 10 for all PCBs in the calibration standard (Sections 10.2.3 and 15.3.3).

16.3 The ratio of the integrated areas of the two exact m/z's specified in Table 8 must be within the limit in Table 9, or within ±10 percent of the ratio in the midpoint (CS3) calibration or calibration verification (VER), whichever is most recent.

16.4 The relative retention time of the peak for a toxic PCB must be within 5 percent of the relative retention times listed in Table 2. The retention time of peaks representing PCBs other than the toxic PCBs must be within the retention time windows established in Section 10.3.

16.5 Confirmatory analysis—Isomer specificity for PCBs 156 and 157 cannot be achieved on the SPB-Octyl column. Therefore, any sample in which these PCBs are tentatively identified by analysis on the SPB-Octyl column and when rigorous identification is required must have a confirmatory analysis performed on a DB-1 or equivalent GC column. The operating conditions in Section 10.1.1 may be adjusted to optimize the analysis on the second GC column, but the GC/MS must meet the mass resolution and calibration specifications in Section 10.

16.6 If the criteria for identification in Sections 16.1-16.5 are not met, the PCB has not been identified and the results may not be reported for regulatory compliance purposes. If interferences preclude identification, a new aliquot of sample must be extracted, further cleaned up, and analyzed.

17.0 Quantitative Determination

17.1 Isotope dilution quantitation—By adding a known amount of a labeled compound to every sample prior to extraction, correction for recovery of the PCB can be made because the native compound and its labeled analog exhibit similar effects upon extraction, concentration, and gas chromatography. Relative response (RR) values are used in conjunction with the initial calibration data described in Section 10.5 to determine concentrations directly, so long as labeled compound spiking levels are constant, using the following equation:

$$C_{ex}\ (ng/mL) = \frac{(A1_n + A2_n)\ C_l}{(A1_l + A2_l)\ RR}$$

where:
C_{ex} = *The concentration of the PCB in the extract.*
The other terms are as defined in Section 10.5.2

Any peaks representing the other congeners are quantitated using an average of the response factors from all of the labeled PCBs isomers at the same level of chlorination.

17.2 Internal standard quantitation and labeled compound recovery.

17.2.1 Compute the concentrations of labeled analogs (including the cleanup standard) in the extract using the response factors determined from the initial calibration data (Section 10.6) and the following equation:

$$C_{ex}\ (ng/mL) = \frac{(A1_s + A2_s)\ C_{is}}{(A1_{is} + A2_{is})\ RF}$$

where:
C_{ex} = *The concentration of the labeled compound in the extract.*
The other terms are as defined in Section 10.6.1

17.2.2 Using the concentration in the extract determined above, compute the percent recovery of the labeled compounds (including the cleanup standard) using the following equation:

$$Recovery\ (\%) = \frac{Concentration\ found\ (\mu g/mL)}{Concentration\ spiked\ (\mu g/mL)} \times 100$$

17.3 The concentration of a native PCB in the solid phase of the sample is computed using the concentration of the compound in the extract and the weight of the solids (Section 11.2.2.3), as follows:

$$Concentration\ in\ solid\ (ng/kg) = \frac{(C_{ex} \times V_{ex})}{W_s}$$

where:
C_{ex} = *The concentration of the compound in the extract.*
V_{ex} = *The extract volume in mL.*
W_s = *The sample weight (dry weight) in kg.*

17.4 The concentration of a native PCB in the aqueous phase of the sample is computed using the concentration of the compound in the extract and the volume of water extracted (Section 11.4), as follows:

$$Concentration\ in\ aqueous\ phase\ (pg/L) = \frac{(C_{ex} \times V_{ex})}{V_s}$$

where:
C_{ex} = *The concentration of the compound in the extract.*
V_{ex} = *The extract volume in mL.*
V_s = *The sample volume in liters.*

17.5 If the SICP area at either quantitation m/z for any compound exceeds the calibration range of the system, a smaller sample aliquot is extracted.

17.5.1 For aqueous samples containing 1% solids or less, dilute 100 mL, 10 mL, etc., of sample to 1 L with reagent water and re-prepare, extract, clean up, and analyze per Sections 11 - 14.

17.5.2 For samples containing greater than 1% solids, extract an amount of sample equal to 1/10, 1/100, etc., of the amount used in Section 11.5.1. Re-prepare, extract, clean up, and analyze per Sections 11-14.

17.5.3 If a smaller sample size will not be representative of the entire sample, dilute the sample extract by a factor of 10, adjust the concentration of the instrument internal standard to 100 pg/µL in the extract, and analyze an aliquot of this diluted extract by the internal standard method.

17.6 Results are reported to three significant figures for the PCBs and labeled compounds found in all standards, blanks, and samples.

17.6.1 Reporting units and levels.

17.6.1.1 Aqueous samples—Report results in pg/L (parts-per-quadrillion).

17.6.1.2 Samples containing greater than 1% solids (soils, sediments, filter cake, compost)—Report results in ng/kg based on the dry weight of the sample. Report the percent solids so that the result may be corrected.

17.6.1.3 Tissues—Report results in ng/kg of wet tissue, not on the basis of the lipid content of the sample. Report the percent lipid content, so that the data user can calculate the concentration on a lipid basis if desired.

17.6.1.4 Reporting level.

17.6.1.4.1 Standards (VER, IPR, OPR) and samples—Report results at or above the minimum level (Table 2). Report results below the minimum level as not detected or as required by the regulatory authority.

17.6.1.4.2 Blanks—Report results above the MDL or as required by the regulatory authority. Do not blank-correct results. If a blank accompanying a sample result shows contamination above the MDL for the congener, flag the sample result and report the results for the sample and the accompanying blank.

17.6.2 Results for PCBs in samples that have been diluted are reported at the least dilute level at which the areas at the quantitation m/z's are within the calibration range (Section 17.5).

17.6.3 For PCBs having a labeled analog, results are reported at the least dilute level at which the area at the quantitation m/z is within the calibration range (Section 17.5) and the labeled compound recovery is within the normal range for the method (Section 9.3 and Tables 6, 6a, 7, and 7a).

17.6.4 Additionally, if requested, the total concentration of all congeners at a given level of chlorination (i.e., total TCB, total PeCB, total HxCB, etc.) may be reported by summing the concentrations of all congeners identified in that level of chlorination, including both the toxic and other congeners.

18.0 Analysis of Complex Samples

18.1 Some samples may contain high levels (>10 ng/L; >1000 ng/kg) of the compounds of interest, interfering compounds, and/or polymeric materials. Some extracts will not concentrate to 10 µL (Section 12.7); others may overload the GC column and/or mass spectrometer.

18.2 Analyze a smaller aliquot of the sample (Section 17.5) when the extract will not concentrate to 10 µL after all cleanup procedures have been exhausted.

18.3 Several laboratories have reported that elimination of several of the toxic PCBs, particularly non-coplanar congeners 105, 114, 118, 123, 156, 157, and 167 is difficult. Backgrounds of these congeners can therefore interfere with the determination of these congeners in environmental samples. Care should therefore be exercised in the determination of these congeners.

18.4 Recovery of labeled compounds—In most samples, recoveries of the labeled compounds will be similar to those from reagent water or from the alternate matrix (Section 7.6).

18.4.1 If the recovery of any of the labeled compounds is outside of the normal range (Table 7), a diluted sample shall be analyzed (Section 17.5).

18.4.2 If the recovery of any of the labeled compounds in the diluted sample is outside of normal range, the calibration verification standard (Section 7.13) shall be analyzed and calibration verified (Section 15.3).

18.4.3 If the calibration cannot be verified, a new calibration must be performed and the original sample extract reanalyzed.

18.4.4 If the calibration is verified and the diluted sample does not meet the limits for labeled compound recovery, the method does not apply to the sample being analyzed and the result may not be reported for regulatory compliance purposes. In this case, alternate extraction and cleanup procedures in this method must be employed to resolve the interference. If all cleanup procedures in this method have been employed and labeled compound recovery remains outside of the normal range, extraction and/or cleanup procedures that are beyond this scope of this method will be required to analyze these samples.

19.0 Method Performance

For this draft version of Method 1668, performance was validated and preliminary data were collected in a single laboratory.

20.0 Pollution Prevention

20.1 The solvents used in this method pose little threat to the environment when managed properly. The solvent evaporation techniques used in this method are amenable to solvent recovery, and it is recommended that the laboratory recover solvents wherever feasible.

20.2 Standards should be prepared in volumes consistent with laboratory use to minimize disposal of standards.

21.0 Waste Management

21.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance is also required with any sewage discharge permits and regulations.

21.2 Samples containing HCl or H_2SO_4 to pH <2 are hazardous and must be neutralized before being poured down a drain or must be handled as hazardous waste.

21.3 The PCBs decompose above 800°C. Low-level waste such as absorbent paper, tissues, animal remains, and plastic gloves may be burned in an appropriate incinerator. Gross quantities (milligrams) should be packaged securely and disposed of through commercial or governmental channels that are capable of handling extremely toxic wastes.

21.4 [This section may need to be modified to accommodate the PCBs: Liquid or soluble waste should be dissolved in methanol or ethanol and irradiated with ultraviolet light with a wavelength shorter than

290 nm for several days. Use F40 BL or equivalent lamps. Analyze liquid wastes, and dispose of the solutions when the PCBs can no longer be detected.]

21.5 For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" and "Less is Better–Laboratory Chemical Management for Waste Reduction," available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

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13 "Methods 330.4 and 330.5 for Total Residual Chlorine," USEPA, EMSL, Cincinnati, OH 45268, EPA 600/4-70-020, March 1979.

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23.0 Tables and Figures

Table 1. Toxic Polychlorinated Biphenyls Determined by Isotope Dilution and Internal Standard High Resolution Gas Chromatography (HRGC)/High Resolution Mass Spectrometry (HRMS)

PCB[1]	Native compound CAS Registry No.	IUPAC No.	$^{13}C_{12}$ analog CAS Registry No.
3,3',4,4'-TCB	32598-13-3	77	160901-67-7
2,3,3',4,4'-PeCB	32598-14-4	105	160901-70-2
2,3,4,4',5-PeCB	74472-37-0	114	160901-72-4
2,3',4,4',5-PeCB	31508-00-6	118	160901-73-5
2',3,4,4',5-PeCB	65510-44-3	123	160901-74-6
3,3',4,4',5-PeCB	57465-28-8	126	160901-75-7
2,3,3',4,4',5-HxCB	38380-08-4	156	160901-77-9
2,3,3',4,4',5'-HxCB	69782-90-7	157	160901-78-0
2,3',4,4',5,5'-HxCB	52663-72-6	167	161627-18-5
3,3',4,4',5,5'-HxCB	32774-16-6	169	160901-79-1
2,2',3,3',4,4',5-HpCB	35065-30-6	170	160901-80-4
2,2',3,4,4',5,5'-HpCB	35065-29-3	180	160901-82-6
2,3,3',4,4',5,5'-HpCB	39635-31-9	189	160901-83-7
Cleanup standards			
$^{13}C_{12}$-3,4,4',5-TCB		81	160901-68-8
$^{13}C_{12}$-2,3,3',5,5'-PeCB		111	160901-71-3
Internal standards			
$^{13}C_{12}$-2,2',5,5'-TCB		52	160901-66-6
$^{13}C_{12}$-2,2',4,4,5'-PeCB		101	160901-69-9
$^{13}C_{12}$-2,2',3,4,4',5'-HxCB		138	160901-76-8
$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB		178	160901-81-5
Final eluter standard			
$^{13}C_{12}$-DCB		209	160901-84-8

[1] Polychlorinated biphenyls:
TCB = Tetrachlorobiphenyl
PeCB = Pentachlorobiphenyl
HxCB = Hexachlorobiphenyl
HpCB = Heptachlorobiphenyl
DCB = Decachlorobiphenyl

Table 2. Retention Time (RT) References, Quantitation References, Relative Retention Times (RRTs), Estimated Method Detection Limits (EMDLs), and Estimated Minimum Levels (EMLs) for the Toxic PCBs[a]

IUPAC[1] No.	Labeled or native PCB	IUPAC[1] No.	Retention time and quantitation reference	RT (min)[2]	RRT	Matrix and Concentration: Water (pg/l) EMDL	Water (pg/l) EML	Other (ng/Kg) EMDL	Other (ng/Kg) EML	Extract (pg/µL) EML
52L	13C12-2,2',5,5'-TCB	52L	13C12-2,2',5,5'-TCB	12.87	1.000					
81L	13C12-3,4,4'5-TCB[4]	52L	13C12-2,2',5,5'-TCB	19.65	1.527					
77L	13C12-3,3',4,4'-TCB	52L	13C12-2,2',5,5'-TCB	20.15	1.566					
77	3,3',4,4'-TCB	77L	13C12-3,3',4,4'-TCB	20.18	1.002	5	20	0.5	2	1
Penta congeners using 13C12-2,2',4,5,5'-PeCB (#101L) as the injection internal standard										
101L	13C12-2,2',4,5,5'-PeCB	101L	13C12-2,2',4,5,5'-PeCB	17.83	1.000					
111L	13C12-2,3,3',5,5'-PeCB[4]	101L	13C12-2,2',4,5,5'-PeCB	20.12	1.128					
123	2',3,4,4',5-PeCB	118L	13C12-2,3',4,4',5-PeCB	21.98	0.987	40	100	4	10	5
118L	13C12-2,3',4,4',5-PeCB	101L	13C12-2,2',4,5,5'-PeCB	22.27	1.249					
118	2,3',4,4',5-PeCB	118L	13C12-2,3',4,4',5-PeCB	22.30	1.001	60	200	6	20	10
114	2,3,4,4',5-PeCB	105L	13C12-2,3,3',4,4'-PeCB	22.82	0.974	600	2000	60	200	100
105L	13C12-2,3,3',4,4'-PeCB	101L	13C12-2,2',4,5,5'-PeCB	23.42	1.313					
105	2,3,3',4,4'-PeCB	105L	13C12-2,3,3',4,4'-PeCB	23.43	1.000	400	1000	40	100	50
126L	13C12-3,3',4,4',5-PeCB	101L	13C12-2,2',4,5,5'-PeCB	26.55	1.489					
126	3,3',4,4',5-PeCB	126L	13C12-3,3',4,4',5-PeCB	26.56	1.000	40	100	10	4	5
Hexa congeners using 13C12-2,2',3,4,4',5'-HxCB (#138L) as the injection internal standard										
138L	13C12-2,2',3,4,4',5'-HxCB	101L	13C12-2,2',4,5,5'-PeCB	25.35	1.422					
167L	13C12-2,3',4,4',5,5'-HxCB	138L	13C12-2,2',3,4,4',5'-HxCB	28.50	1.124					
167	2,3',4,4',5,5'-HxCB	167L	13C12-2,3',4,4',5,5'-HxCB	28.52	1.001	60	200	6	20	10
156L	13C12-2,3,3',4,4',5-HxCB	138L	13C12-2,2',3,4,4',5'-HxCB	29.77	1.174					
157L	13C12-2,3,3',4,4',5'-HxCB	138L	13C12-2,2',3,4,4',5'-HxCB	29.77	1.174					
156	2,3,3',4,4',5-HxCB	156L	13C12-2,3,3',4,4',5-HxCB	29.80	1.001	60	200	6	20	10
157	2,3,3',4,4',5'-HxCB	157L	13C12-2,3,3',4,4',5'-HxCB	29.80	1.001	60	200	6	20	10
169L	13C12-3,3',4,4',5,5'-HxCB	138L	13C12-2,2',3,4,4',5'-HxCB	33.38	1.317					
169	3,3',4,4',5,5'-HxCB	169L	13C12-3,3',4,4',5,5'-HxCB	33.42	1.001	60	200	6	20	10

[a] Continued on next page

Table 2. *(cont.)* Retention Time (RT) References, Quantitation References, Relative Retention Times (RRTs), Estimated Method Detection Limits (EMDLs), and Estimated Minimum Levels (EMLs) for the Toxic PCBs

IUPAC[1] No.	Labeled or native PCB	IUPAC[1] No.	Retention time and quantitation reference	RT (min)[2]	RRT	Matrix and Concentration: Water (pg/l) EMDL	Water (pg/l) EML	Other (ng/Kg) EMDL	Other (ng/Kg) EML	Extract (pg/µL) EML
Hepta and deca congeners using 13C12-2,2',3,3',5,5',6-HpCB (#178L) as the injection internal standard										
178L	13C12-2,2',3,3',5,5',6-HpCB	101L	13C12-2,2',4,5,5'-PeCB	25.78	1.446					
180L	13C12-2,2',3,4,4',5,5'-HpCB	178L	13C12-2,2',3,3',5,5',6-HpCB	31.30	1.214					
180	2,2',3,4,4',5,5'-HpCB	180L	13C12-2,2',3,4,4',5,5'-HpCB	31.32	1.001	60	200	6	20	10
170	2,2',3,3',4,4',5-HpCB	180L	13C12-2,2',3,4,4',5,5'-HpCB	32.75	1.046	60	200	6	20	10
189L	13C12-2,3,3',4,4',5,5'-HpCB	178L	13C12-2,2',3,3',5,5',6-HpCB	36.32	1.409					
189	2,3,3',4,4',5,5'-HpCB	189L	13C12-2,3,3',4,4',5,5'-HpCB	36.35	1.001	60	200	6	20	10
209L	13C12-DCB[5]	178L	13C12-2,2',3,3',5,5',6-HpCB	43.48	1.687					

[1] Suffix "L" indicates labeled compound
[2] Retention time data are for SPB-octyl column sorted in ascending retention time order within each congener group
[3] Some EMDLs and EMLs have been set above the lowest calibration point (Table 4) because backgrounds of these particular congeners are difficult to eliminate from laboratory analytical systems
[4] Cleanup standard
[5] Final eluter

Table 3. Concentrations of Stock and Spiking Solutions Containing the Native PCBs and Labeled Compounds

PCB congener	IUPAC No.	Labeled compound		Precision and Recovery	
		Stock[1] (ng/mL)	Spiking[2] (ng/mL)	Stock[3] (ng/mL)	Spiking[4] (ng/mL)
3,3',4,4'-TCB	77	-	-	220	0.4
2,3,3',4,4'-PeCB	105	-	-	1000	20.0
2,3,4,4',5-PeCB	114	-	-	1000	20.0
2,3',4,4',5-PeCB	118	-	-	1000	20.0
2',3,4,4',5-PeCB	123	-	-	1000	20.0
3,3',4,4',5-PeCB	126	-	-	100	2.0
2,3,3',4,4',5-HxCB	156	-	-	1000	20.0
2,3,3',4,4',5'-HxCB	157	-	-	1000	20.0
2,3',4,4',5,5'-HxCB	167	-	-	1000	20.0
3,3',4,4',5,5'-HxCB	169	-	-	200	4.0
2,2',3,3',4,4',5-HpCB	170	-	-	200	4.0
2,2',3,4,4',5,5'-HpCB	180	-	-	1000	20.0
2,3,3',4,4',5,5'-HpCB	189	-	-	200	4.0
13C12-3,3',4,4'-TCB	77L	1000	2.0	-	-
13C12-2,3,3',4,4'-PeCB	105L	1000	2.0	-	-
13C12-2,3',4,4',5-PeCB	118L	1000	2.0	-	-
13C12-3,3',4,4',5-PeCB	126L	1000	2.0	-	-
13C12-2,3,3',4,4',5-HxCB	156L	1000	2.0	-	-
13C12-2,3,3',4,4',5'-HxCB	157L	1000	2.0	-	-
13C12-2,3',4,4',5,5'-HxCB	167L	1000	2.0	-	-
13C12-3,3',4,4',5,5'-HxCB	169L	1000	2.0	-	-
13C12-2,2',3,4,4',5,5'-HpCB	180L	1000	2.0	-	-
13C12-2,3,3',4,4',5,5'-HpCB	189L	1000	2.0	-	-
13C12-DCB	209L	2000	4.0	-	-
Cleanup standards[5]					
13C12-3,4,4',5-TCB	81L	200	1.0	-	-
13C12-2,3,3',5,5'-PeCB	111L	1000	5.0	-	-
Internal standards[6]					
13C12-2,2',5,5'-TCB	52L	1000	-	-	-
13C12-2,2',4,5,5'-PeCB	101L	1000	-	-	-
13C12-2,2',3,4,4',5'-HxCB	138L	1000	-	-	-
13C12-2,2',3,3',5,5',6-HpCB	178L	1000	-	-	-

[1] Section 7.10-prepared in nonane and diluted to prepare spiking solution
[2] Section 7.10.3-prepared in acetone from stock solution daily
[3] Section 7.9-prepared in nonane and diluted to prepare spiking solution. Concentrations are adjusted for expected background leve
[4] Section 7.14-prepared in acetone from stock solution daily. Concentrations are adjusted for expected background levels.
[5] Section 7.11-prepared in nonane and added to extract prior to cleanup
[6] Section 7.12-prepared in nonane and added to concentrated extract prior to injection

Table 4. Concentrations of PCBs in Calibration and Calibration Verification Solutions

Toxic PCB congener	IUPAC[1] No.	CS1 (ng/mL)	CS2 (ng/mL)	CS3[2] (ng/mL)	CS4 (ng/mL)	CS5 (ng/mL)
3,3',4,4'-TCB	77	0.5	2	10	40	200
2,3,3',4,4'-PeCB	105	2.5	10	50	200	1000
2,3,4,4',5-PeCB	114	2.5	10	50	200	1000
2,3',4,4',5-PeCB	118	2.5	10	50	200	1000
2',3,4,4',5-PeCB	123	2.5	10	50	200	1000
3,3',4,4',5-PeCB	126	2.5	10	50	200	1000
2,3,3',4,4',5-HxCB	156	5	20	100	400	2000
2,3,3',4,4',5'-HxCB	157	5	20	100	400	2000
2,3',4,4',5,5'-HxCB	167	5	20	100	400	2000
3,3',4,4',5,5'-HxCB	169	5	20	100	400	2000
2,2',3,3',4,4',5-HpCB	170	5	20	100	400	2000
2,2',3,4,4',5,5'-HpCB	180	5	20	100	400	2000
2,3,3',4,4',5,5'-HpCB	189	5	20	100	400	2000
Labeled congener						
13C12-3,3',4,4'-TCB	77L	100	100	100	100	100
13C12-2,3,3',4,4'-PeCB	105L	100	100	100	100	100
13C12-2,3',4,4',5-PeCB	118L	100	100	100	100	100
13C12-3,3',4,4',5-PeCB	126L	100	100	100	100	100
13C12-2,3,3',4,4',5-HxCB	156L	100	100	100	100	100
13C12-2,3,3',4,4',5'-HxCB	157L	100	100	100	100	100
13C12-3,3',4,4',5,5'-HxCB	169L	100	100	100	100	100
13C12-2,2',3,4,4',5,5'-HpCB	180L	100	100	100	100	100
13C12-2,3,3',4,4',5,5'-HpCB	189L	100	100	100	100	100
13C12-DCB	209L	200	200	200	200	200
Cleanup standards						
13C12-3,4,4',5-TCB	81L	0.5	2	10	40	200
13C12-2,3,3',5,5'-PeCB	111L	2.5	10	50	200	1000
Internal standards						
13C12-2,2',5,5'-TCB	52L	100	100	100	100	100
13C12-2,2',4,5,5'-PeCB	101L	100	100	100	100	100
13C12-2,2',3,4,4',5'-HxCB	138L	100	100	100	100	100
13C12-2,2',3,3',5,5',6-HpCB	178L	100	100	100	100	100

[1] Suffix "L" indicates labeled compound
[2] Section 15.3, calibration verification solution

Table 5. GC Retention Time Window Defining Solution and Congener Specificity Test Standard[1,2] (Section 7.15)

Congener Group	First eluted[3]		Last eluted	
TCB	54	2,2',6,6'	77	3,3',4,4'
PeCB	104	2,2',4,6,6'	126	3,3',4,4',5
HxCB	155	2,2',4,4',6,6'	169	3,3',4,4',5,5'
HpCB	188	2,2',3,4',5,6,6'	189	2,3,3',4,4',5,5'

SPB-Octyl resolution test compounds

123	2',3,4,4',5-PeCB
118	2,3',4,4',5-PeCB
114	2,3,4,4',5-PeCB

DB-1 column resolution test compounds

156	2,3,3',4,4',5-HxCB
157	2,3,3',4,4',5'-HxCB

[1] All compounds are at a concentration of 100 ng/mL in nonane.
[2] It is not necessary to monitor for the earliest eluted compounds if the toxic PCBs only are to be determined. If the co-planar PCBs (77, 126, 169)only are to be determined, it is necessary to resolve these co-planar PCBs and potentially interfering compounds only; i.e., use of the compounds listed in this. Table is not required.
[3] The earliest eluted compound in each congener group is provided for those instances in which all PCBs in that congener group are to be determined. If the toxic PCBs only (Table 1) are to be determined, use of the first eluted compounds is not required.

Table 6. Preliminary Acceptance Criteria for Performance Tests When All Toxic PCBs are Tested[1]

Congener	IUPAC No.	Test (ng/mL)	IPR s[2] (ng/mL)	IPR X[3] (ng/mL)	OPR (ng/mL)	VER (ng/mL)
3,3',4,4'-TCB	77	20	5.6	16-26	14-32	16-26
2,3,3',4,4'-PeCB	105	1000	172	720-1500	680-1600	780-1300
2,3,4,4',5-PeCB	114	1000	390	160-2800	130-3300	770-1300
2,3',4,4',5-PeCB	118	1000	172	720-1500	680-1600	780-1300
2',3,4,4',5-PeCB	123	1000	390	160-2800	130-3300	770-1300
3,3',4,4',5-PeCB	126	100	17	72-150	68-160	78-130
2,3,3',4,4',5-HxCB	156	1000	222	740-1600	640-1700	780-1300
2,3,3',4,4',5'-HxCB	157	1000	222	740-1600	640-1700	780-1300
2,3',4,4',5,5'-HxCB	167	1000	222	740-1600	640-1700	780-1300
3,3',4,4',5,5'-HxCB	169	200	45	148-320	128-340	156-260
2,2',3,3',4,4',5-HpCB	170	200	33	152-260	140-280	172-232
2,2',3,4,4',5,5'-HpCB	180	1000	165	760-1300	700-1400	860-1160
2,3,3',4,4',5,5'-HpCB	189	200	33	152-260	140-280	172-232
13C12-3,3',4,4'-TCB	77L	100	37	28-134	20-175	71-140
13C12-2,3,3',4,4'-PeCB	105L	100	39	16-279	13-328	77-130
13C12-2,3',4,4',5-PeCB	118L	100	39	16-279	13-328	77-130
13C12-3,3',4,4',5-PeCB	126L	100	39	16-279	13-328	77-130
13C12-2,3,3',4,4',5-HxCB	156L	100	34	24-157	17-205	70-143
13C12-2,3,3',4,4',5'-HxCB	157L	100	43	24-157	17-205	70-143
13C12-3,3',4,4',5,5'-HxCB	169L	100	43	24-157	17-205	70-143
13C12-2,2',3,4,4',5,5'-HpCB	180L	100	41	28-141	20-186	72-138
13C12-2,3,3',4,4',5,5'-HpCB	189L	200	82	56-282	40-372	144-276
Cleanup standards						
13C12-3,4,4',5-TCB	81L	20	7.2	8-31	6-38	15-26
13C12-2,3,3',5,5'-PeCB	111L	100	36	39-154	31-191	79-127

Table 6a. Preliminary Acceptance Criteria for Performance Tests when PCBs 77, 126, and 169 only are Tested[1]

Native PCB	IUPAC	Test (ng/mL)	IPR s[2] (ng/mL)	IPR X[3] (ng/mL)	OPR (ng/mL)	VER (ng/mL)
3,3',4,4'-TCB	77	20	5.4	17-25	15-30	16-25
3,3',4,4',5-PeCB	126	100	17	72-150	68-160	78-130
3,3',4,4',5,5'-HxCB	169	200	45	148-320	128-340	156-260
13C12-3,3',4,4'-TCB	77L	100	34	32-115	25-141	76-131
13C12-3,3',4,4',5-PeCB	126L	100	17	72-150	68-160	78-130
13C12-3,3',4,4',5,5'-HxCB	169L	200	45	148-320	128-340	156-260
Cleanup standards						
13C12-3,4,4',5-TCB	81L	20	7.2	8-31	6-38	15-26
13C12-2,3,3',5,5'-PeCB	111L	100	36	39-154	31-191	79-127

[1] Preliminary criteria transferred from Method 1613. All criteria given as concentration in the final extract, assuming a 20-µL volume.
[2] s=standard deviation
[3] X=average concentration

Table 7. Labeled Compound Recovery in Samples When All PCBs are Tested

Labeled PCB	IUPAC No.	Test conc (ng/mL)	Labeled compound recovery (ng/mL)	(%)
13C12-3,3',4,4'-TCB	77	100	24-169	24-169
13C12-2,3,3',4,4'-PeCB	105	100	21-178	21-178
13C12-2,3',4,4',5-PeCB	118	100	21-178	21-178
13C12-3,3',4,4',5-PeCB	126	100	21-178	21-178
13C12-2,3,3',4,4',5-HxCB	156	100	26-152	26-152
13C12-2,3,3',4,4',5'-HxCB	157	100	26-152	26-152
13C12-2,3',4,4',5,5'-HxCB	167	100	26-152	26-152
13C12-3,3',4,4',5,5'-HxCB	169	100	26-152	26-152
13C12-2,2',3,4,4',5,5'-HpCB	180	100	23-143	23-143
13C12-2,3,3',4,4',5,5'-HpCB	189	100	23-143	23-143
Cleanup standards				
13C12-3,4,4',5-TCB	81	20	7-40	35-197
13C12-2,3,3',5,5'-PeCB	111	100	35-197	35-197

Table 7a. Labeled Compound Recovery When PCBs 77, 126, and 169 Only are Tested[1]

Labeled PCB	IUPAC No.	Test conc (ng/mL)	Labeled compound Recovery (ng/mL)	(%)
13C12-3,3',4,4'-TCB	77	100	29-140	29-140
13C12-3,3',4,4',5-PeCB	126	100	21-178	21-178
13C12-3,3',4,4',5,5'-HxCB	169	100	26-152	26-152
Cleanup standards				
13C12-3,4,4',5-TCB	81	20	8-33	42-164
13C12-2,3,3',5,5'-PeCB	111	100	35-197	35-197

[1] Preliminary criteria transferred from Method 1613. Criteria are given as concentration in the final extract, assuming a 20 uL volume.

