Quality Assurance Project Plan
for
Laboratory Sample Preparation and Analysis Activities in the National Pilot Study of Pharmaceuticals and Personal Care Products (PPCPs) in Fish Tissue

October 19, 2006

Prepared for

United States Environmental Protection Agency
Office of Water
Office of Science and Technology
Standards and Health Protection Division

Prepared jointly by

Computer Sciences Corporation (CSC) Environmental Programs Group
Prepared under
Office of Superfund Remediation and Technology Innovation (OSRTI) Sample Management Office
Contract No. EP-W-06-046

and

Tetra Tech, Inc.
Prepared under
Office of Science and Technology (OST)
Contract No. EP-C-04-030

EPA 820-F-23-004

Quality Assurance Project Plan for Laboratory Sample Preparation and Analysis Activities in the National Pilot Study of Pharmaceuticals and Personal Care Products (PPCPs) in Fish Tissue

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This Quality Assurance Project Plan (QAPP) presents performance and acceptance criteria and measurement quality objectives (MQOs) established for the analysis of environmental samples collected during the *National Pilot Study of Pharmaceuticals and Personal Care Products (PPCPs) in Fish Tissue* (hereafter referred to as either the "PPCP Fish Pilot Study," or more simply "the study"). This QAPP also describes the methods and procedures that will be followed to ensure these criteria and MQOs are met. This document addresses only the sample analysis effort; performance criteria and procedures related to sample collection are described in EPA's *Quality Assurance Project Plan for Sample Collection Activities for a Pilot Study to Investigate the Occurrence of Pharmaceuticals and Personal Care Products (PPCPs) in Fish Tissue* [1].

This document was prepared in accordance with and contains each of the elements described in the most recent version of EPA QA/R-5, *EPA Requirements for Quality Assurance Project Plans* [2]. To improve clarity, the order of certain elements in this QAPP has been modified slightly from the order presented in EPA QA/R-5. For example, in this QAPP the Project Organization section (element "A4" in QA/R-5) follows the Project Background and Project Description sections (elements "A5" and "A6" in QA/R-5).

In accordance with the instructions provided in EPA QA/R-5, this QAPP is considered to be a dynamic document that is subject to change as sample collection and analysis progresses. All changes to procedures described in this QAPP will be reviewed by the EPA Study Manager and the EPA Quality Assurance Manager to determine if the changes significantly impact the technical and quality objectives of the project. If changes are deemed to be significant, the QAPP will be revised accordingly, circulated for approval, and provided to all project participants listed in the QAPP distribution list.

1.0 PROJECT BACKGROUND

EPA's Office of Science and Technology (OST) within the Office of Water (OW) is initiating a pilot study to investigate the occurrence of pharmaceutical and personal care product (PPCP) chemicals in fish tissue called the National Pilot Study of Pharmaceuticals and Personal Care Products in Fish Tissue (hereafter referred to as the "PPCP Fish Pilot Study"). Increasing evidence indicates widespread occurrence of PPCP chemicals in surface water, sediments, and municipal effluent, but data on the accumulation of PPCP chemicals in fish tissue are scarce. This study was planned to respond to EPA's new priority of obtaining environmental data on emerging contaminants and to increase the data available on the occurrence of PPCP chemicals in fish. The proposed targeted study design calls for collecting fish samples from five effluent-dominated streams in the vicinity of wastewater treatment plant (WWTP) discharges and one reference site. Tissue fractions from each fish sample (fillets and livers) will be analyzed for 39 PPCP chemicals. These analyses will provide data to determine the potential of the target chemicals to survive the wastewater treatment process and to bioaccumulate in the tissue of fish.

Tetra Tech has been tasked with planning, implementing, and managing sample collection, preparation and analysis, and forwarding pilot project results to Computer Sciences Corporation (CSC) for data review and database development. Tetra Tech will collect all samples and will subcontract with Baylor University's Center for Reservoir and Aquatic Systems Research (hereafter referred to as "Baylor") for the preparation and analysis of fish samples. Baylor will be subcontracted to analyze PPCP Fish Pilot Study samples for the 39 target chemicals using analytical methods, such as high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), gas chromatography/mass spectrometry selective ion monitoring (GC/MS-SIM), or other methods that can achieve the method detection limits specified by EPA.

2.0 PROJECT DESCRIPTION

The study design reflects the study goal and objectives defined by USEPA. The study goal can be stated simply — *to investigate the occurrence of a broad suite of PPCPs (39 chemicals) in the tissue of adult freshwater fish that are typically consumed by humans and/or wildlife.* Performance and acceptance criteria and measurement quality objectives (MQOs) established for the analysis of environmental samples collected during this study are addressed in this document; those methods and procedures used to collect and ship fish tissue samples for the PPCP Fish Pilot Study are documented in a separate QAPP (*Quality Assurance Project Plan for Sample Collection Activities for a Pilot Study to Investigate the Occurrence of Pharmaceuticals and Personal Care Products (PPCPs) in Fish Tissue[1]*).

In consultation with the USEPA Office of Science and Technology, Tetra Tech will coordinate with USEPA headquarters and regional staff to collect fish tissue samples from targeted effluent-dominated streams. Field teams will sample five sites where waters are dominated by wastewater treatment plant (WWTP) effluent, along with one reference quality site (Appendix B). The samples will be collected between August and November of 2006. The fish tissue samples will be collected based on a targeted design to provide information on the occurrence of PPCP contaminants in fish. Upon collection, fish samples will be shipped to the laboratory where they will be weighed, composited, sub-sampled into liver tissue and muscle tissue (fillet) fractions, homogenized, and divided into aliquots for analysis and archiving, if sufficient tissue is available. The aliquots for each tissue fraction will then be analyzed for the PPCP chemicals listed in Table 1. Details regarding the study design, including how streams are to be sampled, how they were selected, etc., can be found in reference [1].

Table 1. PPCP Pilot Study Target Chemicals and Corresponding Analysis Methods

Analysis Method	Chemical (CAS Number)
Pharmaceuticals and Personal Care Products (PPCPs) by High Performance Liquid Chromatography - Tandem Mass Spectrometry *(Method HPLC-MS/MS)*	1,7-Dimethylxanthine (611-59-6) Acetominophen (103-90-2) Atenolol (29122-68-7) Caffeine (58-08-2) Carbamazepine (298-46-4) Cimetidine (51481-61-9) Clofibric Acid (882-09-7) Codeine (76-57-3) Diltiazem (42399-41-7) Erythromycin (114-07-8) Fluoxetine (54910-89-3) Gemfibrozil (25812-30-0) Ibuprofin (15687-27-1) Lincomycin (154-21-2) Metoprolol (37350-58-6) Miconazole (22916-47-8) Norfluoxetine (83891-03-6) Propranolol (525-66-6) Sertraline (79617-96-2) Sulfamethoxazole (723-46-6) Thiabendazole (148-79-8) Trimethoprim (738-70-5) Tylosin (1401-69-0) Warfarin (81-81-2)
Pharmaceuticals and Personal Care Products (PPCPs) by Gas Chromatography/Mass Spectrometry - Selective Ion Monitoring *(Method GC/MS-SIM)*	4-Methylbenzylidine camphor or 4-MBC (36861-47-9) Benzophenone (119-61-9) Celestolide (13171-00-1) Galaxolide (1222-05-5) *m*-Toluamide (618-47-3) Musk ketone (81-14-1) Musk xylene (81-15-2) Nonylphenol monoethoxylate, isomer 1 (27986-36-3A) Nonylphenol monoethoxylate, isomer 2 (27986-36-3B) Nonylphenol monoethoxylate, isomer 3 (27986-36-3C) Octocrylene (6197-30-4) *p*-Nonylphenol (104-40-5) *p*-Octylphenol (1806-26-4) Tonalide (1506-02-1 or 21145-77-7) Triclosan (3380-34-5)

3.0 PROJECT ORGANIZATION

The Office of Science and Technology's (OST's) Standards and Health Protection Division (SHPD) is responsible for overall management of the PPCP Fish Pilot Study, including day-to-day responsibility for managing various aspects of the study. SHPD is responsible for managing all sample collection, data and laboratory analyses, and data verification (data review) activities. SHPD is also responsible for day-to-day interaction with contractors and with other federal, state, and local authorities involved in the project.

SHPD has contracted Tetra Tech to provide support for project planning, sample collection, and procurement and oversight of all laboratory services necessary for this study. SHPD has also contracted with CSC for general technical support, data verification and validation, and data management. To minimize variability that could arise from sample preparation and analysis, SHPD requested that all laboratory activities be conducted in a single laboratory for the duration of the project. This laboratory must verify the absence of laboratory contamination for target chemicals during sample preparation, have sufficient capacity to receive and process all fish collected during the study, and be capable of analyzing each sample while adhering to all quality assurance/quality control (QA/QC) procedures in an environment that is free from detectable levels of all target chemicals.

Figure 1 illustrates the project organization and relationships between groups participating in the major activities to be conducted under this study. Sections 3.1 through 3.4 describe the roles and responsibilities of the individuals involved in study activities related to sample preparation and analysis. Details regarding roles of individuals involved in sample collection and handling can be found in the *Quality Assurance Project Plan for Sample Collection Activities for a Pilot Study to Investigate the Occurrence of Pharmaceuticals and Personal Care Products (PPCPs) in Fish Tissue*. Section 3.1 of this QAPP describes the responsibilities of EPA staff; Section 3.2 describes the responsibilities of the Computer Sciences Corporation (CSC) staff; Section 3.3 describes the responsibilities of Tetra Tech staff; and Section 3.4 describes the responsibilities of staff at the contract laboratory that will support this study.

3.1 EPA Management and Staff

3.1.1 OST Director

The OST Director, Ephraim King, is responsible for providing financial and staff resources necessary to meet study objectives and implement study requirements described in this QAPP.

3.1.2 OST Quality Assurance Officer and SHPD QA Coordinator

The OST Quality Assurance Officer is Marion Kelly, who will be responsible for reviewing and approving all Quality Assurance Project Plans (QAPPs). The SHPD Quality Assurance Coordinator is Robert Shippen, who will be responsible for reviewing and recommending approval of all QAPPs. Additional OST QA Officer and SHPD QA Coordinator responsibilities include the following:

- reviewing and evaluating field procedures,
- conducting external performance and system audits of the procedures, and
- participating in Agency QA reviews of the study.

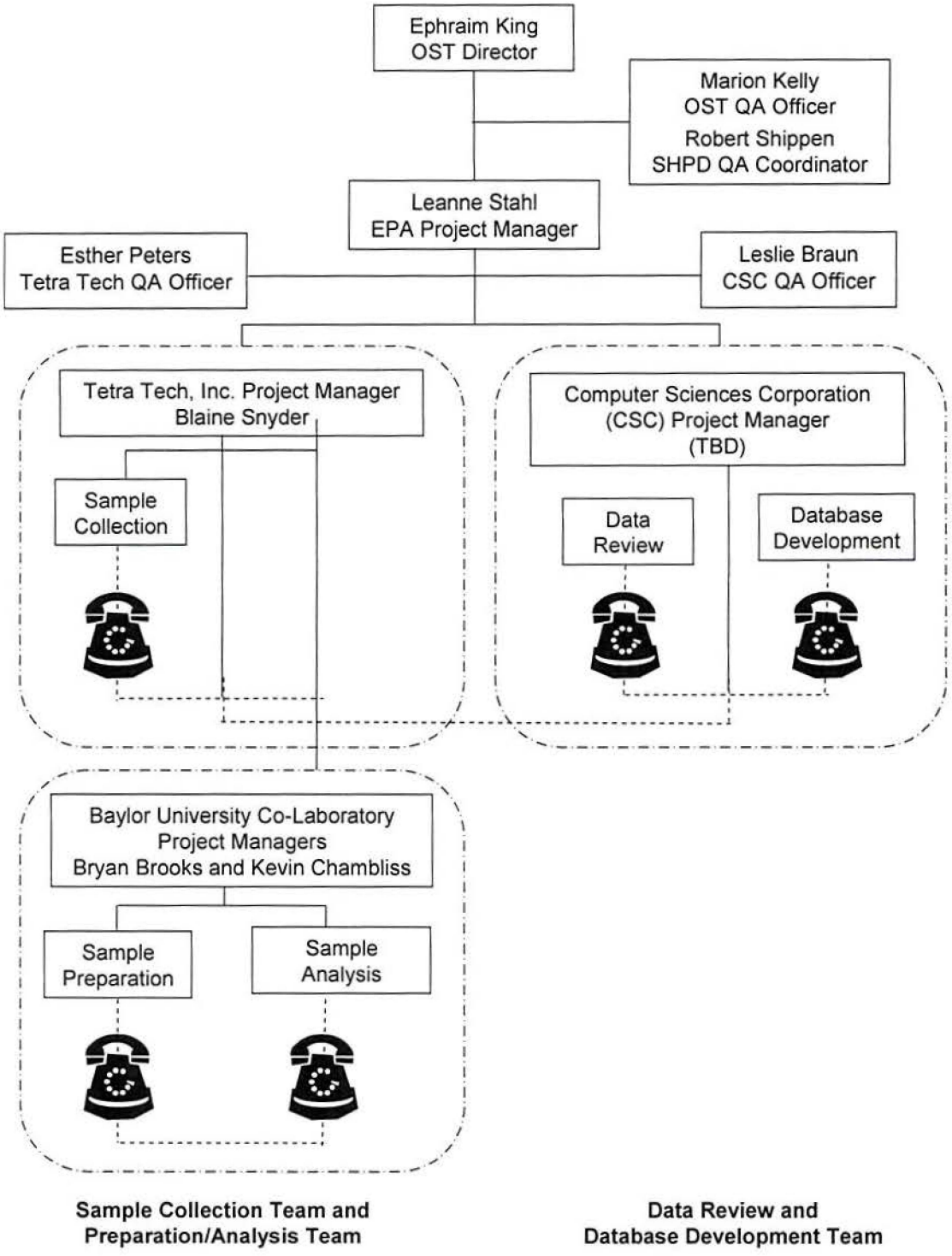

Figure 1. Project Organization and Relationships

3.1.3 EPA Project Manager

The EPA Project Manager, Leanne Stahl, reports to the OST Director and is responsible for providing overall direction concerning the study to the Tetra Tech and Computer Sciences Corporation Project Managers shown in Figure 1. The EPA Project Manager also is responsible for:

- Overseeing the development, approval, and implementation of QAPPs for all phases of the PPCP Fish Pilot Study.
- Communicating study objectives to the contract Project Managers shown in Figure 1.
- Reviewing and approving all major work products associated with the study.
- Providing oversight of all contractor activities related to collection and analysis of samples for this study.
- Participating in meetings with the contract Project Managers, other EPA staff, and staff from other organizations and contractors concerning the study.
- Working with QA staff to identify corrective actions necessary to ensure that study objectives are met.
- Reviewing and approving major deliverables related to the analysis of samples collected in this study.

