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HERRINGTON LAKE, KENTUCKY



Quality Assurance Project Plan (QAPP)
 Field Sample Collection, Preparation, and Analysis

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ACRONYMS AND ABBREVIATIONS

AMEC	Amec Foster Wheeler
ALS	Analytical Laboratory Services
Cabinet	Kentucky Energy and Environment Cabinet
CAP	Corrective Action Plan
CAR	Corrective Action Request
DQI	Data Quality Indicator
DOO	Data Quality Objective
ERA	Ecological Risk Assessment
ESV	Ecological Screening Values
FTL	Field Team Leader
HASP	Health and Safety Plan
HHRA	Human Health Risk Assessment
HSC	Health and Safety Coordinator
ID	Identifier
ICV	Initial Calibration Verification
KAR	Kentucky Administrative Regulation
KDOW	Kentucky Department for Environmental Protection Division of Water
KU	Kentucky Utilities
MS/MSD	Matrix Spike/Matrix Spike Duplicate
N/A	Not Applicable
NOV	Notice of Violation
PPE	Personal Protection Equipment
PBRCC	Precision, Bias, Representativeness, Comparability, Completeness, and Sensitivity
QA/QC	Quality assurance/Quality control
QAPP	Quality Assurance Project Plan
RPD	Relative Percent Difference
SOP	Standard Operating Procedure
USEPA	United States Environmental Protection Agency

DISTRIBUTION LIST

This section provides a list of all the individuals (along with their titles, organizations, and contact information) who will receive the approved Quality Assurance Project Plan (QAPP) and any subsequent revisions. This list also includes all those responsible for project implementation (including project managers, QA managers, field staff, and representatives of all groups/agencies involved).

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PROJECT / TASK ORGANIZATION

This section lists the individuals and organizations participating in the project, and their specific roles and responsibilities.

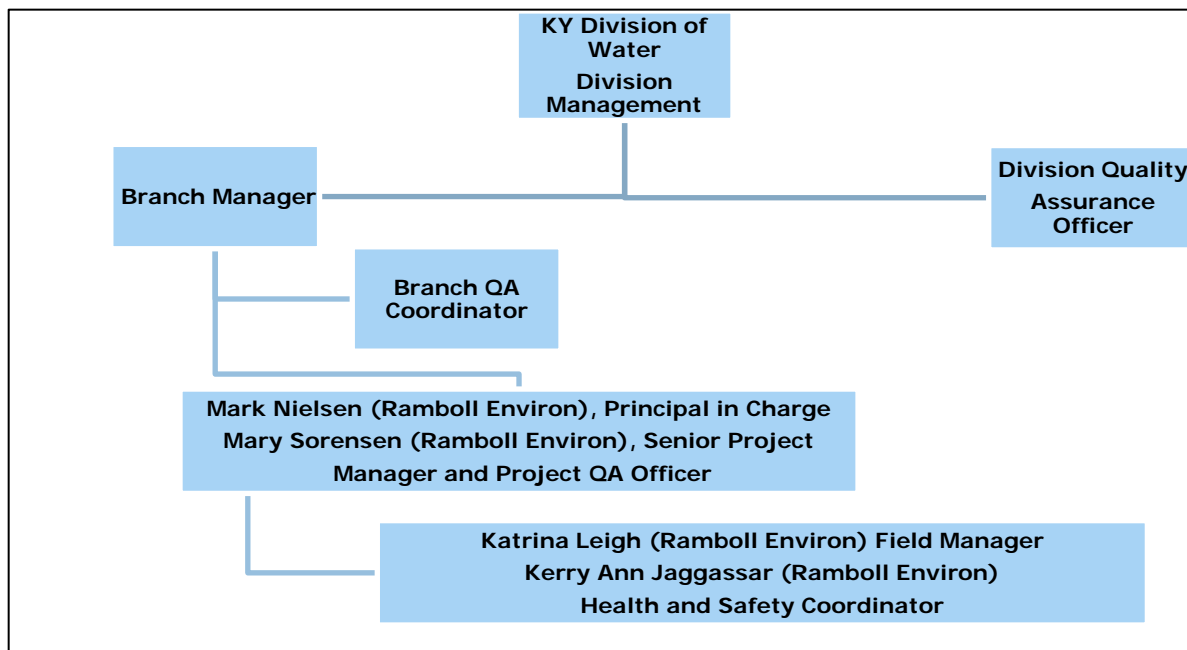


Figure 1. E.W. Brown Station Corrective Action Plan Management Team

Mark Nielsen (Principal in Charge)

Responsible for oversight of the project, the project team, the project contracts, the project QAPP elements, laboratory coordination, field operations, data management and report preparation.

Mary Sorensen (Senior Project Manager and Quality Assurance Officer)

Responsible for the day to day project direction and management of all project tasks, the project QAPP elements, laboratory coordination, field operations, data management and report preparation. She will oversee field collection efforts in direct collaboration with the Field Managers. She is responsible for reviewing and approving the QAPP, ensuring that all quality assurance elements are accounted for and are relevant to other documents, such as Standard Operating Procedures (SOPs).

Kerry Ann Jaggassar (Health and Safety Coordinator)

Responsible for reviewing and approving the Health and Safety Plan associated with this field effort.

Katrina Leigh (Field Managers)

Field Sampling Leader who will be responsible for assigning field samplers specific tasks. Ms. Leigh has overall responsibility for all field activities in coordination with Mary Sorensen. Field Personnel led by Ms. Leigh will be responsible for carrying out all field activities, and assisting the field sampling lead in assigned tasks.

1 PROJECT BACKGROUND AND OVERVIEW

This Quality Assurance Project Plan (QAPP) describes the Data Quality Objectives (DQOs) and the implementation of Quality Assurance (QA), and the Quality Control (QC) for the field data collection, laboratory analytical methods, scientific assessments, and reporting related to the collection of sediment, surface water, and biological samples for the analysis of chemical concentrations as described in the E.W. Brown Corrective Action Plan (CAP) prepared by Ramboll Environ in 2017 (Ramboll Environ 2017).

1.1 Background

On January 11, 2017, the Kentucky Energy and Environment Cabinet (Cabinet) issued a Notice of Violation (NOV) to the Kentucky Utilities Company (KU) due to the detection of selenium in whole body fish tissue from Herrington Lake at concentrations above Kentucky's water quality standard for protection of aquatic life. In order to resolve the NOV, as well as to address other constituents of potential concern related to coal combustion residues, KU entered into an Agreed Order with the Cabinet on January 30, 2017 that required an investigation of sediment and surface water in Herrington Lake.

The CAP was submitted to the Cabinet after multiple iterations of review and comment in August. The Cabinet requested development of this QAPP and specific SOPs for the sampling program described in the CAP. This QAPP generally follows the Cabinet requested format (KDOW 2016b), as appropriate for the Herrington Lake effort, and cross references the CAP sections to avoid unnecessary duplication of CAP text that has already been reviewed and is also subject to public comment. The CAP is informative of sampling collection and handling procedures. The following SOPs, along with this QAPP, developed at the request of the Cabinet, provide additional collection and handling procedures:

- SOP: Fish Tissue Collection and Analysis for Herrington Lake (Ramboll Environ 2017b)
- SOP: Aquatic Vegetation and Aquatic Invertebrate Tissue Collection and Analysis for Herrington Lake (Ramboll Environ 2017c)
- SOP: Sediment Pore Water Collection Approach for Herrington Lake (Ramboll Environ 2017d)

1.2 Project Overview

The goal of the Herrington Lake sampling program is to characterize the nature and extent of chemicals in surface water, sediment, sediment pore water, and biological media from the Herrington Lake Study Area. One key goal of sampling and analyses is to identify and characterize sources and transport mechanisms, if any, for selenium and other metals beyond those identified in previous studies conducted at the Site. An additional goal of the sampling program is to evaluate if selenium in sediment is a source for selenium in the food web of Herrington Lake (e.g. the fish that anglers may catch and eat).

Human health (HHRA) and ecological (ERA) risk assessments will also be conducted for the Site. The overall goal of the ERA is to assess the potential for ecological risks for selenium and other constituents of potential ecological concern within the Herrington Lake Study Area. The overall goal of the screening level ERA is to determine whether (1) there is a high probability that there are no significant ecological risks; or (2) there is a need for additional evaluation of potential risks (USEPA 1997, 2000a, 2000b).

The purpose of the baseline HHRA is to present an assessment of the theoretical human health risks associated with potential exposure to chemicals of potential concern at the site now or in the future.

Risk managers can use the HHRA findings to evaluate the need for further characterization and to determine whether the risks require mitigation. The need for supplemental remedial actions will depend upon the results of ongoing performance monitoring, and the findings of the field-sampling program.

Specifically, the ERA and HHRA may identify one or more of the following:

- Any data gaps requiring further investigation
- Biological monitoring that can be conducted to evaluate the potential trajectory of improving conditions from those remedial actions already implemented
- Physical and chemical monitoring of natural processes that may augment any current remedial actions

1.3 Ramboll Environ (2017a) Corrective Action Plan (CAP) - Brief Overview

The E.W. Brown Station Corrective Action Plan (CAP) prepared by Ramboll Environ during 2017 provides a detailed overview of the environmental monitoring work and the schedule for implementation including:

- Geographic boundary of the study (provided in Figures 1-1A and 1-1B of the CAP).
- Field activities to be conducted (Section 2 of the CAP)
 - Herrington Lake Field Program Overview (Figure 2-1 of the CAP) and detailed discussion of the phased approach to sampling
 - Types of samples to be collected
 - Measurements/analyses to be obtained
 - Data analysis to be performed
- Products/reports to be generated (Section 2.5 of the CAP)
- A targeted schedule for each activity/report including scheduling milestones (Section 6 of the CAP)

Appendix A of this QAPP provides identifies the E.W. Brown Station and the Herrington Lake region (Figure A1-1) an overview of the Herrington Lake Field Sampling Program (A1-2) as it was presented the CAP. Appendix A also provides an overview of the schedule (A-2) as it was presented in Figure 6-1 of the CAP (Ramboll Environ 2017a). The schedule includes a complete timeline for the sampling events, the laboratory analysis, and turnaround times for data and the schedule for the review of activities.

The sampling program and analytical methods identified for the sample media will ensure that data are of sufficient quality and quantity to be used for the HHRA and ERA. The CAP also contains a summary of the studies previously conducted in Herrington Lake and the findings from those studies. The CAP also identifies the existing data that will be used in this project, the new data that is intended for the CAP, and the relevance to this study. The QAPP cross references sections of the CAP, as appropriate, in efforts to minimize duplication but provide the Cabinet with this QAPP, as requested.

2 DATA QUALITY OBJECTIVES AND MEASUREMENT DATA CRITERIA

DQOs are presented in order to ensure adequate data quality during the field sampling. The overall objective of the field sampling at Herrington Lake is to characterize the nature and extent of chemicals in surface water, sediment, sediment pore water, and biological media from the Herrington Lake Study Area Project. Specific DQOs are as follows:

- Characterize the sediment, sediment pore water, and surface water, to determine if concentrations of metals are potentially impacting the Herrington Lake aquatic community.
- Characterize concentration of metals in tissues of aquatic plants, aquatic invertebrates, and fish to determine potential impacts to aquatic wildlife.
- Measure metal concentrations in tissue to provide a greater understanding of the selenium cycle in Herrington Lake.
- Characterize exposure to metals and potential health risks from the consumption of Herrington Lake fish.

The specific field sampling DQOs for each sample location are provided in Appendix B of this QAPP and in Table 2-2 of the Ramboll Environ 2017 CAP (Ramboll Environ 2017a). These DQOs describe the type of data to be collected, the intended use of the data to be collected, and the conditions under which the data should be collected.

Most potential decision error is typically sourced to sample collection variability, conditions, scenarios, or procedures. Analytical error typically contributes much less to total environmental measurement error. Nevertheless, laboratories must still report the analytical data with sufficient precision and reliability to allow comparison to the existing, datasets and standards as presented in Appendix C.

Specified limits of decision errors need to be developed that are indicative of how much uncertainty will be tolerated in decision(s). Location information (x and y) must be determined to within 1 meter. Use of a digital global positioning system onboard the sampling vessel(s) will achieve the specified horizontal accuracy limits. The specific number of samples, sample locations, and rationale are presented in the FSP.

2.1 Action Level vs. Method Detection (MDL) and Reporting Limit (RL)

2.1.1 Method Detection Limit (MDL) and Reporting Limit (RL)

Sampling parameter laboratory reporting limits (RLs) are based upon laboratory method detection limits (MDLs), or the minimum concentration of a compound or analyte that can be measured using a specific laboratory analytical method. Unlike the MDL, the RL also considers sample size, matrix effects, and any dilutions made during the analysis of that particular sample which can vary from sample to sample. The MDL and RL often contribute to project decisions related to the use or exclusion of reported results (e.g. not-detected (ND)).

2.1.2 Action Level

Action levels apply to decision-level project outcomes, and can be determined by regulation, scientific standard, or other established, accepted literature within the scientific community. As the term implies, Action Levels typically related to laboratory results that are high enough to warrant action or to trigger a response. An action level should never be lower than the laboratory-method reporting limit of the parameters in question.

2.1.3 Ecological Screening Values (ESVs) that may be considered

Ecological screening values will be considered among the evaluations of data that will be conducted for the Herrington Lake data. Example ESVs that may be considered include:

- USEPA Region 4 Ecological Risk Assessment Supplemental Guidance, including surface water and sediment ecological screening values (USEPA 2015)
- Kentucky Surface Water Quality and Fish Tissue Standards (401 Kentucky Administrative Regulation (KAR; 10:031) (KDOW 2016a)
- Kentucky Guidance for Ambient Background Assessment (Cabinet 2017). Site data for metals in sediments will be compared with State of Kentucky background concentrations in soil from Table 2 of the Cabinet (2004) document.

2.2 Measurement and Performance Criteria / Acceptance Criteria

Appendix C of this QAPP provides Kentucky Reporting Limits to meet Sufficiently Sensitive Permit Requirements and specified surface water, sediment, and tissue laboratory methods detection limits provided by the selected lab.

Measurement and performance criteria can be stated as data quality indicators (DQIs): the primary indicators are precision, bias, representativeness, comparability, completeness, and sensitivity, referred to as the PBRCC parameters (USEPA 2012). An example table of DQIs is included below for reference, as shown in the EPA 2012 guidance document for developing quality assurance project plans.