Table 8. Descriptors, Exact m/z's, m/z Types, and Elemental Compositions of the PCBs

Descriptor	Exact m/z[1]	m/z type	Elemental composition	Substance[2]
1.	289.9224	M	C12 H6 35Cl4	TCB
	291.9194	M+2	C12 H6 35Cl3 37Cl4	TCB
	292.9825	Lock	C7 F11	PFK
	301.9626	M	13C12 H6 35Cl4	PeCB[3]
	303.9597	M+2	13C12 H6 35Cl3 37Cl	PeCB[3]
	325.8804	M+2	C12 H5 35Cl4 37Cl	PeCB
	327.8775	M+4	C12 H5 35Cl3 37Cl2	PeCB
	330.9792	QC	C7 F13	PFK
2.	325.8804	M+2	C12 H5 35Cl4 37Cl	PeCB
	327.8775	M+4	C12 H5 35Cl3 37Cl2	PeCB
	337.9207	M+2	13C12 H5 35Cl4 37Cl	PeCB[3]
	339.9178	M+4	13C12 H5 35Cl3 37Cl2	PeCB[3]
	354.9892	Lock	C9 F13	PFK
	359.8415	M+2	C12 H4 35Cl5 37Cl	HxCB
	361.8385	M+4	C12 H4 35Cl4 37Cl2	HxCB
	371.8817	M+2	13C12 H4 35Cl5 37Cl	HxCB[3]
	373.8788	M+4	13C12 H4 35Cl4 37Cl2	HxCB[3]
	393.8025	M+2	C12 H3 35Cl6 37Cl	HpCB
	395.7996	M+4	C12 H3 35Cl5 37Cl2	HpCB
	405.8428	M+2	13C12 H3 35Cl6 37Cl	HpCB[3]
	407.8398	M+4	13C12 H3 35Cl5 37Cl2	HpCB[3]
3.	442.9728	Lock	C10 F17	PFK
	509.7229	M+4	13C12 35Cl10 37Cl2	DCB[3]
	511.7199	M+6	13C12 35Cl9 37Cl3	DCB[3]
	513.7170	M+8	13C12 35Cl8 37Cl4	DCB[3]

[1] Nuclidic masses used were:
H = 1.007825 C = 12.00000
13C = 13.003355 35Cl = 34.968853 37Cl = 36.965903

[2] TCB =Tetrachlorobiphenyl
PeCB = Pentachlorobiphenyl
HxCB = Hexachlorobiphenyl
HpCB = Heptachlorobiphenyl
DCB =Decachlorobiphenyl

[3] 13C labeled compound

Table 9. Theoretical Ion Abundance Ratios and QC Limits

Clorine atoms	m/z's forming ratio	Theoretical ratio	QC Limit[1] Lower	QC Limit[1] Upper
4	M/(M+2)	0.77	0.65	0.89
5	(M+2)/(M+4)	1.55	1.32	1.78
6	M/(M+2)	0.51	0.43	0.59
6	(M+2)/(M+4)	1.24	1.05	1.43
7	M/(M+2)	0.44	0.37	0.51
7	(M+2)/(M+4)	1.05	0.88	1.20
8	(M+2)/(M+4)	0.89	0.76	1.02

[1] QC limits represent +/- 15 % windows around the theoretical ion abundance ratio. These limits are preliminary.

Table 10. Suggested Sample Quantities to be Extracted for Various Matrices[1]

Sample matrix[2]	Example	Percent solids	Phase	Quantity extracted
Single-phase				
Aqueous	Drinking water Groundwater Treated wastewater	<1	—[3]	1000 mL
Solid	Dry soil Compost Ash	>20	Solid	10 g
Organic	Waste solvent Waste oil Organic polymer	<1	Organic	10 g
Tissue	Fish Human adipose	—	Organic	10 g
Multi-phase				
Liquid/Solid				
Aqueous/Solid	Wet soil Untreated effluent Digested municipal sludge Filter cake Paper pulp	1–30	Solid	10 g
Organic/solid	Industrial sludge Oily waste	1–100	Both	10 g
Liquid/Liquid				
Aqueous/organic	In-process effluent Untreated effluent Drum waste	<1	Organic	10 g
Aqueous/organic/solid	Untreated effluent Drum waste	>1	Organic & solid	10 g

[1] The quantitity of sample to be extracted is adjusted to provide 10 g of solids (dry weight). One liter of aqueous samples containing one percent solids will contain 10 grams of solids. For aqueous samples containing greater than one percent solids, a lesser volume is used so that 10 grams of solids (dry weight) will be extracted.

[2] The sample matrix may be amorphous for some samples. In general, when the PCBs are in contact with a multiphase system in which one of the phases is water, they will be preferentially dispersed in or adsorbed on the alternate phase because of their low solubility in water.

[3] Aqueous samples are filtered after spiking with the labeled compounds. The filtrate and the materials trapped on the filter are extracted separately, and the extracts are combined for cleanup and analysis.

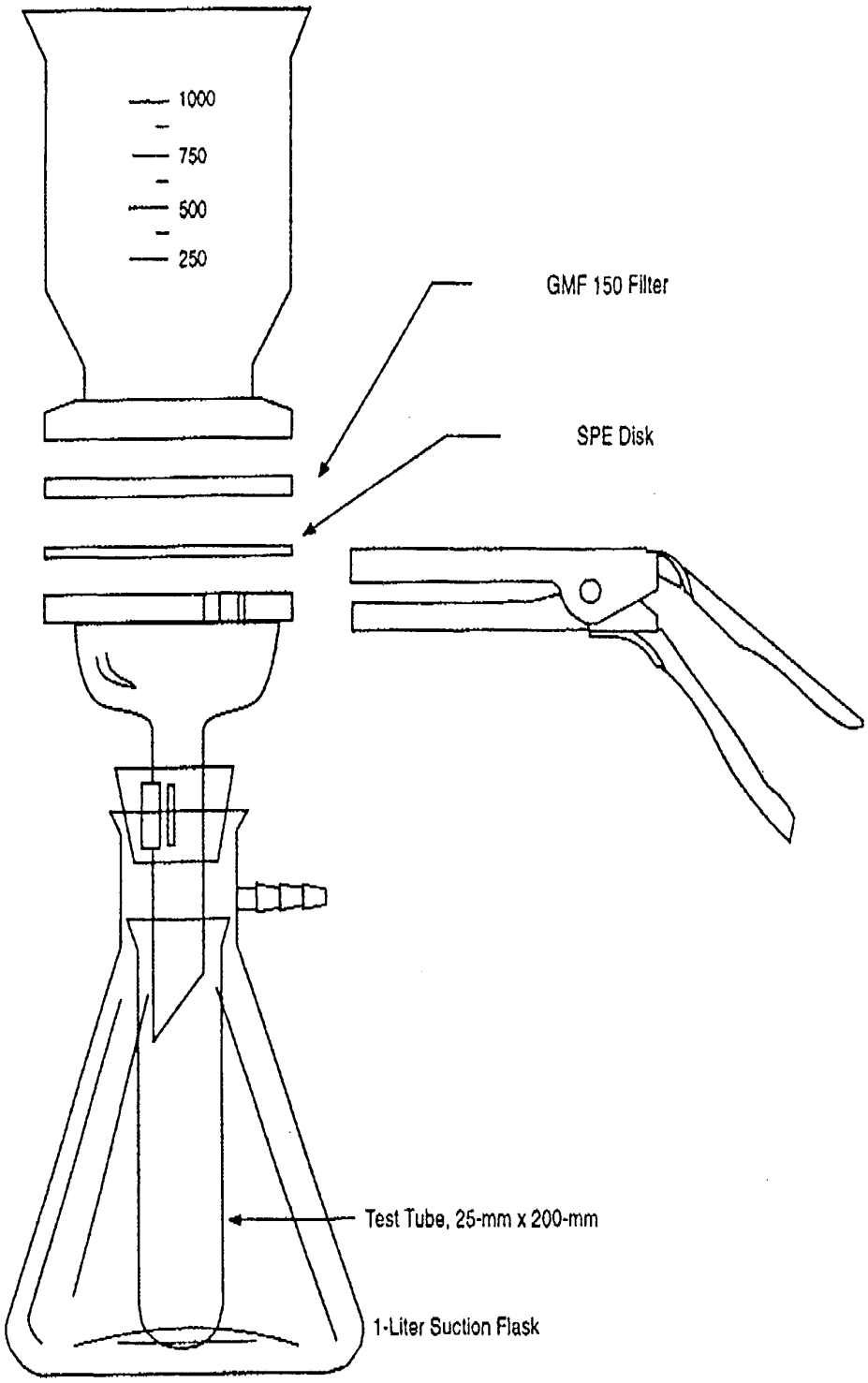
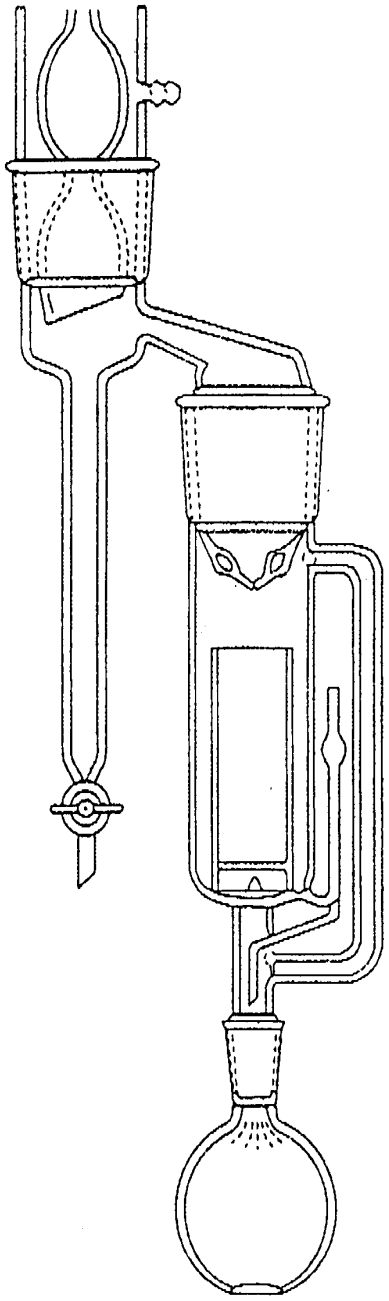


Figure 4 Solid-phase Extraction Apparatus



52-027-02

Figure 5 Soxhlet/Dean-Stark Extractor

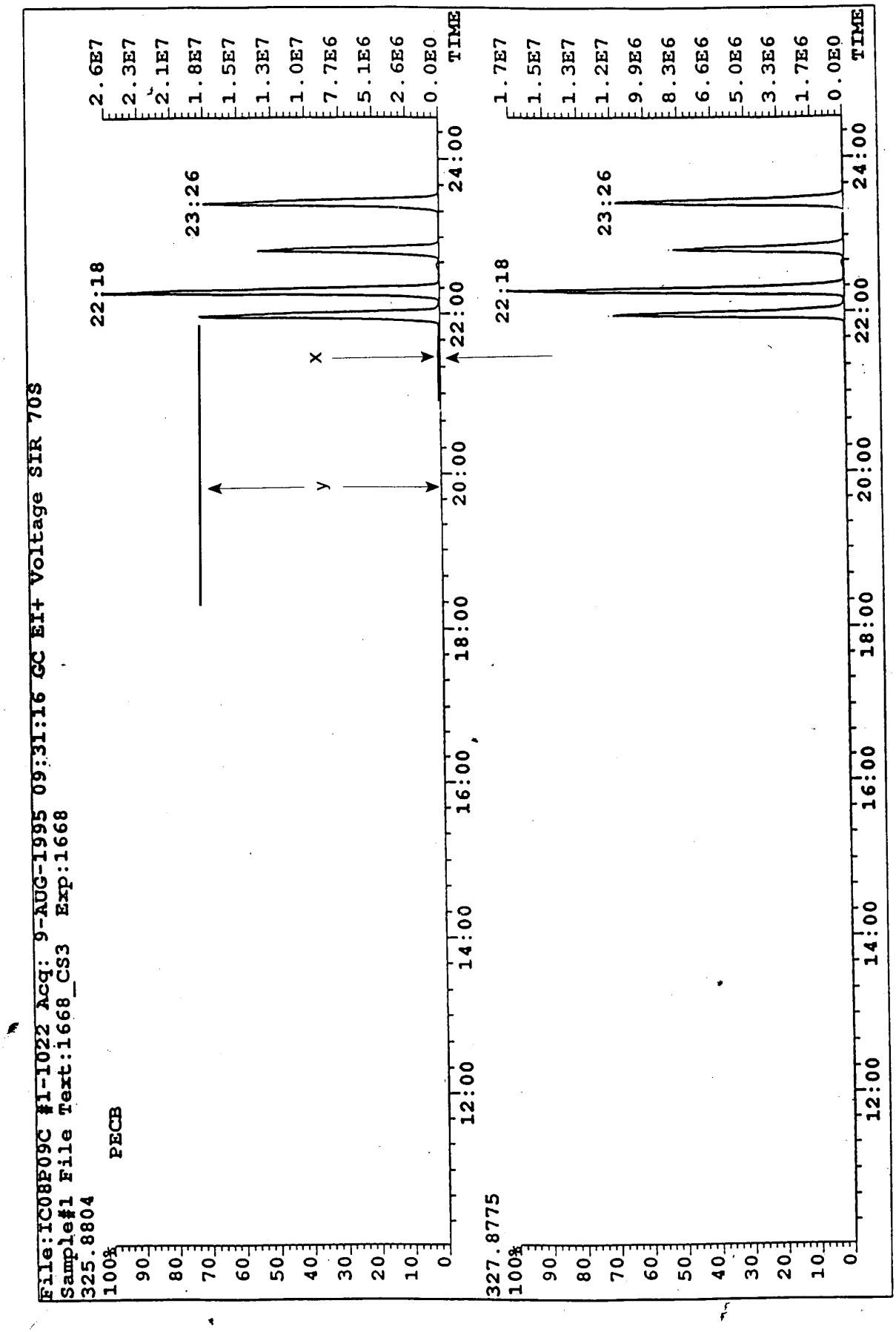


Figure 6 Congener-Specific Separation of Resolution Test Compounds on SPB-Octyl Column

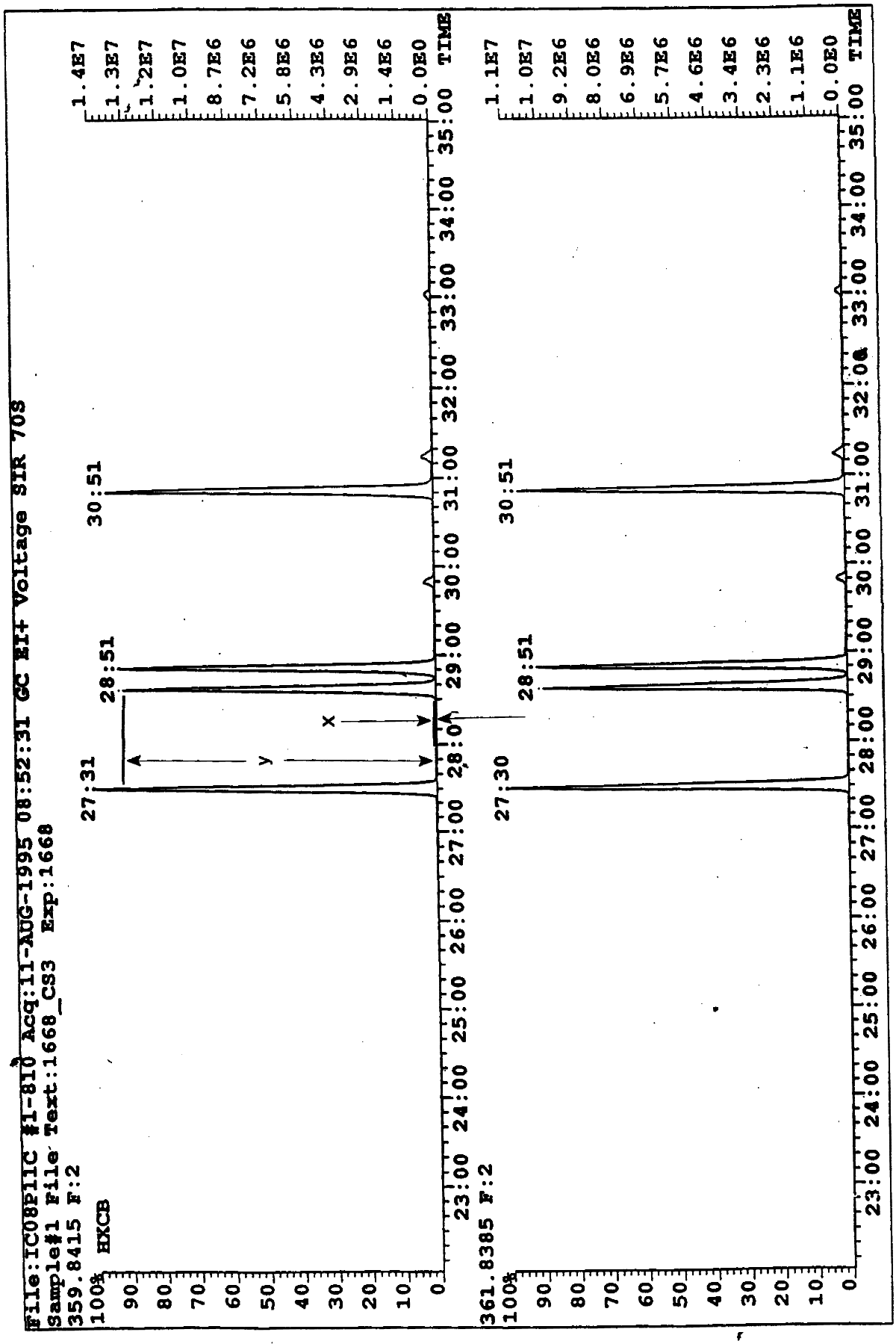


Figure 7 Congener-SpecificSseparation of PCBs 156 and 157 on DB-1 Column

24.0 Glossary of Definitions and Purposes

These definitions and purposes are specific to this method but have been conformed to common usage as much as possible.

24.1 Units of weight and measure and their abbreviations

24.1.1 Symbols

°C	degrees Celsius
µL	microliter
µm	micrometer
<	less than
>	greater than
%	percent

24.1.2 Alphabetical abbreviations

amp	ampere
cm	centimeter
g	gram
h	hour
ID	inside diameter
in.	inch
L	liter
M	Molecular ion
m	meter
mg	milligram
min	minute
mL	milliliter
mm	millimeter
m/z	mass-to-charge ratio
N	normal; gram molecular weight of solute divided by hydrogen equivalent of solute, per liter of solution
OD	outside diameter
pg	picogram
ppb	part-per-billion
ppm	part-per-million
ppq	part-per-quadrillion
ppt	part-per-trillion
psig	pounds-per-square inch gauge
v/v	volume per unit volume
w/v	weight per unit volume

24.2 Definitions and acronyms (in alphabetical order).

Analyte: A PCB tested for by this method. The analytes are listed in Table 1.

Calibration standard (CAL): A solution prepared from a secondary standard and/or stock solutions and used to calibrate the response of the instrument with respect to analyte concentration.

Calibration verification standard (VER): The mid-point calibration standard (CS3) that is used in to verify calibration. See Table 4.

CS1, CS2, CS3, CS4, CS5: See Calibration standards and Table 4.

DCB: Decachlorobiphenyl (PCB 209)

Field blank: An aliquot of reagent water or other reference matrix that is placed in a sample container in the laboratory or the field, and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the field blank is to determine if the field or sample transporting procedures and environments have contaminated the sample.

GC: Gas chromatograph or gas chromatography.

GPC: Gel permeation chromatograph or gel permeation chromatography.

HpCB: Heptachlorobiphenyl

HPLC: High performance liquid chromatograph or high performance liquid chromatography.

HRGC: High resolution GC.

HRMS: High resolution MS.

HxCB: Hexachlorobiphenyl

IPR: Initial precision and recovery; four aliquots of the diluted PAR standard analyzed to establish the ability to generate acceptable precision and accuracy. An IPR is performed prior to the first time this method is used and any time the method or instrumentation is modified.

K-D: Kuderna-Danish concentrator; a device used to concentrate the analytes in a solvent.

Laboratory blank: See Method blank.

Laboratory control sample (LCS): See Ongoing precision and recovery standard (OPR).

Laboratory reagent blank: See Method blank.

May: This action, activity, or procedural step is neither required nor prohibited.

May not: This action, activity, or procedural step is prohibited.

Method blank: An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with samples. The method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

Minimum level (ML): The level at which the entire analytical system must give a recognizable signal and acceptable calibration point for the analyte. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed.

MS: Mass spectrometer or mass spectrometry.

Must: This action, activity, or procedural step is required.

OPR: Ongoing precision and recovery standard (OPR); a laboratory blank spiked with known quantities of analytes. The OPR is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

PAR: Precision and recovery standard; secondary standard that is diluted and spiked to form the IPR and OPR.

PFK: Perfluorokerosene; the mixture of compounds used to calibrate the exact m/z scale in the HRMS.

Preparation blank: See Method blank.

Primary dilution standard: A solution containing the specified analytes that is purchased or prepared from stock solutions and diluted as needed to prepare calibration solutions and other solutions.

Quality control check sample (QCS): A sample containing all or a subset of the analytes at known concentrations. The QCS is obtained from a source external to the laboratory or is prepared from a source of standards different from the source of calibration standards. It is used to check laboratory performance with test materials prepared external to the normal preparation process.

PeCB: Pentachlorobiphenyl

PCB: Polychlorinated biphenyl

Reagent water: water demonstrated to be free from the analytes of interest and potentially interfering substances at the method detection limit for the analyte.

Relative standard deviation (RSD): The standard deviation times 100 divided by the mean. Also termed "coefficient of variation."

RF: Response factor. See Section 10.6.1.

RR: Relative response. See Section 10.5.2.

RSD: See Relative standard deviation.

SDS: Soxhlet/Dean-Stark extractor; an extraction device applied to the extraction of solid and semi-solid materials (Reference 12 and Figure 5).

Should: This action, activity, or procedural step is suggested but not required.

SICP: Selected ion current profile; the line described by the signal at an exact m/z.

SPE: Solid-phase extraction; an extraction technique in which an analyte is extracted from an aqueous sample by passage over or through a material capable of reversibly adsorbing the analyte. Also termed liquid-solid extraction.

Specificity: The ability to measure an analyte of interest in the presence of interferences and other analytes of interest encountered in a sample.

Stock solution: A solution containing an analyte that is prepared using a reference material traceable to EPA, the National Institute of Science and Technology (NIST), or a source that will attest to the purity and authenticity of the reference material.

TCB: Tetrachlorobiphenyl.

VER: See Calibration verification standard.

EPA Region 10 SOP For the Validation of of Method 1668 Toxic, Dioxin-like, PCB Data

EPA Region 10
Environmental Services Division
1200 Sixth Avenue
Seattle, WA 98101

Revision 1.0
12/8/95

APPROVAL:

Quality Assurance Manager:

_____ _____
Barry Towns Date:

Region 10 Chemists:

_____ _____
Peggy Knight Date:

_____ _____
Robert Melton Date:

TABLE OF CONTENTS

EPA Region 10 SOP For the Validation of of Method 1668 Toxic, Dioxin-like, PCB Data

The Quality Assurance Unit of EPA Region 10 has developed the following guidelines which should be used to access the quality of toxic, dioxin-like PCB data from samples originating from Region 10 sampling sites. This SOP is based upon the data validation principles specified in National Functional Guidelines For Organic Data Review, December, 1990, and the quality control (QC) requirements of EPA Method 1668, Draft Revision, 10/4/95. The validator of toxic, dioxin-like PCB data should obtain a copy of the site-specific Quality Assurance Project Plan (QAPP) and use the Data Quality Objectives and QA requirements of the QAPP to assess the data. This SOP requires that the following criteria be evaluated when determining the quality of toxic, dioxin-like PCB data:

1.0 HOLDING TIME AND PRESERVATION OF SAMPLES

1.1 Objective. To determine the validity of the measurement results based upon EPA requirements for preservation and holding time of the samples from day of collection to day of extraction. EPA also has holding time requirements for extracts which is the time from extraction of the samples to injection of the sample extracts.

1.2 Criteria. Holding time and preservation requirements for the measurement of PCBs as Aroclors in water samples under the CWA (40CFR Part 136), SDWA, and RCRA have been promulgated and codified under 40CFR. These regulations require that water samples be preserved by cooling to 4°C using a holding time of 7 days from day of collection to day of extraction of the sample. In addition, the maximum holding time of extracts is 40 days from day of extraction to day of injection of the extract.

The holding time and preservation requirements of toxic, dioxin-like PCB isomers in non-water matrixes have not been promulgated by EPA. Therefore, the data validator should use the holding time specified in the EPA approved site-specific Quality Assurance Project Plan (QAPP).

> Method 1668, Draft Revision, 10/4/95 recommends different preservation and holding times for PCB congeners. Consult Section 8.0 of Method 1668 for preservation and holding time recommendations.

Section 8.2 states that aqueous samples should be tested for chlorine residual. If chlorine is present, 80mg of sodium thiosulfate should be added per liter of water. Adjust pH to 2-3 with sulfuric acid. Store samples in dark at 0 to 4°C. Method 1668 recommends a holding time of less that one year.

Section 8.3 states that solid, semi-solid, oily, and mixed phase samples should be stored in wide mouth bottle at <4°C. Section 8.3 states that solid, semi-solid, oily, and mixed phase samples should be stored in the laboratory at < -10°C. Method 1668 recommends a holding time of less that one year.

Section 8.4 states that fish and tissue samples should be wrapped in aluminum foil, cooled to <4°C, and shipped to lab. Upon receipt at the lab, tissue samples should be stored in the dark at < -10°C. Method 1668 has recommended a holding time of one year for tissue samples which are frozen at < -10°C. Once frozen tissue samples are thawed, tissue samples must be extracted within 24 hours.

Extracts should be analyzed within 40 days of extraction.