3.2 Computer Sciences Corporation (CSC) Staff

3.2.1 CSC Project Manager

CSC's Project Manager, Erin Salo, is responsible for applying resources needed to ensure that CSC project deliverables are completed on time, within budget and to client satisfaction. Other responsibilities of the CSC Project Manager include:

- Working with the EPA Project Manager to address project requirements in this QAPP.
- Communicating project objectives to CSC staff.
- Ensuring that all QA procedures described in this QAPP related to data review and database development are followed during the study.
- Monitoring performance of CSC staff participating in this study to ensure the quality, timeliness, and responsiveness of work performed.
- Reviewing and approving CSC study deliverables for the analytical activities.
- Participating in meetings with EPA and/or CSC staff concerning study objectives, schedules, and concerns.
- Providing day-to-day oversight of technical activities performed by CSC staff participating in the study.
- Ensuring that all necessary corrective action procedures are documented and implemented in a timely manner.

3.2.2 CSC QA Manager

CSC's QA Manager, Leslie Braun, is independent of the PPCP Study. Ms. Braun is responsible for:

- Assisting CSC's Project Manager with the development and review of this QAPP.
- Overseeing the implementation of QA procedures related to CSC tasks that are described in this QAPP.
- Reporting deviations from this QAPP to the CSC Project Manager and assisting in implementing corrective actions to resolve these deviations.

3.3 Tetra Tech Staff

3.3.1 Tetra Tech Project Manager

Tetra Tech's Project Manager, Blaine Snyder, is responsible for applying resources needed to ensure that Tetra Tech project deliverables are completed on time, within budget and to client satisfaction. Other responsibilities of the Tetra Tech Project Manager include:

- Working with the EPA Project Manager to address project objectives and develop a project schedule.
- Communicating project objectives to Tetra Tech staff.
- Understanding and implementing the requirements described in the Laboratory Sample Preparation and Analysis Activities QAPP and the Sample Collection Activities QAPP.
- Monitoring performance of Tetra Tech staff participating in this study to ensure the quality, timeliness, and responsiveness of work performed.
- Monitoring performance of the contract laboratory participating in this study to ensure the quality, timeliness, and responsiveness of work performed.
- Participating in meetings with EPA and/or Tetra Tech staff concerning study objectives, schedules, and concerns.
- Providing day-to-day oversight of technical activities performed by Tetra Tech staff participating in the PPCP Fish Pilot Study.
- Ensuring that all necessary corrective action procedures are documented and implemented in a timely manner.

3.3.2 Tetra Tech Quality Assurance Officer

Tetra Tech's Quality Assurance Officer, Esther Peters, is responsible for:

- Implementing the QAPP describing the analytical activities for the PPCP Fish Pilot Study.
- Participating in meetings with EPA and/or Tetra Tech staff concerning study objectives, schedules, and concerns.
- Providing oversight of technical activities performed by the analytical laboratory or delegating this responsibility to a qualified Tetra Tech professional.
- Ensuring that all necessary corrective action procedures are documented and implemented in a timely manner.

3.4 Contract Laboratory

All sample preparation and chemical analyses in this study will be performed by an academic research team at Baylor University's Center for Reservoir and Aquatic Systems Research (Baylor). Detailed requirements for sample preparation, tissue analysis, and data management are described in Sections 10.0 and 16.0. Due to the complexity of this study, a Laboratory Project Manager will be available and dedicated to the project. Sections 3.4.1 and 3.4.2 below describe the responsibilities of each of these staff members.

3.4.1 Laboratory Project Manager

Bryan Brooks and Kevin Chambliss will assume the roles of co-Project Managers for Baylor University. They will be responsible for the overall technical laboratory activities under subcontract to Tetra Tech. These individuals will be responsible for planning, conducting, and supervising all laboratory activities to support the PPCP Fish Pilot Study.

3.4.2 Laboratory QA Manager

Kevin Chambliss will be responsible for quality assurance (QA) of all sample preparation and analysis activities performed under the laboratory subcontract. He will review progress, evaluate results, report problems, and implement corrective actions approved by Tetra Tech and EPA.

4.0 QUALITY OBJECTIVES

4.1 Project Quality Objectives

The PPCP Fish Pilot Study will allow EPA to obtain data on the occurrence of certain pharmaceuticals and personal care products in fish tissue. However, there are sources of uncertainty associated with the study, including the compositing and subsampling of fish samples and variability in the laboratory analysis process. The combined variability introduced by compositing fish samples, sub-sampling the composites for analysis, and laboratory analysis can be considered the "index" variability. Rather than prescribing a tolerable limit to "index" variability, the approach for this study is to:

(1) Prescribe sample collection procedures that would minimize "index" variability (e.g., through properly trained sampling crews and standardization of collection methods).
(2) Employ a sample compositing and sub-sampling scheme that will ensure low levels of variability.
(3) Select analytical methods capable of providing the best measurement performance (e.g., highly sensitive measurement systems with relatively low bias and acceptable precision).
(4) Require the analytical laboratory to satisfy certain performance criteria for the proposed analytical methods.

4.2 Measurement Quality Objectives

As mentioned in the previous section, "index" variability can be minimized by selecting analytical methods that provide the best available measurement performance, as gauged by standard data quality indicators (DQIs). The methods to be used in the PPCP Fish Pilot Study reflect state-of-the-art technology that should be able to meet certain performance criteria demonstrated to be attainable in well-operated, controlled laboratory environments. These criteria meet EPA's needs for data quality. Therefore, the general measurement quality objective for this study is to satisfy method-specific performance criteria. The DQIs in this plan are estimates based on available data during method demonstration, and Baylor's methods have been under continued refinement throughout the planning period. Therefore, it may be required (following analysis of actual study samples of fillet and liver tissues) to assess performance through development of control charts developed during the study period to gain a better understanding of measurement system performance. This assessment measure is included in the laboratory procedures to preserve data usability in the event of frequent outliers. The following subsections and Section 11 of this QAPP provide details on how standard DQIs will be monitored and controlled in this study.

4.2.1 Precision

Precision is the degree of agreement among replicate measurements of the same property, under prescribed similar conditions [3]. It can be expressed either as a range, a standard deviation, or a percentage of the mean of the measurements (e.g., relative range or relative standard deviation).

Ideally, precision is measured by subdividing samples in the field, preserving and numbering each split separately, and sending the aliquots to the analysis laboratory as 'blind' duplicates. In this study, however, samples must be homogenized, and composited in a strictly controlled, clean laboratory environment. Therefore,

the laboratory will prepare and analyze duplicate or duplicate spike samples to assess the index variability associated with the subsampling, extraction, and analytical portions of the measurement system. The study measurement quality objective (MQO) for analytical precision is that results from 90% of these duplicates agree within 50% for values greater than 5x the project quantitation limit (PQL) and that 90% of these duplicates agree within 100% for values less than 5x the PQL.

In addition to the use of these duplicates, the laboratory will employ a series of EPA Office of Water (OW) laboratory QC measures (e.g., MDL studies, laboratory control samples, and matrix spiked samples) that provide information about the precision associated with various components of the analytical process. For example, duplicate or spiked duplicate pairs will be prepared and analyzed to assess analytical precision from the sample measurement process. These QC elements and associated requirements are described in more detail in Section 11 (Quality Control Requirements) of this QAPP. It should be noted that performance criteria for this study are based on overall data quality, and failure to meet any single laboratory precision measure does not automatically imply the data are unacceptable for use in this study. Laboratory QC measures are used to monitor and control precision in real time so that overall precision goals are met. Details regarding the data quality assessment process governing use of data in this study are given in Sections 16, 17, and 19.

4.2.2 Bias

Bias is the systematic distortion of a measurement process that causes errors in one direction [3]. In this study, bias from the analytical process will be measured by preparing and analyzing laboratory-spiked field samples with 1) the chemicals of interest (i.e, matrix spike samples), 2) internal standards, and 3) surrogate chemicals that are expected to behave in a manner similar to the target chemicals. The measurement quality objective for overall analytical accuracy in this study is for 80% of the laboratory-spiked field sample results to fall within the acceptance criteria specified for each method.

In addition to the use of laboratory-spiked field samples, the laboratory will employ OW's laboratory QC measures (e.g., instrument calibration standards, method blanks, and laboratory control samples) that provide information about the bias associated with various components of the analytical process. These QC measures and associated requirements are described in more detail in Section 11 (Quality Control Requirements) of this QAPP. It should be noted that performance criteria for this study are based on overall data quality, and failure to meet any single measure of bias does not automatically imply the data are unacceptable for use in this study. Laboratory QC measures are used to monitor and control bias in real time so that overall precision goals are met. Details about the data quality assessment process governing use of data in this study are given in Sections 16, 17, and 19.

4.2.3 Accuracy

Accuracy is a measure of the closeness of an individual measurement or the average of a number of measurements to the true value. Accuracy includes a combination of random error (precision) and systematic error (bias) components that result from sampling and analytical operations. Accuracy is determined by analyzing a reference material of known pollutant concentrations or by reanalyzing a sample spiked with a known amount of pollutant. [3]

In this study, certified reference materials (CRMs), *when available,* will be sent to the laboratory annually to assess bias. CRM results will be pooled at the end of this study to determine overall study accuracy.

4.2.4 Sensitivity

Analytical sensitivity is defined as the minimum concentration of a chemical above which a data user can be reasonably confident that the chemical was reliably detected and quantified. For this study, the method detection limit (MDL) and the PQL will be used to define the sensitivity of each measurement process for

qualification purposes. Since both measurement methods employ mass spectral determinations, any positive sample result that yields identifiable spectra will be reported.

The MDL is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the chemical concentration is greater than zero. The EPA procedures that will be used for determining the MDL are described in 40 CFR 136, Appendix B. [4]

The PQL is defined as the lowest concentration at which the entire analytical system must give a recognizable signal and acceptable calibration point for a chemical. It is often equivalent to the concentrations of the lowest calibration standard analyzed by a specific analytical procedure, assuming that all the method-specified sample weights, volumes, and processing steps have been employed. For this study, additional calibration standards below the PQL will be included in the instrument calibration, however since spectral identification will be used in the analysis of the sample data, all positive sample results will be reported. The calibration standard range will only define the required qualification and the reporting limits for non-detected sample values.

Ideally, the analytical methods to be used in this study will have MDLs that are below all levels of concern for the target chemicals. The measurement quality objective (MQO) for detectability is that 100% of the samples be analyzed by the laboratory and reported down to the MDL-level. Again, since both measurement methods employ mass spectral determinations, any positive sample results will be reported, regardless of their relationship to the MDL or the PQL.

4.2.5 Representativeness

Representativeness is a measure of the degree to which data accurately and precisely represent a characteristic of a population parameter at a sampling point or for an environmental condition. It is a qualitative term that is evaluated to determine whether data appropriately reflect the media and phenomenon measured or studied [3]. This study was designed to provide general information on the occurrence of certain pharmaceuticals and personal care products in fish tissue. A description of this design is given in reference [1].

4.2.6 Completeness

Completeness is defined in terms of the percentage of data that are collected and deemed to be acceptable for use in the study. Three measures of completeness can be defined, as follows:

Sampling Completeness: The number of valid samples collected relative to the number of samples planned for collection;

Analytical Completeness: The number of valid sample measurements relative to the number of valid samples collected; and

Overall Completeness: The number of valid sample measurements relative to the number of samples planned for collection.

The analytical completeness goal in this study is to obtain valid measurements from 95% of the valid samples collected. In theory, however, a lower level of completeness can still lead to a valid study. The effects of insufficient completeness will be evaluated during the data analysis phase of this study.

4.2.7 Comparability

Comparability expresses the confidence that two data sets can contribute to a common analysis and interpolation [3]. The study will require the collection and analysis of numerous samples from various parts of the country. To ensure comparability of data generated during this study, EPA will:

- Employ standard operating procedures for sample collection [1],
- Maintain a consistent field sampling team leader [1],
- Use one laboratory for the preparation (weighing, compositing, homogenizing, and dividing tissue into aliquots) and analysis of samples (i.e., Baylor University),
- Use one method for all analyses of a specific target chemical (Table 1),
- Specify method detection limits and QC acceptance criteria that must be met throughout the study (Appendix A),
- Specify data reporting units and analytical procedures that must be used throughout the study (Section 10.0), and
- Use a standardized data quality assessment process (Section 19.0).

5.0 SPECIAL TRAINING REQUIREMENTS

Each Field Sampling Team is required to have the necessary knowledge and experience to perform all field activities. This includes both knowledge and experience in the collection and identification of fishes and in the use of fisheries sampling gear specified for the study. It also includes training in project-specific sample collection and handling procedures. The field sampling crews will be composed of contracted biologists with a strong technical background in fisheries sampling activities. Each Field Sampling Team will consist of one experienced fisheries biologist (that must have experience with the array of fisheries sampling gear types to be used) and field staff to assist with sample collection and processing. At some sites, the contracted biologists may enlist the aid of WWTP staff, state fisheries biologists, or other local personnel to provide logistical support and assist with sample collection. In these cases, each participant will attend an on-site training session led by the Tetra Tech Task Leader (an experienced fisheries biologist).