Field quality assurance/quality control (QA/QC) samples collected during the investigation include field duplicate samples and equipment blanks. The field duplicate is a replicate sample collected as close as possible to the same time that the primary sample is collected and from the same location, depth, or source, and is used to document analytical precision. Locations for field duplicate analysis will be distributed through the project area; each field duplicate will be generated by collecting one additional sample in the immediate proximity of the primary sediment location and at the same time.

Field duplicate samples will be labelled and packaged in the same manner as primary samples but with "Dup" appended to the sample ID. Field duplicates will be collected at a frequency of one in every 10 primary samples and will be analyzed for the same suite of parameters as the primary sample.

Table 1. EPA Data Quality Indicators (DQIs) (EPA 2002)

DQI	Definition	Determination Methodologies will Include:	QC Samples will Include:
Precision	Measure of agreement among repeated measurements of the same property under similar conditions. Usually expressed as a range, standard deviation, variance, percent difference in either absolute or relative terms.	Use the same analytical instrument to make the same measurement. Split samples in the field; submit to same circumstances of handling, preservation, and analysis. Use the same method to make repeated measurements of the same sample within a single laboratory, or use two labs to analyze identical samples with same method.	Locations for field duplicate analysis will be distributed through the project area; each field duplicate will be generated by collecting one additional sample in the immediate proximity of the primary sediment location and at the same time.
Accuracy	Measure of closeness of an individual measurement to a known or reference value. Expressed as percent recovery or percent bias.	Use a different method under the same conditions. Analyze a reference material to which a material of known concentration (spike) has been added.	Matrix spike/matrix spike duplicate (MS/MSD) samples will be submitted for laboratory analysis for every 20 sediment samples to evaluate the accuracy of each batch of samples analyzed for this project.
Bias	Systematic or persistent distortion of a measurement process that results in errors in one direction.	Measurement of materials with a known concentration.	Field spikes, matrix spikes: (MS/MSD) procedures are used as a laboratory control measure, and while not defined as field QA/QC samples, they do require additional sample volume. MS/MSD procedures are performed on field samples at a frequency of 1 per 20 samples.
Representative-ness	A qualitative measure of the degree to which data accurately and precisely represent a characteristic of a population parameter.	Evaluate whether measurements are made and physical samples collected in a way that the resulting data reflect the environment or condition being studied.	Describes how relevant the data are to the actual environmental condition. This can be controlled by careful site selection, which is discussed in the CAP (Ramboll Environ 2017).
Comparability	A qualitative term that describes the degree to which different processes, methods, or data, agree, or can be similarly represented. Expresses the measure of confidence that two data sets can contribute to a common analysis.	Compare the following: sample collection, sample handling, sample preparation, sample analytical procedures, holding times, and QA protocols.	Data comparability will be based on quality control samples already described (e.g., duplicates and MS/MSDs).
Completeness	An evaluation of the amount of data needed to be obtained from a measurement system. Expressed as a percentage of the number of measurements that should have been collected or planned to be collected.	Evaluation of the number of measurements needed to make a determination of the project results and comparison of this to the planned number of samples to be collected.	It is expected that all samples planned for collection will be collected per the CAP and all samples sent to the laboratory will be analyzed meeting hold times for the media of the project. Any samples not collected, not analyzed, or not meeting analytical hold times will be described in the CAP reporting, with rationales for why such conditions were not met and what implications, if any, those may have on the overall project understanding and interpretation. The CAP indicates that field collection will be opportunistic and will be based on field conditions at the time of sampling.
Sensitivity	Capability of a method to discriminate between measurement responses representing different levels of the variable of interest. Terms used to describe sensitivity include: method detection limit, limit of detection, and limit of quantification.	Determine the minimum concentration or attribute that can be measured by a method (method detection limit) by an instrument (instrument detection limit) or by a laboratory (quantitation limit.) with 99% confidence that the analyte concentration is greater than zero.	The methods employed by the laboratory support the reporting limits shown in Appendix C. The laboratory will report data to method detection limits. The reporting limits shown in these tables are on weight/weight basis for biological tissues. However, all biological tissues will be reported in both wet weight and dry weight by the laboratory. Fish tissues will also include lipid measurements so that lipid normalized data can be presented as well, if needed. The reporting limit achieved on individual samples will vary in accordance with percent moisture.

2.2.1 Equipment Blanks and MS/MSD Procedures

2.2.1.1 Equipment blanks

Equipment blank samples are used to assess the effectiveness of decontamination procedures. Equipment blank samples are obtained by pouring deionized water over or through the decontaminated sampling equipment and then collecting and submitting for analysis. Equipment blanks will be collected once per field event, if necessary.

2.2.1.2 Matrix spike/matrix spike duplicates (MS/MSD)

MS/MSD procedures are used as a laboratory control measure, and while not defined as field QA/QC samples, they do require additional sample volume. MS/MSD procedures are performed on field samples at a frequency of one per 20 samples.

The precision of the laboratory analysis for a given matrix is also measured by analyzing laboratory replicates, or laboratory matrix spike and matrix spike duplicate for the project specific analyses. Acceptable precision is defined as relative percent difference (RPD) between these replicates.

The methods employed by the laboratory will support the reporting limits shown in Appendix C. The reporting limits achieved on individual samples will vary in accordance with percent moisture. Reporting limits supported by the laboratory are elevated when dilutions are performed or reduced sample volume is analyzed. In order to avoid this type of reporting limit elevation, specific cleanup steps are specified in the analytical methods for metals. If matrix interferences result in QC outliers and the specified cleanups were performed, the laboratory may perform dilutions as needed, or contact the project chemist for further guidance. The reporting limits established by the laboratory will need to be sufficiently lower than the DQOs described above.

2.3 Special Training Requirements

The health and safety coordinator (HSC), Kerry Ann Jaggassar, will chiefly serve as an advisor to the on-site safety and health matters, and will determine the appropriate personal protection equipment (PPE) levels for worker protection. The HSC will review and approve the Ramboll Environ site-specific Health and Safety Plan (HASP). The HASP will be provided under separate cover. The personnel engaged in field activities will have completed the proper U.S. Occupational Safety and Health Administration health and safety training as indicated in the HASP. Ramboll Environ and their subcontractors will abide by U.S. Occupational Safety and Health Administration regulations and the site-specific HASP (Ramboll Environ 2017e). The HASP will be kept on-site during field activities, and a copy will be maintained in the project files.

Personnel shipping samples must complete the United States Department of Transportation hazardous materials transportation training and certification, including training in specific International Air Transport Association regulations (air shipments).

Analytical Laboratory Services (ALS), Kelso, Washington will be providing the analytical services. ALS is a certified laboratory with a current National Environmental Laboratory Accreditation Conference certification for all of the certifiable methods performed as part of the investigation. The laboratory managers will be responsible for ensuring that all personnel have been properly trained and are qualified to perform their assigned tasks.

2.3.1 Documentation and Records

The purpose of this section is to define the records that are critical to the project, the information to be included in reports, the format for reporting the data, and the appropriate document control procedures.

2.3.2 Field Documentation and Records

Field sampling activities will be recorded in field logbooks following procedures outlined in the field standard operating procedures provided in Appendix D. Field logbooks will be bound field survey books or notebooks. Logbooks will be assigned to the field crew, but stored in a secure location when not in use. The project name will identify each logbook, the title page of which will contain the following information:

- o Name of the person to whom the logbook is assigned
- o Logbook number
- o Project name
- o Project start date
- o Project end date

Logbook entries will provide as much detail as possible so personnel going to the site can reconstruct a particular situation without reliance on memory. Modifications to field sampling protocols must be documented in the field logbook.

At the beginning of each entry, the date, start time, weather, names of all sampling team members present, and the signature of the person making the entry will be documented. Measurements and samples collected will be recorded with a detailed description of the location of the station. The number of photographs taken also will be noted.

Entries will be made in indelible ink, and no erasures will be allowed. If an incorrect entry is made, then the information will be crossed out with a single strike mark, initialed, and dated. Blank pages will be noted as being intentionally blank.

Sample collection equipment will be identified, along with the time of sampling, sample description, parameters being analyzed, and number of containers. Unique sample identification (ID) will be assigned to each sample, including field duplicate samples, and will be noted in the field logbook or field forms.

Field personnel will provide comprehensive documentation of the various aspects of field sampling, field analysis, and sample chain-of-custody. This documentation constitutes a record that allows reconstruction of the field events to aid in the data review and interpretation process. Documents, records, and information relating to the performance of the fieldwork will be retained in the project file.

2.4 Data Reporting

Analytical data will be submitted in accordance with the laboratory contract. A data usability report will be completed and submitted as part of the project data deliverables by the project chemist. The report will include a review of the merged field and laboratory data, an assessment of field sample precision, a statement about data set completeness, and an assessment of overall usability that explains concerns about data usability for the intended purpose.

2.4.1 Field Data Reporting

Information collected in the field through visual observation, manual measurement, or field instrumentation will be recorded in field logbooks or forms and stored electronically in Adobe® (portable document format [PDF]) in the project file. The Field Team Leader (FTL) or project chemist will review the data for adherence to this QAPP and for consistency. Any concerns identified from the review will be discussed with the Ramboll Environ project manager, corrected if possible, and incorporated into the data evaluation process.

Field data calculations, transfers, and interpretations will be conducted by the field team and reviewed for accuracy by the FTL, project chemist, or appropriate designee. The data logs and documents will be checked for the following:

- General completeness
- Readability
- Use of appropriate procedures
- Clearly stated modifications to sampling procedures
- Reasonableness of data collected
- Correctness of sample locations
- Correctness of reporting units, calculations, and interpretations

Where appropriate, field data forms and calculations will be processed and included in appendixes to the report. Original field logs, documents, and data reductions will be kept in the project file. Field activities and field conditions will be selectively photographed to complement descriptions of field activities in the field logbook. In addition, photographs of cores from each location will be collected as part of the project record.

Standard field forms will be used in addition to the field logbooks to ensure necessary data are recorded consistently and to provide a more detailed record. No blank spaces will appear on completed forms. If the requested information is not applicable, the space will be marked with a dashed line or a "Not Applicable" ("N/A") designation. The forms will be completed in the field and placed in the project files. Field data sheets are provided with specific SOPs, as appropriate.

3 DATA COLLECTION

3.1 Sampling Experimental Design

The CAP provides the project's data collection activities and rationale for the design and selection of sampling locations, measurements/analytical parameters, matrix/media to be sampled, etc. and any supporting assumptions. Section 2 of the Ramboll Environ 2017 CAP summarizes the samples, including QC samples to be collected in the field. Table 2-1 of the Ramboll Environ 2017 CAP summarizes matrix/media types and frequencies of to be sampled for each measurement/analytical parameter, types of summarizes the field sampling counts by area; Table 2-2 provides a field sampling plan summary and sampling locations are provided in Figure 1-2 (Ramboll Environ 2017a).

Sampling includes the collection of sediment, surface water, pore water, invertebrate tissue, aquatic vegetation, and fish tissue for the analysis of metals. Additional analysis includes total and dissolved organic carbon in sediment and water, hardness in water, percent solids in sediment samples, percent lipid in tissue samples, and percent moisture in sediment and tissue samples. Water quality metrics will include pH, dissolved oxygen, oxidation reduction potential, specific conductivity, temperature, and turbidity which to be recorded at each surface water, sediment, and pore water sampling location using a multi meter.

3.2 Sampling Procedures and Requirements

Section 2 of the CAP provides detailed description of the Herrington Lake field sampling plan and reporting procedures. As was mentioned in Section 1 of this QAPP, the Cabinet requested development of this QAPP and specific SOPs for the sampling program described in the CAP. This QAPP generally follows the Cabinet requested format, as appropriate for the Herrington Lake effort, and cross references the CAP sections to avoid unnecessary duplication of CAP text that has already been reviewed and is also subject to public comment. The CAP is informative of sampling collection and handling procedures. The following SOPs, along with this QAPP, developed at the request of the Cabinet, provide additional collection and handling procedures:

- SOP: Fish Tissue Collection and Analysis for Herrington Lake
- SOP: Aquatic Vegetation and Aquatic Invertebrate Tissue Collection and Analysis for Herrington Lake
- SOP: Sediment Pore Water Collection Approach for Herrington Lake

This QAPP is intended to provide general overview that supplements the CAP and SOPs. The sections herein briefly describe procedures for collecting field samples and the associated field QC samples including sampling methods and equipment needed to complete the field sample collection. Sample nomenclature will be based on the sample transect, the media or tissue sample type, the sample depth, any quality assurance samples, and sample date.

3.3 Decontamination Procedures

To avoid cross-contamination, disposable or dedicated PPE and sampling equipment will be used when possible. All sampling equipment will be cleaned between sample locations. Decontamination will be conducted in a central location, upwind and away from suspected contaminant sources. The following procedures will be used for all equipment used to collect routine samples undergoing trace organic or inorganic constituent analyses:

- Wash and scrub all sampling equipment with a recommended laboratory critical-cleaning detergent (Alconox®)/distilled water mix, since the samples will be analyzed for trace metals.

- Rinse thoroughly with tap water.
- Rinse thoroughly with distilled water or deionized water (whichever is readily available).
- Rinse again with distilled/deionized water.
- Air-dry until completely dry.
- Remove the equipment from the decontamination area and cover with plastic. Equipment stored overnight should be wrapped in aluminum foil and covered with clean, unused plastic.

3.4 Waste Disposal Procedures

IDW are materials that are known or suspected to be contaminated with hazardous substances through the actions of sample collection or personnel and equipment decontamination. Appendix D-4 contains the USEPA SOP for IDW. IDW are classified into three categories:

- Solid materials consisting of sediments, used PPE, and other materials used in the handling, processing, and storage of sediment
- Liquid wastes such as waste Study Area water and decontamination water
- Spent and residual chemicals (liquids) from decontamination

All waste generated during field activities will be stored, transported, and disposed of according to Kentucky state, federal, and local regulations. All wastes classified as hazardous will be disposed of at a licensed treatment storage and disposal facility or managed in other approved manners. In general, waste disposal should be carefully coordinated with the facility receiving the waste.

Facilities receiving waste have specific requirements that vary even for non-hazardous waste, so characterization should be conducted to support both applicable regulations and facility requirements.

3.5 Corrective Actions

This section identifies corrective actions when equipment or materials are compromised, or when sample methods are altered, temporarily or permanently. Corrective Action Request (CAR) Forms are provided in Appendix E.