1.3 Action. If **40CFR Part 136** and the QAPP for the samples do not specify a holding time, then the holding time which is recommended by applicable EPA method -- Method 1668 should be used. Whenever samples or extracts are analyzed after holding time expiration date, the results should be considered to be minimum concentrations and must be qualified with a "J3". Samples which are not preserved correctly should be qualified with a "J" flag.

2.0 GC/MS PERFORMANCE CHECK

2.1 Objective. Gas chromatograph/mass spectrometer (GC/MS) instrument performance checks stated in Method 1668 Section 10.0 are performed to ensure mass resolution, identification, and calibration. Conformance is determined using standard materials, therefore, these criteria should be met in all circumstances.

2.2 Criteria. For the PFK molecular leak, the resolution must be greater than or equal to 10,000. The deviation between the exact mass and the theoretical mass for each of the three to five ions monitored must be less than 5 ppm. If the mass spectrometer is adjusted the resolution must be tested again and the resolution documented.

The mass spectrometer shall be operated in a mass-drift correction mode using PFK to provide lock-masses. Each lock-mass shall be monitored and shall meet the QC requirements of Section 7.1 of Method 1668.

Ion abundance ratios. All labeled and unlabeled PCB congeners in the CS1 standard shall be within the QC limits described in Section 10.2 and in Table 9 for their respective ion abundance ratios.

The HRGC/HRMS must meet the minimum levels in 1668 Table 2. All labeled and unlabeled analytes in the CS1 calibration standard must have signal to noise ratios greater than or equal to 10.0. (see Method 1668/Section 10.2)

The absolute retention time of PCB 169 shall exceed 20.0 minutes on the SPM-Octyl column, and the retention time of PCB 157 shall exceed 25.0 minutes on the DB-1 column. (see Method 1668/Section 10.2.4)

The compound pairs in the window defining mixtures shall be determined. (see Method 1668/Section 10.3)

The isomer specificity requirements stated in Method 1668 Section 10.4 shall be met.

2.3 Action. Failure to meet either the resolution or the retention window criteria invalidates all calibration or sample data collected during the 12 hour time window.

3.0 INITIAL CALIBRATION

3.1 Objective. Compliance requirements for satisfactory instrument calibration are established to ensure that the instrument is capable of producing acceptable qualitative and quantitative data for PCBs. Initial calibration demonstrates that the instrument is capable of producing a linear calibration curve.

3.2 Criteria. There shall be an initial calibration curve consisting of five points for each analyte. The initial calibration curve shall be determined less than 30 days from the

time the first samples of a Sample Delivery Group (SDG) are measured by the lab. The lab shall use the same calibration standards with the same lot number, for all internal standards, and labeled standards used in measuring the initial calibration curve, verification standards, field samples, and method blanks on both the primary GC column and on the secondary confirmation GC column. If an analyte is calculated by the isotope dilution method, an averaged response factor may be used if the RSD is less than 20% For analytes calculated by the internal standard method, an averaged response factor may be used if the RSD is less than 35%. Otherwise, for either calculation method, the complete curve must be used (see Method 1668/Sections 10.5 and 10.6).

3.3 Action. If the Initial Calibration Curve is older that 30 days, or if internal standards or labeled standards used in measuring of the initial calibration curve, verification standards, field samples, and method blanks on both the primary GC column and on the secondary confirmation GC column or not from the same lot number, then all measurement data should be qualified with a "J" qualifier.

If the RSD exceeds 20% for those analytes analyzed by isotope dilution or 35% for those analytes analyzed by the internal standard method, qualify positive results with "J", and non-detected analytes using professional judgement. At the reviewer's discretion, a more in-depth review may be conducted to minimize data qualification by examining the entire curve and the quantitation method used.

4.0 CALIBRATION VERIFICATION MEASUREMENTS

4.1 Objective. Compliance requirements for satisfactory instrument calibration are established to ensure that the instrument remains capable of producing acceptable qualitative and quantitative data each day that samples are measured.

4.2 Criteria. Native and labeled PCB congeners in the calibration verification standard (CS3) and in the Ongoing Precision and Recovery Standard (OPR) shall meet the acceptance criteria which are specified in Method 1668, Section 15.0.

4.3 Action. The reviewer should use professional judgement to determine if it is necessary to qualify the data. The following are guidelines:

If the %D for an analyte is outside the acceptance window, qualify positive results "J" and non-detected "UJ" for that

analyte. If the ion abundance criteria are not met results qualify all results for that analyte "R".

5.0 SYSTEM PERFORMANCE

5.1 Objective. The performance of the method by the Laboratory is examined by determination of the Laboratory's ability to perform the method (Initial Precision and Recovery (IPR) study) and to demonstrate the Laboratory's continuing ability to perform the analysis. See Section 9.0 of Method 1668, Draft Revision, 10/4/95 for initial and ongoing QA and QC requirements.

As part of measuring system performance, Method 1668 require that samples and standards be measured within require QC limits. QC criteria such as required relative retention times of labeled and native isomers, theoretical ion abundance ratios, recovery limits for OPR and VER standards, and recovery limits for spiked labeled target compounds must be met in order to demonstrate that the measurement system is within the specified control limits of Method 1668. In addition, all samples will be spiked with the labeled compound spiking solution described in Section 7.10.3.

5.2 Criteria. Initial precision and accuracy (IPR). All cleanup steps used in processing samples shall be included in the IPR study. All analytes shall be within the IPR limits in Table 6 of Method 1668 (use Table 6a if only PCBs 77, 126, and 169 are determined). There will be one PAR sample for each sample set analyzed. The recovery of labeled spiked isomers in samples shall be within the QC limits specified in Table 7 (use Table 7a if only PCBs 77, 126, and 169 are measured).

QC limits such as required relative retention times of labeled and native isomers, theoretical ion abundance ratios, recovery limits for OPR and VER standards, and recovery limits for spiked labeled target compounds must be within control limits of Method 1668.

5.3 Action. Results for analytes which do not meet either IPR or PAR requirements should be qualified with either "J" or "UJ". If an analyte is not recovered for an PAR sample, results must be qualified with an "R" for that analyte. Failure to meet QC limits of the method may result in measurement values which are qualified with a "J" or "UJ". In specific cases where major QC limits are exceeded, the data validator may determine that the measurement system is out of control, which would require that all measurement results for a sample be qualified with a "J", "UJ", or "R" flag.

6.0 METHOD BLANKS

6.1 Objective. To determine the existence and magnitude of contamination of samples resulting from laboratory activities. The criteria for evaluation of blanks will apply to any blank associated with the samples, including any method blanks, instrument blanks, field equipment blanks, transfer blanks, trip blanks, or solvent blanks.

6.2 Criteria.

1. The criteria for the frequency of extraction and analysis of method blanks as stated in Section 9.5 of Method 1668 shall be followed and demonstrated in the documented data. The maximum amount of toxic, dioxin-like PCB isomer contamination in method blanks is stated in Table 2 of Method 1668.

2. A method blank must be measured on each GC/MS system which is used to measure a group of samples. This requirement includes measuring method blanks for PCBs 156 and 157 on the secondary GC confirmation column (DB-1) if PCBs 156 and 157 are detected on the primary GC column, SPB-Octyl (see GC confirmation requirements in Method 1668, Section 16.5).

6.3 Action. If the maximum contamination requirements of specific PCB congeners stated in Table 2 of Method 1668 are not met, then all isomers in all samples associated with a method blanks shall be qualified with a "J1" flag. If the frequency of measuring method blanks is not met by the laboratory in the data submitted, then the results of all samples which do not meet the frequency of extraction and measurement of method blanks shall be qualified with a "R" flag. Any measurement of PCB congeners in a sample that is also measured in any associated blank, is qualified with a "U" flag if the sample concentration is less than 5 times the blank concentration.

7.0 RECOVERY OF SPIKED C-13 LABELED PCB CONGENERS

7.1 Objective. Labeled PCB congeners are added to each sample and method blank prior to extraction. The role of these C-13 labeled spiked compounds is to be an internal standard for the quantitation of native toxic, dioxin-like PCB isomers, and to serve as surrogates for the assessment of method performance in the sample matrix.

7.2 Criteria. The recovery of each C-13 labeled toxic, dioxin-like PCB isomer using Method 1668 must be within recovery limits

specified in Table 7 (see Table 7a if PCBs 77, 126, and 169, only, are measured).

7.3 Action. If any of the labeled percent recoveries are outside the guideline windows for individual analytes listed in Table 7 (see Table 7a if PCBs 77, 126, and 169, only, are measured), the individual isomer for that sample will be qualified with a "J" flag. For non-detected toxic, dioxin-like PCB compounds whose percent recoveries are outside the guideline windows for individual analytes, these will be qualified with a "UJ" flag.

8.0 RECOVERY OF C-13 LABELED INTERNAL STANDARDS

8.1 Objective. The purpose of adding four labeled internal standards (see Method 1668, Section 7.12) prior to injecting sample extracts and standards into the GC/MS is to determine the recovery efficiency of the extraction and cleanup procedures, to determine if the GC/MS sensitivity and response are stable during every analytical run, and to determine if the same amount of extract was injected into the GC/MS.

8.2 Criteria. The sum of the area counts of two masses for each of the two cleanup standards for samples, blanks, and standards must not vary by more than a factor of four (-25% to +200%) from the sum of the associated average areas from the five initial calibration standards.

8.3 Action. The reviewer should use professional judgement to determine if it is necessary to qualify the data. The following are guidelines:

1. If the sum of the two quantitation area counts of each internal standard in samples or blanks are outside a -25% to +200% window which is determined by averaging the sum of the area counts present in the five initial calibration standards, then positive measurement results for native compounds should be qualified with a "J".

2. If the sum of the two quantitation area counts is greater that 200%, then non-detected compounds should not be qualified.

3. If the sum of the two area counts is less than 25%, then non-detect compounds should be qualified with a "UJ".

4. If the sum of the area counts is less than 10%, then non-detect target compounds should be qualified with a "R".

9.0 PROJECT AND REGIONAL QUALITY ASSURANCE SAMPLES

9.1 Objective: The data validator should consider the data of samples which are identified as field duplicates, transfer blanks, trip blanks, blind spikes, blind blanks, and performance evaluation (PE) samples.

9.2 Criteria. If QA samples are included among the field samples for measurement by the laboratory, then the data validator should refer to the applicable QAPP for any QA requirements regarding QA samples. Results from the measurement of project and regional QA samples will reflect upon the ability of the laboratory to report analytical results of known and documented quality which meet the PARCC requirements of the QAPP.

9.3 Action. The data validator should recommend action in accordance with Regional specifications, QAPP specifications, or criteria for acceptable PE sample results. Poor performance by the laboratory on blind PE samples may indicate that the laboratory analytical system is out of control, or that the amount of toxic, dioxin-like PCB isomers reported by the laboratory is an estimated quantity. The data validator should use her/his professional judgement to assess if "J" or "R" qualifiers should be placed upon the data due to the measurement of QA or PE samples.

10.0 COMPOUND IDENTIFICATION

10.1 Objective. The qualitative criteria for target compound identification have been established by EPA Method 1668 to minimize the number of erroneous identifications. An erroneous identification can be either a false-positive (reporting a target compound when it is not present in the sample), or false-negative (not reporting a compound that is present in the sample). The addition of single or double blind PE samples among field samples provides ancillary data to support the laboratory's ability to meet QAPP objectives.

10.2 Criteria. EPA Method 1668 specifies certain requirements and guidelines for the positive identification of certain toxic, dioxin-like PCB isomers such as PCBs 156 and 157 (see Section 16.5). The most frequently encountered interfering compounds to the measurement of toxic, dioxin-like PCB isomers are chlorinated substances such as other PCB congeners, Polychlorinated dioxins and furans (PCDDs/PCDFs), methoxy biphenyls, hydroxydiphenyl ethers, benzylphenyl ethers, polynuclear aromatics, and pesticides that may be found at concentrations several orders of magnitude higher than the analytes of interest. Method 1668 requires that if certain PCB congeners such as PCB 156 and 157

are measured on the primary GC column, SPB-Octyl, that PCBs 156 and 157 must be confirmed using second dissimilar GC column (DB-1) before specific identifications can be made.

In this part of the SOP for the validation of toxic, dioxin-like PCB data, the qualitative identification criteria specified in Method 1668, Section 16.0 must be met for a GC peak to be identified as a PCB congener:

1. The signals for the two exact m/z's listed in Table 8 must be present, and must maximize within plus or minus 2 seconds of one another (see 1668/Section 16.1).

2. The signal-to-noise ratio (S/N) of each of the two exact m/z's must be greater than or equal to 2.5 for a sample extract, and greater than or equal to 10 for a calibration standard (see 1668/Section 16.2).

3. The ratio of the integrated ion currents (EICPs) of both the exact m/z's monitored must be within the limits which are listed in Table 9 of the method (see 1668/Section 16.3).

4. The relative retention time (RRT) of the peaks representing a unlabeled PCB congener must be within 5% of the limits listed in Table 2 (see Method 1668, Section 16.4).

5. The measurement of PCBs 156 and 157 on the primary SPD-Octyl GC column must be confirmed by analysis on a confirmatory column such as DB-1. All QC requirements of the method must be met on both the primary and secondary GC columns (see 1668/Section 16.5).

10.3 Action. The validator of the data must use his/her professional judgement in evaluating the data using the above identification criteria. Generally, if all of the above criteria for the identification of toxic, dioxin-like PCB isomers are not met, then each reported positive measurement of a PCB congener should be considered a non-detect, and therefore flagged with a "R" flag. The "R" flag in this case is based upon the fact that the presence of the isomer in the sample can not be corroborated by the laboratory data.

11.0 LABORATORY CONTACTS

Provide and attached to the validation memo a copy of all telephone logs and correspondence with the laboratory concerning the quality of data submitted by the laboratory.

12.0 OVERALL ASSESSMENT OF THE QUALITY OF THE DATA

12.1 Objective. The overall assessment of a data package is a brief narrative in which the data reviewer expresses concerns and comments of the quality of the data. The overall assessment of the data should be made after the data validator considers the DQOs and other QA requirements of the site-specific QAPP. It should be noted that the data reviewer does not determine or report the useability of the data. This determination is made by the Site Manager and by the other users of the data.

12.2 Criteria. The criteria for overall assessment is the QA and DQO criteria of the QAPP and the criteria listed above in this data validation SOP.

12.3 Action. Use professional judgement to determine if there is a need to further qualify the data. Write a brief narrative to give the user an indication of any analytical limitations of the data. Note if there are any inconsistencies observed between the raw data and the laboratory reported sample results.

DATA QUALIFIER DEFINITIONS

U - The analyte was analyzed for, but was not detected above the sample quantitation limit. The associated numerical value indicates the approximate concentration necessary to detect the analyte in this sample.

If a decision requires quantitation of the analyte below the associated numerical level, reanalysis or alternative analytical methods should be considered.

J - The analyte was analyzed for and was positively identified, but the associated numerical value may not be consistent with the amount actually present in the environmental sample.

A subscript may be appended to the "J" that indicates which of the following quality control criteria were not met:

J1 Blank Contamination: indicates possible high bias and/or false positives.

J2 Calibration range exceeded: indicates possible low bias.

J3 Holding times not met: indicates low bias for most analytes.

J4 Other QC parameter outside control limits: bias not readily determined.

J5 Other QC parameter outside control limits. The reported results appear to be biased high. The actual value of target compound in the sample may be lower than the value reported by the laboratory.

J6 Other QC parameter outside control limits. The reported results appear to be biased low. The actual value of target compound in the sample may be higher than the value reported by the laboratory.

R - The sample results are rejected due to serious deficiencies in the ability to analyze the sample and meet critical quality control criteria. The presence or absence of the analyte cannot be verified.

Resampling and reanalysis are necessary to confirm or deny the presence of the analyte.

UJ - The analyte was analyzed for and was not detected above the reported quantitation limit. However, the reported quantitation limit is approximate and may or may not represent the actual limit of quantitation necessary to accurately and precisely measure the analyte in this sample.

If a decision requires quantitation of the analyte close to the associated numerical level, reanalysis or alternative analytical methods should be considered.

United States Environmental Protection Agency Region 10 Laboratory

7411 Beach Drive East, Port Orchard, WA 98366 (360) 871-8700 Fax: (360) 871-8747

EPA SAMPLE ALTERATION FORM FOR FISH CONTAMINANT STUDY COLUMBIA RIVER BASIN

Sample Alteration Form

Project Name and Number:

_____

Material to be Sampled:

_____
_____

Measurement Parameter:

_____
_____
_____

Standard Procedure for Field Collection & Laboratory Analysis (cite reference):

_____
_____
_____

Reason for Change in Field Procedure or Analysis Variation:

_____
_____
_____

Variation from Field or Analytical Procedure:

_____
_____
_____

Special Equipment, Materials or Personnel Required:

_____
_____
_____

Initiators Name: _____ **Date:**_____

Project Officer: _____ **Date:**_____

QA Officer: _____ **Date:**_____

United States Environmental Protection Agency Region 10 Laboratory

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EPA CORRECTIVE ACTION FORM FOR FISH CONTAMINANT STUDY COLUMBIA RIVER BASIN

Corrective Action Form

Project Name and Number: _____

Sample Dates Involved: _____

Measurement Parameter:

_____
_____
_____

Acceptable Data Range:

_____
_____

Problem Areas Requiring Corrective Action:

_____
_____
_____
_____
_____

Measures Required to Correct Problem:

_____
_____
_____
_____
_____
_____

Means of Detecting Problems and Verifying Correction:

_____
_____
_____
_____
_____

Initiators Name: _____ **Date:** _____

Project Officer: _____ **Date:** _____

QA Officer: _____ **Date:** _____

United States Environmental Protection Agency Region 10 Laboratory

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EPA FIELD RECORD FOR FISH CONTAMINANT STUDY COLUMBIA RIVER BASIN

EPA SAMPLING SITE INFORMATION

Sampling Date: _____ Sampling Time: _____

Sampling Site Name/Number: _____

Lat.: _____ Long.: _____

Species Name: _____ Sample Code: _____

Collection Method: _____ Depth: _____

Sampling Team Names: _____

Sampling Vessel: _____ Weather: _____

FISH COLLECTED

NOTE:
Use Assigned EPA composite number for fish listed below in Table

Assigned EPA Group Composite Sample Number	EPA 96

Species Name:

Fish Number	EPA Individual Sample Number	Length (mm)	Weight (g)	Sex (M,F,U*)	Comments
001	EPA 96				
002	EPA 96				
003	EPA 96				
004	EPA 96				
005	EPA 96				
006	EPA 96				
007	EPA 96				
008	EPA 96				
009	EPA 96				
010	EPA 96				
011	EPA 96				
012	EPA 96				
013	EPA 96				
014	EPA 96				
015	EPA 96				

* Undetermined

United States Environmental Protection Agency Region 10 Laboratory

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EPA FIELD SAMPLE IDENTIFICATION LABEL FOR FISH CONTAMINANT STUDY COLUMBIA RIVER BASIN

EPA SAMPLE IDENTIFICATION LABEL

PROJECT NAME: COLUMBIA RIVER BASIN EPA/CRITFC FISH TISSUE STUDY **PROJECT CODE:**

COLLECTING AGENCY:
US EPA
REGION 10 LABORATORY
7411 BEACH DRIVE EAST
PORT ORCHARD, WA. 98366
PHONE # (360) 871-8700

EPA SAMPLE NUMBER:

SAMPLER NAME / PHONE # / SIGNATURE:

SAMPLE TYPE:
Whole Body
Fillet w/Skin On
Fillet w/Skin Off
Egg Skein

SAMPLING DATE / TIME:

SPECIES NAME:

SAMPLE SITE NAME / NUMBER:

SAMPLE LENGTH (mm):

SAMPLE WEIGHT (gm):

COMMENTS:

United States Environmental Protection Agency Region 10 Laboratory

7411 Beach Drive East, Port Orchard, WA 98366 (360) 871-8700 Fax: (360) 871-8747

EPA LABORATORY RECORD FOR FISH CONTAMINANT STUDY COLUMBIA RIVER BASIN

EPA FISH PROCESSING RECORD

Species Name: _____

Sample Type: Whole Body Fillet w/Skin On Fillet w/Skin Off Eggs

FISH COLLECTED AND PROCESSED

NOTE:
Use Assigned EPA composite number for fish listed below in Table.

Assigned EPA Group Composite Sample Number	EPA

Fish Number	EPA Individual Sample Number	Resection Performed	Fillet Weight (g)	Egg Skein Weight (g)	Sample Condition
001	EPA 97				
002	EPA 97				
003	EPA 97				
004	EPA 97				
005	EPA 97				
006	EPA 97				
007	EPA 97				
008	EPA 97				
009	EPA 97				
010	EPA 97				
011	EPA 97				
012	EPA 97				
013	EPA 97				
014	EPA 97				
015	EPA 97				

Weight of Composite Final Homogenate (g)	

Name of grinder and Model # that was used to prepare tissue samples: _____

Indicate size of orifice plate used in grinder?
Orifice size: 1/8" 3/16" 1/4" or _____

Mark how many times the sample was run through the grinder? 1 2 3 4 5

Indicate that the composite sample was completely mixed and homogenized? YES or NO

Date and Time Homogenate Prepared: _____

Signature of person who prepared homogenate sample: _____

Print name if signature is unreadable: _____

United States Environmental Protection Agency

Region 10, 1200 Sixth Avenue, Seattle WA 98101

QUALITY ASSURANCE

PROJECT PLAN

ASSESSMENT OF CHEMICAL CONTAMINANTS IN FISH CONSUMED BY FOUR NATIVE AMERICAN TRIBES IN THE COLUMBIA RIVER BASIN

Revision 6.0
December 16, 1996

Prepared By

U.S. Environmental Protection Agency (EPA)
Office of Environmental Assessment (OEA)
Region 10

APPROVAL OF QUALITY ASSURANCE PROJECT PLAN:

Project Manager:

_____ _____
Pat Cirone Date:
U.S. EPA, Region 10

Assistant Project Manager and Project Risk Assessor:

_____ _____
Dana Davoli Date:
U.S. EPA, Region 10

Field Operations Manager:

_____ _____
David Terpening Date:
U.S. EPA, Region 10

Project Quality Assurance Manager:

_____ _____
Robert G. Melton Date:
U.S. EPA, Region 10

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ATTACHMENTS

1.0 PROJECT DESCRIPTION

The U.S. Environmental Protection Agency (EPA) has initiated a study to assess chemical contaminant exposure from consumption of Columbia River fish by four Native American Tribes (Nez Perce, Warm Springs, Umatilla, and Yakama). These tribes are also referred to as Columbia River Treaty tribes. The first phase of this study was completed in October of 1994 by the Columbia River Inter-Tribal Fish Commission (CRITFC).

This current phase of the study (referred to as Phase II), will consist of evaluating tissue contaminant data representing resident and anadromous fish species that are caught by tribal fisheries in the Columbia River Basin and consumed by tribal members. This Quality Assurance Project Plan (QAPP) is the overall planning document for Phase II of the study.

The information from both phases of this exposure study will then be used to assess the potential health impacts to the Columbia River Treaty Tribes from consuming contaminants in Columbia River fish.

1.1 HISTORICAL OVERVIEW

Several studies have shown that elevated levels of Polychlorinated Dibenzo-p-dioxins and Polychlorinated Dibenzo-p-furans (PCDDs/PCDFs) are present in the biota of several areas of the Columbia River Basin. Measurements of the levels of PCDDs/PCDFs at pulp and paper mills lead to the conclusion that water discharges from these mills were the primary source of these contaminants. As a result, in 1991, Region 10 EPA established a Total Maximum Daily Load (TMDL) for 2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) which provides a framework to allocate the permissible 2,3,7,8-TCDD loading to the Columbia River Basin. In response to this TMDL, pulp and paper mills in the Columbia River Basin have been required, through permits issued under the National Pollutant Discharge Elimination System (NPDES), to modify their processes to achieve non-detectable levels of 2,3,7,8-TCDD in their wastewater discharges. Despite the expected decrease in discharges of this pollutant from pulp and paper mills, there is still concern about consumption of biota contaminated with PCDDs/PCDFs from the Columbia River. This is because exposures to extremely low levels of the PCDDs/PCDFs may result in adverse impacts on human health and because this class of compounds are highly bioaccumulative and persistent in the environment.

Studies in the Columbia River Basin have also shown that there are elevated levels of other contaminants of concern in the biota and sediments, including polychlorinated biphenyls (PCBs), and chlorinated pesticides, and inorganics. For example, as shown in a study done by the United States Geological Survey (7), DDT and its breakdown products, DDE and DDD, are still elevated in the water, sediment and fish in the Yakima River Basin (which is a part of the Columbia River Basin) despite the fact that two decades have passed since the production and

distribution of DDT was banned in the U.S. This study also concluded that fish in the Yakima Basin have among the highest concentrations of DDT in the Nation.

The fishery resource in the Columbia River Basin is not only a major food source for tribal members but it is also an integral part of the tribes' cultural, economic, and spiritual well-being. Because fish are consumed for both subsistence and ceremonial purposes, there has been concern that tribal members may be highly exposed to contaminants in fish because they consume large amounts of fish and eat fish body parts (e.g., fish eggs) that tend to accumulate fat-soluble toxins, like PCDDs/PCDFs, PCBs, and chlorinated pesticides. The Columbia River Treaty tribes have questioned the adequacy of the 6.5 gram per day fish ingestion rate used by the EPA to develop the TMDL for 2,3,7,8-TCDD in the Columbia River Basin. This value of 6.5 grams per day, which is also used by EPA in developing its national ambient water quality criteria for protection of human health from consumption of aquatic life, is the estimated average national fish consumption rate based upon a U.S. national diet survey.

Because of the elevated levels of contaminants in the Columbia River Basin and because of the importance of fish to the tribes in the Basin, the U.S. EPA initiated a two-phase exposure study to examine the role of fish consumption as an exposure route for waterborne contaminants among individuals of four of the Columbia River tribes.

In Phase I of this exposure study, the U.S. EPA entered into a Cooperative Agreement with the Columbia River Inter-Tribal Fish Commission (CRITFC) in 1990 to formally conduct a fish consumption survey of the four tribes represented by CRITFC - the Umatilla, Nez Perce, Yakama, and Warm Springs. This consumption study, published by CRITFC in October of 1994 (1), documented the types and amounts of fish eaten by tribal members as well as the fish parts consumed and food preparation methods used. The average fish consumption rate of adult tribal members (combining both fish consumers and non-fish consumers) was 58.7 grams per day. This value is about 9 times higher than the national average fish consumption rate (6.5 grams per day) used by the EPA. The 95th percentile of consumption for adult tribal members (combining both fish consumers and non-fish consumers) was approximately 170 grams per day. The location and frequency of use of tribal fishing sites in the Columbia River Basin, which is the source of about 90% of the fish consumed by tribal members, were also documented in the survey.

Phase II of this exposure study will use the information from the consumption study and from existing data on the levels of contaminants in Columbia River fish to design and implement a sampling program to collect tissue contaminant data from resident and anadromous fish species consumed by tribal members. The QAPP detailed in this document will be used for the sampling and analysis program in this second phase. The data from the first (fish consumption survey) and second (tissue contaminant data) phases of this exposure study will provide information that can be used to estimate the potential health impacts from consumption of Columbia River fish for these four tribes.

1.2 SURVEY OBJECTIVES

Phase II is being designed and implemented by EPA with input from representatives of CRITFC and its four member tribes, the U.S. Fish and Wildlife Service, the U.S. Geological Survey, and the Washington and Oregon State health and environmental agencies.

Prior to the development of this QAPP, a preliminary scoping document (2) was prepared for EPA by Tetra Tech, an EPA contractor, using data from: (a) the CRITFC fish consumption study; (b) personal communications with tribal fishery managers and tribal fishers; and © a data base compiled by Tetra Tech which summarized existing contaminant data on biota in the Columbia River Basin. This scoping document included a discussion of study objectives and a preliminary study design. At a design conference held in Portland, Oregon, on October 19-20, 1994, and attended by representatives of the organizations listed above, changes to the preliminary scoping document were recommended. The final scoping document, Assessment of Chemical Contaminants in Fish Consumed by Four Native American Tribes in the Columbia River Basin - Final Draft Study Design, was completed on December 2, 1994 (3) (referred to as "draft study design document" from here on).