The laboratory project team will be comprised of senior research associates, complimented with enough technical staff to ensure sufficient oversight and supervision, as well as adequate skills to maintain consistent measurement system performance throughout the study. Required "skills" will include education and experience in using the procedures and instruments employed in this study.

Because the EPA National Lake Fish Tissue Study (NLFTS) standard data review approach will be implemented during this study (and customized to meet study needs), all CSC staff responsible for reviewing data must be experienced in performing data reviews, trained to review data in accordance with NLFTS's general data review guidelines, experienced in reviewing data generated with the instrumentation that will be used in this study, and familiar with the performance criteria and MQOs established for this study. Each reviewer also must have read and understood the performance criteria and MQOs applicable to this study.

6.0 DOCUMENTATION AND RECORDS

Only documentation and records relevant to sample preparation, analysis and data review are discussed in this section. Documentation and records related to sample collection can be found in reference [1].

6.1 Sample Preparation Records

The laboratory will document sample preparation information in notebooks maintained by laboratory staff. Documentation related to sample preparation includes:

- Copies of the Chain-of-Custody Forms documenting shipment of samples from the field. (Chain-of-Custody Forms are standardized, EPA-generated sample tracking forms.) [1]
- Documentation of sample storage conditions (daily temperature records for sub-zero storage).
- Documentation of daily balance verification.
- Documentation of standard and reagent preparation, including material suppliers, lots, and purity; amount used (mass or volume) and final volume; indemnification of solvent or diluent; final concentration (where applicable for standards); expiration date (considering the shelf life of all intermediate and precursor solutions); and the preparer's initials and date of preparation.
- Standard operating procedure [5] for sample preparation, compositing, homogenization and sub-sampling.
- Sample preparation records documenting the following information for each composite sample prepared:
  - Identification (initials/date) of the person preparing composite samples (filleting, dissecting, homogenizing, and preparing aliquots);
  - Verification of information about individual fish included in samples used to prepare the composite (i.e., weight and length);
  - Nine-digit EPA composite number (as assigned by Tetra Tech; see Section 6.3, Data Compilation, Review, and Validation Records)

For each analytical fraction (LC/MS/MS, GC/MS, and lipids), documentation of the following sample preparation procedures:

- Sample mass used in analysis,
- Extraction date,
- Surrogate and matrix spiking solution identification and volumes used,
- Reagent identification and volumes (minimally initial and final volumes, unless the laboratory identifies reasonable benchmarks or procedural increments which may assist in troubleshooting in the event of measurement system failure), and
- Relevant observation made during sample preparation.

6.2 Analytical Records

Baylor will at a minimum be required to do the following with respect to documentation and record keeping:

- Maintain daily records of storage condition for samples from the pilot study (-20°C) throughout sample analysis and storage.
- Submit summary reports of all analytical results. These summary reports must be provided in both hard copy and electronic format.
- Submit hard copies of all raw data. Raw data will include items such as quantitation reports, strip charts, spectra, bench sheets, and laboratory notebooks showing tare and sample weights, and sample volumes. Raw data also will include any other information that would allow an independent reviewer to verify the calculations performed and trace the final results to the raw data. The laboratory will be required to clearly identify each data element in their data package.
- Submit a written report that details any problems encountered during analysis of the samples. The written report also should include comments on the performance of any part of a method.
- Obtain pre-approval of any modifications to the analytical techniques specified and submit detailed explanations of the changes implemented.

- Report results consistently in the reporting units (e.g., ng/kg or percent) specified in the method (Section 10.0).
- Submit chain-of-custody and other sample tracking information.

6.3 Data Compilation, Review and Validation Records

Tetra Tech and CSC will create and maintain study files that document data compilation and review, which will include separate files for each "episode" (sampling event/site). The field sampling teams will be using a nine-digit field-assigned composite sample ID number to uniquely identify each composite. Tetra Tech will provide the laboratory with a series of five-digit EPA sample numbers that will be assigned to each sample after compositing and aliquotting. The nine-digit fish composite sample identification code will include:

- State of collection (2 character abbreviation)
- Year of collection (2 number abbreviation)
- Site identification (3 character code from Appendix B)
- Composite number (1 through 6)
- Tissue fraction to be completed by laboratory ("L" for liver or "F" for fillet)

Tetra Tech will prepare and maintain the following PPCP Fish Pilot Study records:

- A copy of this QAPP and the sample collection activity QAPP;
- A copy of the laboratory statement of work;
- A summary page that documents the Episode Number, the sample numbers assigned to the Episode, and the date of sample collection and shipment;
- The name, address, phone number and primary contact of the laboratory preparing and analyzing samples in the episode;
- A copy of each Chain-of-Custody Form prepared and sent with each sample;
- A list that cross-references the composite sample identification number assigned to each sample by the sample collection team against the five-digit EPA sample numbers assigned by the laboratory after compositing, homogenizing, and aliquotting;
- A log of all verbal communication with laboratory staff, sampling personnel, and EPA staff regarding the status or problems with the particular Episode/samples;
- Copies of all written correspondence with laboratory staff, sampling personnel, and EPA staff regarding the status or problems with the particular Episode/samples; and
- All records submitted by the laboratory.

CSC will develop and maintain the following PPCP Fish Pilot Study records:

- Complete records regarding the data review process, including a final copy of any written data review assessments and the final data submission from each laboratory; and
- A database of final analytical results associated with each field sample.

CSC will provide copies of any data review assessments (if necessary) and a copy of the final database to EPA (and other appropriate stakeholders) after the data reviews are complete. Tetra Tech will retain the master file containing each episode file, complete copies of each laboratory data submission (including the final laboratory summary reports), and other records listed above. Tetra Tech will provide copies of these materials on an as-needed basis to EPA upon request.

All documents and records prepared for this project will be maintained by Tetra Tech and CSC during the project and retained for a period of three years following completion of the project. EPA will follow federal requirements for retention of project records described online at www.epa.gov/records/.

7.0 SAMPLING PROCESS DESIGN

The objective of the PPCP Fish Pilot Study is to investigate the occurrence of a broad suite of PPCPs (39 chemicals) in the tissue of harvestable sized adult freshwater fish that are typically consumed by wildlife and humans. In so doing, the study will provide the following types of information:

- the potential for the target PPCP chemicals to bioaccumulate in fish muscle and liver tissue, and
- data to answer questions concerning the occurrence of these chemicals in fish and the potential for human exposure through fish consumption.

For the purposes of this study design, the target population will be effluent-dominated streams associated with WWTPs within the contiguous United States. The streams in this study must have a viable fish population of a resident species which will spend most of its life stages within the effluent-dominated waters. A total of five locations will be sampled, plus one reference site.

7.1 Sample Type

To meet the study objectives, the PPCP Fish Pilot Study will include composite sampling of fish fillets and fish livers from each sample site. Six composite samples will be collected at each site. At least three adult individuals will be collected per composite such that the combined biomass of the specimens will be adequate to provide sufficient tissue for analysis of the group of target chemicals. It has been determined that at least 30 grams of edible fillet tissue and 10 grams of liver tissue will be required from the composites to allow for analysis of all target chemicals. Based on the recommendations of USEPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1: Fish Sampling and Analysis, Third Edition* (USEPA 2000) [5], fish used in a composite sample must meet the following criteria:

- all be of the same species,
- satisfy any legal requirements of harvestable size or weight, or at least be of consumable size if no legal harvest requirements are in effect,
- be of similar size so that the smallest individual in a composite is no less than 75% of the total length of the largest individual,
- be collected at the same time (i.e., collected as close to the same time as possible but no more than 1 week apart) [**Note:** This assumes that a sampling crew was unable to collect all fish needed to prepare the composite sample on the same day. If organisms used in the same composite are collected on different days (no more than 1 week apart), individual fish will be frozen until all the fish to be included in the composite are available for delivery to the laboratory.], and
- be collected in sufficient numbers (at least 3 per composite) and of adequate size (at least 3 harvestable size adult specimens that collectively will provide greater than 30 grams of edible tissue and 10 grams of liver tissue) to allow analysis of recommended target chemicals.

Individual organisms used in composite samples must be of the same species because of notable differences in the species-specific bioaccumulation potential. Accurate taxonomic identification is essential in preventing the mixing of closely related species with the target species. Under no circumstance should individuals from different species be used in a composite sample.

Fish for this project are being sampled from wastewater treatment plant effluent-dominated streams. Reconnaissance may indicate that appropriate fish are available at a site, but it is possible that inadequate numbers of target species meeting the sample criteria will be found when the site is sampled. If this situation were to occur, the Field Sampling Leader will contact the USEPA Project Manager to discuss possible options, which

include collecting a different size or species of fish, sampling a site farther downstream, or sampling an alternate location.

7.2 Sampling Period

Field sampling will be conducted during the period when water and weather conditions are conducive to safe and efficient field sampling. For this study, the sampling period is from summer to early fall, since lipid content is usually highest and water levels are usually lowest at that time. Where possible, sampling should not occur during the spawning period of the particular target species being sought. With these recommendations in mind, and considering the geographic extent of the study area (i.e., range of latitudes and longitudes) the field sampling period will begin in August and last through November. Any adjustments to this schedule must be approved by the USEPA Project Manager.

7.3 Sample Frame

For the purposes of this study, the target population will be effluent-dominated streams which serve as receiving waters for WWTPs within the contiguous United States. WWTPs using primary, secondary, and tertiary treatment methods and discharging to a stream or river are included in the sample frame. The streams in this study must also have a viable fish population of a resident species that is subject to effluent exposure for most of its life cycle.

7.4 Selection of Sampling Sites

Sites were targeted (Appendix B) in mid- to large-sized cities representing diverse geographic regions of the country. Information on WWTP design capacity, average discharge, and in-stream waste concentration was collected for each candidate site through research of publicly accessible data (i.e., NPDES permits, WWTP websites, and USGS flow data) and through phone calls to state officials and permitting agencies. Once this information was compiled, the list of candidate sites was used by the EPA project team to select a group of priority sites. The site selection criteria that were used are:

- High effluent flow versus ambient flow
- High population density
- Large fraction of elderly residents
- Large volume of PPCP sales/consumption (higher income brackets as surrogate)
- Fish availability

In addition, fisheries information was compiled for each candidate site. This was accomplished by reviewing published fisheries reports and obtaining first-hand information from state fisheries personnel. The site list was further narrowed down to 12 priority sites which could potentially support (via availability of resident species and tissue biomass) the intended sampling. The five top priority sites were selected from the 12 priority candidates to represent diverse geographic regions of the country.

8.0 SAMPLING METHOD REQUIREMENTS

Sampling method procedures and requirements are detailed in the Sample Collection Activities QAPP [1]. Some of the key requirements are summarized below.

8.1 Target Species

A single species of fish will be collected from each site. Suggested target species are listed in the Sample Collection Activities QAPP (Table 3) in order of preference (adapted from [5]). Additional target species may be added to the list of preferred targets on an as-needed basis, following discussion with the USEPA Project Manager and/or the Tetra Tech Project Manager. For a detailed description of target species criteria, refer to the Sample Collection Activities QAPP [1].

8.2 Composite Sampling

The PPCP Fish Pilot Study will involve composite sampling of fish. Composite samples are cost-effective for estimating average tissue concentrations of target chemicals in target species populations, and compositing ensures adequate sample mass for analysis of all target chemicals. Six single-species composites will be collected from each target stream. Each composite will consist of at least three fish of adequate size (i.e., adult specimens that collectively will provide at least 30 grams of edible tissue and 10 grams of liver tissue) to allow analysis of the target chemicals. Fish retained for a composite sample must meet the criteria listed in Section 7.1. For a more detailed description of composite samples, refer to the Sample Collection Activities QAPP [1].

8.3 Sample Collection

The field objective is for sampling teams to obtain six representative composite samples from each stream selected for the PPCP Fish Pilot Study. Prior to sampling, field teams will determine habitats suitable for target species, then sample those habitats in the stream reach located downstream from the WWTP outfall. Sampling teams will be equipped with an array of both active and passive gears to ensure the collection of the desired target numbers and species of fish. Selection of the most appropriate gear type(s) for a particular target stream will be at the discretion of the experienced on-site fisheries biologist. For a detailed description of sample collection methods, refer to the Sample Collection Activities QAPP [1].

9.0 SAMPLE HANDLING AND CUSTODY REQUIREMENTS

Chain-of-Custody Forms will be used for sample documentation and tracking from the field to the sample preparation and analysis laboratory. Detailed sample handling and custody requirements are described in Sections 9.1 and 9.2.

Field personnel are responsible for properly identifying and handling the samples as described in Section 9.1. Laboratory personnel are responsible for receiving and preparing the samples as described in Section 9.2. All parties involved in sample handling and preparation are responsible for using protocols designed to preclude contamination.

9.1 Field Requirements

A more comprehensive description of field sample handling requirements can be found in the Sample Collection Activities QAPP [1]. Key requirements are summarized below.

Species should be identified by experienced personnel as soon as fish are removed from the collection device. Non-target species or specimens of target species that do not meet size requirements will be returned to the water. Individuals of the selected target species will be rinsed in distilled water to remove any foreign material from the external surface. Each fish within the selected target species will be measured and weighed to determine total body length (mm) and total body mass (g). After initial processing, each fish found to be suitable for the composite sample will be assigned a specimen number that can range from one to three (or four). This number

will identify each fish within its respective composite. A nine-character composite sample identification number consisting of the two-character state abbreviation, two-number year abbreviation, three-letter site identification code, composite number (1-6), and tissue type ("F" or "L" for fillet or liver) will be assigned by the field teams for each composite collected. The composite sample identification number and information about individual fish specimens will be recorded on Field Record Forms.

Each fish selected for the composite sample will be individually wrapped in cleaned (rinsed in methylene chloride and dried at 450°C for a minimum of one hour) extra heavy duty aluminum foil. Each individually wrapped fish will be placed into food-grade plastic tubing and sealed on each end with a nylon cable tie. The fish sample identification label containing the specimen number, composite sample identification number, stream or river, and date of collection will be attached to the outside of each sample using one of the nylon cable ties used to seal the plastic tubing. All of the foil-wrapped and plastic tubing-sealed specimens intended for a composite sample will be kept together when possible in a large plastic bag in the same shipping container (ice chest) for transport.