3.5.1 Field Corrective Actions

When a significant condition adverse to quality is noted at the site, corrective action procedures are as follows:

- FTL shall submit a CAR form, or site personnel shall identify and document activities or documents ascertained to be noncompliant with QA requirements.
- The QA manager, project manager, and FTL shall be promptly notified of any significant problems or discrepancies.
- Corrective actions require a written record including condition identification and cause, any reference documents, and the planned corrective action.
- FTL shall update CAR form to verify that corrective action has been implemented.
- The project manager will be responsible for ensuring that recommended corrective actions are implemented, documented, and approved.

Any significant corrective actions taken in the field will be documented in writing in field logbooks and reported to the project manager as well as any field conditions encountered that may compromise data usability. For example, if a certain fish collection method not addressed herein is required in

order to sample a specific habitat or collect a specific species, reasons for the change in sampling method will be recorded in the field notebook, and detailed field procedure implementation notes will be documented.

3.5.2 Laboratory Corrective Actions

If a significant condition adverse to quality is noted at ALS, corrective action procedures are as follows:

- ALS is responsible for reporting any significant problems or discrepancies that occur as analyses are conducted to the project manager or other identified project contact.
- ALS is responsible for assuring that corrective action is taken where appropriate to prevent the reoccurrence of similar problems or discrepancies.
- Each analytical/processing data report will include a case narrative that discusses any problems or discrepancies, and sufficient calibration and QC information to verify that the method was in control at the time that the samples were analyzed.
- The case narrative will include a discussion of any corrective action taken by the subcontractor laboratory to prevent the reoccurrence of similar problems or discrepancies.

In the event that the nonconformance condition is identified and investigated but there is no assignable cause, this finding should be documented and the condition should be monitored to determine if the condition was an isolated event.

3.6 Sample Handling and Custody Requirements

This section presents sample custody procedures for the field, transport, and laboratory activities. Implementation of proper custody procedures for samples generated in the field is the responsibility of field personnel. The primary objective of chain-of-custody procedures is to provide an accurate written or computerized record that can be used to trace the possession and handling of a sample from sampling through completion of all required analyses. A sample is in custody if it is any one of the following:

- in a team member's physical possession;
- in a team member's view locked up or locked and secured nearby
- kept in a secured area that is restricted to authorized personnel

Both laboratory and field personnel involved in the chain-of-custody and transfer of samples will be trained as to the purpose and procedures prior to implementation.

3.7 Sample Containers, Preservation, and Holding Times

The relevant container, preservation, and hold time procedures are as follows:

- The analytical laboratory will supply the contaminant-free sample containers used for chemical analysis.
- Sample containers for laboratory analyses will meet or exceed the requirements in Specifications and Guidance for Obtaining Contaminant-Free Containers (USEPA 1992).
- Containers used for sampling will not contain target organic and inorganic contaminants exceeding the levels specified. No sample containers will be reused during collection activities for this project.
- In addition to the samples for analysis, some samples may be submitted for archive. Archival samples will be maintained by the laboratory at 4°C (exception - all fish will be

kept frozen until extracted for analysis) until notified that the samples are to be analyzed or disposed

A summary of sample containers, preservation, holding times are provided in media specific SOPs. Analyte detection limits are presented in Appendix C of this QAPP.

3.8 Sample Packaging and Handling

Sample handling, packaging, and shipping procedures are described in briefly as follows:

- Sample coolers will be shipped to arrive at the analytical laboratory the morning after sampling (priority overnight) or sent by courier to arrive the same day.
- The laboratory will be notified of the sample shipment and the estimated date of arrival of the samples being delivered.
- Chain-of-custody documentation will be transmitted to the analytical laboratory along with the samples.
- The transportation and handling of samples must be accomplished in a manner that not only protects the integrity of samples but also prevents any detrimental effects due to the possible hazardous nature of the samples.

Regulations for packaging, marking, labeling, and shipping of hazardous materials are promulgated by the DOT in 49 CFR 171 through 177.

3.9 Sample Custody

Formal sample custody procedures begin when the pre-cleaned sample containers leave the laboratory or upon receipt from the container vendor. Sample identification documents must be carefully prepared so that sample identification and chain-of-custody can be maintained and sample disposition controlled. Sample identification documents include the following:

- Field logbooks
- Field forms
- Sample labels
- Custody seals
- Chain-of-custody records

3.9.1 Field Custody Procedures

Pre-cleaned sample containers will be relinquished by the laboratory to the FTL. The FTL will record receipt of the sample containers in the project logbook. The following field custody procedure will be used for collection of samples:

- As few people as possible should handle samples
- Coolers or boxes containing cleaned bottles should be sealed with a custody tape seal during transport to the field or while in storage prior to use
- The sample collector is personally responsible for the care and custody of samples collected until they are relinquished to another custodian or dispatched properly under chain-of-custody rules
- Chain-of-custody forms will be signed and dated by the appropriate personnel to document the custody transfer

- The sample collector will record sample data in the field logbook
- Original chain-of-custody forms will accompany samples to the laboratory, and copies will be forwarded to the project files
- The FTL will determine whether proper custody procedures were followed during the fieldwork and decide if additional samples are required

3.9.2 Custody Seals

Custody seals are preprinted, adhesive-backed seals with security slots designed to break if the seals are disturbed. DOT-approved sample shipping containers are sealed in as many places as necessary to ensure security. Seals must be signed and dated before placement on the coolers. Upon receipt at the laboratory, the custodian must check and document on a cooler receipt form that seals on boxes are intact.

3.10 Laboratory Custody Procedures

- Upon receipt of samples, the laboratory sample custodian will verify package seals, open the packages, check temperature blanks (and record temperatures), verify sample integrity, and inspect contents against chain-of-custody forms.
- Cooler temperature is checked and documented on the laboratory's cooler receipt form. If the sample temperatures are outside the required range, then the laboratory will contact the QA/QC manager for direction on the proper course of action.
- At this point, any discrepancies and nonconforming conditions are addressed, and documented on the cooler receipt form and must be resolved before samples are released to the laboratory for analysis. The laboratory project manager will contact the Ramboll Environ field manager or her designee to resolve discrepancies and report nonconforming conditions prior to releasing the samples for analysis.
- When the shipment and the chain-of-custody agree, the custodian enters the sample and analysis information into the Laboratory Information Management System and assigns each sample a unique laboratory number. This number is affixed to each sample bottle.
- The original of the chain-of-custody form is then given to the data management group, the information it contains is copied to the appropriate laboratory operation areas.
- These login procedures are documented in each lab's sample management SOPs.

3.11 Sample Storage and Security

- With the exception of the fish samples, which will be frozen before transportation to the laboratory, all field samples will be placed into respective labeled sample containers and stored on ice in coolers at 4°C.
- While in the laboratory, the samples that require storage at approximately 4°C will be maintained in a secured refrigerator unless they are being analyzed. All of the refrigerators/freezers in the laboratory used for storage of samples have restricted access, are numbered, and the actual storage location is traceable at all times.
- In the lab, there are dedicated refrigerators designated for extracts and analytical standards. The sample storage areas are within the laboratory to which access is limited to laboratory chemists and controlled by assigned passkeys.

For this project, all remaining sample volume and associated extracts will be held in storage for 60 days after submittal of the final analytical report, unless longer retention is specified by KDOW and

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USEPA. If entire samples are depleted during analysis, a notation of "sample depleted" or "entire sample used" will be made on the internal chain-of-custody forms.

4 ANALYTICAL METHODS

The analytical methods, method detection limits, and reporting limits for each parameter are provided in Appendix B.

- All Analytical methods used by the selected lab are EPA-approved methods. The lab will address any issues of failed calibration checks or contamination in the analytical data and report those issues to the project lead.
- All inorganic compounds for this project are determined using the methods listed in Appendix B and described in the laboratory's analytical SOPs. The project MDLs and reporting limits are listed in Appendix B-2.

4.1 Quality Control Requirements

This section summarizes the required QC samples for the field sampling methods; reference lab SOPs for analytical QC. State the frequency for each type of QC sample, the acceptance criteria for each QC process, as well as the associated corrective action if the acceptance criteria are not met.

QC data are necessary to determine precision and accuracy and to demonstrate the absence of interferences and/or contamination of glassware and reagents. Field QC will include duplicates samples. Field QC samples will be preserved, documented, and transported in the same manner as the samples they represent. Laboratory-based QC will consist of standards, replicates, spikes, and blanks. Method QC limits for analyses are provided in Appendix C.

The QC analysis should include the calculations for percent-difference, standard deviation, or for whatever appropriate measure was outlined in the section. The table below describes example QC control checks on a project basis.

Table 2. Project Quality Control Checklist. Source: Kentucky Department for Environmental Protection Division of Water (KDOW 2016a)

QC Sample	Information Provided
<u>Blanks</u> <ul style="list-style-type: none"> • bottle blank • field blank • reagent blank • rinseate or equipment blank • method blank • trip blank 	<ul style="list-style-type: none"> • cleanliness of sample bottles • transport, storage and field handling bias • contaminated reagent • contaminated equipment • response of a laboratory analytical system • contamination during shipment/transport
<u>Spikes</u> <ul style="list-style-type: none"> • matrix spike • matrix spike replicate • analysis matrix spike • surrogate spike 	<ul style="list-style-type: none"> • analytical (preparation + analysis) bias • analytical bias and precision • instrument bias • analytical bias
<u>Calibration Check Samples</u> <ul style="list-style-type: none"> • zero check • span check • mid-range check 	<ul style="list-style-type: none"> • calibration drift and memory effect • calibration drift and memory effect • calibration drift and memory effect
<u>Replicates, splits, etc.</u> <ul style="list-style-type: none"> • field collocated samples • field replicates • field splits • laboratory splits • laboratory replicates analysis replicates	<ul style="list-style-type: none"> • sampling + measurement precision • precision of all steps after acquisition • shipping + inter-laboratory precision • inter-laboratory precision • analytical precision • instrument precision

4.2 Field Quality Control Samples

The collection of field QC samples and the conditions, under which the samples were collected, will be documented in the field logbook.

4.2.1 Field Duplicate Samples

Field QA/QC samples collected during the proposed investigation include field duplicate and equipment blank samples. The field duplicate is a replicate sample collected as close as possible to the same time that the primary sample is collected and from the same location, depth, or source, and is used to document analytical precision. Field duplicate samples will be labelled and packaged in the same manner as primary samples but with "Dup" appended to the sample ID. Field duplicates will be collected at a frequency of one (1) in every 10 primary samples and will be analyzed for the same suite of parameters as the primary sample.

Duplicate samples will be shipped with the samples they represent, and will be analyzed in the same manner. The RPD between the concentration in the original and duplicate sample measures the overall precision of the field sampling. Field duplicates are evaluated by using two times the laboratory QC criteria for duplicates (i.e., RPD of 70% for sediment). If all other laboratory QC criteria are met, RPD results outside control limits indicate potential matrix effects. Significant deviations in RPD results of field duplicates will be assessed to evaluate whether data met all quality objectives for the project.

4.2.2 Field Equipment Blanks

Equipment blank samples are used to assess the effectiveness of decontamination procedures. Equipment blank samples are obtained by pouring deionized water over or through the decontaminated sampling equipment and then collecting and submitting for analysis. Equipment blanks will be collected once per field event, if necessary. In addition, MS/MSD procedures are used as a laboratory control measure, and while not defined as field QA/QC samples, they do require additional sample volume. MS/MSD procedures are performed on field samples at a frequency of one (1) per 20 samples, or 5%.

4.2.3 Laboratory Quality Control Analyses

QC samples will be analyzed at the frequency stated below for each matrix.

4.2.3.1 Independent Calibration Verification

A mid-level standard from a second source containing all the target analytes is analyzed as the initial calibration verification (ICV). The ICV measured concentration must be within +25% of its expected value for organics, and within +10% of its expected value for metals. The analytical results for the solid reference materials used as ICVs are evaluated against their certified concentrations.

4.2.4 Method Blanks

The method (reagent) blank is used to monitor laboratory contamination. A laboratory standard solid material will be processed through the same analytical procedure as the sample (i.e., digested, extracted, and distilled). One method blank is analyzed at a frequency of one (1) per every analytical preparation batch of 20 or fewer samples.

4.2.5 Laboratory Control Sample

The laboratory control sample is a fortified method blank consisting solid fortified with the analytes of interest for single-analyte methods and selected analytes for multi-analyte methods according to the

appropriate analytical method. They are prepared and analyzed with each analytical batch, and analyte recoveries are used to monitor analytical accuracy and precision.

4.2.6 Matrix Spike/Matrix Spike Duplicate (MS/MSD)

A fortified sample (MS) is an aliquot of a field sample that is fortified with the analyte(s) of interest and analyzed to monitor matrix effects associated with a particular sample. Samples to be spiked are chosen at random. The final spiked concentration of each analyte in the sample should be at least 10 times the calculated MDL. A duplicate fortified sample (MSD) will be performed for every batch of 20 or fewer samples.

4.3 Equipment Testing, Calibration, and Maintenance Requirements

Equipment and instruments will be inspected and maintained in accordance with the manufacturer's specified recommendations to ensure quality.

4.3.1 Instrument Calibration, Testing, and Maintenance

This section summarizes the instrument calibration procedures, outlining how it will be performed and documented in the field.

4.3.2 Field Calibration Procedures

Calibration checks of field instruments will be conducted as needed where readings are suspect to produce accurate and reproducible data. Field instruments will be recalibrated at least once per day prior to the beginning of the sampling activities to confirm that the instrument functioned properly throughout the day and to provide information to assess drift, if any, occurring the period of operation.

4.3.3 Laboratory Calibration Procedures

Instruments and equipment used in the laboratory should be calibrated regularly under a program that verifies that equipment is of the proper type, range, accuracy, and precision to provide data compatible with specified requirements. All instruments and equipment that measure a quantity, or whose performance is expected at a stated level, are subject to calibration. Written procedures should be used by the laboratory for all instruments and equipment subject to calibration. Two types of calibration are discussed in this section:

- Operational calibration, which is routinely performed as part of an analytical procedure or test method, such as the development of a standard curve for use with an atomic absorption spectrophotometer. Operation calibration is generally performed for instrument systems.
- Periodic calibration, which is performed at prescribed intervals for equipment, such as balances and thermometers. In general, equipment that can be calibrated periodically is a distinct, singular purpose unit and is relatively stable in performance.