The objectives for Phase II, as discussed in the draft study design document, are to:

- Measure fish contaminant levels for species and fishing locations being utilized by CRITFC member tribes to provide, in conjunction with the CRITFC fish consumption report, an assessment of fish consumption as an exposure route for waterborne chemical toxics among individuals of these tribes.

- Use the information derived from the exposure assessment to estimate potential health risks to fish consumers in the four CRITFC member tribes.

The discussion surrounding these two objectives is discussed in more detail in the draft study design document.

1.3 DOCUMENT PURPOSE AND SCOPE

This document provides technical and procedural guidance and requirements to ensure that a well-planned scientific investigation is conducted, and that the field measurements and analytical data obtained serve the project objectives described above. The content and structure of this QAPP are based upon requirements and guidelines in Quality Management Program Plan For Region 10, EPA Region 10, Seattle, WA, RQMP-001/92, January 23, 1993, which requires the use of Interim Guidelines and Specifications For Preparing Quality Assurance Project Plans, QAMS-005/80, December 29, 1980, for the preparation of QAPPs involving sampling and analysis projects in EPA Region 10. Specifications for data quality are presented in Section 3.0. Preparation of this QAPP helps the project manager focus on the factors affecting data quality during the planning stage of the project. The completed plan defines field and laboratory procedures, and facilitates project implementation and communication among field, laboratory, and management staff.

Communication is extremely important for this project because of the number of different organizations and individuals involved as shown in Figure 1. The Project Manager at EPA Region 10 in Seattle, Washington, is also the Work Assignment Manager (WAM) for the EPA Contractor, Tetra Tech. CRITFC's Water Quality Manager is the Tribal Manager for this project.

The EPA Field Operations Manager (FOM) in Region 10 will coordinate a field crew of EPA and tribal staff to collect fish samples. Fish samples to be analyzed as whole fish will be sent to a laboratory which is a subcontractor to the EPA Contractor, Tetra Tech. For those fish in which fillets and/or eggs are to be measured, fish will first be filleted and the eggs collected by the EPA and other field crew members and these fillets and eggs will then be sent to the subcontract laboratory. The subcontract laboratory will be responsible for homogenizing all of the fish and egg samples and for preparing sample aliquots for all analyses. The subcontract laboratory will also analyze the fish and egg samples for chlorinated dioxins and furans and toxic, dioxin-like, PCBs (often referred to as coplanar PCBs).

The subcontract laboratory will also send samples of the homogenized samples to the EPA Region 10 Laboratory for analysis (pesticides/PCBs, semivolatiles including polyaromatic hydrocarbons, and inorganics) and for archiving. Some of these archived samples may be used for analysis of radionuclides at another laboratory if resources become available.

Data quality review of all analytical data will be performed by EPA Region 10. Analytical data and documentation for toxic, dioxin-like PCBs and chlorinated dioxins and furans generated by the subcontract laboratory will be sent to the Contractor, Tetra Tech. Tetra Tech will then send copies of these data and documentation to EPA, Region 10, where a validation of the data will be conducted by the EPA Project QA Manager. Validation of data from the EPA Region 10 Laboratory (pesticides/PCBs, semi-volatiles and inorganics) will be conducted by the Region 10 Laboratory. The EPA Project QA Manager will also perform a data quality review of radionuclide data if resources are found for these analyses. All validated data will then be sent to Tetra Tech where it will be compiled into a summary data report and entered into the Columbia River contaminant database which was previously developed for EPA by Tetra Tech.

This QAPP details procedures for field sampling, filleting and homogenization of fish, and chemical analyses. In addition, protocols for documentation, labeling, handling, chain of custody, storage and shipping, and analytical QA procedures are discussed. Field and laboratory procedures are described in Sections 4.0 through 9.0. Sections on data validation and review (10.0); quality control procedures (9.0); preventive maintenance (12.0); data assessment and reporting(14.0); and corrective actions (13.0) provide sufficient detail to direct activities of project participants and provide interested readers with an understanding of how analytical data will be used in project decision-making processes.

A Cooperative Agreement (see Attachment 1) has been developed between CRITFC and U.S. EPA, Region 10. The purpose of this Agreement is to set forth the relationship and nature of cooperation between CRITFC and EPA in all aspects of the Phase II study including, but not limited to, sample collection, tissue analysis, data assessment, and data release. The work done

in this QAPP will be done in cooperation with CRITFC as written in the Cooperative Agreement.

A project schedule of major milestones for sample collection, data analysis, validation and assessment of data, and final project report preparation can not be accurately specified for the project due to major variables such as multi source funding and variations in fish populations. Table 7 provides a schedule of sampling activities for the project. Tables 7 and 8 from the previous revision of the QAPP (Revision 5.1, June 17, 1996) are provided in Attachments 19 and 20 to this QAPP. The project sampling schedule will need to be flexible due to variations in fish populations. A Sample Alteration Form (see Attachment 17) will be prepared and approved when the sampling schedule requires changing. Reservations for laboratory measurements will be made by the FOM with the EPA Manchester Laboratory 45 days prior to shipment of samples. Validation of project data will be completed within 60 days of receipt of laboratory reports. Final data assessment and submission of the draft final report for the project will be completed on or before June 1, 1998.

2.0 PROJECT ORGANIZATION

The managerial organization of the project is shown in Figure 2. Project Managers have the following assigned responsibilities:

- **Project Manager: Pat Cirone**

  USEPA, Region 10
  (206) 553-1597
  fax: (206) 553-0119

  Pat Cirone will be responsible for the overall quality of data and project activities for EPA Region 10. She will be responsible for ensuring that Region 10 project staff clearly understand their responsibilities and authority on the project. The Regional Project Manager consults with the Project Leader and approves all deviations from the QAPP. The Regional Project Manager reviews all audit reports and ensures that corrective actions or non-conformances are taken in a timely and appropriate manner. The Project Manager is responsible for ensuring that the QAPP is adequately reviewed prior to implementation of the project.

  Pat Cirone is also the Work Assignment Manager (WAM) for the contract with Tetra Tech/Redmond. As the Work Assignment Manager for Tetra Tech, she is responsible for ensuring that Tetra Tech and all subcontractors of Tetra Tech, implement the specifications and requirements of the QAPP. Tetra Tech's role in the project is to carry out the requirements of the Work Assignment which is issued and managed by Pat Cirone.

- **Regional Project Leader: Dana Davoli**

  USEPA, Region 10
  (206) 553-2135
  fax: (206) 553-0119

  The Regional Project Leader reports directly to the Region 10 Project Manager. All other Regional Project staff report directly to the Regional Project Leader. The Regional Project Leader is responsible to the Regional Project Manager for implementing and carrying out the requirements of the QAPP for Region 10. All information concerning project activities is transmitted by Region 10 staff through the Regional Project Leader to the Regional Project Manager.

- **Field Operations Manager: David Terpening**

USEPA, Region 10
(206) 553-6905
fax: (206) 553-0119

The FOM is responsible for planning and implementing field activities, including fish collection, fish filleting and egg collection, and shipment of samples to the contract lab. In order to carry-out these responsibilities, the FOM will communicate frequently and periodically with the Regional Project Leader and the Project Manager concerning field activities. The FOM will report to the Project Leader.

- **Regional Lab Project Coordinator: Peggy Knight**

USEPA, Region 10
(360) 871-8713
fax: (360) 871-8747

The Regional Laboratory Project Coordinator will be responsible for coordination and oversight of the EPA Region 10 Laboratory's work on the Project. Peggy Knight will monitor laboratory activities and coordinate communications of laboratory activities and laboratory reports to the Regional Project Leader. The Regional Lab Coordinator will report to the Project Leader.

- **Project QA Manager: Robert G. Melton**

USEPA, Region 10
(206) 553-2147
fax: (206) 553-8210

The Project QA Manager is responsible for implementation of all QA requirements of the QAPP. He will be the primary data quality reviewer of the analytical results (PCDD/PCDF congeners and dioxin-like PCBs) from the subcontract laboratory and, if resources become available, from the laboratory(s) conducting radionuclide analyses. He oversees laboratory performance and quality control requirements of the QAPP. The Project QA Manager is responsible for documenting to the Project Leader and Project Manager that corrective actions have been implemented. The Project QA Manager must review and approve the QAPP before the QAPP can be implemented. The Project QA Manager reports on routine project matters to the Project Leader.

- **Regional QA Manager: Barry Towns**

USEPA, Region 10
(206) 553-1675
fax: (206) 553-0119

The Region 10 Quality Assurance Unit (QAU)is responsible for to review and concur on the approval/disapproval of QAPPs required by EPA Order 5360.1 (see Region 10's Quality Management Plan of 1992). Final approval/disapproval of this QAPP lies with project management personnel. In executing his QA and oversight responsibilities, the Regional QA Officer reports to the Regional Administrator.

- **Columbia River Inter-Tribal Fish Commission (CRITFC):**

CRITFC
(503) 238-0667
fax: (503) 235-4228

As discussed above, a Cooperative Agreement has been developed between CRITFC and U.S. EPA, Region 10 which sets forth the relationship and nature of cooperation between CRITFC and EPA in all aspects of the Phase II study.

Figure 1. Flow Diagram of Project Tasks

3.0 DATA QUALITY OBJECTIVES

The overall QA objective for analytical data is to ensure that data of known and acceptable quality are produced so that potential health risks to fish consumers in the four CRITFC member tribes can be estimated. Data quality objectives (DQOs) for the project are discussed below, in Table 1, in the attachments to the QAPP, and in other sections of the QAPP. Project DQOs include:

- 1. The selection of the appropriate chemical target compounds to be measured and the appropriate quantitation limits for these compounds, and,

- 2. Analytical objectives as defined by measurement of PRECISION, ACCURACY, REPRESENTATIVENESS, COMPLETENESS, and COMPARABILITY of quality assurance samples such as field duplicate samples, performance evaluation samples, and laboratory quality control samples. These QA samples will be used to evaluate project data to determine if data meets the specified DQOs of the QAPP.

3.1 SELECTION OF TARGET COMPOUNDS AND DETECTION LIMITS

As discussed in Section 1.2, the objectives for Phase II are to measure fish contaminant levels for species caught at fishing locations being utilized by CRITFC tribal members. These data will then be used to provide information on potential exposures and health impacts from waterborne chemical toxics for these tribes.

The selection of target compounds and the risk-based detection limit goals were determined in the draft study design document prepared for this project by Tetra Tech (3). In this document, target analytes were selected by considering guidance provided by the U.S. EPA (4) and by performing a health risk-based screening analysis of tissue contaminant data collected within the Columbia River Basin during the last ten years (1984-1994).

Screening for carcinogenic effects was performed for a 70 kg adult using a target cancer risk of 1×10^{-6}. Screening for non-cancer effects was performed for a 14.5 kg child using a target hazard quotient of 0.1. Fish consumption rates assumed for adults and children were 194 and 81 g/day, respectively, which correspond to the cumulative 97th percentile consumption rate reported in CRITFC (1). For chemicals that had both slope factors for estimating carcinogenic risk and reference doses for estimating non-carcinogenic impacts, separate tissue screening concentrations (STCs) were calculated and the lower of the two values was used for the screening analysis. These STCs were then compared to the tissue contaminant data collected in the Columbia River Basin in the past ten years.

Only a small number of chemicals from this tissue contaminant data did not exceed the STCs. Chemicals that exceeded the STCs included dioxins/furans, PCBs, organochlorine and

organophosphorus pesticides, PAHs and other semivolatiles, trace metal and radionuclides. Based on this risk screening analysis, a decision was made to measure for the contaminant classes listed in Table 1. Table 2, and Tables 4 through 6 provide a listing of individual contaminants in each of these classes.

After the draft study design document was completed, an analytical method (Method 1668) became available for measuring toxic dioxin-like PCBs. These compounds were added to the list of analytes and are shown in Table 3.

The STCs calculated in the study design document were selected as the risk-based detection limit goals for this project with the following exception. In the design document, the fish consumption rates used to calculate the STCs were the 97th percentile from the CRITFC study. Because use of the 95th percentile is more in line with EPA guidance, the STCs in the design document were recalculated using the 95th percentile consumption rates. Table 2 and Tables 4 through 6 contain the risk-based detection limit goals (formally the STCs)(shown in the tables as the "risk levels") calculated using the 95th percentile fish consumption rates. The quantitation limits that will be achieved in this project are also included in these tables. Analytical detection limits for the toxic, dioxin like PCBs are shown in Table 3.

Radionuclides are not included in Table 6 (inorganics) due to lack of resources to pay for analytical measurements at this time. However, it was agreed at the scoping meeting that EPA would attempt to find resources for these analyses.

As shown in Tables 2-6, several chemicals have detection limits that are above the risk level goals that were calculated. For this project, the analytical methods being used were chosen to provide detection or quantitation limits which are as low as possible given available analytical methods and resources.

3.2 MEASUREMENT OBJECTIVES

The following objectives are measurement goals for this project:

3.2.1 Precision

Precision is the measurement of agreement among repetitive measurements of the same sample. Precision will be evaluated in two ways:

(1) The relative percent difference (RPD) between matrix spike/matrix spike duplicate (MS/MSD) samples will be calculated. As shown in Table 1, MS/MSD measurements will be made at a frequency of one per twenty samples/composites. Since a total of 122 fish samples are expected to be measured in this project, this results in a total of approximately 7 MS/MSD samples for each analytical group.

(2) The relative percent difference (RPD) between field duplicate samples will be calculated. As shown in Table 7, for one composite sample of steelhead and one of spring chinook, separate composites of fillets will be prepared from each side of the fish. The comparison of the analytical results from both sides will serve as field duplicates. In addition, two blind duplicate field samples will be selected by the Project Manager for complete target compound analysis.

For field duplicate samples and for matrix spiked and matrix spiked-duplicate samples, precision will be measured as Relative Percent Difference (RPD).

$$RPD = \frac{ABS\,(R1 - R2)}{((R1 + R2)/2)} \times 100$$

R1 = Recovery for MS or duplicate 1, R2 = Recovery for MSD or duplicate 2

Precision required for the analysis of project MS/MSD samples is specified in Table 1. Precision required for the analysis of field duplicates (consisting of the opposite fillets of the same fish) shall be less than 40 relative percent difference.

3.2.2 Accuracy

Accuracy is the degree of agreement of an experimental measurement with an accepted standard reference. Accuracy will be evaluated by calculating the percent recovery (%R) of target analytes or isotope-labeled target compounds in spiked samples, and by the measurement of known target compounds in Performance Evaluation (PE) tissue samples.

$$\%\,Recovery = \frac{SQ - NQ}{S} \times 100$$

SQ = quantity found in spiked sample,
NQ = quantity found in native (unspiked) sample,
S = quantity of spike added to native sample

The accuracy requirements for MS/MSD samples for each measurement method are presented in Table 1. As discussed above, MS/MSD samples will be measured at a frequency of 1:20 for a total of 7 per analytical group.

As shown in Table 1, six Performance Evaluation (PE) samples (PE samples EDF-2524, EDF-2525, and EDF-2526) will be measured for chlorinated dioxins/furans and for the toxic, dioxin-like, PCBs. Accuracy requirements of acceptable recovery ranges for these PE samples have been documented by Cambridge Isotope Laboratories. These acceptable accuracy recovery ranges will be required by the laboratory which measures PCDDs/PCDFs and toxic, dioxin-like, PCBs. Blind PE samples for PCDDs/PCDFs and toxic, dioxin-like, PCBs measurements will not be used in this study because none are available in one kilogram quantities. However, two blind duplicate field samples will be selected by the Project Manager for complete target compound analysis.

3.2.3 Representativeness

Representativeness is the degree to which data from the project accurately represents a particular characteristic of the environmental matrix which is being tested. For example, representativeness is the degree to which data accurately and precisely represents a characteristic of a population, a matrix, a natural variation at a sampling location, or an environmental condition. Acceptable representativeness is achieved through adequate sampling program design and QAPP design. Goals for representativeness are primarily met by ensuring that, given available resources, sampling locations are properly selected and that a sufficient number of tissue types and fish species are collected. Sections 4 through 8 of the QAPP specify procedures which will be used to ensure that samples are representative of Columbia River basin fish which are consumed by the Columbia River Treaty tribes.

3.2.4 Completeness

Completeness is the percentage of valid results obtained as compared to the total number of samples taken for a parameter. Completeness requirements for this project are presented in Table 1.

$$\% \text{ Completeness} = \frac{\text{\# of valid results}}{\text{\# of samples taken}}$$

Figure 2. Project Organization

3.2.5 Comparability

Comparability is a qualitative characteristic expressing the confidence with which one data set can be compared with other data sets. In this regard, measurements of PCDDs/PCDFs and toxic, dioxin-like, PCBs from this project may not be comparable with PCDD/PCDF and toxic, dioxin-like, PCB data measurements from previous projects because new and improved state-of-the-art methods such as Methods 1613B and 1668 are used in this project to measure samples. In addition, data from previous projects have not always been validated and qualified by a chemist to determine data quality and data useability. Therefore, a comparability goal for the measurement of PCDDs/PCDFs and non-coplanar PCBs for this project cannot be set. By contrast, project data for the measurement of metals, pesticides/PCBs and semi-volatile organics should be more comparable to previous data from the analysis of Columbia River basin fish. A comparability goal of 70% is set for these non-PCDD/PCDF and non-coplanar PCB data.

The QA data quality objectives outlined above, will be evaluated in conjunction with the data validation process, and will be documented in the Final Summary Data Report for the project.

3.3 OTHER DATA QUALITY OBJECTIVES

In addition to the specific measurement objectives discussed above, Section 9.4 of the QAPP specifies that all quality control requirements of each method which is referenced in Table 1 shall be obtained and reported by each analytical laboratory. These laboratory QC measurements include the use of surrogate compounds, internal standards, recovery standards, matrix spike compounds, isotope dilution labeled internal standards, instrument calibrations, and method blanks.

As shown in Table 7 and discussed in Section 4.3, for all species except sturgeon, three composite samples will be collected at each sampling site for each species. These composites will be composed of different individual fish of the same species at a location close to the specified sampling station. The QAPP does not have a data quality objective for expected precision of these three composite samples of the same species. As discussed in Section 4.3, EPA guidance regarding numbers of fish per composite and length of fish will be followed if possible. However, these goals may not be possible if there is there is difficulty in catching fish. Relative Standard Deviations (RSDs) between the three composite samples collected at each sampling station will be calculated after analyses are completed.

An additional very important data quality objective of the project is to obtain validated PCDD/PCDF data which is free of expected chlorinated chemical interferences to the measurement of PCDD/PCDF target compounds, such as Polychlorinated Diphenyl Ether (PCDPE) interferences. Therefore, one of the additional primary data quality objectives in this QAPP is for the subcontract laboratory to remove chemical interferences to the measurement of 2,3,7,8-TCDF, which is the PCDD/PCDF isomer which has historically been found in the highest concentrations in fish tissue in the Columbia River system. Previous data from the Columbia River system has often been contaminated with PCDPE chemical interferences.

Table 1. Sampling and Measurement Objectives and Requirements For the Project

Analytical Group	List of Target Com-pounds	Tentative Number of Field Samples[1,6]	Number of QA Samples: PE[2], MS/MSD[2] Dups[1]	Matrix	Method	Accuracy[3]	Precision[4] (RPD)	Complete-ness	Preser-vation	Containers (Field/Lab)	Holding Time For Project Samples
PCDDs/ PCDFs/ % Lipids	Table 2	251	6 PE's 8 Dups	Fish Tissue	1613B + SOW	70 to 140%	40%	90%	-20°C	Al foil/ 2x2ozWM	1 yr. (sample) 40 days (extract)
Toxic, Dioxin-Like, PCBs	Table 3	251	6 PE's 8 Dups	Fish Tissue	1668	70 to 140%	40%	90%	-20°C	Al foil/ 2x2ozWM	1 yr. (sample) 40 days (extract)
Chlorinated Pesticides/ Aroclors	Table 11	251	12 MS/MSDs 8 Dups	Fish Tissue	8081 Florisil/Acetonitrile Partitioning/Florisil	30-150%	50%	90%	-20°C	Al foil/ 2x2ozWM	1 yr. (sample) 40 days (extract)
AED/ Pesticides	Table 12	60 to 120	6 MS/MSDs 4 Dups	Fish Tissue	8085 Acetonitrile Partitioning	30-150%	50%	90%	-20°C	Al foil/ 2x2ozWM	1 yr. (sample) 40 days (extract)
Neutral Semivol.	Table 4	251	12 MS/MSDs 8 Dups	Fish Tissue	8270/ GPC/SG	10-150%	50%	90%	-20°C	Al foil/ 2x2ozWM	1 yr. (sample) 40 days (extract)
PAHs	Table 4	251	12 MS/MSDs 8 Dups	Fish Tissue	8270/SIM GPC/SG	30-140%	50%	90%	-20°C	Al foil/ use SV ext	1 yr. (sample) 40 days (extract)
Chlorinated Phenolics	Table 5	251	12 MS/MSDs 8 Dups	Fish Tissue	1653 Modified GPC/Acetylation	20-150%	50%	90%	-20°C	Al foil/ 2x2ozWM	1 yr. (sample) 40 days (extract)
Metals	Table 6	251	8 Dups	Fish Tissue	200.3 & 200.8[5]	60-140%	30%	90%	-20°C	Al foil/ 2x2ozWM	2 yrs.
Mercury	Table 6	251	8 Dups	Fish Tissue	251.6[5,] Rev. 2.3	60-140%	35%	90%	-20°C	Al foil/ use ICP WM	86 days
Archive Samples		16 per sample		Fish Tissue					-20°C	16x2ozWM	

[1] - The total number of samples in column 3 does not include QA samples such as PE samples and Matrix Spike/Matrix Spike Duplicate (MS/MSD) samples. The number of blind field duplicate (Dup) samples are included in the total number of samples in column 3. For example, of the 251 samples which will be measured for metals, 8 of the 251 samples will be blind field duplicate samples.
[2] - PE = Performance Evaluation Samples; MS/MSD = Matrix Spike/Matrix Spike Duplicate Sample; Dups = Blind Field Duplicate Samples.
[3] - Accuracy as measured in MS (matrix spike) and MSD (matrix spike duplicate) samples, which are measured at a frequency of 1:20 samples.
[4]- Precision as measured in MS (matrix spike) and MSD (matrix spike duplicate) samples, which are measured at a frequency of 1:20 samples.
[5] - Methods for the Determination of Metals in Environmental Samples, EPA/600/4-91/010, June 1991.
[6] - The number of filed samples will be revised by project members after January 6, 1997.

Table 2. Method 1613B PCDD/PCDF Target Compounds

Target Compound	CAS Number	Screening Tissue Concentration (STC) ng/Kg	Quantitation Limit[1] ng/Kg
2,3,7,8-TCDD	1746-01-6	0.002	0.2
1,2,3,7,8-PeCDD	40321-76-4	0.005	5
1,2,3,4,7,8-HxCDD	39227-28-6	0.024	5
1,2,3,6,7,8-HxCDD	57653-85-7	0.024	5
1,2,3,7,8,9-HxCDD	19408-74-3	0.024	5
1,2,3,4,6,7,8-HpCDD	35822-46-9	0.024	5
OCDD	3268-87-9	2.4	10
2,3,7,8-TCDF	51207-31-9	0.024	0.2
1,2,3,7,8-PeCDF	57177-41-6	0.048	5
2,3,4,7,8-PeCDF	57117-31-4	0.005	5
1,2,3,4,7,8-HxCDF	70648-26-9	0.005	5
1,2,3,6,7,8-HxCDF	57117-44-9	0.024	5
1,2,3,7,8,9-HxCDF	72918-21-9	0.024	5
2,3,4,6,7,8-HxCDF	60851-34-5	0.024	5
1,2,3,4,6,7,8-HpCDF	67562-39-4	0.24	5
1,2,3,4,7,8,9-HpCDF	55673-89-7	0.24	5
OCDF	39001-02-0	2.4	10

[1] - Quantitation limits listed for fish tissue samples are based on wet weight. A 50 gram fish tissue sample is used for extraction purposes.

Table 3. Method 1668 Toxic, Dioxin-Like, PCB Target Compounds

Target Compound[1]	Congener Number	CAS Number	Quantitation Limit[2] ng/Kg
3,3',4,4'-TCB	77	32598-13-3	2
2,3,3',4,4'-PeCB	105	32598-14-4	100
2,3,4,4',5-PeCB	114	74472-37-0	200
2,3',4,4',5-PeCB	118	31508-00-6	20
2',3,4,4',5-PeCB	123	65510-44-3	10
3,3',4,4',5-PeCB	126	57465-28-8	10
2,3,3',4,4',5-HxCB	156	38380-08-4	20
2,3,3',4,4',5'-HxCB	157	69782-90-7	20
2,3',4,4',5,5'-HxCB	167	52663-72-6	20
3,3',4,4',5,5'-HxCB	169	32774-16-6	20
2,2',3,3',4,4',5-HpCB	170	35065-30-6	20
2,2',3,4,4',5,5'-HpCB	180	35065-29-3	20
2,3,3',4,4',5,5'-HpCB	189	39635-31-9	20

[1] - Nomenclature for Polychlorinated Biphenyls:
TCB = Tetrachlorobiphenyl
PeCB = Pentachlorobiphenyl
HxCB = Hexachlorobiphenyl
HpCB = Heptachlorobiphenyl

[2] - Quantitation limits listed for fish tissue samples are based on wet weight. Quantitation Limits listed are estimated values due to high background levels of some selected standards and due to the lack of maturity of the method which was first proposed on October 4, 1995.

Table 4. Neutral Semivolatile Target Compound List

Target Compound	CAS Number	Risk Level [1] ug/Kg	Quantitation Limit ug/Kg [2]
1,2-Dichlorobenzene	95-50-1	1611.1	330
1,2,4-Trichlorobenzene	120-82-1	179	330
1,4-Dichlorobenzene	106-46-7	15.03	330
1,3-Dichlorobenzene	541-73-1	1593.2	330
2,2'-oxybis (1-Chloropropane) [3]	108-60-1	NC	330
2-Methylnaphthalene	91-57-6	NC	330
2-Chloronaphthalene	91-58-7	NC	330
2,4-Dinitrotoluene [3]	121-14-2	35.8	330
2,6-Dinitrotoluene [3]	606-20-2	0.53	330
4-Bromophenyl-phenylether	101-55-3	NC	330
4-Chlorophenyl-phenylether	7005-72-3	NC	330
Acenaphthene	83-32-9	1074.1	59
Acenaphthylene	208-96-8	NC	76
Anthracene	120-12-7	5370.4	22
Benzo(a)anthracene	56-55-3	0.34	10
Benzo(a)pyrene	50-32-8	0.049	10
Benzo(b)fluoranthene	205-99-2	0.4	10
Benzo(g,h,i)perylene	191-24-2	2.3	10
Benzo(k)fluoranthene	207-08-9	0.4	10
bis(2-Chloroethyl) ether	111-44-4	0.33	330
Chrysene	218-01-9	0.049	10
Dibenzo(a,h)anthracene	53-70-3	0.045	10
Dibenzofuran	132-64-9	NC	330
Fluoranthene	206-44-0	716	10
Fluorene	86-73-7	716	10
Hexachlorobutadiene [3]	87-68-3	4.6	330
Hexachloroethane [3]	67-72-1	17.9	330
Indeno(1,2,3-cd)pyrene	193-39-5	0.18	10
Naphthalene	91-20-3	716	59
Nitrobenzene	98-95-3	9	330
Phenanthrene	85-01-8	519.1	21
Pyrene	129-00-0	537	10

[1] - NC = Not Calculated due to lack of toxicity value for compound.
[2] - Quantitation limits listed for fish tissue samples are based on wet weight.
[3] - It is uncertain if this target compound will survive clean-up procedures.