Once packaged, samples should be placed on dry ice for shipment. The sampling personnel must ship the samples with enough dry ice to ensure temperatures of <-20°C during shipment. Dry ice sublimes at a rate of approximately ½ lb per hour. Therefore, a minimum of 36 lbs of dry ice is recommended to ensure that the fish remain frozen for at least a 48-hour period, in case of shipping problems. If space, funds, and logistics permit, 50 lbs of dry ice is preferred.

Samples will be shipped via Federal Express, using priority overnight service. In addition, a member of the field staff should telephone the laboratory to alert them about the anticipated delivery time of the samples. Field collection staff should avoid shipping samples for weekend delivery to the laboratory unless prior plans for such a delivery have been agreed upon with the laboratory. The field sampling team will ship one copy of the Field Record Form to the laboratory. Upon arrival of the samples, the laboratory will contact Tetra Tech to confirm that the samples are in good condition.

9.2 Laboratory Requirements

Upon receipt of the fish samples, the laboratory will record the arrival time on the Chain-of-Custody Form. The laboratory will document any observations regarding the shipment (e.g., torn or damaged packaging and/or evidence of spoilage) on the Chain-of-Custody Form, as well as on the sample preparation records.

The laboratory will decontaminate any filleting instruments and surfaces as appropriate. Laboratory staff will measure and weigh each fish, rinse the fish with distilled water, remove scales, fillet (including skin and belly flap), remove (and weigh) the liver from each specimen, composite tissue from all specimens for each tissue fraction, and homogenize the tissue following the procedures specified in *U.S. Environmental Protection Agency (USEPA 2000) Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1: Fish Sampling and Analysis*, Third Edition. (EPA 823-B-00-007) [5]. All samples will be composited using the "batch" method, in which like tissue from all of the individual specimens that comprise a sample are homogenized together, regardless of each individual's proportion to one another (as opposed to the "individual" method, in which equal weights of each specimen are added together).

After compositing, the laboratory will prepare the number of aliquots from each composite sample as needed and appropriate for PPCP Fish Pilot Study analytical methods and QA procedures. Each aliquot will be placed in an appropriately pre-cleaned glass jar with a FEP-lined cap. The laboratory will label each sample container with the nine-digit composite number and the five-digit EPA sample number (both assigned by Tetra Tech) and appropriately store the samples at -20°C until analysis. The laboratory will store any leftover composite sample in a solvent-rinsed glass jar labeled with the nine-digit composite number and sealed with a

foil-lined plastic cap labeled with the nine-digit composite number. The sample must be maintained at -20°C until archival in a sample repository designated by EPA.

10.0 ANALYTICAL METHODS REQUIREMENTS

As indicated in Table 1, the target chemicals can be measured using HPLC-MS/MS and GC-MS methodologies. Since no established EPA methods exist for the target chemicals, they will be measured using current literature techniques modified to include many of the EPA 600-series and SW846 QA/QC elements (see Section 11.0). Sections 10.1 and 10.2 summarize the methodologies that EPA expects to use in the PPCP Fish Pilot Study.

10.1 PPCPs by HPLC-MS/MS

Baylor's current method for determination of select pharmaceuticals in fish tissue by High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS) includes sample extraction and concentration, followed by analysis by reverse phase HPLC-MS/MS for detection and quantitative measurement of target chemicals. Due to significant matrix effects for a number of chemicals, external calibration is not appropriate for quantitative determinations using this method. Instead, standards are prepared by spiking known concentrations of the target chemicals into 'clean' fish tissue, and all standards are subjected to the entire sample preparation procedure prior to analysis. Baylor's method was developed and has been used to support aquatic systems and resources research and investigations; however, the method continues to evolve in its use and practical application. During the course of this pilot investigation, Baylor will be developing a detailed method SOP that will follow the basic requirements described in *Guidelines and Format for Methods to be Proposed at 40 CFR Parts 136 or Part 141, EPA 821-D-96-003,* July 1996 and the description in *EPA's Guidance for Preparing Standard Operating Procedures (SOPs) EPA QA/G-6, EPA/240/B-01/004*, March 2001. The final method SOPs will include any requirements for specialized equipment maintenance or corrective action beyond those described in the laboratory's routine procedures and instrument manufacturers' instructions which may arise due to the unique analyte and matrix combination measurements being performed.

10.1.1 Reference Tissue Specimens

Until such time as the PPCP Fish Pilot Study sampling program is able to supplement supplies, Baylor will continue to use their own control matrix supply. Fish from the genus *Lepomis* were collected from Clear Creek (Denton, TX, USA) to serve as control samples in previous work, and remain Baylor's source of control matrix. Clear Creek is not impacted by effluent discharges and is routinely used as a local reference stream by the City of Denton, Texas Watershed Protection program.

10.1.2 Sample Preparation

All tissue specimens are prepared and extracted using the following procedures. Muscle and liver tissues are dissected, composited, and homogenized using standard procedures described in USEPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume I: Fish Sampling and Analysis, Third Edition* (USEPA 2000). Care must be taken during homogenization to ensure the sample is uniformly mixed and to ensure minimal loss of tissue mass. Following homogenization, approximately 1.0 g of control tissue homogenate is prepared for each calibration standard, blank and blank spike, along with 1.0 g of fish sample homogenate for each of the samples and sample QC (duplicates and spikes). Calibration standards are spiked with multiple levels of target chemicals and surrogates to facilitate multipoint calibration for each of the target chemicals and for the laboratory QC spikes. Each sample and QC aliquot is also spiked with a known amount of surrogate spike, and laboratory blank spikes and spiked samples are further fortified with target chemicals. The spiked blank is at the project quantitation limit, while the spiked sample is equivalent to the upper third of the calibration range. Internal standards 7-aminoflunitrazapam-D7 (ESI+), fluoxetine-D6 (ESI+) and ibuprofen-

propionic 13C3 (ESI−) are added to each field, QC, and calibration sample. Calibration samples and matrix spikes are also prepared by spiking the mixture with variable amounts of pharmaceutical standards.

Samples and standards are then combined with 4 mL extraction solvent (1:1 mixture of 0.1 M aqueous acetic acid and methanol) in 20 mL borosilicate glass vials, shaken vigorously, and mixed on a rotary extractor for five minutes. Following extraction, 4 mL extraction solvent is used to quantitatively transfer residues (with rinsing) into individual 50 mL centrifuge tubes. The samples, calibration standards, and QC samples are then centrifuged at 16,000 rpm for 40 min at 4 °C, the supernatant transferred into clean glass culture tubes, and the solvent is evaporated to dryness under a stream of nitrogen at 45 °C. Samples are reconstituted in 0.5mL of water, sonicated for 1 min, and filtered prior to analysis.

10.1.3 HPLC-MS/MS Analysis

Baylor uses a Varian ProStar Model 210 binary pump system and a Model 410 autosampler for all analyses. For each calibration standard, QC sample, and field sample, 10 µl are injected onto an Extend-C18 (Agilent Technologies, Palo Alto, CA) guard cartridge measuring 12.5 mm x 2.1 mm (5 µm, 80 Å), which is serially connected to a 15 cm × 2.1 mm (5 µm, 80 Å) Extend-C18 column for separation using a non-linear gradient program of 0.1% (v/v) formic acid in water and 100% methanol at 350 µL/min and 30 °C. The gradient profile yields chromatographic conditions suitable for separation of the 24 target chemicals in approximately 50 minutes. Eluted chemicals are monitored by MS/MS using a Varian model 1200L triplequadrupole mass analyzer equipped with an electrospray interface (ESI).

To determine the best ionization mode (ESI + or −) and optimal MS/MS parameters for target analytes, each drug was independently infused into the mass spectrometer as a 1 µg/mL solution in 0.1% (v/v) formic acid at a flow rate of 10 µL/min. All analytes were initially tested using both positive and negative ionization modes while the first quadrupole was scanned from *m/z* 50 to [M + 100]. This enabled identification of the optimal source polarity and most intense precursor ion for each compound. Once these parameters were defined, the collision energy at the second quadrupole was varied, while the third quadrupole was scanned to identify and optimize the most efficient MS/MS transition for each compound. Additional instrumental parameters held constant for all analytes were as follows: nebulizing gas, N_2 at 60 psi; drying gas, N_2 at 19 psi; temperature, 300 °C; needle voltage, 5000 V; declustering potential, 40 V; collision gas, argon at 2.0 mTorr.

The resultant retention time profile and identification of precursor and quantitation ions are indicated in Table 2. Quantitation and qualifier ions were selected based on full-scan analyses of commercial standards and typically represent the most abundant fragment ions for each compound. Sample analyses are evaluated using an internal standard quantitation method. Internal standard calibration assesses the response of a target chemical in the reference material against the response of an internal standard solution added prior to analysis.

10.2 PPCPs by GC-MS SIM

Baylor's *Determination of Select Personal Care Products in Fish Tissue by Gas Chromatography- Mass Spectrometry (GC-MS)* provides procedures for sample extraction, clean-up, and concentration, as well as gas chromatographic conditions for detection and quantitative measurement of target chemicals. While matrix effects are commonly encountered in the analysis of fish tissues, experimentally derived data did not demonstrate a clear advantage toward the extraction of standards. Therefore, this method includes application of more conventional calibration and quantitation techniques than does the LC-MS/MS method. However, the preparation for the GC-MS method includes silica gel clean-up and derivitization to facilitate analyses. Baylor's method was developed and has been used to support aquatic systems and resources research and investigations; however, the method continues to evolve in its use and practical application. During the course of this pilot investigation, Baylor will be developing a detailed method SOP that will follow the basic requirements described in *Guidelines and Format for Methods to be Proposed at 40 CFR Parts 136 or Part 141, EPA 821-D-96-003,* July 1996 and the description

in *EPA's Guidance for Preparing Standard Operating Procedures (SOPs) EPA QA/G-6, EPA/240/B-01/004*, March 2001. The final method SOPs will include any requirements for specialized equipment maintenance or corrective action beyond those described in the laboratory's routine procedures and instrument manufacturers' instructions which may arise due to the unique analyte and matrix combination measurements being performed.

10.2.1 Sample Preparation

All tissue specimens are prepared and extracted using the following procedures. Muscle and liver tissues are dissected, composited, and homogenized using standard procedures described in USEPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume I: Fish Sampling and Analysis, Third Edition* (USEPA 2000). Care must be taken during homogenization to ensure the sample is uniformly mixed and to ensure minimal loss of tissue mass. Following homogenization, approximately 1.0 g of control tissue homogenate is prepared for blank and blank spike, along with 1.0 g of fish sample homogenate for each of the samples and sample QC (duplicates and spikes). Each sample and QC aliquot is then spiked with a known amount of surrogate spike solution, containing 2 deuterated target chemicals (benzophenone and p-N-nonylphenol) and three other chemicals which distribute throughout the retention time profile, and laboratory blank spikes and spiked samples are further fortified with target chemicals. The spiked blank is at the project quantitation limit, while the spiked sample is equivalent to the upper third of the calibration range.

Samples are subsequently combined with a 10 mL aliquot of acetone and shaken vigorously for 5 minutes. The sample residues are then quantitatively transferred (with rinsing) to a 50 mL centrifuge tube and centrifuged at 16,000 rpm for 40 minutes at 4 °C. Each of the sample supernatants are then transferred into a clean culture tube, and evaporated to dryness under a stream of dry nitrogen at 35 °C. Evaporated samples are reconstituted in ca. 200 µL acetone and loaded onto a silica gel column that has been preconditioned with 8 mL hexane:acetone (65:35). The target chemicals are subsequently eluted from the column with 10 mL of the hexane:acetone mixture, and the volume of the collected eluate is reduced to ca. 200 µL under nitrogen. At this point, 100 µL of derivatizing agent (*N*-methyl-*N* (trimethylsilyl)-trifluoroacetamide) is added to each field sample, blank, and QC extract, and the mixture is heated at 60 °C for 45 minutes. Samples are evaporated to dryness and 100 ng Mirex is added as an internal standard. Samples are reconstituted in 180 µL hexane and 20 µL acetone and immediately analyzed.

Table 2 - Retention Times and Ions Specified for Selected Ion Monitoring of PPCPs by HPLC-MS/MS – Baylor University

Chemical	RT (min)	Precursor ion	Quantification ion
Acetaminophen	5.4	152	110
Atenolol	6.1	267	145
Cimetidine	6.2	253	159
Codeine	7.9	300	165
1,7-dimethylxanthine	9.5	181	124
Lincomycin	11.2	407	359
Trimethoprim	12.5	291	261
Thiabedazole	12.5	202	175
Caffeine	14.6	195	138
Sulfamethoxazole	18.4	254	156
Metoprolol	19.5	268	191
Propranolol	24.0	260	116
Diltiazem	26.9	415	178
Carbamazepine	29.6	237	194
Tylosin	31.4	916	174
Fluoxetine	32.7	310	148
Norfluoxetine	32.9	296	134

Sertraline	34.7	306	275
Erythromycin	37.2	716	558
Clofibric Acid	38.6	-309	163
Warfarin	40.4	417	161
Miconazole	41.7	-213	127
Ibuprofen	44.8	-205	161
Gemfibrozil	46.7	-249	121

10.2.2 GC-MS Analysis

Chemicals are separated and measured using a Varian Model CP3800 gas chromatograph interfaced with a Varian Model 1200 triple-quadrupole mass spectrometer. Chemicals are separated on a 30 m × 0.25 mm × 0.25 um XTI-5 capillary column using a temperature gradient from 100-180 °C at 15 °C/min. The temperature is then raised to 290°C at 6 °C/min, and the final temperature is held for 6 min with helium as the carrier gas at approximately 1.0 mL/min. The spectrometer is operated in selected ion monitoring (SIM) mode, and all chemicals are ionized at 250 °C with an electron impact source operated at a potential of 70 eV. The chromatographic conditions result in the following retention time profile, with the identification and quantitation ions identified in Table 3. Quantitation and qualifier ions were selected based on full-scan analyses of commercial standards and typically represent the most abundant fragment ions for each compound. Additional instrumental parameters held constant for all analytes are as follows: mobile phase/flow rate, He at 1.0 mL/min; injector temperature, 275 °C; injection volume, 1 μL; split ratio, 20:1; transfer line temperature, 280 °C; and source temperature, 250 °C.