4.3.4 Instrument Testing and Maintenance

Major instruments in the laboratory are covered by annual service contracts with manufacturers. Under these agreements, regular preventive maintenance visits are made by trained service personnel. Maintenance is documented and maintained in permanent records by the individual responsible for each instrument. Scheduled preventive maintenance for laboratory equipment and their frequency of performance are documented on laboratory maintenance logs, which are standardized and controlled by the QA manager (or designee).

4.4 Supplies and Consumables

Sampling, preparation/processing, and shipment supplies will be provided as specified as follows:

- Each field team is responsible for gathering and inspecting the sampling gear and the received sample packaging and shipping supplies prior to the sampling event.
- Defective packaging and shipping supplies (e.g., broken glassware) will be discarded, and, if necessary, the field team will contact the supplier to obtain replacement supplies.

4.5 Data Acquisition Requirements for Non-direct Measurements

Section 1.2 of the CAP provides a detailed summary of the Herrington Lake Study Area and existing information (Ramboll Environ 2017a). Existing information for Herrington Lake and the E.W. Brown Generating Station were summarized from a variety of sources. Data were used from these existing studies to inform the field-sampling program described in Section 2 of the Ramboll Environ 2017 CAP. The studies included the Groundwater Assessment Report (GWAR, AMEC 2013), Groundwater Assessment Update (GWAR Update, AMEC 2015b), the KPDES Discharge Monitoring Reports, spring sampling data, sediment data (AMEC 2013), and fish tissue data (Kentucky Division of Water [KDOW] 2016a).

Additional non-direct measurements that apply to the Herrington Lake CAP are observations made during the implementation of the field effort. Example field data sheets are provided as part of this QAPP and are provided as part of specific SOPs. The field data sheets will be included in the project record as either scanned pages or with specific data entry, as appropriate, given the nature of the field data sheet.

A photographic record of sample collection will also be provided. Photographs will be reported as appropriate and logical given the amount of information needed to convey CAP results.

4.6 Data Management Requirements

Data will be managed using Microsoft Excel, Microsoft Access, and a geographic information system, to the extent logical for the particular category of data. QC queries will be run to ensure that sample results have an associated entry. In addition, a completeness check will be performed to ensure that requested analyses were completed. Ramboll Environ staff will review all laboratory data packages for completeness, for deviations from QC procedures, and for general data quality. Metrics, if calculated by the laboratory, will be rechecked. These procedures will ensure that preliminary review of pre-validated data can be accomplished in a timely manner to reduce the potential for errors not related to validation-related changes.

4.6.1 Field Records

Field notebooks and datasheets will be scanned and permanently archived in computer files at the Atlanta, Georgia office where this project will be managed on a day to day basis. All electronic files will be backed up separately as part of normal business procedures, including scanned copies of field notes.

4.6.2 Laboratory Records

The raw data from the subcontractor laboratories will be submitted to Ramboll Environ electronically as well as in hard copy. Electronic versions of raw data shall be permanently archived in computer files at the Atlanta, Georgia Ramboll Environ office. All electronic files will be backed up separately as part of normal business procedures, including scanned copies of field notes.

The laboratory will maintain calibration, analysis, and corrective action documentation associated with the sample analysis for a minimum of five years. A separate file will be maintained for each analytical procedure. The documentation maintained must be sufficient to document factors used to derive the final (reported) value for each sample. Calculations performed by hand will be documented in writing, signed, and dated by the person performing the hand calculations and stored with the raw data.

Calculations performed by the data system will be documented and stored as electronic and hard copy data. Any photo documentation data will be included in the data deliverable and retained as an electronic file by the laboratory for a minimum of five years.

4.6.3 Electronic Data Deliverables

The laboratory will provide Ramboll Environ with electronic data deliverables and all reporting for this project will include electronic data that can be readily provided to the Cabinet. Analytical data that has undergone the QA/QC process will be loaded into a database and reported in tabular format. Database fields will be provided for information regarding the field sample number, analytical/processing results, and validation qualifiers.

4.6.4 Project Files

Project documentation will be maintained in general accordance with USEPA guidelines. A project file will be maintained that will contain appropriate project documentation. Some of this documentation may be retained electronically in lieu of paper copies. This file at a minimum will include these records:

- Project plans and specifications
- Field logbooks and data records
- Photographs, maps, and drawings
- Sample identification documents
- Chain-of-custody records
- The entire analytical data package provided by the laboratory, including QC documentation
- Data review notes
- Progress and technical reports
- Correspondence and other pertinent information

4.6.5 Document Retention

Data generated by the subcontractor laboratories will be produced electronically and in a hard copy report. Electronic versions of raw data shall be permanently archived in computer files at the Ramboll Environ Atlanta, Georgia office. The hard copies of laboratory records will be held separately at Ramboll Environ offices for backup protection against fire, vandalism, and computer failure. Electronic and hard copies shall be retained for at least five years following project completion.

5 ASSESSMENTS

5.1 Technical Assessments

The data collected during this project will undergo a systematic review for compliance with the DQOs and performance objectives as stated in Section 1.7. In particular, laboratory and field data will be reviewed by the QA/QC manager for compliance with the method QC criteria, including performance and accuracy. In cases where data may be considered not usable (i.e., rejected during data validation), resampling may be required at a specific location due to exceedances of holding times or poor laboratory performance.

5.1.1 Quality Assurance Performance Audits and System Audits

Field and/or laboratory QA audits will be implemented to assure that the project work is conducted in the manner specified within the QAPP. An on-site field audit will be conducted on a weekly basis by the FTL to assess compliance of sampling methods with the methods specified in this QAPP and good field practices. Audit results, including observations on compliance with, or deviations from, the QAPP, will be documented in a field logbook. If deviations occur, which in the opinion of the FTL will not adversely affect the results, and then the FTL shall document the recommendation of “no corrective action” in the notes. Where deviations require corrective actions, those recommendations shall be recorded on the corrective action request (CAR) forms (Appendix E). The CAR identifies the out of compliance condition, reference document(s), and recommended corrective action(s) to be administered. The CAR is issued to the personnel responsible for the affected item or activity and to the project manager and/or QA/QC manager for approval. Ramboll Environ 2017 CAP Section 2.2.3, describes the corrective action procedures in detail.

During the on-site field audit, the FTL should document any deviations that will substantially affect the usefulness or the quality of the data and/or the interpretations of those data. The audit findings, along with the necessary opinions and rationale and the records of requested corrective actions, shall be provided to the project manager. The project manager will be responsible for addressing the findings of the on-site field audit. On-site audits of ALS laboratories may be conducted internally if deemed warranted based on communication with laboratory personnel. Laboratories shall maintain documentation of internal quality control procedures, audit results, and any corrective actions that were necessary.

Additionally, the laboratories shall provide interpretation of data quality based on their QA/QC procedures and criteria, and, where warranted, will provide data qualifiers. The project manager has overall responsibility to ensure that corrective actions necessary to resolve audit findings are acted upon promptly and satisfactorily. Serious deficiencies will be reported to the project manager within 24 hours. CAR forms, if needed, audit findings, and acceptable resolutions need to be approved by the project manager and/or QA/QC manager prior to issue.

5.2 Reports to Management

Reports to management include the submission of weekly progress reports, data usability summaries, and project status reports.

The ‘data usability summary report’ will be completed by the QA/QC manager. Impacts on the usability of data will be tracked by adding qualifiers to individual data points.

Project status reports are completed by the project manager or by FTL to document the overall assessment of the project. Upon completion of a project, sampling effort, and analytical and QC data will be included in a report that summarizes field activities and provides data evaluation. A discussion of the validity of results in the context of QA/QC procedures will be included. Management personnel receive QA reports appropriate to their level of responsibility. The project manager receives copies of QA documentation. QC documentation is retained within the department that generated the product or service except where this documentation is a deliverable for a specific contract. QC documentation is also submitted to the QA manager for review and approval. Previous sections detailed the QA activities and the reports. Serious analytical or QA problems will be reported immediately to the project manager. Corrective action full documentation may include procedural alterations to field methods, or laboratory protocol modifications.

6 REVIEW, EVALUATION, AND REPORTING

6.1 Initial Data Review

The CAP and the SOPs summarize the constituents to be measured and the laboratory methods that will be used. All documentation and records used throughout the project, including field and lab records will be included in the investigation report.

6.2 Validation and Verification Methods

The QA/QC manager will conduct data validation, including a review of field sampling forms, custody documentation, and laboratory QC methods and documentation, Level II data validation will be performed on all samples using USEPA functional guidelines for inorganics (USEPA 2014). Level IV data validation will be performed on approximately 20% of the samples. Validation will be conducted in accordance with USEPA guidelines. Any discrepancies between the forms and the QAPP or the reported data will be reconciled with the appropriate field personnel and will be documented. Calibration documentation of laboratory instruments will be reviewed as warranted. The laboratory will review chain-of-custody forms upon receipt of samples to ensure that holding times have not been exceeded. Violations of holding times will be reported by the laboratory. Data entered into computer files will be validated by comparison to each laboratory data sheet. Appendix C summarizes laboratory and field-data validation qualifiers. The project manager and the QA manager will address other data validation issues that may arise individually.

6.3 Laboratory Data Reporting

Analytical data will be transferred directly from the instrument to a computerized data system. Raw data will be stored electronically. Laboratory data entry will be sufficient to document information used to arrive at reported values.

Types of records to be maintained by the laboratory and to be provided as part of the laboratory report include the following:

- Complete chain-of-custody records from sample receipt to destruction. Sample destruction records must contain information on the manner of final disposal
- Supporting documentation for any nonconformance or corrective action forms supplied in the analytical report or related to the analysis of project samples
- Computer records with magnetic tape backup of cost information, scheduling, laboratory
- Chain-of-custody transfers, and laboratory management records
- Laboratory notebooks including raw data such as readings, calibration details, and QC results
- Hard copies of data system printouts (i.e., chromatograms, mass spectra, and inductively coupled plasma [ICP] data files).

6.4 Evaluation of Data Usability

Data usability report will be completed and submitted as part of the project data deliverables by the QA/QC manager. The report will include a review of the merged field and laboratory data, an assessment of field sample precision, a statement about data set completeness, and an assessment of overall usability that explains concerns about data usability for the intended purpose.

6.5 Reconciliation with Project Requirements

The QA/QC manager will be responsible for evaluating precision, accuracy, representativeness, comparability, and completeness of data using procedures described. Any deviations from analytical performance criteria or quality objectives for the project will be documented in the data usability summary report provided to the data users for the project.

The QA/QC manager will work with the final users of the data in performing data quality assessments. The data quality assessment may include some or all of the following steps:

- Data that are either determined to be incomplete, or not usable for the project, will be discussed with the project team. If critical data points are involved which impact the ability to complete project objectives, data users will report immediately to the project team, discuss resolution of the issue and implement necessary corrective actions (for example resampling);
- Data that are non-detect but have elevated reporting limits due to blank contamination or matrix interference will be compared to site-specific remedial goals. If reporting limits exceed the screening values, then results will be handled as incomplete data as described above; and
- Data that are qualified as estimated will be used for all project decision making. If an estimated result is close to a site-specific remedial goal, then there is uncertainty in any conclusions as to whether the result exceeds the remedial goals. The data user must evaluate the potential uncertainty in developing recommendations for the site.

6.6 Reports to Management

Section 6 of the Ramboll Environ 2017 CAP provides a list of specific milestones and reports:

- Draft CAP submitted to the Cabinet on April 14, 2017.
- Phase I of Herrington Lake sampling will be conducted in late 2017 and in the first or second quarter of 2018,
- Two surface water field-sampling events in summer stratification and fall/winter Lake overturn timeframes.
- Preparation of a Phase I Technical Memorandum will be provided to the Cabinet
- Laboratory analyses, reporting, and data validation will follow each effort,
- Two Herrington Lake Corrective Action Reports

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https://www.epa.gov/sites/production/files/2015-09/documents/r4_era_guidance_document_draft_final_8-25-2015.pdf

HERRINGTON LAKE QAPP APPENDICES

Appendix A. Herrington Lake Study Area and Project Schedule

Appendix B. Data Quality Objectives

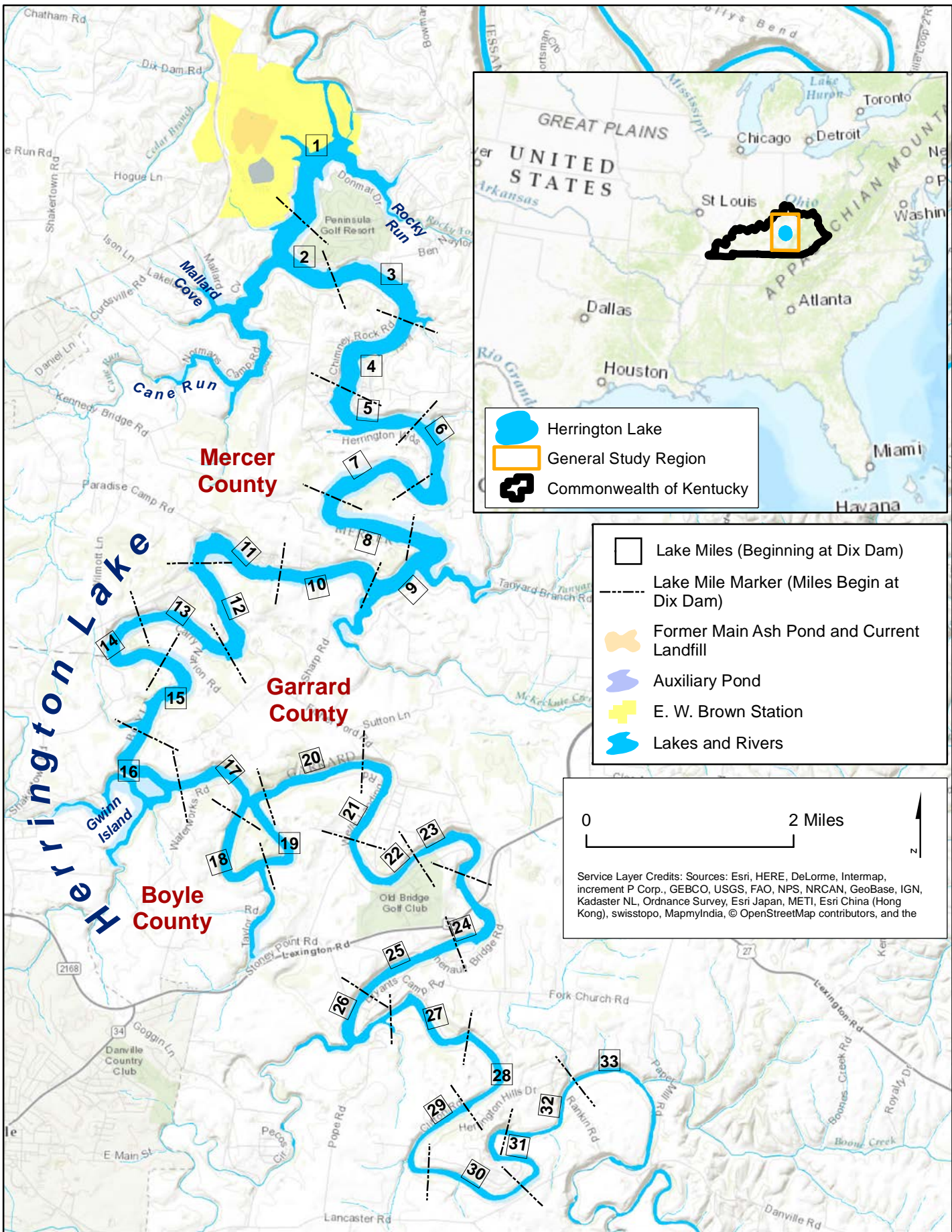
Appendix C. Laboratory Detection and Reporting Limits