Table 5. Chlorinated Phenolics Target Compound List

Target Compound [1]	CAS Number	Risk Level [2] ug/Kg	Quantitation Limit ug/Kg [3]
2-Chlorophenol	95-57-8	89.5	300
2,4,6-Trichlorophenol	88-06-2	32.6	300
2,4,5-Trichlorophenol	95-95-4	1790.1	300
2,4-Dichlorophenol	120-83-2	53.7	300
2,3,4,6-tetrachlorophenol	58-90-2		300
2,6-dichlorophenol	87-65-0		300
3,4-dichloroguaiacol	77102-94-4		300
3,4,6-trichloroguaiacol	60712-44-9		300
3,4,5-trichloroguaiacol	57057-83-7		300
4,6-dichloroguaiacol	16766-31-7		300
4,5,6-trichloroguaiacol	2668-24-8		300
4,5-dichloroguaiacol	2460-49-3		300
4-Chloro-3-methylphenol	59-50-7	NC	300
4-chloroguaiacol	16766-30-6		300
Pentachlorophenol	87-86-5	3	300
Tetrachloroguaiacol	2539-17-5		300

[1] -- Some compounds in this target compound list are expected to be lost during extract clean-up procedures.
[2] -- NC = Not Calculated due to lack of toxicity value for compound.
[3] -- Quantitation limits listed for fish tissue samples are based on wet weight.

Table 6. Inorganic Target Analyte List*

Target Compound	CAS Number	Risk Level [1] (mg/Kg)	Detection Limit (mg/Kg) [2]
Aluminum	7429-90-5	NC[2]	40
Antimony	7440-36-0	7.2	12
Arsenic	7440-38-2	0.21	2
Barium	7440-39-3	1253.1	40
Beryllium	7440-41-7	0.084	1
Cadmium	7440-43-9	9.0	1
Chromium	7440-47-3	89.5	2
Cobalt	7440-48-4	NC	10
Copper	7440-50-8	662.3	5
Lead	7439-92-1	7.7	0.6
Manganese	7439-96-5	89.5	3
Mercury	7439-97-6	5.4	0.1
Nickel	7440-02-0	358.0	8
Selenium	7782-49-2	89.5	1
Silver	7440-22-4	89.5	2
Thallium	7440-28-0	NC	2
Vanadium	7440-62-2	0.13	10
Zinc	7440-66-6	5370.4	4

[1] - NC = Not Calculated due to lack of toxicity value for compound.
[2] - Detection Limits listed for fish tissue samples are based on wet weight.

4.0 FIELD SAMPLING PROCEDURES

This section identifies the station locations (Section 4.1), target species and sample types (Section 4.2), sampling strategy (Section 4.3), field collection methods (Section 4.4), and handling of samples and documentation in the field (Section 4.5).

All of the field sampling for this project will be coordinated and conducted by EPA, Region 10, with assistance from the CRITFC tribes. The project leader and EPA FOM will thoroughly review the QAPP before sampling begins. Prior to sampling, the field team members will be familiar with:

- The responsibilities of each member of the field team
- Study objectives and time commitments for this project
- Collection permit requirements
- Site locations and collection equipment and gear needed at each site
- Proposed sampling dates and species of interest for each site location
- Composite sample size for each species and sample type
- Fish handling procedures and storage requirements.

4.1 STATION LOCATIONS

The CRITFC fish consumption survey (1) identified 102 fishing sites used by the four tribes in the Columbia River Basin. Due to resource constraints, all of these sites could not be sampled in Phase II of EPA's exposure study. The draft study design document referred to in Section 1.2 discusses in detail the process that was used to reduce the number of sites to be sampled to 13 sites. Initially, fishing sites that represented greater than 40 percent of each tribe's fishing use for resident and anadromous fish species were identified. This number of fishing sites (24 sites) was reduced to 8 sites by (1) selecting one site at the base of a watershed to represent the entire watershed for the Deschutes (site 98), Clearwater (site 96), and Umatilla (site 30) Rivers and (2) limiting the number of sites on the mainstem Columbia River to be sampled to sites 6, 7, 8, 9 and 18. Additional sites were added because they: are near local pollution sources of concern to the tribes (sites 48 and 49 on the Yakima River, and site 79 on the Salmon River); contain species of special concern to the tribe such as smelt (site 57 on the Cowlitz River); or provide needed geographical coverage (site 21 on the Willamette River). Use of this decision tree resulted in the selection of 13 sites for sampling.

Subsequent to the completion of the draft study design document, additional discussions were held with CRITFC tribal fisheries program managers and tribal staff. In these discussions, it was decided that for sites 9, 18, and 21, it would be easier to collect samples of salmon from nearby salmon hatcheries that supply salmon to the tribes. This is because recent data on fish runs suggested that low numbers of salmon may return to sites 9, 18, and 21. Also, using the fish returning to the hatcheries will help reduce some of the field collection time and sampling effort for this project. Therefore, at site 21, no salmon will be caught; they will instead be taken at site 21A (Dexter Hatchery on the McKenzie River). Salmon that were to be caught at site 9 will now be taken at site 14 (Priest Rapids Hatchery on the Columbia River); salmon that were to be caught at site 18 will now be taken at site 51 (Icicle Hatchery on the Wenatchee River). Other species will still be caught at sites 9, 18, and 21. An updated decision tree is shown in Figure 3 and now includes 16 sites . Site 14 will provide information on a local pollution source of concern, while sites 21A and 51 will provide the geographical coverage used in the decision tree. All of the fish species of interest and sampling locations are shown in Figure 4. The map of sampling locations from the June 17, 1996 revision of the QAPP is provided in Attachment 21.

The sampling locations in Figure 4 are not precise but rather indicate that area of the river system where fish will be collected. If an insufficient number of fish for a given species are collected from within the identified location, collection efforts may be extended to additional sections of the river as close as possible to the original location.

Whenever possible, the Global Positioning System (GPS) will be used to locate the sampling location (e.g. latitude and longitude) during fish collection efforts and this information will be transferred on to USGS topographical maps. If GPS positions cannot be obtained, then sampling locations will be determined using USGS topographical maps and the latitude and longitude recorded for this site . This information will be compiled in an appendix which will be included with the data report.

Figure 3. Decision Tree For Selection of Tissue Sampling Sites

Figure 4. Proposed Sampling Locations

4.2 TARGET SPECIES AND SAMPLE TYPE

Table 7 shows the locations, species, and sample types that will be measured during the entire 1996-1997 study. The selection of species to be collected was based primarily on consumption data presented in the CRITFC Fish Consumption Report. Input during the design conference in Portland and from the CRITFC tribal members was also considered. The primary target species selected are listed below:

Chinook salmon	**Oncorhynchus tshawytscha**
Coho salmon	**Oncorhynchus kisutch**
Steelhead trout	**Oncorhynchus mykiss**
Rainbow trout	**Oncorhynchus mykiss**
Mountain whitefish	**Prosopium williamsoni**
Lake whitefish	**Coregonus clupeaformis**
White sturgeon	**Acipenser transmontanus**
Walleye	**Stizostedion vitreum**
Largescale sucker	**Catostomus macrocheilus**
Bridgelip sucker	**Catostomus columbianus**
Eulachon (smelt)	**Thaleichthys pacificus**
Pacific lamprey	**Lampetera tridentata**

Table 9 shows the fish species that are consumed by tribal members and the fishing sites where fish are to be collected. Tissue samples for all consumed species except northern squawfish (Ptychocheilus oregonensis) and American shad (Alosa sapidissima) will be measured. These two species are consumed by only a small fraction (<2.7 percent) of adult tribal members.

If the primary species of aquatic organism can not be obtained, other species of fish will/may be substituted after consultation between the Project Manager, the Project Leader, the FOM, and CRITFC.

Four types of samples will be measured: whole-body (WB), fillet with skin (F_s), fillet without skin (F_w), and eggs (E). Whole-body samples were selected for several species to maximize the chances of measuring detectable levels of contaminants of concern and because data presented in the CRITFC fish consumption study show that tribal members may consume several fish parts in addition to the fillet (Table 10). Eggs from spring chinook, fall chinook, and steelhead will be measured because consumption data shows that salmonid eggs are widely consumed by tribal members (Table 10). Because of the high lipid levels in eggs, concentrations of hydrophobic organic chemicals may reach substantially higher levels than in other fish tissues. Salmonid heads were not designated as a matrix for compositing and analysis due to limited project resources and because the CRITFC fish consumption study did not indicate that most tribal members consumed large amounts of Salmonid heads on a frequent basis.

Table 7. Revised Sampling Design For the CRITFC Exposure Study [a]

Site No.	Location	Fish Species [b]	Res /Anad	Sample Type	Collection Period [c]	Collection Method	Repli-cates [d]	Number of Fish
57	Cowlitz River, lower	smelt	Anad	whole body	January 1997	dipnet	3	300
8	Columbia River, John Day Pool	steelhead	Anad	whole body	February 1997	gillnet	3	15
8	Columbia River, John Day Pool	steelhead	Anad	fillet with skin	February 1997	gillnet	3	15
8	Columbia River, John Day Pool	steelhead	Anad	eggs	February 1997	gillnet	3	0
56A	Klickitat River, lower	steelhead	Anad	whole body	February 1997	gillnet	3	15
56A	Klickitat River, lower	steelhead	Anad	fillet with skin	February 1997	gillnet	3	15
93	Snake River	steelhead	Anad	whole body	February 1997	gillnet	3	15
93	Snake River	steelhead	Anad	fillet with skin	February 1997	gillnet	3	15
6	Columbia River, Bonneville Pool	sturgeon	Res	fillet without skin	February 1997	deep water gillnet	3	3
7	Columbia River, Dalles Pool	sturgeon	Res	fillet without skin	February 1997	deep water gillnet	3	3
8	Columbia River, John Day Pool	sturgeon	Res	fillet without skin	February 1997	deep water gillnet	3	3
8	Columbia River, John Day Pool	sturgeon	Res	eggs	February 1997	deep water gillnet	3	0
96	Clearwater River, lower	sturgeon	Res	fillet without skin	March 1997	deep water gillnet	3	3
96	Clearwater River, lower	mountain whitefish	Res	whole body	March 1997	gillnet	3	45
96	Clearwater River, lower	mountain whitefish	Res	fillet with skin	35489	gillnet	3	45
98	Deschutes River	mountain whitefish	Res	whole body	35489	boat electrofish	3	45
98	Deschutes River	mountain whitefish	Res	fillet with skin	March 1997	boat electrofish	3	45
96	Clearwater River, lower	rainbow trout	Res	whole body	March 1997	boat electrofish, gillnet	3	45
96	Clearwater River, lower	rainbow trout	Res	fillet with skin	March 1997	boat electrofish, gillnet	3	45
98	Deschutes River	rainbow trout	Res	whole body	March 1997	boat electrofish	3	45
98	Deschutes River	rainbow trout	Res	fillet with skin	March 1997	boat electrofish	3	45
48	Yakima River, lower	steelhead	Anad	whole body	March 1997	dipnet (fish facility)	3	15
48	Yakima River, lower	steelhead	Anad	fillet with skin	March 1997	dipnet (fish facility)	3	15
96A	Clearwater River, lower	steelhead	Anad	whole body	March 1997	dipnet (hatchery)	3	15
96A	Clearwater River, lower	steelhead	Anad	fillet with skin	March 1997	dipnet (hatchery)	3	15
8	Columbia River, John Day Pool	sturgeon	Res	whole body	March 1997	deep water gillnet	3	3
98	Deschutes River	sucker	Res	whole body	March 1997	boat electrofish	3	30
98	Deschutes River	sucker	Res	fillet with skin	March 1997	boat electrofish	3	30
57A	Cowlitz River, upper	spring chinook	Anad	whole body	April 1997	dipnet (hatchery)	3	15
57A	Cowlitz River, upper	spring chinook	Anad	fillet with skin	April 1997	dipnet (hatchery)	3	15
18	Columbia River, at Rocky Reach	steelhead	Anad	whole body	April 1997	gillnet	3	15
18	Columbia River, at Rocky Reach	steelhead	Anad	fillet with skin	April 1997	gillnet	3	15
8	Columbia River, John Day Pool	lake whitefish	Res	whole body	May 1997	gillnet	3	45
8	Columbia River, John Day Pool	lake whitefish	Res	fillet with skin	May 1997	gillnet	3	45
8	Columbia River, John Day Pool	spring chinook	Anad	whole body	May 1997	gillnet	3	15
8	Columbia River, John Day Pool	spring chinook	Anad	fillet with skin	May 1997	gillnet	3	15
8	Columbia River, John Day Pool	spring chinook	Anad	eggs	May 1997	gillnet	3	0
21B	Willamette River, Middle Fork	spring chinook	Anad	whole body	May 1997	dipnet (hatchery)	3	15
21B	Willamette River, Middle Fork	spring chinook	Anad	fillet with skin	May 1997	dipnet (hatchery)	3	15
30	Umatilla River, lower	spring chinook	Anad	whole body	May 1997	gillnet	3	15
30	Umatilla River, lower	spring chinook	Anad	fillet with skin	May 1997	gillnet	3	15
56A	Klickitat River, lower	spring chinook	Anad	whole body	May 1997	gillnet	3	15
56A	Klickitat River, lower	spring chinook	Anad	fillet with skin	May 1997	gillnet	3	15
48	Yakima River, Prosser	spring chinook	Anad	whole body	June 1997	dipnet (fish facility)	3	15
48	Yakima River, Prosser	spring chinook	Anad	fillet with skin	June 1997	dipnet (fish facility)	3	15
9	Columbia River, Hanford	catfish	Res	whole body	July 1997	gillnet	3	30
9	Columbia River, Hanford	catfish	Res	fillet with skin	July 1997	gillnet	3	30

Table 7. Revised Sampling Design For the CRITFC Exposure Study [a]

Site No.	Location	Fish Species [b]	Res /Anad	Sample Type	Collection Period [c]	Collection Method	Repli-cates [d]	Number of Fish
9	Columbia River, Hanford	lake whitefish	Res	whole body	July 1997	gillnet	3	45
9	Columbia River, Hanford	lake whitefish	Res	fillet with skin	July 1997	gillnet	3	45
93	Snake River	rainbow trout	Res	whole body	July 1997	boat electrofish	3	45
93	Snake River	rainbow trout	Res	fillet with skin	July 1997	boat electrofish	3	45
51	Wenatchee River	spring chinook	Anad	whole body	July 1997	dipnet (hatchery)	3	15
51	Wenatchee River	spring chinook	Anad	fillet with skin	July 1997	dipnet (hatchery)	3	15
203	Palouse River	lake whitefish	Res	whole body	August 1997	gillnet	3	45
203	Palouse River	lake whitefish	Res	fillet with skin	August 1997	gillnet	3	45
24	Fifteen Mile Creek	lamprey	Anad	whole body	August 1997	dipnet	3	60
56	Klickitat River, upper	rainbow trout	Res	whole body	August 1997	backpack electrofish	3	45
56	Klickitat River, upper	rainbow trout	Res	fillet with skin	August 1997	backpack electrofish	3	45
79	South Fork Salmon River	rainbow trout	Res	whole body	August 1997	backpack electrofish	3	45
79	South Fork Salmon River	rainbow trout	Res	fillet with skin	August 1997	backpack electrofish	3	45
203	Palouse River	rainbow trout	Res	whole body	August 1997	boat electrofish	3	45
203	Palouse River	rainbow trout	Res	fillet with skin	August 1997	boat electrofish	3	45
9	Columbia River, above Snake	sturgeon	Res	fillet without skin	August 1997	deep water gillnet	3	3
203	Palouse River	sucker	Res	whole body	August 1997	boat electrofish	3	30
203	Palouse River	sucker	Res	fillet with skin	August 1997	boat electrofish	3	30
8	Columbia River, John Day Pool	walleye	Res	whole body	August 1997	gillnet	3	24
8	Columbia River, John Day Pool	walleye	Res	fillet with skin	August 1997	gillnet	3	24
8	Columbia River, John Day Pool	fall chinook	Anad	whole body	September 1997	gillnet	3	15
8	Columbia River, John Day Pool	fall chinook	Anad	fillet with skin	September 1997	gillnet	3	15
8	Columbia River, John Day Pool	fall chinook	Anad	eggs	September 1997	gillnet	3	0
56A	Klickitat River, lower	fall chinook	Anad	whole body	September 1997	gillnet	3	15
56A	Klickitat River, lower	fall chinook	Anad	fillet with skin	September 1997	gillnet	3	15
14	Columbia River, near Priest Rapids	fall chinook	Anad	whole body	October 1997	dipnet (hatchery)	3	15
14	Columbia River, near Priest Rapids	fall chinook	Anad	fillet with skin	October 1997	dipnet (hatchery)	3	15
30A	Umatilla River, lower	fall chinook	Anad	whole body	October 1997	dipnet (holding pond)	3	15
30A	Umatilla River, lower	fall chinook	Anad	fillet with skin	October 1997	dipnet (holding pond)	3	15
48	Yakima River, Prosser	fall chinook	Anad	whole body	October 1997	dipnet (fish facility)	3	15
48	Yakima River, Prosser	fall chinook	Anad	fillet with skin	October 1997	dipnet (fish facility)	3	15
8A	Columbia River, at Umatilla River	walleye	Res	whole body	July 10, 1996	boat electrofish, gillnet	1	8
8A	Columbia River, at Umatilla River	walleye	Res	fillet with skin	July 10, 1996	boat electrofish, gillnet	3	24
101	Umatilla River, upper	mountain whitefish	Res	whole body	July 11, 1996	backpack electrofish	3	27
101	Umatilla River, upper	mountain whitefish	Res	fillet with skin	July 11, 1996	backpack electrofish	3	27
101	Umatilla River, upper	rainbow trout	Res	whole body	July 11, 1996	backpack electrofish	2	40
8A	Columbia River, at Umatilla River	sucker	Res	whole body	July 11, 1996	boat electrofish, gillnet	3	36
8A	Columbia River, at Umatilla River	sucker	Res	fillet with skin	July 11, 1996	boat electrofish, gillnet	1	4
101	Umatilla River, upper	rainbow trout	Res	whole body	July 13, 1996	backpack electrofish	2	60
48	Yakima River, lower	sucker, bridge lip	Res	whole body	July 15, 1996	dipnet (fish facility)	3	21
48	Yakima River, lower	sucker, large scale	Res	whole body	July 15, 1996	dipnet (fish facility)	3	21
21	Willamette River, lower	lamprey	Anad	whole body	June 20, 1996	dipnet	3	60
49	Yakima River, upper	rainbow trout	Res	fillet with skin	September 11, 1996	boat electrofish	3	21
49	Yakima River, upper	rainbow trout	Res	whole body	September 12, 1996	boat electrofish	3	21
49	Yakima River, upper	sucker	Res	whole body	September 12, 1996	boat electrofish	3	15

Table 7. Revised Sampling Design For the CRITFC Exposure Study [a]

Site No.	Location	Fish Species [b]	Res /Anad	Sample Type	Collection Period [c]	Collection Method	Repli-cates [d]	Number of Fish
49	Yakima River, upper	sucker	Res	fillet with skin	September 12, 1996	boat electrofish	3	15
8A	Columbia River, at Umatilla River	sucker	Res	fillet with skin	September 9, 1996	boat electrofish	3	24
						Sample Total	276	2560

[a] -Table 7 has been modified to reflect what EPA field crews will attempt to complete without additional resources, equipment, and ESA collection permit from the CRITFC organization. This field sampling effort will require the EPA field crew to acquire state collection permits to complete these objectives.

[b] - Samples from all species are composites (composites samples consist of 20 lamprey each and 8 each for other fish species).

[c] - Dates reflect suggested sampling periods.

[d] - Number of samples assumes each tissue sample is performed in triplicate.

Table 9. Percentage of Adult Tribal Members Consuming Proposed Target Species and Species Collection Sites

Species	Weighted Percent That Consume the Species	Proposed Fishing Sites	
		Site Numbers	Site Locations (Rivers)
Salmon	92.4%	8, 9, 14*, 21A*, 30, 51*	Columbia, McKenzie, Umatilla, Wenatchee,
Lamprey	54.2%	21, 6	Willamette, Columbia
Trout [a]	70.2%	98, 8, 18, 30, 48, 49, 96*, 79	Deschutes, Columbia, Umatilla, Yakima, Clearwater, Salmon
Smelt	52.1%	57	Cowlitz
Whitefish	22.8%	8, 30, 96	Columbia, Umatilla, Clearwater
Sturgeon	24.8%	6, 7, 8, 9, 96	Columbia, Clearwater
Walleye	9.3%	8	Columbia
Sucker	7.7%	98	Deschutes
Squawfish	2.7%	none	none
Shad	2.6%	none	none

Source: Modified from CRITFC (1).

[a] Rainbow Trout and Steelhead.
* Hatchery Site.

Table 10. Columbia River Inter-Tribal Fish Commission Exposure Study: Adult Consumption of Fish Parts

Species	Parts											
	Fillet		Skin		Head		Eggs		Bones		Organs	
	N	Weighted % That Consume	N	Weighted % That Consume	N	Weighted % That Consume	N	Weighted % That Consume	N	Weighted % That Consume	N	Weighted % That Consume
Salmon	473	95.1%	473	55.8%	473	42.7%	473	42.8%	473	12.1%	470	3.7%
Lamprey	249	86.4%	251	89.3%	250	18.1%	250	4.6%	250	5.2%	250	3.2%
Trout	365	89.4%	365	68.5%	365	13.7%	364	8.7%	365	7.1%	362	2.3%
Smelt	209	78.8%	209	88.9%	210	37.4%	209	46.4%	210	28.4%	206	27.9%
Whitefish	125	93.8%	124	53.8%	125	15.4%	125	20.6%	125	6.0%	124	0.0%
Sturgeon	121	94.6%	121	18.2%	121	6.2%	121	11.9%	121	2.6%	121	0.3%
Walleye	46	100%	46	20.7%	46	6.2%	46	9.8%	46	2.4%	46	0.9%
Sucker	15	89.7%	15	34.1%	15	8.1%	15	11.1%	15	5.9%	15	0.0%
Squawfish	42	89.3%	42	50.0%	42	19.4%	42	30.4%	42	9.8%	42	2.1%
Shad	16	93.5%	16	15.7%	16	0.0%	16	0.0%	16	3.3%	15	0.0%

Source: CRITFC (1).

Contaminant levels in various fish parts (i.e., whole-body, fillet, and eggs) will be estimated so that this information can be used to provide guidance on how to prepare fish, or what parts should be avoided, in the event that contaminant levels exceed levels that warrant concern. In addition, the conversion factors developed from these data (e.g., whole-body-fillet and whole-body-egg ratios) may assist in the comparison of the data from this study with other historical data that exist from the Columbia River Basin. Table 7 indicates that most of the comparisons of contaminant levels in different fish body parts will occur at Site 8 in the Columbia River between the McNary and John Day dams. This site was selected because of its importance as a fishing site for all four CRITFC member tribes.

4.3 SAMPLING STRATEGY

The sampling strategy proposed for this study design is consistent with guidance provided in the document entitled: **Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume I: Fish Sampling and Analysis** (4). For all fish species except white sturgeon, three replicate composite samples will be measured from each collection site. For white sturgeon, composite samples will not be taken. Instead three individual fish will be measured from each collection site. The planned number of fish per composite will likely vary for different species: 100 individuals per composite for smelt, 20 individuals per composite for lamprey, 8 individuals per composite for resident (non-salmonid) species, and 5 individuals per composite for salmon and steelhead. U.S. EPA (4) recommends that 3 to 10 individuals should be collected for a composite sample for each target species and that the same number of individual organisms should be used to prepare all replicate composite samples for analysis of contaminants for a given target species at a given site. Several ongoing fish contaminant studies in the Columbia River Basin are compositing 8 individuals per sample, so the use of this number would simplify comparisons with other available data. Because of the small size of lamprey and smelt, a composite of 8 individuals would not provide enough tissue for all chemical analyses; therefore a nominal value of 20 individuals per composite was suggested by the Design Conference attendees for smelt and lamprey, respectively. To ensure adequate sample volume for analyses, EPA, Region 10, decided to increase the composite size for smelt to 100 fish. Design Conference attendees felt that the number of individuals per composite for salmon and steelhead should be reduced from 8 to 5 (some individuals suggested 3) because of concerns about the ability to collect sufficient numbers of fish, and because it was felt that the study should strive to minimize impacts on these fish stocks.

At the Scoping Meeting, it was recommended that if possible, all fish used in a composite be female. This recommendation was made because eggs are to be collected for some of the sampling locations and because it was thought that females have a higher lipid content (and, therefore, potentially a higher contaminant level for lipid soluble contaminants). However, recent data collected by the Lower Columbia Bistate Program suggest that, for chinook, coho, and steelhead, males have the higher lipid content. The Bistate Program measured the lipid content and contaminant levels for male and female fish for these three species. For all three species, percentages of lipids were substantially higher in male fillet as is specified in the following table.

SPECIES	PERCENT LIPIDS (MALE)	PERCENT LIPIDS (FEMALE)
Chinook	3.51%	0.72%
Coho	1.67%	0.85%
Steelhead	4.06% 4.82%	2.87%

Based upon this lipid data and upon the fact that the Native Americans eat what they collect (i.e. both males and females), the decision was made to collect random samples of fish (by sex) for each composite rather than all females. The exception to this will be at site #8. At this site, for fall and spring chinook and steelhead, the fish to be used for the fillet with skin composites will be as follows:

Composite 1 - 5 fish, all male
Composite 2 - 5 fish, all female
Composite 3 - random

This will provide information on the lipid content of males and females of these three species. Eggs that are to be collected from these species at this location will be taken from Composite Number Two, above.

Collection periods for each species have been tentatively assigned and are given in Table 7. According to U.S. EPA guidance (4), the collection period should ideally avoid the spawning period of the target species because many fish are subject to stress during spawning. However, because eggs will be collected from salmonid species and because the CRITFC tribes fish for salmonids when they are spawning, the typical spawning period for these species will be targeted. For resident species, collection periods have been proposed so that spawning periods can be avoided. For white sturgeon, the proposed collection period is consistent with seasons established in previous years.

For each target species composite, a single size class will be targeted at the site. Because the concentrations in fish for some pollutants (e.g., PCBs and mercury) have been shown to increase with age and size, an attempt will be made to collect a composite that represents the larger fish being caught at the sampling site during the sampling period. Therefore, the selection of fish for the composite will, when possible, adhere to the following two criteria:

(1) Composites will be comprised of fish that are in the upper 75% of fish length of those fish being caught by the CRITFC tribes near the sampling location, and;

(2) Composites will comply with EPA guidance (reference 4) which recommends that the smallest individual in a composite be no less than 75% of the total length of the largest individual.

Replicate composite samples for a target species should be as similar to each other as possible. Therefore, if possible, the relative difference between the average length of individuals within

any composite sample from a given site as well as the average of the average lengths of individuals in all composite samples from that site will not exceed 10 percent.

This goal may not be possible for composite samples if (1) fish populations are low and (2) endangered species considerations for salmon limit the number of fish that should be caught. In those cases where the above goal is unattainable during the time scheduled for sampling, composites will be prepared using available fish. These composites will represent all sizes of fish captured at the different sampling sites. In all cases, the total length and weight of each fish in the composite sample will be recorded.

4.4 FIELD COLLECTION METHODS

Sampling methods for finfish include: electrofishing, hand collection, hatchery collection, trapping at dams, dip netting, and gill netting. The preferred method will be dependent on the conditions at the sampling site, selected species, and legal constraints. Collection of fish by any techniques will be controlled by the stipulations of the federal, state and tribal permits. Copies of permits should be in the possession of the field sampler at all times.

The EPA FOM and his EPA alternate are qualified boat operators as defined in EPA Region 10's "Boat Operating Policy" (Attachment 2). Both will ensure that the necessary safety equipment is available for all sampling team members on all EPA boats used and that emergency information is available (e.g., local hospitals and police). Sampling team members will also be briefed on boat safety prior to launching any EPA vessel. The safety procedures that will be followed for electrofishing are provided in Attachment 3. At some locations, boats and equipment owned by the CRITFC tribes may be used for sampling.

4.4.1 Electrofishing

Electrofishing is considered to be the most efficient method for collecting a variety of species in large rivers because it is easily standardized and less selective than alternative gear. However, electrofishing is generally not effective in capturing fish that are at depths greater than about 10 feet, therefore alternative methods, such as gillnetting, will need to be used for some species. In this project the boat mounted electro shocker will be used in the deeper rivers. Some of the smaller rivers selected (i.e., Deschutes, Umatilla, South Fork of the Salmon) may not be deep enough to use the boat for electrofishing. In these smaller rivers, sampling will be done using electro backpack shocking equipment allowing for the selection of the fish species of interest. It is anticipated that steelhead, rainbow trout, whitefish, and sucker from selected locations (as shown in Table 7) will be captured by electrofishing. Only fish that appear to be in the desired target size range (see below) will be brought aboard using a dip net. The fish that are not netted will be allowed to recover from the electroshocking pulse by shutting off electrofishing equipment until fish swim away from the boat.