Table 3 - Retention Times and Ions Specified for Selected Ion Monitoring of PPCPs by GC-MS SIM – Baylor University

Chemical	RT (min)	Qualifier ions	Quantification ion
m-Toluamide	6.79	91, 190	119
Benzophenone	7.59	77, 105	182
p-Octylphenol	10.74	165, 180	278
Galaxolide	11.69	213, 258	243
Tonalide	11.93	201	243
Musk xylene	11.94	297	282
p-Nonylphenol	12.92	149, 179	292
Musk ketone	16.32	217, 265	261
Triclosan	17.09	345, 362	200
Octocrylene	25.05	177, 249	361
Nonylphenol monoethoxylate isomer 1	15.97	120, 207	265
Nonylphenol monoethoxylate isomer 2	16.53	207, 265	251
Nonylphenol monoethoxylate isomer 3	16.73	149, 221	265
Celestolide	8.81	173, 244	229
4-MBC	15.76	115, 211	254

10.3 Lipids in fish tissues

Baylor's method for determination of lipids follows those specific procedures described in reference [1] for the gravimetric determination of lipids in fish tissue. The procedure includes extraction of a known mass of fish tissue (fillet or liver) with methylene chloride, filtration of the solvent/tissue mixture, and drying of the

solvent in a tared vessel to determine the mass of lipid residue remaining. The amount of residue divided by the initial sample mass is the percent lipid.

11.0 QUALITY CONTROL REQUIREMENTS

Data quality is addressed, in part, by consistent performance of valid procedures established in laboratory methods (Section 10.0). It is enhanced by the training and experience of project staff (Section 5.0) and documentation of project activities (Section 6.0). This QAPP and other supporting materials will be distributed to all project personnel. The Laboratory QC Officer(s) will ensure that all analytical data and sample results are reviewed, calculations verified, and that report compilations are free from transcription errors. The laboratory manager shall certify results in the analytical narrative along with descriptions of any areas of departure from the laboratory methods or the requirements of the project QAPP.

In preparation for use of Baylor University methods, Baylor defined and Tetra Tech approved a minimum set of standard QC elements, including development of experimentally derived MDLs, initial and continuing calibration acceptance criteria, spiking levels and acceptance criteria (percent recovery) for laboratory-spiked control samples (LCS) and field samples (MS/MSD), precision acceptance criteria for duplicate or laboratory-spiked duplicate (MS/MSD) samples, and surrogate spikes and acceptance criteria based on the requirements in EPA 600 series methods, and those presented in SW846 guidance. These QC elements will be used as the basis for assessment of all contaminant analyses performed in this study, and they include the following:

Procedural requirements:

- Use of pure and traceable reference standards.

- Demonstration of instrument calibration and system performance. For this study, a minimum of five concentrations of calibration standards will be prepared (extracted for LC-MS/MS) and analyzed in advance of sample analyses. Internal standard calibration will be used in both methods. Internal standard calibration assesses the response of a target chemical in the reference material against the response of an internal standard added just prior to analysis. Internal standard calibrations are used to generate relative response factors (RRFs) which are used to evaluate calibration curve linearity and subsequently, to calculate sample concentrations. Following is the formula to be employed in internal standard calibration evaluations and in evaluation of continuing calibration results.

  Relative Response Factor (RRF)

$$RRF = \frac{R_x}{R_{is}} x \frac{A_{is}}{A_x}$$

  where R_x is the response of the target chemical and R_{is} is the response of the internal standard, and A_{is} is the amount (or concentration) of the internal standard and A_x is the concentration of the target chemical in the calibration standard.

  Calibration curve RRFs are then be plotted to assess linearity. Linearity of calibration is expressed in percent relative standard deviation.

Percent Relative Standard Deviation (%RSD)

$$\%RSD = \frac{s}{\bar{\chi}} \times 100$$

where s is the standard deviation, and $\bar{\chi}$ is the average concentration of the replicate samples, and standard deviation is defined through the following:

Standard Deviation

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(\chi_i - \bar{\chi})^2}{n-1}}$$

where χ_i is the measured value of the replicate, $\bar{\chi}$ is the mean of the measured values, and n is the number of replicates. In most analytical methods, an acceptable %RSD qualifies the curve for use of the average response, calibration, or relative response factors, depending on the method of quantitation. Alternatively a curve must be plotted, and results generated against the calibration plot. For this study <30%RSD will constitute a linear response, and the average RRF can be used for sample calculations. Alternatively, a plot of the linear regression curve can be used for sample analyses.

- Periodic calibration verification. Calibration verification criteria are generally expressed as an acceptable percent difference (%D), which is calculated from either concentration, calibration, or response factors, or from the relative response factor (RRF). It is calculated as follows:

$$\%D_{amt} = \frac{(A_{obs} - A_{nom})}{A_{nom}} x100\%$$

where A_{obs} is the amount observed and A_{nom} is the nominal (known) amount of the standard, calculated as a direct percent difference in concentration. Results of $\leq 25\%$ difference will demonstrate acceptable calibration for continued sample analysis.

Sensitivity requirement:

- Verification that the laboratory can achieve required MDLs and PQLs. With each analytical batch, the laboratory will provide evidence of method performance at the project quantitation limit. Data qualification will be based on performance of the LCS and on the sample concentrations observed relative to the calibrated linear range. The pilot study will include calibration below the PQL, hence results reported to approximately 2.5 times below the PQL can be reported from within the linear range without qualification for calibration.

Precision and accuracy (bias) requirements:

- Development of initial acceptance criteria for the PPCP Fish Pilot Study. Available data were not adequate to fulfill the requirements for the initial precision and recovery (IPR) established in many

methods. Rather, the initial acceptance windows were developed from MDL study data, most of which were spiked at 2-10 times the Project Quantitation Limit (PQL). As very little work has been conducted with these chemicals in edible fish or in livers, the initial effort was deemed sufficient to establish initial acceptance criteria for the pilot program; however, batch-specific laboratory control samples (LCS) and surrogate recoveries will be collected and plotted into control charts for ongoing assessment of performance and potential trend analysis.

- Analysis of laboratory control samples to demonstrate the laboratory can achieve precise and accurate results with the method prior to use on field samples. For this study, Baylor will include analysis of LCS prepared by adding known amounts of all target chemicals to control tissue matrix at the PQL. Unlike standard LCS analyses, this modification reinforces report limits with each analytical batch. The purpose of the study is to clearly demonstrate whether or not there is a statistical difference in the concentrations of PPCPs in fish from effluent-dominated streams when compared to those from a reference stream representing a control condition. Therefore, LSC results will be evaluated as a range of acceptable recovery and will be calculated as follows:

  % Recovery (surrogates and LCS):

$$\%R = \frac{analyticalresult}{truevalue} \times 100\%$$

  where *analyticalresult* is the observed concentration, and *truevalue* is the amount added during sample preparation. Recovery limits of 60% to 150% have been proposed as acceptable performance until such time as control charts indicate revised limits are necessary (See Section 4).

- Recovery of surrogate or labeled chemicals, where available, spiked into the sample to assess the effect of matrix interferences on compound identification and quantitation. Surrogate recoveries will be assessed using the same equation presented above for LCS evaluation. Recovery limits of 60% to 150% have been proposed as acceptable performance until such time as control charts indicate revised limits are necessary.

- Duplicate matrix spike analyses (MS/MSD, where sample mass is sufficient) to assess the effect of matrix interferences on sample quantitation, and to assess precision of the entire measurement system, including sample homogenization and extraction. Matrix spikes will be prepared at concentrations in the upper third of the calibration range, at concentrations corresponding to those presented in Appendix A. MS/MSD recoveries must take into account the target chemical results native to the unspiked sample, if any. Therefore, the calculation of MS/MSD recoveries is performed as follows:

  % Recovery (MS/MSD):

$$\%R = \frac{(spikedsampleresult - sampleresult)}{amountspiked} \times 100\%$$

  where spiked *spikedsampleresult* is the observed concentration of target chemical in the MS or MSD analysis, the *sampleresult* is amount of target chemical in the unspiked sample analysis, and *amountspiked* is the amount of target chemical spiked into the MS or MSD aliquot. Recovery limits of 60% to 150% have been proposed as acceptable performance until such time as control charts or trend analyses indicate revised limits are necessary.

Precision, whether on laboratory duplicates or on MS/MSD pairs, is assessed through calculation of relative percent difference. Larger sets of replicates are assessed using the %RSD equation, while the following represents a duplicate precision evaluation:

Relative Percent Difference (RPD)

$$RPD = \frac{|C_1 - C_2|}{(C_1, C_2)} x100\%$$

where C_1 is the first of two measurements and C_2 is the second of 2 measurements. The absolute difference of the two values is divided by the mean.

Or

$$RPD = \frac{|C_1 - C_2|}{(C_1 + C_2)/2} x100\%$$

where C1 is the first of two measurements and C2 is the second of 2 measurements.

If tissue mass is limited, the laboratory will analyze one spiked sample, and one laboratory duplicate, rather than MS/MSD to preserve mass. Duplicate RPDs should be less than 40 percent; however, for values less than 5 times the PQL, a broader acceptance of 100% RPD will apply.

- Analysis of blanks to demonstrate freedom from contamination. Blanks should be free of target chemicals above the PQL. Sample results > 5x the blank values will be reported without qualification. Results greater than the PQL, but less than 5x the blank value will be qualified as maximum quantities, while any sample values at or below the blank concentration will be reported as non-detected at the reported level.

Method performance statistics will be calculated for both of the methods to be used in this study. Table 4 summarizes the method performance statistics and criteria and information on how they will be used to control data quality for this study. Appendix A indicates the method/chemical acceptance criteria.

Table 4 - Method Performance Criteria

Statistic	*Description*	*Required frequency*
Method Detection Limit (MDL) and Project Quantitation Limit (PQL)	MDL to be obtained following procedures described in 40 CFR part 136 Appendix B.	The laboratory participating in the study will be required to do one MDL study prior to the analysis of actual field samples. The resulting MDL must support the PQL. The lowest standard used to calibrate the instrument must be below the PQL.
Labeled or surrogate compound recovery	Recovery of labeled and surrogate chemicals spiked into all samples.	Labeled and surrogate standards are spiked into every sample analyzed. Recoveries must be within the acceptable range given in the method.
LCS recovery	Measured concentration or recovery of a laboratory control sample (an aliquot of control matrix spiked at the Project Quantitation Limit)	One typically required per analytical batch (specific frequency provided in the methods). Recovery must be within the acceptable range given in the method (60%-150% at the PQL).
Matrix spike (MS) recovery	Recovery from spiked samples (MS) and/or spiked duplicate samples (MS/MSD).	The laboratory will be required to spike one sample per batch (possibly in duplicate, depending on sampling success) of samples received for analysis. The laboratory will determine specific samples to spike. Calculated spike recoveries (60%-150%) and RPDs (40%) must be within the acceptable range given in the method.
Matrix spike (MS) precision	RPD between measured concentrations in duplicate spiked field samples (MS/MSD).	
Analytical precision (includes sample compositing, homogenization, and aliquotting)	RPD between duplicate analyses of a composite sample.	Alternatively, if only one sample is selected for matrix spiking, the laboratory will be required to analyze in duplicate 5% of the composite samples (to be specified by Tetra Tech). The RPD of the duplicate measurements must be less than 40% for values greater than 5 times the MDL, and must be less than 100% for values less than 5 times the MDL.

12.0 INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE REQUIREMENTS

The laboratory contracted in this study will be responsible for testing and inspecting the equipment used in this study. The laboratory also will be responsible for implementing preventative and corrective maintenance necessary to produce precise and accurate data that meets the measurement quality objectives listed in this QAPP. Specific requirements for maintaining the equipment at the laboratory will be documented in the laboratory QA plan, Baylor-CRASR QMP 2005, Quality Management Plan for the Center for Reservoir and Aquatic Systems Research (CRASR) Baylor University, September 2005, and in the method SOPs being developed during the course of this study. Specific records of preventative maintenance, problems, and corrective actions will be documented by the laboratory in instrument logbooks maintained on-site in the laboratory. These logbooks will

be periodically reviewed by a laboratory manager/supervisor and will be available to an external audit team upon request.

13.0 INSTRUMENT CALIBRATION AND FREQUENCY

The laboratory supporting this study will be required to calibrate instruments used in the study prior to analysis of field samples and to periodically verify calibration during the course of the study. Calibration standards used by the laboratory will need to be certified as to purity, concentration, and authenticity, or prepared from materials of known purity and composition. Detailed instrument calibration procedures will be specified in the laboratory's analytical method SOPs, which are being developed during the course of this study.

The methods employed in this study require a multi-point calibration prior to use of the instrument for analysis of field and QC samples. The frequency of this initial, multi-point calibration varies between methods due to variations in instrument stability and calibration procedures. The methods require the laboratory to verify instrument calibration against a valid multi-point calibration curve, or valid NIST class "S" weights for lipids, at least once per working shift during which samples are analyzed.

14.0 INSPECTION/ACCEPTANCE REQUIREMENTS FOR SUPPLIES AND CONSUMABLES

The laboratory participating in this study will not be providing supplies or consumables to EPA. The laboratory will be required to adhere to the inspection and acceptance requirements outlined in Section 4.0 of their approved quality management plan, *Baylor-CRASR QMP 2005, Quality Management Plan for the Center for Reservoir and Aquatic Systems Research (CRASR) Baylor University, September 2005*, which reliably meets or surpasses the measurement performance requirements of this study. Section 4.0 describes Baylor's purchasing process in general terms, and identifies the person initiating a procurement request as being responsible for its acceptance. Further the QMP indicates that "laboratory personnel assess all items required for specific activities prior to data collection." The laboratory must have a comprehensive QA program in place and operating at all times during the performance of the contract. In performing work for this study, the laboratory shall adhere to the requirements in the analytical methods described in this QAPP, to *EPA Requirements for Quality Assurance Project Plans for Environmental Data Operations,* and to the general laboratory procedures specified in the *Handbook for Analytical Quality Control in Water and Wastewater* (EPA-600-4-79-019). Cumulatively, these sources provide guidelines concerning laboratory inspection and acceptance of chemical standards.