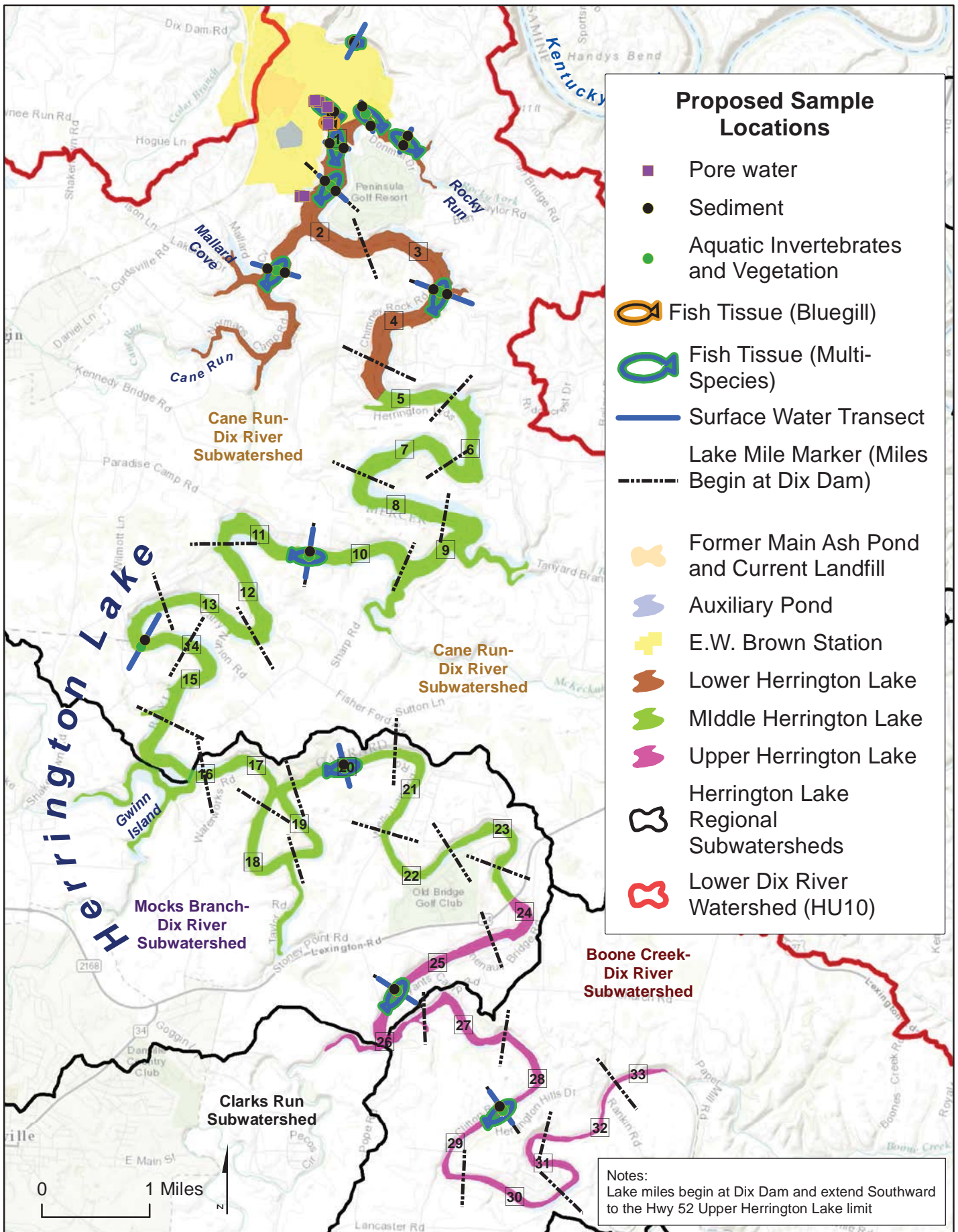
Appendix D. KY Division of Water Standard Operating Procedures

APPENDIX A
HERRINGTON LAKE STUDY AREA AND PROJECT SCHEDULE

Appendix A-1. Herrington Lake Study Area

Appendix A-2. Project Schedule





**Table A-2. Proposed Approximate Schedule for Activities and Reporting Through Phase I
Herrington Lake Corrective Action Plan
Mercer County, Kentucky**

Tasks and Proposed Schedule Assuming Phase I Sampling Is Sufficient for Remedial Decision-making	Estimated Time By Task	2017						2018						2019																
		Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Public Review & Cabinet Approval	30 days	█																												
Field Planning	60 days	█	█																											
Phase I Field Sampling Effort (Summer Stratification Sampling Event)	30-45 days			█	█																									
Laboratory Analysis	30-45 days			█	█																									
Data Validation & Data Mgt	30 days					█																								
Data Analysis	60 days						█	█																						
Draft Phase I Tech Memo	60 days								█	█																				
Phase I Field Sampling Effort (Overturn Water Sampling Event)	7 days										←	→																		
Laboratory Analysis and Validation	30 days											█																		
Data Analysis	14 days												█																	
Draft Phase I Tech Memo	30 days													█																
Draft Phase I Tech Memo to Cabinet	~June 15														X															
Cabinet Review	30 days															█														
Respond to Cabinet Comments and Submit Final Memo	45 days																█													
Cabinet Review and Approval of Final Memo	30 days																	█												
Develop Draft Investigation, Risk Assessment, Source Identification Report	90 days																		█	█	█									
Submit Draft Report to Cabinet	~Jan 15																				X									
Cabinet Review	45 days																					█								
Response to Cabinet Comments and Submit Final Report	60 days																						█	█						
Develop Draft Corrective Action Remedy Evaluation Report	120 days																													
Submit Draft Corrective Action Remedy Evaluation Report to Cabinet	~Aug 1																												X	
Cabinet Review	45 days																											█	█	
Response to Cabinet Comments and Submit Final Report	60 days																												█	█

O Overturn
SS Summer Stratification assumes that sampling will include surface water, sediment, pore water, and biological tissues.

█ Schedule that involves Cabinet Reviews
█ Schedule that involves field or reporting efforts
← Schedule notation reflects that overturn sampling is not certain.



**APPENDIX B
DATA QUALITY OBJECTIVES**

Appendix B-1. Sample Location Data Quality Objectives

Appendix B-2. Data Quality Characteristics Formula

**Table B-1. Herrington Lake Field Sampling Plan Summary
Herrington Lake Corrective Action Plan
Mercer County, Kentucky**

Herrington Lake Areas	Sample Location Data Quality Objectives
Phase I: Lower Herrington Lake (LHL) (Figure 2-1, 2-2A, 2-2B, 2-2C, 6-1A, 6-1B)	
Rocky Run Embayment	This location is northeast from the plant and will be used to characterize the influence of flow from the Rocky Run portion of the Can Run-Dix River Sub watershed. There are no known KPDES permitted dischargers in this area. KYDOW identified fish tissue samples were collected in this area in 2016, but no fish tissue data were available for review at the time of CAP plan development.
Dix River Dam	This location is near the plant and near the dam. Data from this location will be used to characterize the potential influence from the plant. KYDOW fish sampling in this area did not include bluegill.
Dix River Main Channel	There are 3 samples on the main channel of the Dix River in the LHL area at approximately RM 0.5, 1, and 3. Data from these locations will be used to identify a gradient of concentration with distance from Curds Inlet, if such a gradient exists, particularly for small home range fish (bluegill) and the other biological samples (aquatic vegetation and aquatic invertebrates).
Cane Run Embayment	This sample location reflects a large embayment to the lake. The location is placed near a permitted KPDES outfall location, with two additional KPDES permits also present upgradient from this location. Data from this location will be used to characterize the potential influence from the other sources.
Curds Inlet	Curds Inlet reflects the area proximate to the plant. This area has the highest density of sediment sampling, augmenting the sediment locations sampled in 2017. In addition, this is the key area of focus for the pore water selenium speciation. This area reflects one fish collection area for three species of fish. Three aquatic vegetation and three aquatic invertebrate samples are planned from this area as well. Data from the sampling of the inlet will be used to evaluate the potential contribution of sediment to the food web of Herrington Lake. Data from this inlet will also be used to identify the potential sources of selenium to the lake from the plant.
HQ Inlet	The HQ Inlet is also proximate to the plant. Surface water, sediment and vegetation sampling is identified for this inlet. Data from the sampling of the inlet will be used to evaluate the potential contribution of sediment to the food web of Herrington Lake. Data from this inlet will also be used to identify the potential sources of selenium to the lake from the plant.

**Table B-1. Herrington Lake Field Sampling Plan Summary
Herrington Lake Corrective Action Plan
Mercer County, Kentucky**

Herrington Lake Areas	Sample Location Data Quality Objectives
Hardin Inlet	Hardin Inlet reflects a small inlet removed from the plant. The focus of sampling in this area is surface water and pore water to augment the sediment samples collected in this area in 2017. The data from the sampling of this inlet will be used to compare against surface water, sediment, and sediment pore water from Curds Inlet and the HQ Inlet to understand potential influences from the plant to the lake.
Phase II: Middle Herrington Lake (MHL) (Figure 2-1, 2-2D, 6-1A, 6-1B)	
Cane Run SW	The samples in this portion of the MHL include a transect at RM 10 (including fish) and a transect at RM 14. Data from these locations will be used to characterize influence of the flow from the Can Run-Dix River Subwatershed, including potential influence from KPDES permitted locations.
Mocks Branch SW	The samples in this portion of the MHL include a transect at RM 20 (including fish) and a transect at RM 23. Data from these locations will be used to characterize influence of the flow from the Mocks Branch Subwatershed, including potential influence from KPDES permitted locations.
Phase II: Upper Herrington Lake (UHL) (Figure 2-1, 2-2E, 6-1A, 6-1B)	
Clarks Run SW	The sample location at RM 25 (including fish) was placed just downgradient of the Clarks Run Subwatershed. Data from this location will be used to evaluate flow from the subwatershed, including potential influence from 8 KPDES permitted dischargers.
Lower Hanging Fork Creek SW	The sample location at RM 28 reflects the furthest upgradient Herrington Lake location. Data from this location will be used to evaluate flow from the subwatershed, including potential influence from a KPDES permitted discharger.
Phase I: Dix River Downstream from Herrington Lake Dam (Figure 2-1, 2-2F)	
Dix River	The sample location is downgradient from the dam and the dam overflow. Three locations along the transect will be sampled. Data from these locations will be used to evaluate the potential flow of selenium out of Herrington Lake (if any).

Notes:

RM

River mile, with the mileage estimates starting at the Herrington Lake dam.

Table B-2
Data Quality Characteristics Formulas
Buffalo River Verification Monitoring Field Sampling Plan - Sediment QAPP

Characteristic	Formula	Symbols
Precision (as relative percent difference, RPD)	$\text{RPD} = \frac{ X1 - X2 }{(X1 + X2)/2} \times 100$	X1, X2 = duplicate values
Precision (as relative standard deviation, %RSD)	$\%RSD = \frac{S}{A} \times 100$	s = standard deviation A = mean of the measurements
Accuracy (as percent recovery (%R) for samples without a background level of the analyte, such as reference materials, laboratory control samples, and performance evaluation samples)	$\%R = \frac{X}{T} \times 100$	X = found concentration T = true or assumed concentration
Accuracy (as percent recovery (%R) for measurements in which a known amount of analyte (a spike) is added to an environmental sample)	$\%R = \frac{X}{T} \times 100$	X = found concentration T = true or assumed concentration
Completeness	$C = \frac{N}{S} \times 100$	C = completeness (%) N = number of valid data S = number of samples collected