4.4.2 Gillnetting

Gill nets capture fish by entanglement. They are particularly well-suited for the capture of highly mobile fish (e.g., salmonids) which are not easily captured by electrofishing. For this

project, sinking gill nets (approximately 100 ft long by 6 ft or 12 ft deep) will be used, each of which consists of variable mesh (2 to 6 inch diameter) monofilament line attached to cork and lead lines. The nets will be anchored with lead mushroom weights and marked with the appropriate information identifying who the nets belong to and how they are being used (i.e., research). Flashing lights should be attached to either end to help mark net deployment areas.

Gillnets will be deployed and monitored during the fishing efforts for both day and night operations. After several hours the nets will be retrieved and the captured fish collected. All non-target fish species and all targeted fish species that are not within the desired size category will be returned to the water, whether dead or alive. A record will be kept of the catch of each gillnet set. It is anticipated that chinook, sturgeon, steelhead, whitefish, walleye, and rainbow trout from selected sampling sites will be captured by gillnetting (see Table 7).

4.4.3 Trap/Dam

At the barrier dam on the Umatilla River, fish have no access through the dam and are trapped behind weirs. Samples of selected fish species (e.g., steelhead and salmon) may be taken from these weirs using dipnets.

4.4.4 Dipnet

Dipnets may be used in areas where the migrating fish, such as smelt, steelhead, salmon and lamprey, are following the shoreline of the river. Dipnets are usually made with small cotton mesh (e.g. ½" to 3") and used to dip up fish in small confined areas such as shallow pools or water falls. The sampling nets will need to be monitored at all times to be most effective. Once a fish is caught, the dip net will be pulled to the surface and the fish removed. Only the fish selected for the project will be retained and other species will be released.

4.4.5 Hatchery

Specific fish returning to the hatchery can be targeted for collection and retrieved from the holding pond. This sampling effort will be coordinated with the hatchery management personnel so that the fish can be taken from the holding pond area before their eggs and sperm are removed and before any type of chemical treatment has been applied.

4.4.6 Hand Collection

The hand collection method of sampling will be used in and around the Willamette Falls for lamprey. As the lamprey migrate over the falls area, they can be collected off the rocks and from shallow pools with small nets or by hand.

4.5 FISH SAMPLE HANDLING IN THE FIELD

4.5.1 Sample Integrity

The EPA FOM or his EPA alternate will be present at all times when fish are collected in order to assure sample integrity.

Sample integrity requires that fish be handled in a manner that prevents loss of contaminants already in the fish and prevents extraneous tissue contamination. Loss of contaminants already in the fish tissue will be prevented in the field by ensuring that the skin on fish specimens has not been lacerated by the sampling gear. Sources of extraneous tissue contamination include contamination from dirty hands, sampling gear, greasy cables, spilled engine fuel, engine exhaust, dust, ice chests and ice used for cooling.

The FOM will identify all potential sources of contamination in the field and take appropriate steps to minimize or eliminate them. The FOM will observe the following practices (and others as indicated by professional judgement) as well as provide training to tribal members who are assisting in fish collection in these practices: (1) Caught fish will only be placed on clean surfaces, such as aluminum foil. (2) Ice chests will be cleaned prior to any sampling activities. (3) Samples will be placed in waterproof plastic bags to avoid contamination from melting ice. (4) Sampling equipment, such as gillnets and dipnets, will be free from contaminants such as oils, grease and fuels. (5) All utensils or equipment used directly in handling fish (e.g., such as fish hooks, measuring boards and fish clubs) will be cleaned in the laboratory prior to each field sampling effort and placed in aluminum foil. (6) The field collection team will clean this equipment between sampling sites by rinsing with ambient water and rewrapping in aluminum foil.

4.5.2 Handling Of Field Samples During Collection

Upon retrieval from the sampling equipment, each fish will be identified by species by personnel familiar with the taxonomy of
the fish in the Columbia River Basin. The FOM will assure that a taxonomic key is readily available at all times. Once a target species is caught, the length of the fish will be measured to ensure that it meets the target size class as defined in Section 4.3. Based upon size of fish caught in the field, the acceptable size range of fish will be determined by the FOM and documented using a Sample Alteration Form (see Attachment 17). Those fish that do not meet the target size class will be released unharmed. The fish that do meet the target size class will be subdued by a sharp blow or blows to the base of skull. All individual fish (with the exception of lamprey and smelt) that are kept will be assigned a unique identification number (EPA Sample #) consisting of an numeric eight digit code XXXXXXXX. The fish will then be assigned to one of the three composite samples for that location which will also have a unique identification number. These numbers will be chosen to be consistent with EPA Region 10's sample management tracking system. Selected specimens will be photographed. For lamprey and smelt, each fish will be placed into one of three composite groups (approximately 20 per composite for lamprey and 100 per composite for smelt) and each composite group assigned an identification number.

The FOM will wrap each whole fish (with the exception of lamprey and smelt) in clean heavy-duty aluminum foil. The whole fish will then be placed into a plastic bag and the bag will be tied.

For lamprey and smelt, the composite group will be wrapped in aluminum foil and tied in a plastic bag. The FOM will immediately pack the bagged fish sample on ice (preferably dry ice) in clean ice chests to start cooling the fish down.

4.5.3 Documentation During Fish Collection

The FOM will be personally responsible for the care and custody of the fish samples until they are properly transferred or dispatched to the storage and/or filleting facility or to the subcontract laboratory. He will also determine whether custody procedures are followed properly during the field work and will decide if additional samples are required.

Documentation for fish collection consists of information that must be provided: (1) on the Field Record Form; (2) in the Sampler's Notebook; (3) on the Sample Identification/Chain of Custody Tag, and (4) on the Chain-of-Custody Form.

Field Record Form - EPA has developed a standard Field Record Form (attachment 4) that will be filled out by EPA at each sampling location. The information listed below will be included on this Field Record Form:

Geographic location (latitude and longitude) using Global Positioning System
Species name
Date and time
Method of collection (e.g., gill net, trap, electrofish, etc.)
Station number
Sample identification number / numbers
Composite sample number
Weather conditions (e.g., cloud cover, rain or shine, windy)
Water depth of capture (feet)
Sex of species
Evidence of hatchery markings (e.g.., fin clips, tags)(under "Comments")
Total fish length (in metric units)
Total fish weight (in metric units to the nearest gram)
Sampling crew names
Type of vessel
External marks or gross physiological abnormalities noted (under "Comments")

Sampler's Notebook - The sampler's notebook will include the same information that is on the Field Record Form. In addition, the Sampler's Notebook will be used to document any unusual activities or problems encountered in the field that would be useful for the Project Leader and Manager to be aware of when data quality is being evaluated. It will also include a record of any photographs taken in the field.

Sample Identification/Chain of Custody Tag - A waterproof Sample Identification/Chain of Custody Tag (SI/COC Tag) (Attachment 5) should be completed in indelible ink for each individual fish (or composite for lamprey and smelt) and taped to each aluminum-foil-wrapped specimen(s) before placing the specimen(s) in a plastic bag in the field. This tag will include the

following information: the project name/code, station location/number, sampling date and time, species name, fish sample and/or composite number, sample length and weight, and the name, phone number, and signature of the sampler.

If a fish sample tag is lost during shipment or a tag is never created, the FOM will write a statement detailing how the sample was collected, stored, and transferred to the laboratory. The statement will include all pertinent information, such as entries in field logbooks regarding the sample, whether the sample was in the sample collector's physical possession or in a locked compartment until hand transported to the laboratory, etc.

Chain-of Custody Form - A Region 10 Chain-of-Custody Form (COC Form) (Attachment 6) will be completed in indelible ink for each shipment that is made. These COC forms will be enclosed in plastic and taped to the inside lid of the cooler. The information on this form will be used to track all samples from field collection to receipt at the subcontract laboratory.

5.0 SAMPLE STORAGE

5.1 STORAGE PROCEDURES

Once fish are caught, the FOM will immediately pack the bagged fish samples in ice (preferably dry ice) to start cooling the fish down. If fish are to be filleted the same day they are caught, they will not be frozen. Fish that will not be filleted the day they are caught or whole fish samples that are not shipped to the subcontract laboratory the day they are caught will be transported to the EPA Laboratory or to another prearranged locations (e.g. local fish hatcheries) having freezer space available. This freezer must have a temperature less than or equal to $\leq$-20 ^{o}C and must be secured. Fish will be completely frozen before any shipping occurs.

5.2 DOCUMENTATION

The COC Forms and SI/COC Tags described in section 4.5.3 will remain with the stored samples until samples are removed for filleting and/or shipping. In addition, the FOM will include information in the Sampler's Notebook on:

- Sample storage location and contact person
- Compliance of storage location with EPA Chain of Custody procedures if storage location is not the Region 10 EPA laboratory
- Freezer temperature

6.0 FILLETING OF FISH

The FOM or EPA Region 10 staff (with oversight by the FOM) will fillet selected fish samples. The samples to be filleted are identified in Table 7. Filleting will be done at the EPA Laboratory or at a field laboratory that allows for the appropriate quality control procedures to be followed (e.g., a fish hatchery or EPA's mobile trailer.)

6.1 FILLETING PROCEDURES

Fish will be handled following the guidance provided in sections 7.2.1 (General Considerations) and 7.2.1.3 (Samples for Both Organics and Metals Analyses) in Reference 4 (see Attachment 7 for a copy of these sections of Reference 4). Fish will be partially thawed prior to filleting. If rupture of organs is noted for an individual fish, the specimen will be eliminated from the composite sample. For scaling and filleting, the methods described in sections 7.2.2.6 and 7.2.2.7 and illustrated in Figure 7-3 of Reference 4 will be followed (see Attachment 8 for a copy of these sections and the figure in Reference 4). Labeling of fish filleted for compositing will be done as described in the next paragraph.

The FOM will create composites of fillets (with and without skin) using the fillet from the right side of each fish (the "F1s")(right side to be determined from the perspective of the direction in which the fish would swim). This composite will be wrapped in clean aluminum foil and placed in a plastic bag. The FOM will wrap the left side fillet from each fish separately in heavy duty aluminum foil and add the two digit identifier "F2" to the end of the sample number for this fillet. The individual fillets (the "F2"s) that will not be ground and composited will be placed in individual plastic bags with the composite identification number, the individual identification numbers, and the date of resection. The FOM will arrange for shipment of the F2s to the EPA Region 10 laboratory for storage.

6.2 DOCUMENTATION PROCEDURES DURING FILLETING

Documentation for fish filleting consists of information that must be provided: (1) on a Sample Processing Record; (2) in the Filleter's Notebook; and (3) on the Sample Identification/Chain of Custody Tag.

6.2.1 Sample Processing Record

Sample processing records will be kept for each individual sample (sturgeon) or composite of whole fish, fillets and eggs. The record (Attachment 9) will include the following information:

- Information on sample type and species name
- Unique sample number for individual fish and/or fish (egg) composite number (identical to that number assigned during sampling)
- Weights of unprocessed individual fillets and egg skeins

(Additional information to be added after sample homogenation is included on these forms - this will be completed by the subcontract laboratory)

6.2.2 Filleter's Notebook

The filleter (the FOM or Region 10 designee) will record the information described above for the Sample Processing Record in a Filleter's Notebook. Additional information to be recorded in the Filleter's Notebook is as follows:

- Evidence of hatchery markings on fish (e.g., fin clips) in addition to those noted in the field
- Incidence of external abnormalities (e.g., fin erosion, skin ulcers, skeletal anomalies, tumors) in addition to those noted during field sampling
- Incidence of internal abnormalities if any
- Record of scales and/or pectoral fins collected.

Scales for age determination will be collected for all fish, except for smelt, lamprey, and sturgeon. Lamprey do not have scales and smelt are too small to obtain scales. For sturgeon, one pectoral fin will be removed by EPA prior to filleting and this fin will be shipped to Oregon Department of Fish and Wildlife for ageing. Otoliths may be also taken for selected fish to verify aging done using scales. Scales (and otoliths if collected) will be placed in small jars and preserved with ethanol. Pectoral fins will placed in plastic bags and frozen until ageing. Each scale or fin sample taken will be given a matching EPA sample number.

6.2.3 Sample Identification/Chain-of-Custody Tag

After filleting, SI/COC Tags (containing the information described in section 4.5.3) will be attached to the aluminum foil on individual fish fillet (sturgeon and the individual "F2s" from fish forming a composite) and on the combined composite fillets (the combined "F1s"). These will then be placed in plastic bags.

7.0 SHIPMENT OF SAMPLES AND RECEIPT BY SUBCONTRACT LABORATORY

In preparation for shipping, the FOM will pack whole fish, fillets, and egg samples securely inside a cooler with dry ice. The cooler will be closed, fiber tape will be wrapped completely around it, and a custody seal (shown in Attachment 10) will be attached so that it must be broken when the cooler is opened. All fish samples will be packaged and shipped to the subcontract laboratory (for further processing) or to the Region 10 EPA Laboratory (for storage of the "F2" fillets and of pectoral fins and scales) via overnight delivery using Federal Express.

As identified in "Dangerous Goods Regulations" (36th Edition, January, 1995, International Air Transport Association), the FOM will assure that appropriate dry ice labels (shown in Attachment 10) will be affixed to each shipping container. These same procedures must be followed by the subcontract laboratory when sending processed samples to the EPA laboratory for analysis and archiving (see Section 8.6).

The EPA FOM will notify the Tetra Tech contact person when samples will be shipped. The contact person will be given sample ID numbers, number of ice chests being sent and species of fish being sent in each mailing. Upon arrival at the laboratory, fish tissue may be distributed immediately to a technician for processing. If they are not processed immediately, they must be stored in a freezer at $\leq$-20^{o} C until they are removed for processing.

7.1 DOCUMENTATION REQUIREMENTS

The original Region 10 Chain-of-Custody Forms will be signed by the FOM and enclosed in plastic and taped to the inside lid of one cooler of each group of coolers shipped at one time. A custody seal will be attached to each cooler so that it must be broken when the cooler is opened. The Sample Processing Records and the SI/COC Tags for each sample will be shipped at the same time. In addition, one photocopy of all of the paperwork sent to the subcontract laboratory will be sent to the Tetra Tech contact person via Federal Express or FAX and one copy will be retained by the FOM.

Upon receipt by the subcontract laboratory, the return delivery receipts and chain-of-custody procedures listed in Attachment 11 should be followed. The return delivery receipt will be sent to Tetra Tech. A copy of the Chain-of-Custody Form for each shipment will be delivered by the subcontract laboratory to EPA (the WAM) within 7 calendar days of receipt of each shipment of samples.

In addition to the written record required by Attachment 11, the subcontract laboratory will contact the FOM after they have received the samples to let the FOM know if sample integrity was maintained during shipment. The following information will be communicated to the FOM by telephone or FAX within 24 hours after samples are received: (1) condition of the samples upon arrival at the laboratory (e.g. to ensure sample degradation has not occurred during shipment); (2) time delays (e.g., not arriving the next day); (3) condition of chain-of-custody

seals. A project file including a copy of all Chain of Custody forms, field notebooks, *etc.*, will be maintained by the Project Manager at the EPA Region 10 Seattle office.

8.0 HOMOGENIZATION OF INDIVIDUAL FISH AND COMPOSITES AND DISTRIBUTION OF HOMOGENIZED SAMPLES

Upon receipt of fish and egg samples from the FOM, the subcontract laboratory will homogenize the samples, prepare sample aliquots, and distribute these aliquots to the appropriate analytical laboratories for analyses.

8.1 GENERAL CONSIDERATIONS FOR HANDLING SAMPLES

Fish samples and homogenized samples will be handled following the guidance provided in sections 7.2.1 (General Considerations) and 7.2.1.3 (Samples for Both Organics and Metals Analyses) of Reference 4 (see Attachment 7 of this QAPP for a copy of these sections of Reference 4). An additional requirement is that the Hobart grinder specified below must be completely taken apart and the auger, auger housing, orifice plate, and any implement used to push tissue through the grinder be cleaned after each sample (individual fish or fish/egg composite) has been homogenized.

8.2 GENERAL CONSIDERATIONS FOR PREPARING COMPOSITES

Composite samples may be prepared using two different methods. In the first method (the "individual" method), each individual fish or fish fillet that is to be part of a composite is homogenized separately. Equal weights of each individual fish homogenate are then compiled into a composite and homogenized again. The individual method is designed to provide information on the mean concentration of contamination in fish tissue for the fish population that is being sampled. In the second method (the "batch method"), all of the fillets or whole fish that are to be part of a composite are homogenized together. The batch method provides information on the weighted mean of the concentration in the batch sampled.

For this project, composites will be homogenized by the subcontract laboratory using the batch method. Information on the fish consumption habits of tribal members suggest that once fish are caught, the entire fish is consumed. Therefore, the information on contaminant levels provided by the batch method (which includes information from the entire fillet of each fish in a composite) will provide a more appropriate estimate of exposure for the Native Americans. It is expected that since every attempt will be made to ensure that fish that make up a composite sample will be similar in size (i.e., the smallest individual will be no less than 75% of the total length of the largest individual), the mean concentrations generated by the batch method will likely be similar to that generated using the individual method.

The batch method is also easier to implement in the laboratory because it saves sample preparation time and resources and maximizes the amount of tissue available after grinding smaller fish. This is because tissue from smaller fish often remain inside the grinder due to the small volume of sample going through the grinder.

8.3 SAMPLE HOMOGENIZATION

Whole fish, fish fillets, and eggs should be ground and homogenized using a Hobart Model 84186 commercial meat grinder. If possible, the blades of the Hobart should be made of titanium or tantalum rather than stainless steel since stainless steel blades have been found to be a potential source of nickel and chromium contamination (due to abrasion at high speeds) and should be avoided. While an orifice size of 3/16th inch is recommended, orifice sizes up to 1/4th inch could be used.

Grinding of tissue is easier when it is partially frozen. Chilling the grinder briefly with a few chips of dry ice may also keep the tissue from sticking to it.

For larger fish samples, the fish tissue should first be cut into small pieces no larger than 2.5 cm cubes and then the fish tissue cubes from all of the fish that make up one composite sample should be combined and ground in the equipment specified. After the first grinding, the ground material should be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed together. **At a minimum, each composite sample should be run through the grinder and hand mixed three times**. If chunks of tissue are present at this point, the grinding and homogenization should be repeated until the composite sample appears to be homogenous. No chunks of tissue or pieces of skin should remain because these may not be extracted or digested efficiently.

Egg samples sent to the subcontract laboratory by EPA should be ground using the procedures given in the paragraphs above for fish samples **(i.e., ground a minimum of three times)**.

The subcontract laboratory will prepare an adequate amount of homogenate to meet the requirements for analysis as specified
in Table 1 (column titles "Containers").

8.4 SAMPLE DISTRIBUTION

The subcontract laboratory must prepare sample aliquots (as described in section 8.5) immediately after homogenization is completed and then distribute these sample aliquots to the appropriate laboratory for chemical analyses. Unless aliquots are to be measured immediately, they must be frozen and stored in a secure location at $\leq$-20^{o} C until transfer to the EPA laboratory for analysis or until analyses are begun by the subcontract laboratory. If adequate homogenate is available, approximately 400 grams of the unused portion of each homogenate (i.e., that not put into sample aliquot jars) should be placed into each of two (2) wide mouth glass 16 ounce jars and stored at $\leq$-20^{o} C by the subcontract laboratory for 30 calendar days. This will ensure that adequate sample homogenate is available for EPA analysis and archiving in case the aliquots sent to the EPA laboratory are lost or damaged during shipment. The shipping directions found in Section 7.0 (i.e., overnight shipping on dry ice) should be followed. Glass jars should be securely packed to avoid breakage during shipment.

8.5 SAMPLE CONTAINERS AND LABELS

The laboratory will place approximately fifty (50) grams of homogenized sample into each of 26 properly cleaned wide mouth
2 ounce glass sample jars. The laboratory must leave sufficient headspace in each jar such that expansion during freezing does not cause the jar to break. As shown on Table 1, a total of 4 jars (2 for PCDDs/PCDFs analyses and 2 for dioxin-like PCB analyses) will be retained by the subcontract laboratory for analyses of PCDDs/PCDFs and toxic, dioxin-like, PCBs. The laboratory will send the 22 remaining jars to Region 10 EPA's laboratory.

EPA will use twelve (12) of these jars for the analyses of pesticides/PCBs, semivolatiles, PAHs, Target Analyte List (TAL) inorganics, mercury and arsenic. The sample jar distribution for measurements by the EPA Manchester Laboratory will be as follows: 2 for pest/PCB (100 grams), 2 for PAHs and semi-volatiles (100 grams), 2 for TAL inorganics, arsenic, and mercury (100 grams), and 16 for archive (800 grams).

If resources become available, some of the archived material will be used for analysis of selected radionuclides.

The laboratory must affix bottle labels firmly to each sample container and lid. The laboratory must keep these bottles and lids dry and empty before labeling so that the gummed label can be securely attached to the side of the container and the tape stuck to the lid. Each container label and lid tape should be filled out with the appropriate sampling information. The following list identifies the information that must be written on each container and lid label:

LID LABEL
* EPA Composite Sample Number: (8 digit code) * Sample Processing Date: MM/DD/YY

BOTTLE LABEL
* EPA Composite Sample Number: (8 digit code) * Station Location: * Sample Processing Date: MM/DD/YY * Laboratory Samplers Initials: * Type of Sample: 1. Whole body, 2. Fillet with skin 3. Fillet without skin, 4. Eggs

Each sample container should be labeled before filling the bottles with tissue.

To ensure that the bottle labels are attached firmly and will not come off after the bottles are filled with tissue and frozen, the laboratory must wrap an extra layer of clear strapping tape around the bottle completely sticking the tape to itself. As mentioned before, the bottles should not be filled to the rim in order to leave room for some expansion of the tissue when freezing.

8.6 DOCUMENTATION FOR SAMPLE HOMOGENIZATION, ALIQUOT PREPARATION, AND DISTRIBUTION OF ALIQUOTS

8.6.1 Homogenization

The relevant portions of the Sample Processing Record discussed in Section 6.2 and included in Attachment 9 will be completed by the personnel at the subcontract laboratory responsible for homogenization. Each record should be signed and dated upon completion. Copies of this Record will be forwarded to the EPA Work Assignment Manager within 7 calendar days after each batch of samples has been prepared for analyses. In addition, the laboratory will prepare a narrated video tape showing the procedures and equipment used during each stage of the initial sample processing, including all steps in grinding, mixing and homogenizing, and in cleaning of

all equipment. A copy of this video will be sent to the EPA Project Manager after the first batch of samples has been prepared for analysis.

8.6.2 Preparation of Sample Aliquots

The laboratory must maintain accurate records when samples aliquots are prepared for analysis. The Sample Aliquot Record (included as Attachment 12) must be completed by the subcontract laboratory. The Composite Sample ID used on the Sample Aliquot Form should be the one assigned by EPA on the Sample Processing Record. This Sample Aliquot Record should be used to record the total composite homogenate weight for each composite sample and the total number of bottles filled. This record should be signed and dated.

8.6.3 Sample Aliquot Transfer

Laboratory personnel at the analytical laboratories (subcontract laboratory and EPA lab) will be responsible for the care and custody of sample aliquots from the time they are received until the samples are depleted or disposed of. Well documented chain-of-custody procedures must be in place at the laboratory and should include a COC form which must be signed and the date and time noted each time the samples change hands. For sample aliquots being measured by the subcontract laboratory, COC records must be available for review by EPA. For sample aliquots sent to the EPA Laboratory for analysis or archiving, the original field COC Form(s) corresponding to the samples being sent should be signed, enclosed in plastic, and taped to the inside lid of the cooler in which the samples are sent and COC seals applied to the shipping container. All samples/sample aliquots will be shipped on dry ice using the procedures written in Section 7.0. The subcontract laboratory must coordinate with the FOM or other designated staff at the EPA laboratory when samples are to be sent to EPA. **Samples should be sent to the EPA laboratory Monday through Thursday only since the lab is not open on weekends.**

Upon receipt by the EPA laboratory, the sample receipt and chain-of-custody procedures listed in Attachment 11 of this QAPP should be followed.

In addition to the documentation (sample tracking record) required by Attachment 11, the FOM will communicate with the EPA laboratory after they have received the samples to ensure that sample integrity was maintained during shipping. The following information will be communicated to the FOM by telephone or FAX within 24 hours after samples are received: (1) condition of the samples upon arrival at the laboratory (e.g. to ensure sample degradation has not occurred during shipment); (2) time delays (e.g., not arriving the next day); (3) condition of chain-of-custody seals.

The unused portion of the sample will be retained by the analytical laboratories until data validation is completed for the analyses and the Project Manager determines that the DQOs for the samples held at the laboratories have been met without additional analyses. Once the Project Manager has made that determination, the laboratories may dispose of the archived material.

9.0 LABORATORY ANALYSES

As previously discussed, the analysis of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo-p-furans isomers (PCDDs/PCDFs), percent lipids, and toxic, dioxin-like, PCB congeners will be conducted by a laboratory which is subcontracted by the primary Contractor, Tetra Tech. Pat Cirone will be the Work Assignment Manager (WAM) for this part of the project. The remaining analyses will be measured by the EPA Region 10 laboratory at Manchester, WA.

Laboratory analytical protocols specified for this project are referenced in Table 1 and in the specifications below. Each analytical laboratory which measures project samples will group analytical reports into Sample Delivery Groups (SDGs) as designated by the FOM. SDGs will usually be groups of samples of 20 or less samples. The FOM will designate SDG sizes of 20 samples whenever field conditions permit such a size designation.

Each analytical laboratory which measures project samples will use the following procedure prior to removing a ground sample from a sample bottle for analysis of target compounds:

- Place sample container containing ground fish tissue/eggs in a 34^{o}F to 40^{o}F refrigerator 24 hours prior to removing sample.

- Remove sample bottle from the refrigerator and place on the lab bench at room temperature until all ice crystals in the sample bottle have melted.

- Hand stir the thawed tissue vigorously with a 1/4 inch solid glass rod for 1 minute.

- Immediately remove sample containing tissue and liquid from sample bottle for weighing and laboratory analysis.

- Fill out a Corrective Action Form (see Attachment 18) if any sample bottles contain either chunks of fish tissue or pieces of fish skin. A copy of this Corrective Action Form must be sent to the Project Manager and the Project QA Manager.

9.1 TARGET ANALYTES

For the measurement of PCDDs/PCDFs, target isomers are listed in EPA Method 1613B and in Table 2. Table 3 lists the PCB toxic, dioxin-like, congeners which will be measured using Method 1668.

The EPA Region 10 Laboratory will measure the classes of organics and inorganics listed in Table 1. Target compounds for each class of compounds are listed in Tables 4, 5, 6, 11, and 12. For this project which requires the measurement of pesticide and semi-volatile (SV) organics in fish tissue, it has been difficult to specify the list of target compounds in Tables 4 and 5, because some project samples such as Pacific Lamprey are expected to be composed of 25% by wet weight of lipid compounds. These naturally occurring lipids and fatty acids must be removed from all sample extracts before organic target compounds can be measured. Extract cleanup procedures such as the use of Florisil and silica gel are expected to remove some target compounds listed in Tables 4 and 5. Project quality control measurements for the recovery of laboratory matrix spiked target compounds will provide critical information on the loss of target compounds due to the required use of lipid removing cleanup procedures.

9.2 ANALYTICAL METHODOLOGY

9.2.1 PCDDs/PCDFs

Tetra Tech will be responsible for subcontracting to an analytical laboratory which will be responsible for analysis of PCDDs/PCDFs isomers and toxic, dioxin-like, PCBs according to specifications stated in this QAPP and the following document which is included as Attachment 13:

> EPA Region 10 Statement of Work (Revision 2.1, 6/6/96) For the Measurement of 17 Polychlorinated Dibenzo-p-dioxins and Polychlorinated Dibenzo-p-furans (PCDDs/PCDFs) In Fish Tissue By High Resolution GC/High Resolution Mass Spectrometry Using Method 1613B.

The above Statement of Work (SOW) provides QAPP specifications for the subcontract laboratory in order to permit the measurement of PCDDs/PCDFs in the presence of expected chlorinated chemical interferences, and to provide documented data which will permit EPA to validated PCDD/PCDF data according to the following data validation guidelines (included as Attachment 14):

> EPA Region 10 SOP For the Validation of Polychlorinated Dibenzofuran (PCDD) and Polychlorinated Dibenzofuran (PCDF) Data, Revision 1.4, December 7, 1995.