15.0 REQUIREMENTS FOR ACQUISITION OF NON-DIRECT MEASUREMENT DATA

The analytical phase of this study will not involve the collection of data obtained from non-measurement sources such as computer databases, spreadsheets and programs, and literature files.

16.0 DATA MANAGEMENT

Data management practices employed in this study will be based on standard data management practices used for the National Study of Chemical Residues in Lake Fish Tissue. These practices (i.e., sample tracking, data tracking, data inspection, data quality assessment, database development) are described in USEPA's *Quality Assurance Report for the National Study of Chemical Residues in Lake Fish Tissue: Analytical Data for Years 1 through 4* (USEPA 2005) [7]. The data management procedures to be implemented for the PPCP Fish Pilot Study are effective and efficient, and auditors conducting internal and external reviews have evaluated them and found them to be successful. They are summarized below.

16.1 Laboratory Data Management

Laboratory data management procedures will include the following:

- The laboratory will be required to maintain all records and documentation associated with the preparation and analysis of samples for a minimum period of five years after completion of the study.
- To facilitate data tracking, the laboratory will be required to use EPA-assigned episode and sample numbers when reporting results.
- All results of sample analyses, labeled and native standards, surrogate chemicals, spike chemicals, and blanks must be reported on hard copy and on electronic media.
- All required reports and documentation, including chromatograms and mass spectra, must be sequentially paginated and clearly labeled with the laboratory name, contract number, episode number, and associated EPA sample numbers. Any diskettes, or other electronic media submitted must be similarly labeled.

16.2 CSC Data Management

Data management procedures employed by CSC will include the use of 1) an automated tracking system to effectively manage data review and database development activities, 2) standardized data review guidelines to promote consistency in data quality audits across reviewers and over time, 3) a multi-stage data review process designed to maximize the amount of useable data generated during the study, and 4) a standardized database development process that facilitates rapid development of a database with at least 99.9% accuracy.

The automated sample tracking system will facilitate development of up-to-date information concerning work in progress, projected delivery dates, and notice of any problems encountered with laboratory analyses or data turnaround times. To ensure that this information is as complete and accurate as possible, entries will be made into the tracking system at each stage of the sample-to-data sequence.

Standardized data review guidelines will be used in this study to facilitate rapid, consistent, accurate, and thorough data quality audits. Data review guidelines already have been developed and are in use for a variety of analyses performed under EPA OST programs. These guidelines are described in CSC's General Data Review Guidelines for Use with 1600 Series Methods and Other Classicals, Metals, and Organic Methods (Draft, January 1999) [8]. They detail method-specific data review procedures for commonly used methods and more general procedures that can be applied to less frequently used methods. Where appropriate, CSC will modify existing data review guidelines as necessary to reflect the methods, method modifications, and data quality objectives for the PPCP Fish Pilot Study. Any modifications deemed to be necessary will be made in accordance with the CSC *Quality Management Plan* (Version 5, June 2006, or subsequent updates, if applicable).

Although each guideline will be written for a specific method, technique, or group of chemicals, all guidelines will specify a general five-stage review process that will ensure data are in proper format, are complete, are contractually compliant, and are usable. CSC chemists will use this multi-stage process to verify the quality of each laboratory submission under the study. If an error is detected in any stage of the review, CSC staff will initiate corrective action procedures to obtain the maximum amount of usable data from the study. These actions may serve to obtain missing data, correct typographical or transcription errors on data reporting forms, or initiate reanalysis of field or QC samples that do not meet the performance criteria for this study.

Concurrent with the performance of data quality audits, CSC staff will develop a database of combined field and analytical results. This database will be formatted in a manner that is consistent with EPA's National Lake Fish Tissue Study database, with modifications in the format necessary to integrate field data. The database also will be compatible with STORET; data will be stored locally in a Microsoft Access database during the study and delivered to EPA upon study completion or at requested intervals. Each record in the database will contain

information pertaining to both a "field sample" and to an "analytical sample." At a minimum, each record should include fields containing the following information:

- Five-digit EPA sample number assigned by Tetra Tech;
- 9-digit composite sample identification number assigned by field teams;
- Sample type (indicates the type of sample – liver or fillet tissue);
- Fish species;
- Length of each fish;
- Weight of each fish;
- Method of collection (active or passive);
- Sample collection date;
- Sample collection time;
- State and county;
- WWTP facility name;
- Stream (receiving water) name;
- Site coordinates (latitude and longitude) for the start and end of the sampling reach;
- Estimated stream width and depth;
- Average stream flow;
- Chemical concentrations; and
- Lipid content measurements.

The data structure listed above for the integrated field and analytical database and the field database (Section 16.1) will allow data users and reviewers to:

(1) Look up individual fish sample data in the database for a given composite sample number.
(2) Extract analytical data from a given sampling location from the database.
(3) Extract fish sample data from a given sampling location from the database.

As with the data quality audits, a multi-stage process of inspections and corrective actions will be used to facilitate timely, efficient construction of databases that are least 99.9% accurate. The database development process will begin with a completeness check to verify the laboratory has submitted all data in an appropriate format. If deficiencies are found, the CSC Project Manger will notify the Tetra Tech Project Manger, who will in turn contact the Baylor Laboratory Manager to obtain information to address the deficiencies. After problems have been identified and resolved, the CSC Database Administrator will prepare a "QC Check Report" that displays the results submitted by the laboratory. The CSC chemist responsible for performing the data quality audit will review this QC Check Report to verify that the electronic data accurately reflect the hard copy submission. Accuracy will be confirmed by spot checking at least 10% of all results that were downloaded directly from an analytical instrument in the laboratory and by performing a 100% QC check of data that were manually entered by the laboratory or CSC. If errors are identified during spot checking of electronic data, the CSC Project Manager will notify the Tetra Tech Project Manager, who will in turn contact the Baylor Laboratory Manager, to specify errors that require correction. If errors are identified during the 100% QC check of manually entered data, CSC will correct the errors in the database. Following completion of the data quality audit, the CSC chemist and the Database Administrator will modify the database to reflect data usability determinations. A report, generated to reflect the modified database, will then be reviewed by the CSC chemist to verify database accuracy before submission to EPA.

17.0 Assessments and Response Actions

The laboratory is required to have a comprehensive QA program in place and operating at all times during the performance of their contract. Baylor's QA Program is described in Baylor-CRASR QMP 2005, Quality Management Plan for the Center for Reservoir and Aquatic Systems Research (CRASR) Baylor University,

September 2005. In performing laboratory work for this study, they shall adhere to the requirements of their QMP, the requirements of the analytical methods described in this QAPP, in the laboratory SOPs being developed during the course of this study, and the general laboratory procedures specified in the *Handbook for Analytical Quality Control in Water and Wastewater* (EPA-600-4-79-019), as well as the data management principles set forth in *EPA 2185- Good Automated Laboratory Practices*, EPA Office of Administration and Resources Management, August 10, 1995. Cumulatively, these sources describe assessment and response actions that will be implemented within the laboratory (e.g., bench level review of results and calculations, independent surveillance by a Quality Assurance Officer (QAO), and periodic in-house audits).

Sections 17.1 - 17.7 describe other types of assessment activities and corresponding response actions identified to ensure that data gathering activities in the PPCP Fish Pilot Study are conducted as prescribed, and that the measurement quality objectives established in this QAPP and the performance criteria defined for the study (see Table 4) are met. Assessment activities and corresponding response actions are summarized in Table 5.

17.1 Surveillance

The Tetra Tech QA Officer or designee is responsible for facilitating sample scheduling and tracking the location of samples and data throughout the study. During sample collection, the Tetra Tech QA Officer or designee will maintain communication with field sampling coordinators and teams to identify and notify the laboratory of any delays or anticipated changes to the sampling plan. In the event these delays or changes impact the laboratory's contract or EPA schedules, the Tetra Tech QA Officer or designee will notify the EPA Project Manager and work with EPA, the sampling teams, and appropriate CSC staff to identify and implement an appropriate solution.

When samples are shipped to the laboratory, Tetra Tech staff will contact designated laboratory personnel to notify them of the forthcoming shipment(s) and request that they contact the Tetra Tech QA Officer or designee if the shipments do not arrive intact as scheduled. Within 24 hours of scheduled sample receipt, the Tetra Tech QA Officer or designee will contact the laboratory to verify that the samples arrived in good condition, and if problems are noted, will work with the laboratory, the sampling team, and EPA to resolve the problem as quickly as possible to minimize data integrity problems. Laboratory notification of sample receipt and condition will be transmitted from the Baylor Laboratory Manager or designated staff to the Tetra Tech Project Manager via an email message.

The Tetra Tech QA Officer or designee also will communicate periodically with laboratory staff to monitor the progress of sample preparation, analysis, and data reporting. If technical problems are encountered during sample preparation and analysis, the Tetra Tech QA Officer or designee will discuss the situation with the EPA Project Manager and CSC staff. If warranted, the Tetra Tech QA Officer or designee will work with a technical expert (if necessary), laboratory staff, and EPA to identify and implement a solution to the problem. If the laboratory fails to deliver data on time, or if the laboratory notifies Tetra Tech of anticipated reporting delays, the Tetra Tech Project Manager will notify the EPA Project Manager and the CSC Project Manager of the situation. To the extent possible, the CSC Project Manager will adjust data review schedules and shift resources within CSC as necessary to minimize the impact of laboratory delays on EPA schedules. The Tetra Tech Project Manager also will immediately notify the EPA Project Manager of any laboratory delays that are anticipated to impact EPA schedules.

Table 5. Assessment and Response Actions

Assessment Measure	Definition	Frequency	Responsible Party	Rationale	Documentation
Surveillance	Continual or frequent monitoring and verification of the status of an entity and the analysis of records to ensure that specified requirements are being fulfilled.	Throughout sample preparation, laboratory analysis, and data review procedures	Tetra Tech QA Officer or designee and CSC Project Manager	Identify and correct analytical problems as soon as they occur to minimize delays and to notify data users of potential delays as early as possible.	Identification and correction of analytical problems or deficiencies will be documented through communication records, where required. Otherwise, data packages will be initialed and dated with notation indicating that they have been reviewed and approved.
Peer Review	A documented critical review of work that is conducted by qualified individuals who are independent, but technically equivalent of those who performed the work.	Performed on at least 2 of the data package audits conducted by CSC data reviewers and 100% of data review narratives prepared by CSC data reviewers	CSC data reviewers not responsible for original data review	Ensure that activities are technically adequate, competently performed, properly documented, and satisfy established technical and quality requirements.	CSC Peer Review Form will be completed after each of the selected packages are reviewed and approved.
Procedural Review	Qualitative assessment of a data collection operation to establish whether the prevailing quality management structure, policies, practices, and procedures are adequate for ensuring that the type and quality of data needed are obtained. Procedural reviews are conducted to implement immediate corrective actions. Immediate corrective action minimizes the impact of deficiencies or inconsistencies, and optimizes valid data collection.	A procedural review will be performed during the processing of the first series of analyses to be conducted at the laboratory.	Tetra Tech WA Officer or designee	The procedural review process focuses on the processes used in the generation of data specific to the project. Deficiencies are identified and, to the extent possible, corrected immediately to ensure consistency throughout the data collection program. Corrective actions are implemented early to limit the collection of quality-limited data, to optimize valid data.	A report of findings and corrective actions implemented during the procedural review will be retained with the project records and distributed to the laboratory staff.
Readiness Review	A systematic documented review of the readiness for the start-up or continued use of a facility, process or activity that is typically conducted before proceeding beyond project milestones and prior to initiation of a major phase of work.	Prior to the laboratory's analysis of field samples collected during each year of the study	Lab staff, Tetra Tech QA Officer or designee and CSC data reviewers	Verify that the laboratory is capable of producing precise and accurate results with the method(s) they will use during the study.	If TSAs are deemed necessary, they will be documented through formalized audit report, including observations and findings, and the laboratory will be afforded a period of time to respond to those findings or to implement appropriate corrective action to address any deficiencies.
Technical Systems Audit	A thorough, systematic, on-site, qualitative audit of facilities, equipment, personnel, training, procedures, record keeping, data validation, data management, and reporting aspects of a system.	As needed basis only; TSAs are not required for this study unless specific concerns are raised through discussions with laboratory staff or during other data assessment activities.	If TSAs are deemed necessary, they will be performed by the EPA QAO or designee, and Tetra Tech QA staff	Ability of the laboratory to adequately analyze and report data will be assessed prior to analysis and continually throughout analyses via other QA/QC measures described in this QAPP.	Data qualifiers will be used where appropriate to identify limitations of the data in the project database. Qualification will follow standard conventions, and data qualifiers will be clearly defined in reference tables.
Audit of Data Quality	Systematic and independent examination to determine if quality activities and related results comply with planned arrangements and whether these arrangements are implemented effectively and are suitable to achieve objectives.	100% of laboratory data packages submitted	CSC Data Reviewers	To verify that all data collected meet MQOs established for this study.	A final report of data quality will be prepared at the culmination of data collection as described in Section 19 under the heading "Reconciliation with user requirements."
Data Quality Assessment	Statistical and scientific evaluation of the data set to determine the validity and performance of the data collection design and statistical test, and to determine the adequacy of the data set for its intended use.	Upon completion of data review and database development	CSC Staff and EPA Data Users		Identification and correction of analytical problems or deficiencies will be documented through communication records, where required. Otherwise, data packages will be initialed and dated with notation indicating that they have been reviewed and approved.