APPENDIX C
LABORATORY DETECTION AND REPORTING LIMITS

Appendix C-1. Kentucky Reporting Limits

Appendix C-2. Analytical Method by Sample Matrix

Appendix C-3. Analytical Methods and Method Detection Limits by Media

to meet

Sufficiently Sensitive Permit Requirements

KY Category	CAS Number	Pollutant / Analyte	RRL	ARRL	Units	Non-Detect Reported as B or <MRL
Inorganic/Metals	7440-39-3	Barium, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Metals	7440-41-7	Beryllium, Total (Analysis)	0.004	N/A	mg/L	<MRL
Inorganic/Metals	7440-42-8	Boron, Total	N/A	N/A	N/A	<MRL
Inorganic/Metals	7440-48-4	Cobalt, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Metals	7440-57-5	Gold, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Metals	7439-88-5	Iridium, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Metals	7439-95-4	Magnesium, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Metals	7439-96-5	Manganese, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Metals	7439-98-7	Molybdenum, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Metals	7440-04-2	Osmium, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Metals	7440-05-3	Palladium, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Metals	7440-06-4	Platinum, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Metals	7440-09-7	Potassium, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Metals	7440-16-6	Rhodium, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Metals	7440-18-8	Ruthenium, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Metals	99439-28-8	Silica, Dissolved	N/A	N/A	N/A	<MRL
Inorganic/Metals	7440-23-5	Sodium, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Metals	7440-31-5	Tin, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Metals	7440-32-6	Titanium, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Metals	7440-62-2	Vanadium, Total (Analysis)	N/A	N/A	N/A	<MRL
Microbiology	N/A	Coliform, fecal	200	N/A	MPN/ 100mL	<MRL
Microbiology	N/A	Coliform, fecal	200	N/A	CFU/ 100mL	<MRL
Microbiology	N/A	Coliform, fecal in the presence of chlorine	200	N/A	CFU/ 100mL	<MRL
Microbiology	N/A	Coliform, fecal in the presence of chlorine	200	N/A	CFU/ 100mL	<MRL
Microbiology	N/A	E. coli	130	N/A	MPN/ 100mL	<MRL
Microbiology	N/A	E. coli	130	N/A	CFU/ 100mL	<MRL
Microbiology	N/A	Coliform, total	N/A	N/A	N/A	<MRL
Microbiology	N/A	Coliform, total in the presence of chlorine	N/A	N/A	N/A	<MRL
Microbiology	N/A	Enterococci	N/A	N/A	N/A	<MRL
Microbiology	N/A	Fecal streptococci	N/A	N/A	N/A	<MRL
Microbiology	N/A	Salmonella	N/A	N/A	N/A	<MRL
ORG/Dioxins	1746-01-6	2,3,7,8-TCDD (Dioxin) (2,3,7,8-Tetrachlorodibenzo-p-dioxin)	1.00E-08	N/A	mg/L	<MRL
ORG/Dioxins	67562-39-4	1,2,3,4,6,7,8-Heptachloro-dibenzofuran	N/A	N/A	N/A	<MRL
ORG/Dioxins	60851-34-5	2,3,4,6,7,8-Hexachloro-dibenzofuran	N/A	N/A	N/A	<MRL
ORG/Dioxins	57117-31-4	2,3,4,7,8-Pentachloro-dibenzofuran	N/A	N/A	N/A	<MRL
ORG/Dioxins	51207-31-9	2,3,7,8-Tetrachloro-dibenzofuran	N/A	N/A	N/A	<MRL

RRL: Required Reporting Limit

ARRL: Alternative Required Reporting Limit

Appendix C-1. Kentucky Reporting Limits
to meet
Sufficiently Sensitive Permit Requirements

Revised: 10/09/2014

KY Category	CAS Number	Pollutant / Analyte	RRL	ARRL	Units	Non-Detect Reported as B or <MRL
Inorganic/Gen Chem	N/A	Hardness (as CaCO ₃) - Calcium & Total	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Hardness (as CaCO ₃) - Total	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Kjeldahl Nitrogen - Total (TKN) (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Kjeldahl Nitrogen - Total (TKN) (Automated)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	14797-55-8	Nitrate (as N)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	C-005	Nitrate-Nitrite (as N)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Nitrogen, Total	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	C-007	Oil and Grease	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	8013-59-0	Organic Nitrogen (as N)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	98059-61-1	Ortho-phosphate	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem		Phosphorus - Elemental	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	7723-14-0	Phosphorus, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	7723-14-0	Phosphorus, Total (Preparation)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Radium	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Radium-Total	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Solids, Settleable (Residue, Settleable)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Solids, Total (Residue-Total)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	10050	Solids, Total Suspended (Residue - non-filterable) (TSS)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Solids, Volatile (Residue, Volatile)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	7664-93-9	Sulfate (as SO ₄)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	9073-75-0	Sulfide (as S)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	80879-60-3	Sulfite (as SO ₃)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Surfactants	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	C-012	Total Organic Carbon (TOC)	N/A	N/A	N/A	<MRL
Inorganic/Metals	7440-36-0	Antimony, Total (Analysis)	0.64	N/A	mg/L	<MRL
Inorganic/Metals	7440-38-2	Arsenic, Total (Analysis)	0.15	N/A	mg/L	<MRL
Inorganic/Metals	7440-43-9	Cadmium, Total (Analysis)	0.00027	N/A	mg/L	<MRL
Inorganic/Metals	7440-50-8	Copper, Total (Analysis)	0.0093	N/A	mg/L	<MRL
Inorganic/Metals	7439-89-6	Iron, Total (Analysis)	1	N/A	mg/L	<MRL
Inorganic/Metals	7439-92-1	Lead, Total (Analysis)	0.0031	N/A	mg/L	<MRL
Inorganic/Metals	7440-02-0	Nickel, Total (Analysis)	0.05	N/A	mg/L	<MRL
Inorganic/Metals	7782-49-2	Selenium, Total (Analysis)	0.005	N/A	mg/L	<MRL
Inorganic/Metals	7440-22-4	Silver, Total (Analysis)	0.003784	0.003	mg/L	<MRL
Inorganic/Metals	7440-28-0	Thallium, Total (Analysis)	0.00047	0.001	mg/L	B
Inorganic/Metals	7440-66-6	Zinc, Total (Analysis)	0.11	N/A	mg/L	<MRL
Inorganic/Metals	7429-0-5	Aluminum, Total (Analysis)	N/A	N/A	N/A	<MRL

RRL: Required Reporting Limit

ARRL: Alternative Required Reporting Limit

Kentucky Reporting Limits

Revised: 10/09/2014

to meet

Sufficiently Sensitive Permit Requirements

KY Category	CAS Number	Pollutant / Analyte	RRL	ARRL	Units	Non-Detect Reported as B or <MRL
Field	7782-50-5	Chlorine - Total Residual	0.02	N/A	mg/L	<MRL
Field	N/A	Dissolved Oxygen	2	N/A	mg/L	<MRL
Field	N/A	Temperature	89.666	N/A	°F	<MRL
Field	7782-50-5	Chlorine - Free Available	N/A	N/A	N/A	<MRL
Field	N/A	pH (Hydrogen Ion)	N/A	N/A	N/A	<MRL
Field	N/A	Specific Conductance	N/A	N/A	N/A	<MRL
Field	N/A	Turbidity	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	7664-41-7	Ammonia (Analysis)	1	N/A	mg/L	<MRL
Inorganic/Gen Chem	C-003	Biochemical Oxygen Demand (BOD5)	5	N/A	mg/L	<MRL
Inorganic/Gen Chem	C-002	Carbonaceous Biochemical Oxygen Demand (CBOD5)	5	N/A	mg/L	<MRL
Inorganic/Gen Chem	16887-00-6	Chloride	600	N/A	mg/L	<MRL
Inorganic/Gen Chem	18540-29-9	Chromium (VI)	0.011	N/A	mg/L	<MRL
Inorganic/Gen Chem	57-12-5	Cyanide, Free	0.0052	0.01	mg/L	B
Inorganic/Gen Chem	7439-97-6	Mercury, Total	0.0000051	N/A	mg/L	<MRL
Inorganic/Gen Chem	22967-92-6	Methylmercury	0.0000004	N/A	mg/L	<MRL
Inorganic/Gen Chem	1594-56-5	Nitrite (as N)	1	N/A	mg/L	<MRL
Inorganic/Gen Chem	N/A	Phenols (Total Phenolics) (Analysis)	0.3	N/A	mg/L	<MRL
Inorganic/Gen Chem	10052	Solids, Total Dissolved (Residue-Filterable)	250	N/A	mg/L	<MRL
Inorganic/Gen Chem	N/A	Acid Mine Drainage	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Acidity as CaCO3	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Adsorbable Organic Halides (AOX)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Alkalinity as CaCO3	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Alpha-Counting error	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Alpha-Total	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	1332-21-4	Asbestos	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Beta-Counting error	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Beta-Total	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	7726-95-6	Bromide	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	C-004	Chemical Oxygen Demand	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	16065-83-1	Chromium (III)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	7440-47-3	Chromium, Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	N/A	Color	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem		Cyanide - Available	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	57-12-5	Cyanide - Total (Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	57-12-5	Cyanide - Total (Preparation & Analysis)	N/A	N/A	N/A	<MRL
Inorganic/Gen Chem	7782-41-4	Fluoride, Total (Analysis)	N/A	N/A	N/A	<MRL

RRL: Required Reporting Limit

ARRL: Alternative Required Reporting Limit

**Table C-2. Analytical Methods Per Sample Matrix
Herrington Lake Corrective Action Plan
Mercer County, Kentucky**

Test	Methods	Surface Water	Sediment	Porewater	Aquatic Vegetation	Aquatic Invertebrates	Fish Tissues
Total Metals (selenium, arsenic, cadmium, lead, zinc, iron, boron, and magnesium)	USEPA 200.8 and 6010/6020	X	X	X			
Dissolved Metals (selenium, arsenic, cadmium, lead, zinc, iron, boron, and magnesium)	USEPA 200.8 and 6010/6021	X		X			
Metals (selenium, arsenic, cadmium, lead, and zinc)	USEPA 6010/6020				X	X	X
Mercury	USEPA 7470 and EPA 7471	X	X	X	X	X	X
Methylmercury	USEPA 1630 and USEPA 1631E	X	X	X	X	X	X
Sulfate	USEPA 300.0	X	X	X			
Total organic carbon	Lloyd Kahn		X	X	X	X	X
Dissolved organic carbon	Lab SOP	X		X			
Hardness	130.2	X		X			
Percent Solids	SM 2540G		X				
Percent Lipids	Lab SOP		X			X	X
Percent Moisture	Lab SOP		X		X	X	X
Speciated selenium	HPLC with ICP-MS			X			
Speciated arsenic	1632			X			X

Notes:
USEPA
HPLC
ICP-MS
SOP

United States Environmental Protection Agency
High performance liquid chromatography
Inductively coupled plasma/mass spectrometry
Standard operating procedure

Appendix C-2

Analytical Methods Per Sample Matrix Herrington Lake Corrective Action Plan Mercer County, Kentucky

Test	Methods	Surface Water	Sediment	Porewater	Aquatic Vegetation	Aquatic Invertebrates	Fish Tissues
Total Metals (selenium, arsenic, cadmium, lead, zinc, iron, boron, and magnesium)	USEPA 200.8 and 6010/6020	X	X	X			
Dissolved Metals (selenium, arsenic, cadmium, lead, zinc, iron, boron, and magnesium)	USEPA 200.8 and 6010/6021	X		X			
Metals (selenium, arsenic, cadmium, lead, and zinc)	USEPA 6010/6020				X	X	X
Mercury	USEPA 7470 and EPA 7471	X	X	X	X	X	X
Methylmercury	USEPA 1630 and USEPA 1631E	X	X	X	X	X	X
Sulfate	USEPA 300.0	X	X	X			
Total organic carbon	Lloyd Kahn		X	X	X	X	X
Dissolved organic carbon	Lab SOP	X		X			
Hardness	130.2	X		X			
Percent Solids	SM 2540G		X				
Percent Lipids	Lab SOP		X			X	X
Percent Moisture	Lab SOP		X		X	X	X
Speciated selenium	HPLC with ICP-MS			X			
Speciated arsenic	1632			X			X

Notes:
USEPA
HPLC
ICP-MS
SOP

United States Environmental Protection Agency
High performance liquid chromatography
Inductively coupled plasma/mass spectrometry
Standard operating procedure

Appendix C-3

Herrington Lake Quality Assurance Project Plan

Table C-3a. Water Analytical Methods

Mercer County, Kentucky

METHOD	ANALYTE	CAS No.	MATRIX	MDL	MRL	UNITS
SM2340 C	Hardness as CaCO ₃	NA	Water	0.8	2	mg/L
9060A	Total Organic Carbon	7440-44-0	Water	0.07	0.5	mg/L
300.0	Sulfate	14808-79-8	Water	0.06	0.2	mg/L
6020A	Arsenic	7440-38-2	Water	0.05	0.5	µg/L
6020A	Boron	7440-42-8	Water	0.07	2	µg/L
6020A	Cadmium	7440-43-9	Water	0.005	0.02	µg/L
6020A	Iron	7439-89-6	Water	0.3	1	µg/L
6020A	Lead	7439-92-1	Water	0.004	0.02	µg/L
6020A	Selenium	7782-49-2	Water	0.4	1	µg/L
6020A	Zinc	7440-66-6	Water	0.09	2	µg/L
6010C	Magnesium	7439-95-4	Water	0.3	5	µg/L
1631E	Mercury	7439-97-6	Water	0.00006	0.0005	ug/L
7470A	Mercury	7439-97-6	Water	0.02	0.2	µg/L
1630	Methyl Mercury	22967-92-6	Water	0.03	0.1	ng/L
1632A	Total Inorganic Arsenic (TIA)	7440-38-2	Water	0.003	0.02	µg/L
1632A	Monomethylarsonic acid (MMA)	7440-38-2	Water	0.002	0.02	µg/L
1632A	Dimethylarsinic acid (DMA)	7440-38-2	Water	0.006	0.05	µg/L
1632A	Arsenite/Arsenate (As(III) & (V))	7440-38-2	Water	0.003	0.02	µg/L
SWA100	Se(IV)	7782-49-2	Water	0.006	0.05	µg/L
SWA100	Se(VI)	7782-49-2	Water	0.006	0.05	µg/L
SWA100	SeCN	7782-49-2	Water	0.005	0.044	µg/L
SWA100	MeSe(IV)	7782-49-2	Water	0.006	0.05	µg/L

MDL Method Detection Limit

MRL Method Reporting Limit

Appendix C-3

Herrington Lake Quality Assurance Project Plan

Table C-3b. Sediment Analytical Methods

Mercer County, Kentucky

METHOD	ANALYTE	CAS No.	MATRIX	MDL	MRL	UNITS
300.0 Mod	Sulfate	14808-79-8	Soil	0.48	2	mg/kg
9030M	Sulfide, Total	18496-25-8	Soil	0.2	0.5	mg/Kg
6020A	Arsenic	7440-38-2	Soil	0.2	0.5	mg/kg
6020A	Boron	7440-42-8	Soil	0.05	0.5	mg/kg
6020A	Cadmium	7440-43-9	Soil	0.009	0.02	mg/kg
6020A	Lead	7439-92-1	Soil	0.02	0.05	mg/kg
6020A	Selenium	7782-49-2	Soil	0.2	1	mg/kg
6020A	Zinc	7440-66-6	Soil	0.2	0.5	mg/kg
6010C	Iron	7439-89-6	Soil	2	4	mg/kg
6010C	Magnesium	7439-95-4	Soil	0.2	2	mg/kg
7471B	Mercury	7439-97-6	Soil	0.002	0.02	mg/kg
ALS SOP	Methyl Mercury	22967-92-6	Soil	0.04	0.4	µg/kg