Tetra Tech shall be responsible for determining if the subcontract laboratory has a QA Program which will support the QA and technical requirements of the QAPP and the analytical

Table 11. Chlorinated Pesticide/Aroclor Target Compound List

Target Compound	CAS Number	Risk Level [1] ug/Kg	Quantitation Limit ug/Kg [2]
4,4'-DDE	72-55-9	1.1	3.3
4,4'-DDT	50-29-3	1.1	3.3
4,4'-DDD	72-54-8	1.5	3.3
Aldrin	309-00-2	0.021	17
alpha-BHC	319-84-6	0.057	1.7
alpha-Chlordane	5103-71-9	0.28	1.7
beta-BHC	319-85-7	0.20	1.7
delta-BHC	319-86-8	NC	1.7
Dieldrin	60-57-1	0.023	33
Endosulfan I	959-98-8	0.90	17
Endosulfan sulfate	1031-07-8	NC	33
Endosulfan II	33213-65-9	0.90	33
Endrin	72-20-8	5.4	33
Endrin aldehyde	7421-36-3	NC	33
Endrin ketone	53494-70-5	NC	33
gamma-Chlordane	5103-74-2	0.28	1.7
gamma-BHC(Lindane)	58-89-9	0.28	1.7
Heptachlor epoxide	1024-57-3	0.040	1.7
Heptachlor	76-44-8	0.080	1.7
Hexachlorobenzene	118-74-1	0.23	1.7
Methoxychlor	72-43-5	89.5	170
Pentachloroanisole	1825214	NC	1.7
Toxaphene	8001-35-2	0.33	170.0
Aroclor-1016	12674-11-2	0.047	33.0
Aroclor-1221	11104-28-2	0.047	67.0
Aroclor-1232	11141-16-5	0.047	33.0
Aroclor-1242	53469-21-9	0.047	33.0
Aroclor-1248	12672-29-6	0.047	33.0
Aroclor-1254	11097-69-1	0.047	33.0
Aroclor-1260	11096-82-5	0.047	33.0

Table 12. AED/Pesticide Target Compound List	
Target Compound[1]	**CAS Number**
Abate (Temephos)[3]	3383-96-8
Alachlor	15972-60-8
Ametryn	834-12-8
Atraton	1610-17-9
Atrazine	1912-24-9
Azinphos Ethyl (Ethyl guhion)	642-71-9
Azinphos methyl (Guthion)	86-50-0
Benfluralin	1861-40-1
Bromacil	314-40-9
Butachlor	23184-66-9
Butylate	2008-41-5
Captafol [3]	2425-06-1
Carbophenothion	786-19-6
Carboxin [3]	5234-68-5
Chlorpropham	101-21-3
Chlorpyrifos	5598-13-0
Chlorthalonil (Daconil)	1897-45-6
Coumaphos	56-72-4
Cyanazine [3]	21725-46-2
Cycloate	1134-23-2
DCPA (Dacthal)	2136-79-0
DEF (Butifos)	78-48-8
Diallate	2303-16-4
Diazinon	333-41-5
Dichlobenil (Casoron)	1194-65-6
Dichlorvos (DDVP)	62-73-7
Dimethoate [3]	60-51-5
Dioxathion [3]	78-34-2
Diphenamid	957-51-7
Disulfoton (Disyston)	298-04-4

Table 12. AED/Pesticide Target Compound List	
Target Compound[1]	**CAS Number**
EPN	2104-64-5
Eptam (EPTC)	759-94-4
Ethalfluralin (Sonalan)	55283-68-6
Ethion	563-12-2
Ethoprop	13194-48-4
Fenamiphos	22224-92-6
Fenarimol	60168-88-9
Fenitrothion	122-14-5
Fensulfothion	115-90-2
Fenthion	55-38-9
Fluridone [3]	59756-60-4
Fonofos	944-22-9
Gardona (Tetrachlovinphos)	961-11-5
Imidan (Phosmet)	732-11-6
Malathion	121-75-5
Merphos	150-50-5
Metalaxyl	57837-19-1
Methyl chlorpyrifos	5598-13-0
Methyl parathion	298-00-0
Metolachlor	51218-45-2
Metribuzin	21087-64-9
Mevinphos	7786-34-7
MGK-264	113-48-4
Mirex	2385-85-5
Molinate	2212-67-1
Napropamide	15299-99-7
Norflurazon [3]	27314-13-2
Oxyfluorfen	42874-03-3
Parathion	56-38-2
Pebulate	1114-71-2
Pendimethalin	40487-42-1

Table 12. AED/Pesticide Target Compound List	
Target Compound[1]	**CAS Number**
Phorate	298-02-2
Phosphamidan [3]	297-99-4
Profluralin	26399-36-0
Prometon (Pramitol 5p)	1610-18-0
Prometryn	7287-19-6
Pronamide (Kerb)	23950-58-5
Propachlor (Ramrod)	1918-16-7
Propargite (S-181)	2312-35-8
Propazine	139-40-2
Propetamidophos	31218-83-4
Ronnel	299-84-3
Simazine	122-34-9
Sulfotepp	3689-24-5
Sulprofos (Bolstar)	35400-43-2
Tebuthiuron	34014-18-1
Terbacil	5902-51-2
Terbutryn (Igran)	886-50-0
Triademefon	43121-43-3
Triallate	2303-17-5
Trifluralin (Treflan)	1582-09-8
Vernolate [3]	1929-77-7

[1] -- Some compounds in this target compound list are expected to be lost during extract clean-up procedures.
[2] -- Quantitation limits are for fish tissue on a wet weight basis.
[3] -- It is uncertain if this target compound will survive clean-up procedures.

Statement of Work, above. In order for Tetra Tech to determine if the subcontract laboratory has an adequate QA Program, Tetra Tech shall review and comment upon the following documents from each subcontract laboratory source which submits a bid proposal for this Task:

1. Results of the measurement of EPA Water Supply performance evaluation (PE) samples over the past 2 years for the measurement of 2,3,7,8-TCDD.

2. Laboratory Quality Assurance Plan.

3. Standard Operating Procedures (SOPs) for the measurement of fish tissue samples using Method 1613B and for the measurement of fish tissue samples which meet the data quality objectives which are specified in the QAPP and the Laboratory SOW for the project. These SOPs must document the procedure that the laboratory will use to obtain an initial calibration of 2,3,7,8-TCDD and 2,3,7,8-TCDF between 0.1 ng/ml and 200 ng/ml.

The above three types of documents will be reviewed by Tetra Tech to determine if the subcontract laboratory(s) has a comprehensive QA program and the facilities, staff, and experience to meet the QA requirements and Data Quality Objectives of the QAPP.

The quantitation limits specified in Table 2 for the measurement of 2,3,7,8-TCDD and 2,3,7,8-TCDF require that the subcontract laboratory achieve a Minimum (Quantitation) Limit (ML) of 0.2 ng/Kg (wet weight) for isomers 2,3,7,8-TCDD and 2,3,7,8-TCDF. This lower ML shall be achieved by the use of a low initial calibration point of 0.1 ng/ml and an ultra-low sensitivity HRMS system.

Tetra Tech will provide a data package which addresses all the data assessment requirements of Method 1613B and the EPA data validation SOP.

9.2.2 Toxic, Dioxin-Like, PCBs

Tetra Tech (Contractor) will be responsible for subcontracting to an analytical laboratory which will be responsible for analyses of toxic, dioxin-like, PCB congeners listed in Table 3. These analyses shall be done according to the specifications stated in this QAPP and the following document (included as Attachment 15):

Draft Method 1668 For the Measurement of Toxic PCB Congeners By Isotope Dilution HRGC/HRMS, October 4, 1995 Draft Revision.

All the required standards and isotopes to measure samples using Method 1668 are currently commercially available. Similar to the measurement of PCDDs and PCDFs, the toxic, dioxin-like, PCBs will be validated by EPA Region 10 according to a validation guidelines (SOP) developed by Region 10 (see Attachment 16).

9.2.3 Pesticides/Aroclors

The Region 10 Laboratory will measure chlorinated pesticides/PCB mixtures (as Aroclors), other pesticides including nitrogen and organo-phosphorous pesticides by AED (Method 8085), neutral SVs, chlorinated phenolics and inorganic target compounds listed in Table 1 and Tables 4, 5, 6, 11, and 12 using EPA Laboratory SOPs.

The homogenized tissue samples will be extracted utilizing the Soxhlet technique as described in the "National Study of Chemical Residues in Fish", EPA 823-R-92-008a, September 1992. This extraction procedure is analogous to SW-846 Method 3540B. The extract volume will be split with one third of the volume used for Semivolatiles (Tables 4 and 5) and two thirds of the volume used for pesticides (Tables 11 and 12). Extracts for pesticides/Aroclors listed in Tables 11 and 12 will require Florisil cleanup (SW-846 Method 3620A), generating two fractions, 0% and 100%.

The 0% fraction will be treated with concentrated sulfuric acid (SW-846 Method 3665), to remove any GC/ECD interferences and analyzed for PCBs, DDE, Heptachlor and Aldrin. These compounds are not acid labile. The 100% fraction will be cleaned up using an acetonitrile partitioning step to remove lipids. After removal of lipids, the extract will be split. The split for AED analysis will not require additional clean up except for possible sulfur removal with elemental mercury, SW-846 Method 3660A. The split for GC/ECD analysis for the remaining Chlorinated Pesticides will be partitioned again using Florisil chromatography, SW-846 Method 3620A, generating a 6% fraction, a 15% fraction and a 50% fraction. All three fractions will receive mercury treatment to remove elemental sulfur. A portion of the 6% fraction will be treated with concentrated sulfuric acid. The 6% fractions, 15% fraction, and 50% fraction will be analyzed primarily by GC/ECD for Chlorinated Pesticides.

All project samples will be measured for the chlorinated pesticides and Aroclors listed in Table 11. Samples will be measured in batches of approximately 20 samples. Each batch will also consist of 2 method blanks and 4 MS/MSD samples.

The split for AED analysis and all Pesticide/Aroclor extracts will be saved for future potential AED analysis. These extracts will be sealed in containers and kept in the freezer. Depending on the results of the chlorinated pesticide/Aroclor analysis and the location of project samples, a subset of the project samples will be analyzed by atomic emmision detector (AED). The subset will include a few samples with low concentrations as well as samples with high chlorinated pesticide/Aroclor concentrations. The Project QAM with concurrence of the Project Manager will designate between 60 to 120 project samples which will be measured for the additional pesticides listed in Table 12 using AED Draft Method 8085. Quantitation limits for AED target compounds listed in table 12 are unknown, because most of these compounds have not been previously measured in the fish matrix using Method 8085.

The spiking protocol for chlorinated pesticides/Aroclors will be as follows:

All extraction sets for chlorinated pesticides and PCBs will receive both the chlorinated-pesticide mix of 19 pesticides and the PCB mix of Aroclors 1242 and 1260 for Table 11 target compounds. In addition the organochlorine spiking mixes #2 and #3, as well as the

organophosphate mixes #1, #2, and #3 and nitrogen-containing pesticide spiking mixes #1, #2, and #3 for the 8085 method will be added on a rotating basis:

- Set 1 (first batch of 20 samples)
  chlorinated pesticide mix #1 and PCB 1242/1260 mix
  O-pesticide/ N-pesticide mix #1

- Set 2 (next set of 20 samples)
  chlorinated pesticide mix #1 and PCB 1242/1260 mix
  Cl/ O-pest/ N-pest mix #2

- Set 3 (next set of 20 samples)
  chlorinated pesticide mix #1 and PCB 1242/1260 mix
  Cl/ O-pest/ N-pest mix #3

Each set of 20 samples will have different target compounds or the same target compound at different spiking concentration levels.

After the third set, the cycle goes back to Set 1 protocol for Cl/ O-pest/ N-pest.

9.2.5 Neutral Semivolatiles

Neutral SV target compounds are listed in Table 4. Extracts will be cleaned up using gel permeation chromatography (GPC) followed by silica gel column chromatography to isolate a neutral fraction containing PAHs and compounds. Target compounds will be measured using HRGC/LRMS/SIM in order to achieve the quantitation limits listed in Table 4.

9.2.6 Chlorinated Phenolics

This group of phenolics listed in Table 5 will be extracted, derivatized by acetylation, and analyzed using a modification to the procedure described in draft Method 1653.

A synopsis of the analytical procedure for the analysis of the chlorinated phenolics is as follows. A portion of the hexane extract prepared from the fish tissue is added to a stir-bar extraction vessel containing one liter of potassium carbonate buffer. Internal standard and surrogate are added and the mixture stirred. Acetic anhydride and hexane are added and the mixture stirred to simultaneously derivatize and extract the derivatives. If necessary, extracts will be cleaned up by either silica gel or alumina chromatography. Additional details are described in Manchester SOP 730016_7/93.

9.2.7 Metals

Cold mercury measurements of project tissue samples are described in EPA Region 10 SOP Automated Mercury Analysis of Tissue Samples by Cold Vapor Atomic Absorption (CVAA) Using Leeman Labs' PS200 or PS200ii, Revision 11/27/96.

Mercury measurements on project tissue samples are described in EPA Region 10 SOP "Automated Mercury Analysis of Tissue Samples by Cold Vapor Atomic Absorption (CVAA) Using Leeman Labs' PS200 or PS200ii, Revision 11/27/96."

The remainder of metals listed in Table 6 will be digested using a modified Method 200.3 and measured by ICP/MS using Method 200.8. A freeze-dried fish reference sample will be measured with each of project samples which are digested.

A summary of the procedure is as follows.

Samples are digested in batches of 20, with duplicate, spike/spike duplicate, spike and method blank and reference material.

Five gram subsamples of homogenized fish tissue are transferred to 250 mL pre-cleaned Teflon beakers. The tissues are digested in a Class 100 hood as specified in the EPA method 200.3. The addition of hydrochloric acid is omitted to avoid interferences produced by the chloride ion during ICP/MS analysis.

Hydrogen peroxide is added to a maximum of six mL and the multi element spike is added to give a concentration of 30 ug/L in the analytical solution for each element.

After a period of cooling, the samples are transferred to 125 mL polyethylene pre-cleaned bottles and diluted with ASTM type I water to 100 mL. The samples are then left to settle any insoluble material and then diluted five times with deionized water.

The reference material used is DORM-2, freeze-dried dogfish muscle and liver, from the National Research Council Canada. The amount of DORM-2 digested is 0.5 grams.

The samples are analyzed as soon as possible after digestion by ICP/MS using the EPA method 200.8. Samples are measured against a linear,four point calibration curve forced through the origin,and results are reported in mg/Kg wet weight.

The reference material is only being analyzed as a measure of precision throughout this long term project. DORM-2 is a different matrix than and no representative of the digested frozen tissue. A frozen tissue reference sample does not exist and this is the next best alternative.

9.3 CALIBRATION PROCEDURES AND FREQUENCY

Calibration and frequency of calibration of laboratory instruments shall be according to the requirements of each method of analysis. These requirements are listed in the methods (Attachments 13 and 15 for PCDD/PCDFs and dioxin-like PCBs, respectively, and in Table 1 for other analytes) for each class of chemicals to be analyzed. Each laboratory shall have a Standard

Operating Procedure (SOP) which describes how each target compound will be measured. The EPA Region 10 Statement of Work (Revision 2.0, 12\5\95) For the Measurement of 17 Polychlorinated Dibenzo-p-dioxins and Polychlorinated Dibenzo-p-furans (PCDDs/PCDFs) In Fish Tissue By High Resolution GC/High Resolution Mass Spectrometry Using Method 1613B, QAPP Attachment 13, sets specifications for calibration of 2,3,7,8-TCDF on a second confirmation column. Tetra Tech shall provide copies the subcontract laboratory's analytical SOPs to EPA.

9.4 LABORATORY QC PROCEDURES

Quality Control procedures specified in the QAPP and in the methods listed in Table 1 shall be followed and documented by each laboratory. In addition, Section 3.0 of the QAPP specifies that all quality control requirements of each method which is referenced in Table 1 shall be obtained and reported by each analytical laboratory, which includes QC requirements for surrogate compounds, internal standards, recovery standards, matrix spike compounds, calibrations, and method blanks.

10.0 ANALYTICAL DATA VALIDATION AND REVIEW

This section describes data validation, which is the process of technically reviewing analytical data using written data validation protocols, and qualifying measurement results using data qualifiers. The primary objective of data validation is to determine if project data meets the data quality objectives which are specified in the QAPP. After the data validation process is completed, data qualifiers are appended to measurement values by the data validation chemist. Final useability of qualified and validated data is determined by data users such as the Project Manager, CRITFC members, and local community members.

10.1 DATA VALIDATION

Data validation of PCDD/PCDF and toxic, dioxin-like, PCB data will be conducted by EPA Region 10. The following written protocols will be used for PCDD/PCDF and toxic, dioxin-like, PCB data:

> EPA Region 10 SOP For the Validation of Polychlorinated Dibenzofuran (PCDD) and Polychlorinated Dibenzofuran (PCDF) Data, Revision 1.4, December 7, 1995. (Attachment 14)
>
> EPA Region 10 SOP For the Validation of Method 1668 Toxic, Dioxin-Like, PCB Data, Revision 1.0, December 8, 1995, (Attachment 16).

The Project QA Manager will provide data validation reports for PCDD/PCDF and toxic, dioxin-like, PCB data to the Project Manager.

EPA Region 10 Laboratory staff will perform a standard laboratory data validation of Region 10 Laboratory data using the following guidelines:

> EPA Contract Laboratory Program National Functional Guidelines for Inorganic Data Review (PB-94-963502)(5)
>
> EPA Contract Laboratory Program National Functional Guidelines for Organic Data Review (PB-94-963501)(6)

The Project QA Manager will provide an assessment and evaluation of data validation reports. Criteria for the assessment and evaluation of data validation reports will be based upon the validation criteria which is specified in the above data validation SOPs and EPA data validation guidelines. Data outliers such as data qualified with "J" and "R" flags will be documented in data validation reports to the Project Manager. Data validation guidelines require that measurement values below the quantitation limit be qualified as an estimated value. Data users

such as risk assessors will determine the useability of such estimated values. If resources are available, the Work Assignment Manager may elect to have "R" qualified samples reanalyzed using archived samples.

10.2 DATA ASSESSMENT PROCEDURES

Following the data validation process, validated data will be assessed by the Project Manager to determine if the data meets the DQOs of the project plan. This assessment of validated data will be reported in the Final Report for the project.

11.0 PERFORMANCE AND SYSTEM AUDITS

Performance and systems audits for field work, filleting, homogenization and analyses will be conducted according to the following schedule:

11.1 AUDITS RELATED TO SAMPLE COLLECTION AND SAMPLE FILLETING

The Project QA Manager or his designee may conduct an on-site systems audit during sample collection and filleting field activities. An oral report of the results of any audits will be made to the Project Manager within 2 days of completion of each audit. A written report will be submitted within two weeks of each field audit.

11.2 AUDITS RELATED TO COMPOSITING AND HOMOGENIZATION OF FISH TISSUE

The FOM at Region 10 Office may conduct an on-site Technical Systems Audit of the subcontract laboratory which is responsible for grinding and compositing project fish samples. If this audit is conducted, written results will be submitted to the Project QA Manager and to the Project Manager within two weeks of the date of the audit.

In addition, the Region 10 FOM or his designate will inspect and document the nature of samples that are composited and ground by the subcontract laboratory when these samples are received by the Region 10 laboratory. The results of this audit and these inspections will be reported orally to the Project QA Manager and the Project Manager within two days of the audit and inspections. A written report will be submitted within two weeks of each inspection.

11.3 AUDITS RELATED TO SAMPLE ANALYSES

Tetra Tech shall conduct a Technical Systems Audit of the analytical subcontract laboratory. Tetra Tech shall develop, with EPA review and approval, an audit checklist that will be used in auditing the subcontract laboratory. The checklist will include QAPP requirements, method requirements, and any additional requirements established by Tetra Tech's work assignment. The subcontract laboratory shall prepare a report for the WAM (EPA Project Manager), based on the audit, which shall identify any instances in which the analytical laboratory work does not meet the requirements specified in the QAPP or Tetra Tech's Work Assignment. Tetra Tech shall provide the WAM with a advance notice of the audit and shall afford EPA and its technical advisors on the project the opportunity to participate in the audit as observers.

The subcontract laboratory which is responsible for measuring PCDDs/PCDFs and toxic, dioxin-like, PCBs will procure and measure PE samples EDF-2524, EDF-2525, and EDF-2526 when the first Sample Delivery Group is measured using Methods 1613B and 1668. Tetra Tech will

designate a second SDG during the latter phase of the project for the subcontract laboratory to measure PE samples EDF-2524, EDF-2525, and EDF-2526. The results of the measurement of PCDDs/PCDFs and toxic, dioxin-like, PCBs in these PE samples will be evaluated by the Project QA Manager within 14 days of receipt of data from Tetra Tech using the data validation report and the mean value and confidence intervals (at the 95% confidence level) of the interlaboratory study. The evaluation of PE measurement results will be summarized in the data validation report which is sent to the Project Manager. The Project Manager (WAM) will require corrective actions of Tetra Tech if the subcontract laboratory submits PE sample results which are determined by EPA to be outside the 95% confidence level of the interlaboratory study.

Other types of Performance or Systems Audits of field or laboratory activities may be scheduled by the Project Manager.

12.0 PREVENTATIVE MAINTENANCE

Preventive maintenance will take two forms: 1) implementing a schedule of preventive maintenance activities to minimize downtime and ensure accuracy of measurement systems, and 2) ensuring stock of critical spare parts and backup systems and equipment. The preventive maintenance approach for specific pieces of equipment used in sampling, monitoring, and documentation will follow manufacturer specifications and method requirements. Performance of these maintenance procedures will be documented in field logbooks and laboratory notebooks.

All laboratories will have service contracts in place for measurement systems which are used to measure project samples. The EPA Manchester Laboratory and Tetra Tech may be required by the Project Manager (WAM) to provide documentation that each laboratory which measures project samples have a preventive maintenance program and service contracts in place for measurement systems which are used to measure project samples.

Each laboratory will follow the preventive maintenance procedures specified in approved SOPs.

13.0 CORRECTIVE ACTIONS

Corrective actions taken during the sample collection and analysis phase of the project fall into two categories: 1) analytical or equipment malfunctions which could affect the ability of project staff or Tetra Tech to meet the stated requirements of the QAPP and 2) nonconformance or noncompliance with QA requirements set forth for the project.

Attached to the QAPP are a SAMPLE ALTERATION FORM (Attachment 17) and CORRECTIVE ACTION FORM (Attachment 18). These forms will be used to report problems that occur in the field, such as changes in the location or nature of samples collected, and in the EPA, Region 10 laboratory. The subcontract laboratory can use these forms or equivalent ones to report to Tetra Tech. Tetra Tech will forward a copy of these forms to the WAM and the Project QA Manager. In addition, each laboratory will provide a Case Narrative with the laboratory Data Report which will specify any problems which occur during the measurement of project samples.

SAMPLE ALTERATION FORMS and CORRECTIVE ACTION FORMS are initiated by any staff member of the Project or any staff member of Tetra Tech or laboratories which process Project samples. All SAMPLE ALTERATION FORMS and CORRECTIVE ACTION FORMS are signed by a Project Manager and the Project QA Manager. A file of all SAMPLE ALTERATION FORMS and CORRECTIVE ACTION FORMS implemented for Project activities will be maintained by the Project QA Manager.

It is the responsibility of the Project QA Manager to ensure that corrective actions are taken and recorded for all problems which are documented by CORRECTIVE ACTION FORMS, by field or laboratory audits, or by data validation evaluations. The Project QA Manager will document and report all of the above project problems to the Project Team Leader and the Project Manager. The Project Manager will initiate corrective actions in the event of lost samples or unusable project samples.

14.0 REPORTING REQUIREMENTS AND DELIVERABLES

This section briefly describes the deliverables and reporting requirements that are expected for this project. Deliverables are required from both Tetra Tech and the subcontract laboratory, as well as from the EPA, Region 10, field staff, laboratory, and QA Unit. Reporting requirements apply to Tetra Tech only. Contractor deliverables and reporting requirements and their due dates are described in detail in the Work Assignment for the EPA Contractor for this project and are summarized below.

14.1 FIELD WORK

Samples (whole fish and fillets, and eggs) will be collected by EPA with help from CRITFC. Fish tissue samples (with or without filleting) will be sent on dry ice via Federal Express to the processing laboratory with the appropriate documentation (i.e., SI/COC Tags, Sample Processing Records, and Chain-of-Custody Forms). The Field Record Form and notebook will be retained by the FOM.

14.2 FISH PROCESSING

Within 7 calendar days of receipt of fish samples, the subcontract laboratory will process the fish samples and distribute the sample aliquots for analysis to both the subcontract laboratory and the EPA, Region 10 laboratory. Deliverables include:

> (1) the sample aliquot jars and the reusable shipping containers to the EPA laboratory,
>
> (2) documentation (the COC Forms to the EPA laboratory with the sample aliquots; the Sample Processing Records, Sample Aliquot Records, and videotapes documenting sample processing) to the WAM.

As discussed above in Section 11.2, the FOM may conduct an on-site audit of the subcontract laboratory to ensure that fish processing is completed according to specifications of the QAPP. In addition, each batch of processed fish and egg samples will be inspected as they arrive at the Region 10 laboratory for analysis. The results of the fish processing laboratory audit, if conducted, and of the inspection of the processing of fish samples at the Region 10 laboratory will be documented and copies will be provided to the Project Manager (WAM) and the Project QA Manager within two weeks of their completion. Any problems noted with fish processing will be reported orally to the Project Manager and the QA Manager within 2 days.

14.3 LABORATORY ANALYSES

Detailed communication logs concerning this project and the preparation and analysis of project samples shall be maintained by Tetra Tech and subcontract laboratory. Copies of these logs shall be submitted to the WAM and Project QA Manager, in addition to any corrective action and sample alteration forms.

Within 35 days of verified time of shipment of homogenized and composited samples from the subcontract laboratory to the EPA Manchester Laboratory (subcontract laboratory must homogenize and composite project samples within 7 days of verified time of sample receipt), analytical results for Methods 1613B and Method 1668 shall be reported to Tetra Tech. Tetra Tech must submit data packages of analytical results from the subcontract laboratory to the WAM within 7 days of receipt from the subcontract laboratory. The Project QA Manager will perform the data validation review.

For PCDDs/PCDFs, a detailed description of required data documentation is given in the analytical SOW (Attachment 13) for the subcontract laboratory. In general, the subcontract laboratory shall provide all original data to document that all requirements of Method 1613B have been met. All raw data shall be submitted, along with example calculations, such that an independent data reviewer may recreate the calculations reported by the laboratory. In order to check for polychlorinated diphenyl ether (PCDPE) interferences, the subcontract laboratory shall submit simultaneous offset display of single ion chromatogram for each GC column for analyte peaks and for PCDPE peaks which may co-elute with native target compounds, according to the specifications of the PCDD/PCDF SOW (Attachment 13). Similar type documentation will be submitted for the analysis of toxic (dioxin-like) PCB congeners as discussed in Attachment 15.

The EPA Region 10 laboratory at Manchester will provide both analytical data from the analyses of other organics and all inorganics, and provide data validation reports. Data analytical reports of Manchester Laboratory data will be due within 45 days from verified time of sample receipt. Validation reports from the Manchester Laboratory will be delivered to the Project Manager within 75 days from verified time of sample receipt.

As described in Section 11.3, an on-site Technical Systems Audit of the subcontract laboratory will be conducted by Tetra Tech. EPA will be afforded the opportunity to observe the audit process. A checklist to be used during the audit will be developed by Tetra Tech with review and approval from EPA. Tetra Tech and any EPA staff participating in the audit will provide separate verbal observation reports to the WAM within 2 working days of the audit. A written report of audit results will be prepared by Tetra Tech and submitted to EPA WAM within 14 working days of the audit. If the written audit report indicates conditions at the laboratory that may compromise the project or DQOs of the QAPP, the WAM will immediately contact Tetra Tech to request corrective actions.