Finally, the CSC Project Manager will monitor the progress of the data quality audits (data reviews) and database development to ensure that each laboratory data submission is reviewed in a timely manner. In the event that dedicated staff is not able to meet EPA schedules, the CSC Project Manager will work to identify additional resources that are qualified and capable of reviewing the data in a timely manner. If such resources cannot be identified, and if training new employees is not feasible, the CSC Project Manager will meet with the EPA Project Manager to discuss an appropriate solution.

17.2 Peer Review

All laboratory results and calculations will be reviewed by the laboratory manager prior to data submission. Any errors identified during this peer review will be returned to the analyst for correction prior to submission of the data package. Following correction of the errors (See Appendix C for Baylor's Corrective Action Procedures), the Laboratory Manager will verify that the final package is complete and compliant with the contract, and will sign each data submission to certify that s/he has reviewed the package and determined it to be in compliance with the terms and conditions of the contract.

Peer reviews also will be performed within CSC to verify that the data quality audits are being performed consistently over time and across peer reviewers, that the audit findings are technically correct, and that the audits are being performed in accordance with this QAPP. These peer reviews of the CSC data quality audit process will be performed on at least one data delivery package from each method/matrix combination submitted as part of this study (i.e., at least one data package for LC/MS/MS determination of pharmaceuticals in liver, one data package for GC/MS determination of personal care products in fillets, etc.). By addressing data validation and review processes on a method/tissue fraction basis, the laboratory will have greater flexibility in its report preparation process, and validation will be conducted on an analytical batch basis. This approach will facilitate a more expeditious review and release process for interim reports and technical discussions. Peer reviewers will be charged with evaluating the completeness of the original data review, the technical accuracy of the reviewer's findings, and the technical accuracy of the analytical database developed to store results associated with the data package. They will use a standard data review form that they complete, sign, and date to document review of the data quality audit process (Appendix D). The CSC Project Manager will be responsible for identifying and assigning qualified peer reviewers and for selecting packages to be peer reviewed. Qualified peer reviewers will include any staff members who have been trained in CSC data review procedures, are experienced in reviewing data similar to those being reviewed, and are familiar with the requirements of the PPCP Fish Pilot Study and this QAPP. To the extent possible, these peer reviews will be performed after the primary data reviewer has drafted a written narrative describing the results of his/her audit, but before this narrative is submitted to EPA.

To ensure the findings of each data quality audit are documented in a consistent and technically accurate manner, CSC staff will conduct a peer review of 100% of the data review summaries (narratives) prepared for this study. Each data review summary will be subjected to at least two levels of peer review, and each peer reviewer will be charged with evaluating the clarity, technical accuracy, and the grammatical quality of the data review summary.

17.3 Quality Systems Audit

A quality systems audit will not be performed during this study (see Table 5).

17.4 Readiness Review

A readiness review of the laboratory's capability to produce precise and accurate results with the methods specified in this study will be performed before the laboratory is allowed to analyze field samples collected during the study. As part of the readiness review, the laboratory will submit data demonstrating that it is capable of analyzing a known, reference matrix with the methods to be used in this study. In most cases, laboratories meet

this requirement by performing IPR tests. IPR tests consist of preparing four replicate aliquots that contain the target pollutants, analyzing these replicate aliquots with the specified method, and calculating the average percent recovery and standard deviation of the measured aliquots. If the average percent recovery and standard deviation meet pre-defined acceptance criteria, the laboratory is considered to be qualified (or ready) to perform the analyses.

On a case-by-case basis, EPA, Tetra Tech, and CSC may decide to accept alternate data in lieu of IPR data for the readiness reviews. In such cases, the alternate data must provide as much information about laboratory readiness as would the IPR samples. Examples of acceptable non-IPR data that might be used for a readiness review include performance evaluation (PE) sample data, ongoing QC data gathered over a period of time, or MDL study data. For this study, due to the highly specialized nature of the target chemicals and matrices, EPA, Tetra Tech, and CSC have accepted alternative method demonstration data developed during method refinement (which has been underway at Baylor since the project planning phase).

Readiness reviews will be performed by CSC data reviewers and Tetra Tech's QA Officer or designee, who will document and forward their findings to the Tetra Tech Project Manager. If problems are identified during these reviews, the Tetra Tech QA Officer (or designee) and CSC Project Manager will work with the laboratory (to the extent possible) to resolve the problem. If the problem cannot be resolved within the time frame required by EPA or within the scope of the laboratory's existing contract, the EPA Project Manager will be contacted immediately.

17.5 Technical Systems Audit

The laboratory must be prepared for an on-site (or technical systems) audit of its facilities, equipment, staff, and procedures for sample analysis, training, record keeping, data validation, data management, and data reporting. Laboratory audits will be conducted only if the results of the readiness reviews, data quality audits, and surveillance suggest serious or chronic laboratory problems that warrant on-site examinations and discussions with laboratory personnel. If such an audit is determined to be necessary, a standardized audit checklist will be used to facilitate an audit walkthrough and to document audit findings. Audit participants may include the EPA Quality Assurance Officer (or a qualified EPA staff member designated by the EPA QAO) and a Tetra Tech staff member (assigned by the Tetra Tech QA Officer) experienced in conducting laboratory audits. One audit team member will be responsible for leading the audit and conducting a post-audit debriefing to convey significant findings to laboratory staff at the conclusion of the audit. The other audit team member will be responsible for gathering pre-audit documentation of problems that necessitated the audit, customizing the audit checklist as necessary to ensure that those problems are addressed during the audit, documenting audit findings on the audit checklist during the audit, and drafting a formal report of audit findings for review by EPA.

17.6 Data Quality Audits

Every laboratory data package submitted under this study will be subjected to a data quality audit. These data quality audits will be performed by qualified CSC data review staff who have been trained in procedures for performing data quality audits and who are familiar with the laboratory methods used to prepare the data packages. These data quality audits will be performed using a multi-stage review process designed to identify and correct data deficiencies as early as possible in order to maximize the amount of usable data generated during this study.

17.7 Data Quality Assessment

Upon completion of each data quality audit, the CSC Data Reviewer will work with CSC's database development staff to create an analytical database that contains all field sample results from the PPCP Fish Pilot Study (see Section 16.2).

At selected intervals and upon completion of the study, CSC's database development staff will perform statistical analyses to verify the accuracy of the database. The statistical procedures will be directed at evaluating the overall quality of the database against data quality objectives established for the study and in identifying trends in field and QC results obtained during the study. CSC staff will document their findings and recommendations concerning this data quality assessment in a written report to EPA.

18.0 REPORTS TO MANAGEMENT

Following the completion of sample analyses and data quality audit and assessment, CSC chemists will prepare a Quality Assurance Report, in narrative format, that describes data quality limitations and CSC recommendations concerning data use.

Upon request, CSC can also provide a weekly report that describes the status of all current data review activities and prepare periodic database status reports that provide up-to-date information concerning database activities that occurred since distribution of previous reports.

19.0 DATA REVIEW, VALIDATION, AND VERIFICATION

Criteria for acceptance:

A multi-stage data review process, as summarized in Section 16 (and detailed below) will be used to evaluate the quality of all data submitted in the PPCP Fish Pilot Study. Acceptance criteria against which data will be evaluated include 1) study performance criteria and MQOs detailed in this and affiliated QAPPs, 2) applicable QC acceptance criteria, and 3) best professional judgment (BPJ) of CSC chemists responsible for performing data quality assessments. The goal of this data review process will be to maximize the amount of useable data gathered in the study. This will be accomplished by 1) performing data reviews promptly so that corrective actions may be taken wherever possible and 2) considering data quality failures in light of the entire analytical sequence rather than as isolated events.

Process:

In the first stage of the data review process, CSC chemists will perform a "Data Completeness Check" in which all elements in each laboratory submission will be evaluated to verify that results for all specified samples are provided, that data are reported in the correct format, and that all relevant information (such as preparation and analysis logs) are included in the data package. Corrective action procedures will be initiated if deficiencies are noted.

The second stage of the data review process will focus on an "Instrument Performance Check" in which the CSC chemists will verify that calibrations, calibration verifications, standards, and calibration blanks were analyzed at the appropriate frequency and met method or study performance specifications. If errors are noted at this stage, corrective action procedures will be initiated immediately.

Stage three of the data review process will focus on a "Laboratory Performance Check" in which CSC staff will verify that the laboratory correctly performed the required analytical procedures and was able to demonstrate a high level of precision and accuracy. This stage includes evaluation of QC elements such as the preparation and laboratory blanks and laboratory control samples. Corrective action procedures will be initiated with the laboratories to resolve any deficiencies identified.

In stage four of the data review process, the CSC chemist will perform a "Method/Matrix Performance Check" to discern whether any QC failures are a result of laboratory performance or difficulties with the method

or sample matrix. Data evaluated in this stage will include matrix spike, matrix spike duplicate, duplicate sample, and surrogate spike results. The CSC chemist also will verify that proper sample dilutions were performed and that necessary sample cleanup steps were taken. If problems are encountered, the CSC chemist will immediately notify the Tetra Tech Project Manager to implement corrective actions.

Reconciliation with user requirements:

Finally, CSC will perform a "Data Quality and Usability Assessment" in which the overall quality of data is evaluated against the performance criteria and MQOs detailed in this QAPP. As noted above, this assessment will strive to maximize use of data gathered in this study based on performance criteria established for this study. This will be accomplished by evaluating the overall quality of a particular data set rather than focusing on individual QC failures. Results of this assessment will be documented in a written QA Report that CSC will submit to EPA. To expedite the process, this report will follow a standardized format developed for EPA's National Lake Fish Tissue Study and, wherever possible, utilize standardized language to communicate data limitations and CSC recommendations concerning data quality.

20.0 REFERENCES

(1) U.S. Environmental Protection Agency (USEPA). 2006. *Quality Assurance Project Plan for Sample Collection Activities for a Pilot Study to Investigate the Occurrence of Pharmaceuticals and Personal Care Products (PPCPs) in Fish Tissue.* EPA Office of Water, Office of Science and Technology, Washington, DC.

(2) U. S. Environmental Protection Agency (USEPA). 2001. *EPA Requirements for Quality Assurance Project Plans, EPA QA/R-5.* EPA Office of Environmental Information, Washington, DC. EPA/240/B-01/003.

(3) U.S. Environmental Protection Agency (USEPA). 2002. *EPA Guidance for Quality Assurance Project Plans, EPA QA/G-5.* EPA Office of Environmental Information, Washington, DC. EPA/240/R-02/009.

(4) Appendix B, 40 CFR part 136

(5) U.S. Environmental Protection Agency (USEPA). 2000. *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Volume 1: Fish Sampling and Analysis.* Third Edition. EPA Office of Water, Washington, DC. EPA 823-B-00-007.

(6) U.S. Environmental Protection Agency (USEPA). 1996. *Guide to Method Flexibility and Approval of EPA Water Methods.* EPA Office of Water, Engineering and Analysis Division (4303), Washington DC. EPA-821-D-96-004.

(7) U.S. Environmental Protection Agency (USEPA). 2005. *Quality Assurance Report for the National Study of Chemical Residues in Lake Fish Tissue: Analytical Data for Years 1 through 4.* EPA Office of Water, Office of Science and Technology, Washington DC. EPA-823-R-05-005.

(8) Computer Sciences Corporation. *General Data Review Guidelines for Use with 1600 Series Methods and Other Classicals, Metals, and Organic Methods* (Draft, January 1999)

Appendix A

Method/Chemical Acceptance Criteria

Appendix A – Method/Chemical Acceptance Criteria

PRELIMINARY ACCEPTANCE CRITERIA LC-MS Analysis of PPCPs in fish tissue

Chemical	MDL 0.5 (ng/g)	PQL (ng/g)	Calibration Standards (ng/g)								Acceptance Criteria			
			CC1	CC2 PQL/LCS	CC3	CC4 (CCV) Spike/MS	CC5	CC6	CC7	CC8 (optional)	ICV %RSD	CCV %D	LCS/MS/MSD UCL	LCS/MS/MSD LCL
propranolol	0.01	**0.05**	0.02	0.05	0.125	0.25	0.5	1.0	2.0	4.0	30%	25%	60%	150%
carbamazepine	0.02	**0.05**	0.02	0.05	0.125	0.25	0.5	1.0	2.0	4.0	30%	25%	60%	150%
clofibric acid	0.06	**0.50**	0.20	0.50	1.25	2.5	5.0	10	20	40	30%	25%	60%	150%
miconazole	0.10	**0.50**	0.20	0.50	1.25	2.5	5.0	10	20	40	30%	25%	60%	150%
diltiazem	0.10	**0.50**	0.20	0.50	1.25	2.5	5.0	10	20	40	30%	25%	60%	150%
warfarin	0.13	**0.50**	0.20	0.50	1.25	2.5	5.0	10	20	40	30%	25%	60%	150%
1,7-dimethylxanthine	0.19	**1.0**	0.40	1.0	2.5	5.0	10	20	40	80	30%	25%	60%	150%
gemfibrozil	0.19	**1.0**	0.40	1.0	2.5	5.0	10	20	40	80	30%	25%	60%	150%
codeine	0.20	**1.0**	0.40	1.0	2.5	5.0	10	20	40	80	30%	25%	60%	150%
metoprolol	0.33	**1.0**	0.40	1.0	2.5	5.0	10	20	40	80	30%	25%	60%	150%
sulfamethoxazole	0.36	**1.0**	2.0	5.0	12.5	25	50	100	200	400	30%	25%	60%	150%
cimetidine	0.68	**5.0**	2.0	5.0	12.5	25	50	100	200	400	30%	25%	60%	150%
erythromycin	0.90	**5.0**	2.0	5.0	12.5	25	50	100	200	400	30%	25%	60%	150%
lincomycin	1.18	**5.0**	2.0	5.0	12.5	25	50	100	200	400	30%	25%	60%	150%
thiabendazole	1.36	**5.0**	2.0	5.0	12.5	25	50	100	200	400	30%	25%	60%	150%
acetominophen	1.44	**5.0**	2.0	5.0	12.5	25	50	100	200	400	30%	25%	60%	150%
sertraline	1.83	**10**	4.0	10	25	50	100	200	400	800	30%	25%	60%	150%
atenolol	2.18	**10**	4.0	10	25	50	100	200	400	800	30%	25%	60%	150%
norfluoxetine	2.20	**10**	4.0	10	25	50	100	200	400	800	30%	25%	60%	150%
caffeine	2.96	**10**	4.0	10	25	50	100	200	400	800	30%	25%	60%	150%
tylosin	3.73	**20**	8.0	20	50	100	200	400	800	1600	30%	25%	60%	150%
ibuprofen	4.57	**20**	8.0	20	50	100	200	400	800	1600	30%	25%	60%	150%
trimethoprim	4.99	**20**	8.0	20	50	100	200	400	800	1600	30%	25%	60%	150%
fluoxetine	8.01	**20**	8.0	20	50	100	200	400	800	1600	30%	25%	60%	150%