MDL Method Detection Limit

MRL Method Reporting Limit

Appendix C-3

Herrington Lake Quality Assurance Project Plan

Table C-3c. Tissue Analytical Methods

Mercer County, Kentucky

METHOD	ANALYTE	CAS No.	MATRIX	MDL	MRL	UNITS
6020A	Arsenic	7440-38-2	Tissue	0.02	0.5	mg/kg
6020A	Cadmium	7440-43-9	Tissue	0.002	0.02	mg/kg
6020A	Lead	7439-92-1	Tissue	0.0005	0.02	mg/kg
6020A	Zinc	7440-66-6	Tissue	0.06	0.5	mg/kg
7742	Selenium	7782-49-2	Tissue	0.05	0.1	mg/kg
7471B	Mercury	7439-97-6	Tissue	0.002	0.02	mg/kg
ALS SOP	Methyl Mercury	22967-92-6	Tissue	3	10	µg/kg
1632A	Total Inorganic Arsenic (TIA)	7440-38-2	Tissue	0.007	0.02	mg/kg
1632A	Monomethylarsonic acid (MMA)	7440-38-2	Tissue	0.004	0.02	mg/kg
1632A	Dimethylarsinic acid (DMA)	7440-38-2	Tissue	0.009	0.04	mg/kg
1632A	Arsenite/Arsenate (As(III) & (V))	7440-38-2	Tissue	0.02	0.04	mg/kg
1631E	Mercury	7439-97-6	Tissue	0.08	1	µg/kg

MDL Method Detection Limit

MRL Method Reporting Limit

APPENDIX D
KY DIVISION OF WATER STANDARD OPERATING PROCEDURES

Appendix D-1. Procedure for Collection of Fish in Large Wadeable and Non-wadeable Streams and Rivers

Appendix D-2. Methods for the Collection of Selenium Residue in Fish Tissue used to Determine KPDES Permit Compliance

Appendix D-3. Preparation and Homogenization of Fish Tissue Samples

Appendix D-1

Standard Operating Procedure for Collection of Fish in Large Wadeable and Non-wadeable Streams and Rivers







Commonwealth of Kentucky
Energy and Environment Cabinet
Department for Environmental Protection
Division of Water

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Scope and Applicability

This manual has been developed by the Kentucky Division of Water (KDOW) as guidance for the uniform and accurate collection, field processing, field handling and quality assurance/quality control (QA/QC) of fish samples collected from the large wadeable and non-wadeable waters of Kentucky. The methods defined herein are required for all fish collection, field processing, field handling and QA/QC activities resulting in information that could be used for water quality assessments. Advantages of using fish as biological indicators include their 1) widespread distribution from small streams to all but the most polluted waters; 2) utilization of a variety of trophic levels; 3) stable populations during summer months; and 4) the availability of extensive life history information (Karr et al. 1986). The methods used for collecting fish community structure data for use in the large river biotic index development are outlined in this manual.

Any data submitted to KDOW for review will undergo QA/QC and those identified as not following the methods set forth in this document will be flagged as not suitable for the Integrated Report to Congress on Water Quality in Kentucky (305[b] and 303[d] Reports). These data may be retained in KDOW files for other data purposes.

Definitions

Anode – the positive electrode.

Backpack Electrofisher– unit designed for electrofishing.

Backpack Electrofishing (BPEF) – electrofishing with a backpack electrofisher.

Barge Electrofishing – use of a small boat to carry a generator and pulsator.

Cathode – The negative electrode.

DC – Direct current

Dip Net – A net (of appropriate size for size fish being collected) with 3/16 inch mesh affixed to a fiberglass handle.

Electrofishing – The use of electricity to provide a sufficient electrical stimulus in fish to permit easy capture by netting.

GPP – Generator powered pulsator electrofisher

KDOW – Kentucky Division of Water

Large Streams = free-flowing streams with catchment areas greater than 150-200 mi², with most of the channel accessible for sampling and with most of the stream depth less than 1 meter. All of sampling reach is wadeable.

Netter – The individual who nets the captured fish during electrofishing operations.

Non-wadeable – stream sections that cannot be traversed by foot and sampling cannot be performed without the aid of a boat.

Probe – Pole fitted with a metal ring or dropper array.

PPE – Personal Protective Equipment

Rat Tail – cable that is dragged behind a backpack electrofisher and serves the cathode.

Restricted flow Non-wadeable river = a flowing river with a catchment area greater than 200mi² and the presence of low-head dams in the system, most areas around the bank may not be wadeable for sampling and with a mean average thalweg depth greater than 4 meters. Most of sampling reach is non-wadeable with generally no areas that are wadeable.

Run-of-river non-wadeable river = a free-flowing stream with a catchment area greater than 150-200 mi², with most areas around the bank that may or may not be wadeable for sampling and with a mean average thalweg depth greater than 1 meter and less than 4 meter. Most of sampling reach is non-wadeable with small areas that are wadeable.

Sample Point – Latitude and longitude that identifies sampling location.

SDS – safety data sheet

Seine – A 10 or 15 foot length by 6 foot width net with 3/16 in mesh affixed to two brails.

Seine effort – One seine effort equal approximately seining 9.2 m² (100ft²) area.

Shocking seconds – time (in seconds) recorded on the electrofisher that the unit is actively electrofishing.

Small River = a free-flowing stream with a catchment area greater than 150-200 mi², with most areas around the bank that are accessible for sampling and with a mean average thalweg depth less than 1 meter. Most of sampling reach is wadeable with small areas that are non-wadeable.

Wadeable – stream locations that can easily be traversed on foot and efficient sampling can be performed.

Health & Safety Policy/Section

Supervisors must make employees aware of proper safety procedures before the employee is engaged in electrofishing. Prior to field work, new crew members should receive orientation on equipment, procedure and risks involved. This orientation should include: explain equipment components and function, demonstration of equipment and hazards associated with electrofishing.

For general safety purposes, field crews should consist of more than one field person. At least two, and preferably all, crew members must have CPR and first aid training.

Members of a field crew should familiarize themselves with the nearest hospital, doctor's office or instant medical care provider.

Each field crew should use the following personal protective equipment (PPEs) (as deemed necessary) for each sampling trip: waders, boots, long pants, hearing protection, eye protection, bug repellent, sunscreen and hand sanitizer. If additional PPE is deemed necessary and not available the site must not be sampled.

Each field crew shall take an inventory/checklist of PPEs before each sampling trip making sure that all equipment is working properly. If any PPE is found to be inadequately working, such as leaking, ripped, etc., it should be repaired or replaced before leaving for the sampling trip.

Field crew allergies, such as bee stings, should be identified before the sampling trip.

Field crews should be properly dressed for the weather conditions. Coats, gloves and head coverings should be used during the late fall, winter and early spring to reduce the threat of hypothermia. Shorts can be worn under waders during the summer to reduce the threat of heat exposure (as deemed necessary).

Drinking water and other liquids should be available to field crews during sampling trips. Water coolers with ice can assist in reducing dehydration and heat exposure illnesses.

When transporting a formaldehyde container inside a vehicle, it must be transported in a secondary leak proof container of sufficient volume to hold the amount in the storage container. When pouring formaldehyde into collection jars, gloves should be worn to prevent skin exposure.

Unless placing a specimen into a collection jar, the lid shall remain closed to prevent the splashing of formalin out of the jar. Jars should be kept away from the facial area to reduce splashing and inhalation exposure. Collection jars should be inspected before use to check for damage. If damage is found, the jar is discarded. Plastic collection jars should be utilized.

Gasoline cans should have tight seals to eliminate the escape of fumes. Electrofisher should be refueled in an open area. Care should be taken when pouring gasoline into the electrofisher so that spillage and inhalation and skin exposure can be reduced.

Field crews should ensure containers are properly sealed before transport to prevent spill and release of fumes.

Personnel Qualifications / Responsibilities

All field crew members will meet at least the minimum qualifications for their job classification. Fish sample collection will be done by Division of Water or partner agency biologists with specialized expertise in fisheries management, fisheries biology, fisheries science or related field. The nature of the sampling protocols for this group requires specialized knowledge of habitats and taxonomy. The fisheries biologist should have knowledge of taxonomy and be familiar with the taxonomic references listed in Appendix A. Fisheries biologist are considered to be qualified if they have specific advanced academic training and/or several years professional experience in field collection of fish assemblages. Division of Water personnel with the required expertise usually holds the title Environmental Biologist Specialist or Environmental Biologist Consultant. Individuals assisting with sampling will be under the direct supervision of a fisheries biologist.

Equipment and Supplies

- Field Datasheet or Waterproof Notebook
- Dipnets
- Electrofisher (DC backpack shocker or GPP)
- Probes
- Anode rings
- Spare probe and rings
- Rat tail
- Boat
- Electrofishing barge
- Fuel: gasoline or batteries
- Field guide (e.g. Peterson's Field Guide Freshwater Fishes)
- Seine (15 foot)
- Formalin and SDS sheet
- Voucher jars (Various sizes)
- 5 gal bucket
- Live well
- Waterproof paper for sample labels

- Lineman's gloves if using non-insulated probes or nets
- Waders and boots
- Polarized sunglasses
- Copies of field protocols
- Pencils
- Clipboard
- First aid kit
- Global Positioning System (GPS) Unit

Methods

Cautions

While following these sampling techniques, it is important to keep the sampling reach intact and undisturbed. Field personnel should not disturb the reach until sampling has occurred. Doing so could result in degradation of the sample. If the sampling reach has been disturbed by other activities, sufficient time should be allowed for the water to clear and fish to settle back into normal habitats. Electrofishing in turbid water can result in underestimates of the fish community. The experience of the crew and their ability to see and net the fish improves the effectiveness of sampling the reach. Polarized sunglasses are recommended when electrofishing, since they will cut down on the glare of the water. In addition, features such as water clarity, flow, depth and time of day need to be considered to obtain optimal success in sampling.

The sampling reach must not be associated within the immediate area (<100 meters) of major tributary confluences or human structural influences, such as bridges, road crossings (fords), low head dams or any other similar structure, unless the purpose of obtaining the fish community data is related to these influences. If these conditions are not adequate or practical, sampling needs to be postponed until an efficient sampling effort can be obtained.

Instrument Calibration

Select the electrofisher settings based on the conductivity of the water. To minimize stress and mortality, it is important to use the minimum amount of electrical energy to stun fish. Select initial voltage setting 150-400 V for high conductivity (>300 $\mu\text{S}/\text{cm}$), 500-800 V for medium conductivity (100 to 300 $\mu\text{S}/\text{cm}$) and 900-1100 V for low conductivity (<100 $\mu\text{S}/\text{cm}$) waters) pulse width (2-6 ms) and pulse frequency (30-120 Hz). Adjust the voltage, pulse width and pulse frequency to efficiently capture fish without inducing excessive stress and mortality.

Type of Collections

To ensure collection of standardized fish community data, stream size (i.e., drainage area) and depth (i.e. wadeable and non-wadeable) have been used to designate streams into four classes: Large Streams, Small River, Run-of-River Non-wadeable Rivers and Restricted Flow Non-wadeable Rivers.

Sampling Periods

The sampling index period is June through October. In some cases, sampling outside of these index periods is necessary to assess immediate impacts (e.g., chemical spills) or to adhere to specific guidelines set forth by the U.S. Fish and Wildlife Service or KDOW for trend monitoring and bioassessment in streams containing federally listed threatened or endangered species. For routine bioassessment or baseline data collection, samples collected outside of these index periods will be considered unacceptable. Also, fish samples should not be collected during periods of excessively high or low flows or within 14 days of a known scouring flow event. Scour events occur when excessive rain fall occurs and river substrates have been altered. In addition, excessive turbid waters should not be sampled.

Sample Reach

Wadeable Large Streams and Small Rivers

- A. At each site, a sampling reach of a 300 m length will be established.
- B. Latitude and longitude will be determined for each site at the downstream location.

Run-of-River and Restricted Flow Non-wadeable Rivers

- A. Sample reaches will be determined by methods presented in Flotemersch et al. (2006).
- B. At each site, a sampling reach of a 500 m length will be established.
- C. Latitude and longitude will be determined for each site at the downstream location.

Sampling Methods

Wadeable Large Streams and Small Rivers

- A. The sampling crew will consist of a minimum of at least three.
- B. A combination of electrofishing and seining techniques will be utilized at all wadeable sites. Dip nets and seines shall have 3/16th inch mesh. Electrofishing and seining collections will be kept separate.

Barge Electrofishing Method

- A. A barge electrofisher is the preferred electrofishing gear in wadeable large streams and small rivers

- B. A tote barge or similar electrofisher capable of producing at least 2,500 watts should be used with a single anode.
- C. One crew member will navigate the barge and operate the electrofishing unit.
- D. The other crew members will work the anode and dip stunned fish.
- E. Stunned fish are placed in a live well carried in the barge.
- F. Anode operator should also carry a dip net (Barbour et al. 1999).
- G. One pass on each bank reach is sampled from the downstream end to the upstream end, with all recognizable habitats thoroughly sampled (Barbour et al. 1999). One pass of the stream channel is allowed if stream width is small enough to allow one zig zag pattern and all habitats to be sampled efficiently.
- H. The sampling zone on each bank extends from the edge of the water to the center of the river or to depth of 3 ft.
- I. Some circumstances (e.g. swift water) may require the use of a seine (rather than a dip net) and electrofishing.
 - 1. The seine may be set perpendicular to the current (to act as a block net) by two crew members.
 - 2. The anode operator(s) applies current upstream to downstream to the seine.
 - 3. Stunned fish are carried by current into the seine where they are captured.
 - 4. The electrofishing operator may need to dislodge specimens caught in the substrate.
- J. Collected fish should be frequently transferred from dip nets and seines to the live well to lessen stress and mortality.
- K. In addition, water in the live well should be changed periodically (warmer water temperatures require more frequent water changes) to reduced stress and mortality of fish.
- L. At the conclusion of each sampling run, record the time spent electrofishing (in seconds) (Appendix B).

Backpack Electrofishing Methods

- A. Note: At large streams and small river sites a single backpack electrofishing unit may not provide sufficient power to collect fish. However, some sampling site may prevent tote barge access. In these situations a backpack electrofisher may be used. Depending on sampling condition, a second backpack unit may be needed in order to provide a sufficient electrical field to collect fish. If two backpack units are used, one unit is designated as the primary unit. The primary unit will be the unit that electrofishing time is recorded from. The secondary unit will provide support to the primary unit by cutting escape routes off from fish fleeing the sampling area.
- B. One member of the field crew operates each backpack electrofishing unit.
- C. The other field crew members dip stunned fish and carry the bucket used to transport captured fish.
- D. The anode operators will also carry a dip net (Barbour et al. 1999).
- M. One pass on each bank reach is sampled from the downstream end to the upstream end, with all recognizable habitats thoroughly sampled (Barbour et al. 1999). One pass

of the stream channel is allowed if stream width is small enough to allow one zig zag pattern and all habitats to be sampled efficiently.