14.4 DATA SUMMARY FINAL REPORT AND DATABASE UPDATE

Data Summary Report - The analytical data generated and validated by the EPA Laboratory will be sent to the Project QA Manager to determine if the data validation meets the DQOs described in the QAPP. The analytical data generated by the subcontract laboratory will be validated by

the Project QA Manager. The validated data from both laboratories will then be sent to Tetra Tech who will compile it into a Final Data Summary Report. A draft summary data report will first be prepared and sent to the WAM for review. Within 2 weeks of receiving comments from EPA, Tetra Tech will submit the final summary data report. This report will summarize all of the analytical data for each target species at each sampling site as well as additional data specified in Tetra Tech's Work Assignment. The format and schedule for preparation of the Data Summary Report are described in Tetra Tech's Work Assignment.

Columbia River Contaminant Database Update - Tetra Tech will enter the validated analytical data from all analyses from this project into the Columbia River Contaminant Database and check the data for errors after data entry is completed. The schedule and deliverables for completion of the database update are described in Tetra Tech's Work Assignment.

15.0 QA REPORTS TO MANAGEMENT

The Region 10 Quality Assurance Unit will provide assistance to the Project Manager in reviewing the QAPP and in performing audits of selected project activities when requested by the Project Manager.

The results of audits specified in Section 10, above, will be submitted within 2 weeks of completion of each requested audit. Problems noted during the audits will be reported orally within 2 working days.

The Project QA Manager will submit written QA reports to the Project Manager when requested. These reports may include the following:

- Project status reports.
- Results of performance and systems audits
- Summary of significant QA problems and the corrective actions taken to correct these problems.
- Requests for changes or modifications to the QAPP.
- Results of data quality assessments of project data.
- Data validation reports for PCDD/PCDF and toxic, dioxin- like, PCBs data.

16.0 REFERENCES

(1) A Fish Consumption Survey of the Umatilla, Nez Perce, Yakama, and Warm Springs Tribes of the Columbia River Basin. CRITFC Technical Report No. 94-3. Portland, Oregon.

(2) Assessment of Chemical Contaminants in Fish Consumed by Four Native American Tribes in the Columbia River Basin - Draft Scoping Document, prepared for the U.S. EPA by Tetra Tech, September 30, 1994.

(3) Assessment of Chemical Contaminants in Fish Consumed by Four Native American Tribes in the Columbia River Basin - Final Draft Study Design, prepared for the U.S. EPA by Tetra Tech, December 2, 1994.

(4) Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume I: Fish Sampling and Analysis (U.S. EPA 1993b).

(5) EPA Contract Laboratory Program National Functional Guidelines for Inorganic Data Review (PB-94-963502).

(6) EPA Contract Laboratory Program National Functional Guidelines for Organic Data Review (PB-94-963501).

(7) Persistence of the DDT Pesticide in the Yakima River Basin Washington, U.S. Geological Survey, Circular 1090, 1993.

ATTACHMENTS

Attachment 1. Cooperative Agreement Between the Columbia River Inter-Tribal Fish Commission and the U.S. EPA

Attachment 2. EPA, Region 10, Boat Operating Policy

Attachment 3. Electrofishing Safety Procedures

Attachment 4. Field Record Form

Attachment 5. Sample Identification/Chain of Custody Tag

Attachment 6. EPA, Region 10, Chain of Custody Form

Attachment 7. Sections 7.2.1 and 7.2.1.3 of "Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1, Fish Sampling and Analysis"

Attachment 8. Sections 7.2.2.6 and 7.2.2.7 and Figure 7-3 of "Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1, Fish Sampling and Analysis"

Attachment 9. Fish Processing Record

Attachment 10. Custody Seal and Hazardous Substances Label

Attachment 11. Sample Receipt and Chain of Custody

Attachment 12. Sample Aliquot Record

Attachment 13. EPA Region 10 Statement of Work (Revision 2.2, 6/17/96) For the Measurement of 17 Polychlorinated Dibenzo-p-dioxins and Polychlorinated Dibenzo-p-furans (PCDDs/PCDFs) In Fish Tissue By High Resolution GC/High Resolution Mass Spectrometry Using Method 1613B.

Attachment 14. EPA Region 10 SOP For the Validation of Polychlorinated Dibenzofuran (PCDD) and Polychlorinated Dibenzofuran (PCDF) Data, Revision 1.4, December 7, 1995.

Attachment 15. Draft Method 1668 For the Measurement of Toxic PCB Congeners By Isotope Dilution HRGC/HRMS, October 4, 1995 Draft Revision.

Attachment 16. EPA Region 10 SOP For the Validation of Method 1668 Toxic, Dioxin-Like, PCB Data, Revision 1.0, December 8, 1995.

Attachment 17. Sample Alteration Form

Attachment 18. Corrective Action Form

Attachment 19. 1996 Summer Sampling Design For the CRITFC Exposure Study

Attachment 20. Previous 6/11/96 Sampling Design For the CRITFC Exposure Study

Attachment 21. Previous Sampling Map From 6/17/96 QAPP

SAMPLE ALTERATION FORM

Sample Number(s): 45 samples from sites 7, 8, 9, "K" Pond at Hanford, and site 96 (upstream) plus 2 laboratory duplicates.

Material to be Sampled: sturgeon, whitefish, and either catfish or sucker.

Measurement Parameter: The following gamma analysis will be preformed on all samples according to the attached 7/31/97 addendum:

Be-7, Na-22, K-40, Mn-54, Co-58, Co-60, Fe-59, Zn-65,
Zr-95/Nb-95, Ru-103, Ru-106, Sb-125, I-131, Cs-134, Cs-137,
Ba-140/ La-140, Ce-141, Ce-144/Pr-144, Eu-152, Eu-154, Am-241

The following additional radionuclides will be on all samples of whole body fish:

Sr-89, Sr-90, Pu-238, Pu-239/Pu-240, U-238, U-234, Ra-226

QAPP Reference or Standard Procedure for Field Collection & Laboratory Analysis (cite reference):

See following documents for specifications for this Sample Alteration Form:

1. Radionuclear Measurements For QAPP For Assessment of Chemical Contaminants in Fish Consumed by Four Native American Tribes In the Columbia River Basin, Addendum Revision 2.0, September 3, 1997.

2. Quality Assurance Project Plan, Assessment of Chemical Contaminants in Fish Consumed by Four Native American Tribes In the Columbia River Basin, Revision 6.0, December 16, 1996.

Reason for Change in QAPP, Field Procedure or Analysis Variation:

Samples for radionuclear measurements have been added to the project according to Section 1.3 of Rev. 6.0 of the QAPP, which states the following:

"Some of these archived samples may be used for analysis of radionuclides at another laboratory if resources become available."

Variation from QAPP, Field or Analytical Procedure:

not applicable

Special Equipment, Materials or Personnel Required:

none

Project Manager For Radionuclear Measurements: _____ Date: _____

Project Manager: _____ Date: _____

Project Risk Assessment Manager: _____ Date: _____

Project QA Officer: _____ Date: _____

RADIONUCLEAR MEASUREMENTS

FOR

QUALITY ASSURANCE

PROJECT PLAN

ASSESSMENT OF CHEMICAL CONTAMINANTS IN FISH CONSUMED BY FOUR NATIVE AMERICAN TRIBES IN THE COLUMBIA RIVER BASIN

Revision 1.1
July 31, 1997

Manager For Radionuclear Measurements: _____ Date: _____

Project Manager: _____ Date: _____

Project Risk Assessment Manager: _____ Date: _____

QA Officer: _____ Date: _____

ADDITIONAL SAMPLES FOR THE QUALITY ASSURANCE PROJECT PLAN FOR ASSESSMENT OF CHEMICAL CONTAMINANTS IN FISH CONSUMED BY FOUR NATIVE AMERICAN TRIBES IN THE COLUMBIA RIVER BASIN REV. 6.0 12/16/97

ADDITION OF RADIONUCLIDE SAMPLE ANALYSIS

1.0 TASK DESCRIPTION

This work will support the Assessment of Chemical Contaminants in Fish Consumed by Four Native American Tribes in the Columbia River Basin by providing laboratory radiological analysis of fish samples collected from the Columbia River basin.

1.1 LABORATORY

Radionuclide analyses will be performed by the EPA National Air and Radiation Environmental Laboratory (NAREL) NAREL is a comprehensive environmental laboratory managed by the U.S. EPA Office of Radiation and Indoor Air. Among its responsibilities, NAREL includes a national program for collecting and analyzing environmental samples from a national network of monitoring stations for the analysis of radioactivity. This network, which has stations in every State, has been used to track environmental releases of radioactivity from nuclear weapons tests and nuclear accidents.

The NAREL radioanalytical program analyzes more than 10,000 samples annually and constitutes EPA's primary laboratory support for evaluation of ionizing radiation. Samples include air, water, soil, vegetation, human tissue, and food. NAREL applies quality assurance standards to all analyses, and routinely participates in laboratory intercomparison quality assurance programs with groups such as the World Health Organization and the International Atomic Energy Agency, as well as with the EPA Quality Assurance Program operated by the Agency's Office of Research and Development.

NAREL supports States in radiological environmental monitoring and has performed radiological surveys for the U.S. Navy. Through a cooperative agreement , NAREL is helping the Agency for Toxic Substances and Disease Registry to monitor radiation levels near major federal facilities.

1.2 TASK NARRATIVE

Samples will be shipped to the NAREL laboratory in Montgomery, AL. All samples will be analyzed by gamma spectrometry. Approximately 50% of all samples will also be analyzed for specific nuclides of uranium, thorium, plutonium, radium and strontium. Laboratory instruments

are maintained, calibrated, and operated in accordance with established NAREL procedures and SOPs.

NAREL quality assurance staff will assess laboratory operations in accordance with *The Quality Assurance Plan for the National Air and Radiation Environmental Laboratory* and written QA/QC policies for radionuclide analysis. Data packages and reports will be thoroughly reviewed in accordance with established NAREL procedures. Analytical data will be reviewed according to the *NAREL Standard Operating Procedure for the Review of Radioanalysis Data* (Draft, June 14, 1996).

This task will involve approximately 40-50 samples. Samples will be analyzed and reported within 24 weeks after the samples arrive at NAREL. A summary analytical report, including results of blanks and other QC samples and a case narrative describing sample exceptions, will be produced for each group of samples analyzed.

1.3 PERSONNEL, TRAINING, AND EQUIPMENT REQUIRED

NAREL personnel including staff from the Monitoring and Analytical Services Branch, and Quality Assurance will participate in each study. All laboratory analysts will be trained and certified for the analytical procedures they conduct at NAREL. NAREL has staff who are knowledgeable and who routinely perform data evaluation and interpretation.

NAREL maintains laboratory equipment used daily in the handling of radioactive samples. Equipment is maintained, calibrated, and used in accordance with NAREL SOPs and written policies and procedures.

2.0 ORGANIZATION AND RESPONSIBILITIES

According to the NAREL Quality Assurance Plan, the Director of the Office of Radiation and Indoor Air (ORIA) is responsible for ensuring that measurements performed within ORIA meet established Data Quality Objectives (DQOs). The Office Director has delegated the responsibility for overseeing quality assurance to the ORIA Quality Assurance Officer and has further delegated to the Director, NAREL, the primary responsibility for quality assurance on measurements in that facility. The Director, NAREL, has appointed a Quality Assurance Coordinator (QAC) to direct and oversee the laboratory's Quality Assurance Program. In addition, the Quality Assurance Forum (QAF) has been formed to focus on all pertinent QA issues. The QAF meets monthly, includes all interested laboratory personnel, and uses open forums and work groups to recommend procedures that will effectively and efficiently resolve an identified problem.

3.0 LABORATORY INSTRUMENTS AND EQUIPMENT

Laboratory instruments and equipment are used, calibrated, and maintained according to accepted good laboratory practices, written NAREL policies, and NAREL SOPs.

Regular efficiency checks are performed on every detector in use at the laboratory. Efficiency checks are performed daily for gas-flow proportional counters, scintillation counters, and germanium detectors. Checks are performed weekly for alpha spectrometers.

The results of efficiency checks are plotted on control charts and compared to warning and rejection limits. If an efficiency check fails on a detector, it is rerun once. If the second result is in the warning or rejection range, the detector is taken out of service and corrective action is initiated.

Regular background measurements are required for all detectors. Backgrounds are measured daily for proportional counters, twice a month for alpha spectrometers, monthly for germanium detectors, and immediately before a sample is counted on a scintillation counter.

Each gross radiation detector has an acceptable range for background levels. Background measurements are evaluated statistically to determine whether the true level is outside the acceptable range. There are warning limits and rejection limits for the test. If the most recent background level is outside the rejection limit, the detector is not used to analyze samples.

All radioactive standard solutions used for calibrations and efficiency checks at NAREL are traceable to the National Institute of Standards and Technology (NIST). Standard reference materials are purchased directly from NIST whenever they are available.

4.0 DOCUMENTATION AND RECORDS

4.1 NAREL DOCUMENT CONTROL SYSTEM

NAREL operates under a formal document control system, described in the *NAREL SOP for Document Control,* which presents the policies and procedures for the production, review, revision, storage, and distribution of documents. Document control policies apply to all printed internal documents that are maintained by or for NAREL personnel on a continuing basis for a period longer than one year. Controlled documents include, but are not limited to, the NAREL QMP, QAMs, QAPPs, SOPs, technical documentation, and forms. The Document Control Officer maintains the NAREL Document Control Logbook, maintains current copies of all controlled documents in hardcopy and electronic forms, approves any new or revised documents in the system, and has primary responsibility for the *NAREL SOP for Document Control.*

4.2 NAREL OPERATING DOCUMENTS

The *NAREL Quality Management Plan* (QMP) describes the Quality System at NAREL in terms of the organizational structure, functional responsibilities of management and staff, lines of authority, and processes for planning, implementing, documenting, and assessing activities. The QMP is the umbrella document for management policies, goals, and processes which incorporate Quality Assurance and Quality Control into all aspects of NAREL's work. The QMP describes how NAREL implements its Quality System and educates its staff about QA and QC processes.

A Quality Assurance Manual (QAM), formerly called a Quality Assurance Plan (QAP), presents technical criteria for analytical and administrative tasks to ensure that all data produced will be of known and desired quality, that all measurements performed at NAREL are valid, scientifically defensible, and of known precision and accuracy. The QAM addresses all phases of the quality control, quality assurance, and quality assessment processes. The manual presents specific and detailed information about tasks, processes, and criteria for various programs and activities. The QAM provides a detailed program for evaluating QC procedures and assessing results produced by the branch or program.

The Quality Assurance Project Plan, Assessment of Chemical Contaminants in Fish Consumed by Four Native American Tribes In the Columbia River Basin, Revision 6.0, December 16, 1996 provides specifications for required QA, QC, and reporting activities that must be implemented to ensure that the work performed on a specific project will satisfy the required performance criteria. Each project conducted by or for NAREL requires a QAPP. This includes projects supported by contract, interagency agreement, or grant. A QAPP is required to ensure that the analytical, sampling, and other programs meet the required DQOs that have been established for the project.

Standard Operating Procedures (SOPs) contain specific details and procedures which ensure that data generated by their use will be of known and adequate quality. All SOPs must be written, reviewed, approved, distributed, and revised in accordance with provisions in the *NAREL SOP for Writing SOPs.* An SOP details the method for an operation, analysis, or action, with thoroughly prescribed techniques and steps.

4.3 DATA PACKAGE DELIVERABLES

For each analytical batch of samples analyzed at the laboratory, a summary data package will be provided for each type of analysis. This summary includes:

- tabulated sample information: NAREL Sample ID, Client Sample ID, matrix, date collected, date received, and date analyzed.
- documentation exceptions
- holding time information if applicable
- sample preparation exceptions
- sample analytical exceptions
- general information unique to the sample batch, the analytical method, or reporting conventions
- individual report forms for each sample which provide
    - sample identification information
    - analytical method
    - detector identification
    - sample weight information
    - activity units
    - nuclides, activity, 2σ uncertainty, and MDC

4.4 RECORDS ARCHIVING AND RETENTION POLICIES

All records pertaining to environmentally related measurements will be archived, retained, and disposed of according to the pertinent EPA records schedule, with concurrence of the Navy and the NAREL project officer. Generally, hard copy records are maintained at NAREL for a minimum of ten years.

5.0 SAMPLE ANALYSIS

All samples will be analyzed for gamma-emitting nuclides with approximately 50% also analyzed for isotopic uranium, thorium, plutonium, radium and strontium. The NAREL Minimum Detectable Concentrations (MDC) for typical nuclides are listed in Tables 1 and 2. Sample size will be a minimum 600 g.

Target radionuclides for analysis were selected based on both on reviews of historical records for radionuclides previously detected or analyzed in the Columbia River and biota, and on the basis of current possible sources of radionuclides. Documents consulted included annual environmental monitoring reports from the Washington State Environmental Radiation Program and the Department of Energy Hanford Site Environmental Monitoring Program. Both short-lived and long-lived radionuclides were included consistent with ongoing reactor operations at the Washington Public Power Supply System nuclear plant as well as historic Hanford reactor operations.

Gamma analysis will be performed on all samples. The energy spectrum collected from the sample will be evaluated for gamma-emitting radionuclides over the energy range 60-2000 keV. Analysis will include naturally-occurring as well as manmade radionuclides. The analysis will quantify any spectrum peaks identified. In addition, minimum detectable concentrations for the following specified gamma emitting radionuclides will be quantified whether or not a peak is identified:

Be-7, Na-22, K-40, Mn-54, Co-58, Co-60, Fe-59, Zn-65,
Zr-95/Nb-95, Ru-103, Ru-106, Sb-125, I-131, Cs-134, Cs-137,
Ba-140/ La-140, Ce-141, Ce-144/Pr-144, Eu-152, Eu-154, Am-241

The following radionuclides will be analyzed in whole fish samples only. Strontium and plutonium, in particular, primarily accumulate in bone and should therefore be evaluated in whole fish rather than fillet.

Sr-89, Sr-90
Pu-238, Pu-239/Pu-240
U-238, U-234, Ra-226

Table 1. NAREL Minimum Detectable Concentration (MDC) for Selected Gamma Emitters Using Gamma Spectrometry with Ge Detector

Selected Gamma Emitters	MDC (pCi/L) for 1 L of Water Counted for 1000 min	MDC (pCi/gwet) for 1500 g of Sediment Counted for 1000 min	MDC (pCi/gwet) for 100 g of Sediment Counted for 1000 min	MDC (pCi/gwet) for 40 g of Sediment Counted for 1000 min	MDC (pCi/gwet) for 1000 g of Biota Counted for 1000 min
Am-241	17.7	0.0179	0.0842	0.168	0.0177
Cd-109	83.0	0.0749	0.424	0.901	0.0830
Th-234	52.5	0.0468	0.270	0.578	0.0525
U-235	56.7	0.0470	0.294	0.684	0.0567
Ra-226	86.1	0.0710	0.446	1.05	0.0861
Th-229	65.6	0.0540	0.340	0.799	0.0656
Pb-212	8.45	0.00689	0.0439	0.104	0.00845
Ra-224	91.0	0.0742	0.473	1.12	0.0910
Ra-223	26.7	0.0216	0.139	0.329	0.0267
Pb-214	11.4	0.00919	0.0601	0.143	0.0114
I-131	5.84	0.00468	0.0307	0.0730	0.0584
Rn-219	69.6	0.0556	0.367	0.873	0.0696
Be-7	45.4	0.0360	0.241	0.574	0.0454
Ba-140	22.2	0.0175	0.119	0.282	0.0222
Rn-220	7110	5.6	38.0	90.4	7.11
Tl-208	6.42	0.00504	0.0344	0.0818	0.00642
Cs-134	6.67	0.00523	0.0357	0.0850	0.00667
Bi-214	13.1	0.0103	0.0704	0.167	0.0131
Cs-137	7.26	0.00567	0.0391	0.0929	0.00726
Bi-212	89.4	0.0696	0.483	1.15	0.0894
Pb-211	188	0.145	1.02	2.42	0.188
Mn-54	7.03	0.00543	0.0382	0.0909	0.00703

Table 1. NAREL Minimum Detectable Concentration (MDC) for Selected Gamma Emitters Using Gamma Spectrometry with Ge Detector

Selected Gamma Emitters	MDC (pCi/L) for 1 L of Water Counted for 1000 min	MDC (pCi/gwet) for 1500 g of Sediment Counted for 1000 min	MDC (pCi/gwet) for 100 g of Sediment Counted for 1000 min	MDC (pCi/gwet) for 40 g of Sediment Counted for 1000 min	MDC (pCi/gwet) for 1000 g of Biota Counted for 1000 min
Ra-228	24.4	0.0188	0.133	0.317	0.0244
Pa-234m	950	0.728	5.20	12.4	0.950
Co-60	10.3	0.00782	0.0566	0.135	0.0103
Na-22	9.38	0.00712	0.0519	0.123	0.00938
K-40	99.3	0.0749	0.552	1.31	0.0993

NOTE: MDCs will vary depending on activity in the sample, density of sample matrix, efficiency of detector, and other counting parameters. The above MDCs were calculated based on a 1000-min count of a 1.0-L Marinelli of deionized water.

Table 2. NAREL Minimum Detectable Concentration (MDC) for Selected Radionuclides Using Various Radiochemical Analyses

Radionuclide	Matrix	Typical Aliquot Size	Count Time (min)	Method	MDC
Gross Alpha	Water	250 mL	100	GFP	6 pCi/L
Gross Beta	Water	250 mL	100	GFP	3 pCi/L
Radium-226	Water	1 L	1000	SC	0.02 pCi/L
	Solids	0.5 g	1000	SC	0.04 pCi/g
Radium-228	Water	1 L	100	GFP	1 pCi/L
	Solids	0.5 g	100	GFP	2 pCi/g
Iodine-131	Water	2 L	1000	GFP	0.7 pCi/L
Uranium-234, 235, 238 Thorium-230, 232 Plutonium-238, 239	Water	1L	1000	AS	0.1 pCi/L
	Solids	0.5 g	1000	AS	0.2 pCi/g
Thorium-227	Water	1L	1000	AS	0.2 pCi/L
	Solids	0.5 g	1000	AS	0.35 pCi/g
Thorium-228	Water	1L	1000	AS	0.15 pCi/L
	Solids	0.5 g	1000	AS	0.3 pCi/g
Tritium	Water	10 mL	50	LS	400 pCi/L

AS Alpha Spectrometry
GFP Gas-Flow Proportional Counting
GS Gamma Spectrometry
LS Liquid Scintillation Counting
SC Scintillation Counting

6.0 SAMPLE LOCATIONS

Samples selected for radionuclide analysis are:

Site 7: sturgeon fillet without skin (3 replicates)

Site 8: sturgeon fillet without skin (3 replicates)
sturgeon whole (3 replicates)
whitefish fillet (3 replicates)
whitefish whole (3 replicates)

Site 9: sturgeon fillet without skin (3 replicates)
whitefish fillet (3 replicates)
whitefish whole (3 replicates)
other fillet (catfish or sucker) (3 replicates)
other whole (catfish or sucker) (3 replicates)

K Pond: sturgeon fillet without skin (3 replicates)
sturgeon whole (3 replicates)

Lab duplicates: 2

In addition: Samples from upstream (location 96)

sturgeon fillet without skin (3 replicates)
whitefish fillet (3 replicates)
whitefish whole (3 replicates)

7.0 SAMPLE MANAGEMENT

Environmental samples are received at NAREL, logged-in, and stored in accordance with the *NAREL SOP for Sample Receipt, Log-in, and Storage*. All samples are received by the Sample Preparation Manager (SPM) or designee. Sample coolers are stored in a secure area until they are surveyed for radioactive contamination. The results of the survey are recorded on the chain-of-custody forms.

The SPM compares the samples received to the chain-of-custody forms. Any discrepancies must be resolved and documented on the COC before sample analysis begins. The samples are then logged-in to the Sample Preparation Logbook and into the NAREL Radioanalytical Database.

After samples are logged-in and numbered, and all documentation is complete, samples are stored at various locations at NAREL, depending on the matrix, analyses requested, and project, until the analyses are performed.

Samples are always stored in a secure area to which only laboratory personnel have access. Access to NAREL is restricted. Outside entrances, laboratories, the counting room, and the sample preparation area require a key-card for entry. Visitors must sign in at the reception area and are escorted while in the laboratory.

The samples are shipped to NAREL where they are received by the NAREL sample preparation staff and transferred to the sample preparation laboratory. The packages are routinely screened, using beta and gamma detection equipment, to check external radiation levels before opening. The shipping containers are opened, the sample containers removed, and checked against the chain-of-custody documentation.

8.0 DATA MANAGEMENT

A sample identification code is assigned to each sample which is recorded in a logbook and entered into the NAREL database. Sample preparation activities are performed and the samples are distributed to analysts. Samples are subjected to appropriate analyses (alpha/beta screening, gamma spectroscopy, radiochemistry/ specific nuclides analysis), and the resulting data are subjected to verification by two independent parties. Following verification, the data are available in the database. At this point, a draft data package is prepared. The data package is reviewed and finalized by incorporating reviewers comments, as appropriate, and is then available for use by harbor studies personnel.

A draft of the *NAREL Standard Operating Procedure for the Review of Radioanalysis Data* includes:

* sample receipt and preparation,
* laboratory data handling (using the Laboratory Information Management System (LIMS),
* instrumentation calibrations and efficiency checks,
* types of sample analyses performed (individual detailed procedures are available for each type),
* data review, error detection and other problem determinations, including requirements for recounting or reanalysis of samples,
* involvement of the project quality assurance officer and the NAREL quality assurance coordinator,

Record keeping is in the form of laboratory logbooks and information and data storage in the NAREL LIMS system (which includes a large database of storage of all data produced in the radioanalytical laboratory). NAREL has a formal document control system which allows for documents to be either controlled or uncontrolled. The procedures used to administer this system are included in the procedure entitled *NAREL Standard Operating Procedure for Document Control*. Data storage and retrieval is via the LIMS system referenced above.

The methods for detecting and correcting errors are used in NAREL data management activities including verification of samples with chain-of-custody records, instrument calibration and

background checks, and extensive review of counting room data before release. Loss of data during entry, reporting or reduction is very unlikely since data are maintained on a local area network where nightly backups are performed, and data are archived to optical disks on a regular basis. Any calculations requiring the data extract a copy from the database, leaving the original electronic record intact. The only chance for temporary data loss is in case of a power failure during sample counting or hardware failure. In those instances, the sample is still available and is simply recounted.

Data handling equipment includes radioanalytical instrumentation, PC's dedicated to counting room applications, and the NAREL local area network. All of this equipment has been thoroughly tested for these applications and proven to be stable and reliable. Commercial software currently used by the radioanalytical laboratory is as listed below:

Program	Analysis System
LB4000	Tennelec LB4000 Gas-Flow Proportional Counters (raw data)
GDR	High-purity Germanium Detectors (raw and reduced data)
AlphaMat	Alpha Spectrometers (raw and reduced data)
G3000	Gamma Products G3000 Automatic Germanium Counting System (control software)
G5000	Gamma Products G5000 Automatic Alpha/Beta Counting System (control software)

In-house software includes the following:

Data Entry and Instrument Control

Program	Analysis System
I131	Tennelec LB4000 Iodine-131
GROSS	Tennelec LB4000 Gross alpha and beta
SR	Tennelec LB4000 Strontium-89 and 90
RA228	Tennelec LB4000 Radium-228
TH234	Tennelec LB4000 Thorium beta tracer
GET4, GET12	Germanium counters

Calculation and Data Review

Program	Analysis System
GAMMARVW	Gamma spectrometry
ALPHARVW	Alpha spectrometry (Am,U,Pu,Th)
I131RVW	LB4000 Iodine-131
GROSSRVW	LB4000 Gross alpha and beta
SRRVW	LB4000 strontium-89 and 90
RA226	Radium-226 by the radon emanation method

Data Management

CAA	NAREL database system
RPT	Interactive database queries
NARSS	Counting Room analysis scheduling
DATAPKG	Data package production

The calculations performed by all in-house analysis software are documented in the software user manuals. Only one NAREL employee is authorized to modify in-house analysis software and electronic and written records of software modifications are maintained. After each modification of an analysis software system, the calculations are checked using a calculator program, which reads equations from a text file in a form similar to that shown in the user's manual. The results generated by the analysis software are checked against the results given by the calculator.