MDL 0.5 represents projected values using 1.0 g tissue and reconstitution in 0.5 mL solvent prior to analysis

PRELIMINARY ACCEPTANCE CRITERIA GC-MS analysis of PPCPs in fish tissue

Chemical	MDL 0.5 (ng/g)	PQL (ng/g)	Calibration Standards (ng/g)							Acceptance Criteria				Precision (%RPD)
			CC1	CC2 PQL/LCS	CC3	CC4 (CCV) Spike/MS	CC5	CC6	CC7	ICV %RSD	CCV %D	LCS/MS/MSD UCL	LCS/MS/MSD LCL	
***p*-nonylphenol**	2.81	**10**	4.0	10	25	50	100	200	400	30%	25%	60%	150%	40%
***p*-octylphenol**	2.92	**10**	4.0	10	25	50	100	200	400	30%	25%	60%	150%	40%
***m*-toluamide**	3.48	**10**	4.0	10	25	50	100	200	400	30%	25%	60%	150%	40%
celestolide	4.03	**20**	8.0	20	50	100	200	400	800	30%	25%	60%	150%	40%
tonalide	4.81	**20**	8.0	20	50	100	200	400	800	30%	25%	60%	150%	40%
NPE #3	4.95	**20**	8.0	20	50	100	200	400	800	30%	25%	60%	150%	40%
triclosan	5.33	**20**	8.0	20	50	100	200	400	800	30%	25%	60%	150%	40%
4-MBC	5.34	**20**	8.0	20	50	100	200	400	800	30%	25%	60%	150%	40%
NPE#2	6.30	**20**	8.0	20	50	100	200	400	800	30%	25%	60%	150%	40%
NPE#1	10.59	**20**	8.0	20	50	100	200	400	800	30%	25%	60%	150%	40%
musk xylene	7.29	**30**	12	30	75	150	300	600	1200	30%	25%	60%	150%	40%
benzophenone	7.46	**30**	12	30	75	150	300	600	1200	30%	25%	60%	150%	40%
galaxolide	9.05	**30**	12	30	75	150	300	600	1200	30%	25%	60%	150%	40%
octocrylene	16.58	**50**	20	50	125	250	500	1000	2000	30%	25%	60%	150%	40%
musk ketone	16.89	**50**	20	50	125	250	500	1000	2000	30%	25%	60%	150%	40%

Appendix B

Sampling Locations

Appendix B – Sampling Locations

St.	City	Facility Name	Treatment	Receiving Water Name	County Name	Pop	PCS Design Capacity	Existing Flow MGD	% Effluent	% 65& older	Median Income
AZ	Phoenix (PHX)	91st Avenue WWTP	secondary	Salt River	Maricopa	1,418,041	165	153	100%[1]	8.1	$41,207
IL	Chicago (CHI)	Northside WRD	Advanced secondary	North Shore Channel	Cook	5,376,741	333	234	100%[3]	10.3	$38,625
TX	Dallas (DAL)	Dallas WWTP	tertiary	Trinity River†	Dallas	3,500,000	150		100%[3]	8.1	$43,324
FL	Orlando (ORL)	Orlando-Iron Br Fac	Advanced Treatment I	Little Econlockhatchee	Seminole	442,542	40.00	36	64%[2]	11.3	$35,732
PA	West Chester (WCH)	Taylor Run WWTP	secondary	Taylor Run	Chester	17,701	1.5		36 - 86%*	9.0	$37,803
NM	Santa Fe (SAF)	Santa Fe WWTP	secondary	Santa Fe River	Santa Fe	68,041	9	8.5	100%	13.9	$49,705
NV	Las Vegas (LAV)	City of Las Vegas WPCF	primary & secondary, removes ammonia	Las Vegas Wash‡	Clark	575,000	110	84	100%[3]	11.6	$44,046
TX	San Antonio (SAN)	Dos Rios WRC	Advanced treatment & disinfection	Medina & San Antonio Rivers	Bexar	1,236,249	125	56.5	100%	10.4	$36,214
VA	Lorton (LOR)	Noman M. Cole, Jr. Pollution Control Plant	tertiary	Pohick Creek	Fairfax	17,786	54	>40	no gauge data	7.9	$81,050
TX	Denton (DNT)	Pecan Creek Water Reclamation Plant	secondary	Pecan Creek	Denton	98,288	21		100%[4]	7.9	$35,422
NC	Raleigh (RAL)	Neuse River WWTP	tertiary	Neuse River	Wake	326,653	60	36.67	48 - 78%	8.3	$46,612
DC	Washington DC (WDC)	Blue Plains STP	tertiary	Potomac River	DC	553,523	370	335	no gauge data ?	12.2	$40,127

= Priority
= Secondary

* Instream waste concentration from Tetra Tech's WERF field sampling data

◊ Gauge is upstream of the Potomac's confluence with the Anacostia River; Blue Plains STP is downstream of this confluence, so Potomac's discharge will be much greater than the gauge shows.

1 Information from Debra Daniel, AZ DEQ

2 Calculation based on data provided by Alex Trounov, Tt Fairfax

3 Flow is primarily made up of effluent discharged from multiple facilities (http://ndep.nv.gov/docs_04/bwpc_nv0000060_fs.pdf & www.epa.gov/osp/regions/emerpoll/howe.ppt).

4 During non-storm conditions, flows are comprised almost entirely of effluent; during summer months, water in Pecan Cr. is exclusively effluent (Brooks et. al., 2005).

‡ Natural drainage in the Las Vegas Valley and the receiving stream for all area surface water dischargers. The water in the wash is primarily treated wastewater from the Clark County Water Reclamation District, City of Las Vegas, & City of Henderson.

† Recommendation from Scott Dyer, PGI

Appendix C

Baylor University's Laboratory Corrective Action Plan

Appendix C – Baylor University's Laboratory Corrective Action Plan

General QA/QC Details

Here we include typical QA/QC information, including requested corrective action procedures[1].

Laboratory Measurement Quality Control Requirements and Acceptability Criteria
Detailed laboratory QC requirements are contained within each individual method and laboratory quality assurance manuals (QAMs). The minimum requirements that all participants abide by are stated below. Lab QC sample results are reported with the laboratory data report.

Lab QC samples are prepared and analyzed in batches, which are defined as follows:

Batches are environmental samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A preparation batch is composed of one to 20 environmental samples of the same matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours. An analytical batch is composed of prepared environmental samples (extracts, digestates or concentrates) that are analyzed together as a group. An analytical batch can include prepared samples originating from various environmental matrices and can exceed 20 samples.

Laboratory duplicate - Laboratory duplicates are used to assess precision. A laboratory duplicate is prepared by splitting aliquots of a single sample (or a matrix spike or a laboratory control standard) in the laboratory. Both samples are carried through the entire preparation and analytical process. Laboratory duplicates are run at a rate of one per preparatory (if applicable) and analytical batch.

Precision is calculated by the relative percent difference (RPD) of duplicate results as defined by 100 times the difference (range) of each duplicate set, divided by the average value (mean) of the set. For duplicate results, X_1 and X_2, the RPD is calculated from the following equation:

$$\mathbf{RPD = \{ (X_1 - X_2)/ (X_1+X_2)/2\}* 100}$$

Performance limits and control charts are used to determine the acceptability of duplicate analyses.

Laboratory Control Standard (LCS)/Laboratory Control Standard Duplicate (LCSD) - LCS/LCSD pairs are analyte-free tissue matrix samples spiked with the analyte of interest prepared from standardized reference material. The LCS/LCSD pairs are generally spiked into analyte-free tissue matrix at a level less than or equal to the mid-point of the calibration curve for each analyte. They are carried through the complete preparation and analytical process. The LCS/LCSD pairs are used to document the bias of the method due to the analytical process. Bias can be assessed by measuring the percent recovery of LCSs and LCSDs, and precision can be assessed by comparing the results of LCS/LCSD pairs. LCS/LCSD pairs are run at a rate of one each per preparatory (if applicable) and analytical batch. Laboratory-specific control limits and charts are calculated and maintained by laboratory staff on a periodic basis.

Bias of LCSs and LCSDs is expressed by percent recovery (%R) where SR is the observed spiked sample concentration, and SA is the spike added:

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[1] References to analyses of metals and field blank samples do not apply to the PPCP Fish Pilot Study.

$$\%R = SR/SA * 100$$

The mean bias of LCS/LCSD pairs is expressed by $\%R_{mean}$, where $\%R_{LCS}$ is the percent recovery of the LCS and $\%R_{LCSD}$ is the percent recovery of the LCSD:

$$\%R_{mean} = (\%R_{LCS} + \%R_{LCSD})/2$$

Precision between LCS/LCSD pairs is expressed by relative percent difference (RPD). For LCS/LCSD results, X_1 and X_2, the RPD is calculated from the following equation:

$$RPD = \{(X_1-X_2)/<(X_1+X_2)/2>\}*100$$

Matrix spikes (MS) - A matrix spike is an aliquot of sample spiked with a known concentration of the analyte of interest. Percent recovery of the known concentration of added analyte is used to assess accuracy of the analytical process. The spiking occurs prior to sample preparation and analysis. Matrix spike samples are routinely prepared and analyzed at a rate of 10% of samples processed or one per preparatory (if applicable) and analytical batch whichever is greater. The MS is spiked at a level less than or equal to the midpoint of the calibration or analysis range for each analyte. The MS is used to document the accuracy of a method due to sample matrix and not to control the analytical process. Percent Recovery (%R) is defined as 100 times the observed concentration, minus the sample concentration, divided by the true concentration of the spike. MS recoveries are indicative of matrix-specific biases and are plotted on control charts maintained by the laboratory. Measurement performance specifications for matrix spikes are not specified in this document, and MS data should be evaluated on a case-by-case basis.

The formula used to calculate percent recovery, where %R is percent recovery; SSR is the observed spiked sample concentration; SR is the sample concentration; and SA is the spike added, is:

$$\%R = (SSR - SR)/SA * 100$$

Method Blank- A method blank is an analyte-free matrix to which all reagents are added in the same volumes or proportions as used in the sample processing and analyzed with each preparatory (if applicable) and analytical batch. The method blank is carried through the complete sample preparation and analytical procedure. The method blank is used to document contamination from the analytical process. The analysis of method blanks should yield values less than the laboratory's reporting limit. For very high level analyses, blank value should be less then 5% of the lowest value of the preparatory (if applicable) and analytical batch or corrective action will be implemented.

Additional method specific QC requirements - Additional QC samples are run (e.g., surrogates, internal standards, continuing calibration samples, interference check samples) and will be specified in the method SOPs being developed during the course of this study. The requirements for these samples, their acceptance criteria, and corrective action are method-specific.

Failures in Quality Control and Corrective Action

Sampling QC excursions are evaluated by the Baylor University Project Manager, in consultation with the Baylor University QAO. In that differences in sample results are used to assess the entire sampling process, including environmental variability, the arbitrary rejection of results based on pre-determined limits is not practical. Therefore, the professional judgment of the Baylor University Project Manager and QAO will be relied upon in evaluating results. Rejecting sample results based on wide variability is a possibility. Field blanks for trace elements and trace organics are scrutinized very closely. Field blank values exceeding the acceptability criteria

may automatically invalidate the sample, especially in cases where high blank values may be indicative of contamination which may be causal in putting a value above the standard. Notations of field split excursions and blank contamination are noted in the quarterly report and the final QC Report. Equipment blanks for metals analysis are also scrutinized very closely.

Corrective action will involve identification of the cause of the failure where possible. Response actions will typically include re-analysis of questionable samples. In some cases, a site may have to be re-sampled to achieve project goals.

Laboratory measurement quality control failures are evaluated by the laboratory staff. The disposition of such failures and conveyance to the project sponsor are discussed below under Failures in Measurement Systems and Corrective Actions.

Failures in Measurement Systems and Corrective Actions

Failures in field measurement systems involve, but are not limited to such things as instrument malfunctions, failures in calibration, contamination, and quality control samples outside defined limits. In many cases, the field technician will be able to correct the problem. If the problem is resolvable, then the technician will document the problem on the field data sheet and complete the analysis. If the problem is not resolvable, then it is conveyed to the Baylor University Field Supervisor, who will make the determination and notify the Baylor University QAO. If the analytical system failure may compromise the sample results, the resulting data will not be submitted for loading and storage in the SWQM portion of the TRACS database, if data is collected in Texas. The nature and disposition of the problem is reported on the data report which is sent to the Baylor University Project Manager. The Baylor University Project Manager will include this information in the CAR and submit with the Progress Report which is sent quarterly to the sponsoring organization's Project Manager.

Appendix D

CSC Data Review Form

FISH STUDY PEER REVIEW RECORD

SAMPLE NOs.: _____ CLASS: _____ INSTRUMENT: _____

Original Reviewer: _____

Second Reviewer: _____

Was the entire data package reviewed? _____
If not, what parameters were reviewed? _____
_____
_____

This review was the result of (check one):

- ☐ data review assignment
- ☐ database investigation # _____
- ☐ client request (please explain below)
- ☐ other (please explain below)

Issues noted during re-review and resolutions:

Signature: _____
Completion Date: _____