- E. The sampling zone on each bank extends from the edge of the water to the center of the river or to depth of 3 ft.
- F. Crew members with dip nets walk alongside and behind the anode operator(s) to collect stunned fish.
- G. Some circumstances (e.g. swift water) may require the use of a seine (rather than a dip net) and electrofishing.
 - 1. The seine may be set perpendicular to the current (to act as a block net) by two crew members.
 - 2. The anode operator(s) applies current upstream to downstream to the seine.
 - 3. Stunned fish are carried by current into the seine where they are captured.
 - 4. The electrofishing operator may need to dislodge specimens caught in the substrate.
- H. Collected fish should be frequently transferred from dip nets and seines to a bucket of water to lessen stress and mortality.
- I. In addition, water in the bucket should be changed periodically (warmer water temperatures require more frequent water changes) to reduced stress and mortality offish.
- J. At the conclusion of sampling, record the time spent electrofishing (in seconds) (Appendix B).

Seining

- A. Habitats not effectively sampled by electrofishing are sampled by seining once electrofishing activities have concluded.
- B. Seining is a better technique for collecting some minnow species that are not as affected by the electric current.
- C. Use a seine that is at least 15 feet long, 6 feet tall and with a mesh size of 3/16th inch. The brails must be sturdy to be used in swift runs.
- D. There are 3 seining techniques that may be utilized at all stations where fish collections are conducted: seine hauls, kick seining and specific habitat seining
 - 1. Seine Hauls
 - i. Seine hauls are used in shallow areas near the shore with very little structure or in swift runs.
 - ii. Seine hauls are generally performed in a downstream direction (Etnier and Starnes 1993, Jenkins and Burkhead 1993 and Hendricks et al. 1980).
 - iii. Seining with the current is more efficient because there is less drag on the net and takes advantage of a fish's tendency to escape upstream. Seine operators can also move more quickly to trap fish, and there is no pressure wave in front of the seine, which can cause fish to move away from the net.
 - iv. Two members of the field crew will each take a brail and begin moving with the current through the targeted habitat.

- v. Make sure that the lead line is down on the bottom, there is an adequate bag in the seine and that the floats at the top of the seine are floating on the surface.
 - vi. When the seine haul is finished, the seine is beached by dragging it onto the shore.
 - vii. When there is only a small shoreline area to beach the seine, the brails are brought close together at the shoreline and the lead line slowly pulled into shore by hand.
 - viii. If the seine cannot be beached, then in one motion, the seine is quickly lifted out of the water and carried onto shore.
2. Kick Seining
- i. Kick seining will be conducted in riffle and run areas of the stream.
 - ii. Kick seining involves two crew members holding the seine in a position downstream of the area to be sampled.
 - iii. The brails are slightly angled downstream so that the flow forms a bag or pocket in the seine.
 - iv. A third crew member disturbs (or kicks) the substrate while moving toward the seine.
 - v. After reaching the seine, crew members lift the seine out of the water.
3. Specific Habitat Seining
- i. Sometimes specific habitat seining might be utilized, if specific habitats within the sampling reach could not be adequately electrofished.
 - ii. Specific habitat seining involves encircling specific habitat (i.e. woody debris pile) with a seine and thrusting the brails into the habitat (or crew member disturbs the habitat) to force fish out.
 - iii. After disturbing the habitat the seine is lifted out of the water.
- E. After each seine effort, fish are briefly examined by the fish biologist for the species present and then placed in a bucket of water.
- F. Large fish are identified, recorded and released immediately after each seine haul.
- G. Smaller fish are identified and released or retained as a voucher after all seining has been completed.
- H. A minimum of five seine efforts will be used and will continue until no new species are collected in three consecutive efforts or until a maximum of 90 minutes of effort is reached.
- I. One seine effort equal approximately seining 9.2 m² (100ft²) area.
- J. If five seine efforts have been expended and no new species were encountered in the last three efforts, seining may cease if all appropriate habitats in the reach have been sampled.
- K. Minimum and maximum times are defined as the start to finish of the seining effort.
- L. Record the time spent seining (in minutes start to finish) and the number of efforts (Appendix B).

Run-of-River and Restricted Flow Non-wadeable Rivers

- The sampling crew will consist of a minimum of two or three. Crew size will depend on the electrofishing boat.
- Electrofishing will be utilized at all non-wadeable sites. Dip nets shall have 3/16th inch mesh. Left and right bank electrofishing collections will be kept separate.

Electrofishing

- A. Collection of fish will follow methods described in Flotemersch et al. (2006) and Flotemersch and Blocksom (2005).
- B. The LR-BP method specifies that a single bank is electrofished for 1000 m or a pair of 500 m banks is sampled. Preferred method is the paired 500 m bank.
- C. This method is appropriate in Run-of-River and Restricted Flow sites.
- D. Daytime electrofishing is conducted at Run-of-River sites
- E. Nighttime electrofishing is conducted at Restricted Flow sites.
- F. Run-of-River and Restricted Flow sites, each bank (500 m left and right) along the riparian habitat in the main channel will be electrofished with boat mounted electrofishing gear.
- G. The electrofishing crew should consist of one boat operator (who maneuvers the boat and controls the electrofishing unit) and one dip-netter (who collects stunned fish and places them in the livewell). Under some circumstances it may be necessary to increase the number of dip-netters (e.g. fast water, low visibility or size of boat).
- H. For each bank, electrofishing will start in the upstream portion of the reach and proceed downstream to the end of the reach.
- I. During electrofishing, the boat should be operated at a speed near, or if velocities are low just above the current of the river and maneuvered in and out of shoreline habitat.
- J. Fish should be dipped from the water and placed into a livewell for processing. In the cases where large quantities of fish are present at once (i.e. when large schools of gizzard shad are encountered), only a representative sample of these fish should be collected (i.e. one or two scoops of the dip net).
- K. If large quantities of fish are collected during the sample run and the live well is at capacity, the sampling run should be suspended. The boat should be maneuvered to a location that will not influence additional sampling. Fish should be processed and sampling should continue after fish have been processed. This will help reduce mortality of released fish.
- L. At the conclusion of each sampling run the electrofishing settings (i.e. pulse width, percent applied and shocking seconds) for each run should be recorded (Appendix B).
- M. Some shallow portions of reaches (e.g. shoals/riffles) may require that the boat be beached and alternate electrofishing techniques employed. Alternate methods include the use of a handheld anode attached to the boat electrofishing unit, hand maneuvering the boat into shallow portions or the use of a backpack electrofisher. In these cases, one crew member operates the anode/boat/backpack electrofisher while the other dips stunned fish with a dip net. The shallow portion of the reach is sampled in this fashion and the electrofishing time is added (if gear other than the electrofishing boat is used)

to the boat electrofishing time to calculate total electrofishing effort. After the shallow portion is electrofished with the alternate methods, the boat is then maneuvered over the shallow portion and boat electrofishing is then continued.

- N. Any deviation from boat electrofishing is recorded on the biological verification form (Appendix B).

Sample Processing and Preservation

- A. Young of the year fish should not be retained as voucher or included in field counts.
- B. Wadeable seining and electrofishing fish collections should be kept separate. Wadeable sites will result in one jar of voucher specimens for electrofishing, one jar for seining and a list of released species.
- C. Non-wadeable paired 500m left and right bank samples should be kept separate. Non-wadeable sites will result in two voucher jars (one for left and right banks) and a list of released species.
- D. Vouchers specimens.
 - 1. A minimum of two specimens of all species will be kept as vouchers from the sample event as either retained specimens or photographs. Voucher specimens will be of at least 2 different age/size classes.
 - 2. Retained specimens are preserved in the field with a 10%-15% buffered formalin solution.
 - 3. Field containers should be large enough to accommodate the largest specimen without distorting it.
 - 4. If at all possible, large specimens will be identified in the field, photographed, recorded and released. Retained large specimens vouchers should have a slit made in the abdomen to permit entrance of preservative into the body cavity. This is particularly important in warm weather to prevent partial decomposition of internal organs.
 - 5. If a specimen represents a significant ichthyological find (e.g., state or drainage record) or the specimen is hard to identify, then they are to be preserved and retained.
 - 6. If a species or genus is viewed but not collected and if positively identified, these records should be noted (i.e., *Hypentelium nigricans*, *Micropterus* spp. or *Lepomis* spp.).
 - 7. Federally protected species must be identified, photographed and released immediately.
- E. While at the sampling location, all fish samples will receive a label.
 - 1. The label is placed in the sample jar (labels placed in the jar will be written in No. 2 pencil on waterproof paper).
 - 2. The label will consist of the following information:
 - a. station ID,
 - b. stream name,
 - c. county,
 - d. date sampled,

- e. collectors' initials and
- f. collection method.

Data and Records Management

Released fish are counted and recorded in the fisheries biologist's field notebook or on the field datasheet (Appendix B). Photographed fish are recorded with the file number from the camera.

Record the time spent electrofishing (in seconds).

Record the time spent seining (in minutes) and the number of efforts.

Completed Chain-of-Custody (KDOW 2009b) if fish samples will not be retained by fish crew leader.

All records are to be stored in project files.

Quality Control and Quality Assurance

A field crew will consist of at least one trained fisheries biologist who is knowledgeable of the identification and nomenclature of Kentucky fishes. This fisheries biologist is to ensure that voucher collections of all fish are taken, specimens are preserved correctly for laboratory examination and sample jars are labeled correctly. All released specimens will be noted in field notebooks or datasheets. After any sampling has been completed, all sampling gear will be thoroughly cleaned to remove all fish so that no fish are carried to the next site. The equipment shall be examined prior to sampling at the next site to ensure that no fish are present.

Five percent of samples taken in a season will be duplicated by a field crew. The samples will be selected randomly by numbering each collected site as 1-X. Sites will be chosen for replication using a random numbers table or other random numbering method. Replicates will be collected by a different fisheries biologist (if possible) within the same index period.

Field data must be complete and legible and entered on field data sheet (Appendix B) or field notebook. While in the field, the field team should possess sufficient copies of standardized field data forms and chains-of-custody for all anticipated sampling sites, as well as copies of all applicable Standard Operating Procedures.

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Appendix B. Fish Verification and Field Datasheet

STREAM NAME:	LOCATION(Lat/Long):
STATION ID:	DATE:

FISH COLLECTION	
Collectors:	River Type: <input type="checkbox"/> Large Streams <input type="checkbox"/> Small River <input type="checkbox"/> Run of River <input type="checkbox"/> Regulated Flow
Time of Electrofishing <input type="checkbox"/> Day <input type="checkbox"/> Night	Type of Electrofishing Unit: <input type="checkbox"/> BPEF _____ # of Units <input type="checkbox"/> 2.5 GPP <input type="checkbox"/> 5.0 GPP
LEFT BANK	RIGHT BANK
Method(s): <input type="checkbox"/> Boat <input type="checkbox"/> BPEF <input type="checkbox"/> Barge <input type="checkbox"/> Seine	Method(s): <input type="checkbox"/> Boat <input type="checkbox"/> BPEF <input type="checkbox"/> Barge <input type="checkbox"/> Seine
Total Time: <u>Start:</u> <u>Finish:</u>	Total Time: <u>Start:</u> <u>Finish:</u>
Voltage Applied:	Voltage Applied:
Amp output:	Amp output:
Percent Applied:	Percent Applied:
Boat Shock Time:	Boat Shock Time:
Alternate Shock Time:	Alternate Shock Time:
Total Shock Time:	Total Shock Time:
Electrofishing Total # of Voucher Jars:	
Left Bank:	Right Bank:
Seine Time:	# of Efforts:
Seining Total # of Voucher Jars:	

Comments:

Stream Name:		Location:			
Station Id:		Date:			
	Species	Left Bank		Right Bank	
		Released	DELT	Released	DELT
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
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Appendix D-2

Methods for the Collection of Selenium Residue in Fish Tissue Used to Determine KPDES Permit Compliance

Commonwealth of Kentucky
 Energy and Environment Cabinet
 Department for Environmental Protection
 Division of Water

Action By	Signature	Date
John Brumley Author, Environmental Scientist III	<i>Randall G. Payne</i>	4-9-14
Randall Payne Reviewed, Environmental Scientist III	<i>Randall G. Payne</i>	4-9-14
Clark Dorman, Approved, Manager Water Quality Branch	<i>Clark Dorman</i> for Clark Dorman	4/9/2014
Lisa Hicks Approved, Quality Assurance Officer, Division of Water	<i>Lisa A Hicks</i>	4/9/14
Peter Goodmann, Director, Division of Water	<i>Peter Goodmann</i>	8/29/2014
Larry Taylor Approved, Quality Assurance Manager, Department for Environmental Protection	<i>Larry Taylor</i>	4/9/14

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**Commonwealth of Kentucky
Steven L. Beshear, Governor**

**Energy and Environment Cabinet
Len Peters, Secretary**

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1. Procedures

1.A. Scope and Applicability

This manual has been developed by the Kentucky Division of Water (KDOW) as guidance for the uniform collection of selenium residue in fish tissue for the purposes of compliance with KPDES permits. The methods set forth herein are required for all activities related to the collection of fish for the determination of selenium residue in fish tissue. Data submitted to KDOW for review will undergo QA/QC review and those data identified as not following the methods set forth in this document will be flagged and shall not be used for purposes of determining compliance with the KPDES permit.

The source for the collection methods in this Standard Operating Procedure (SOP) document are based on the historical methods used by the Division of Water (KDOW 2008).

1.B. Definitions

CFR – Code of Federal Regulations

COC – Chain-of-Custody

DNR – Department for Natural Resources

DW – Dry Weight

EPA – U.S. Environmental Protection Agency

Headwater or Headwater Stream – Stream that is less than 6 square miles in catchment area.

KDFWR – Kentucky Department of Fish & Wildlife Resources

KDOW – Kentucky Division of Water

KPDES – Kentucky Pollutant Discharge Elimination System

QA – Quality Assurance

QC – Quality Control

Sample Reach – the specific length of the stream where fish survey collections are made; it includes the entire width of the stream within that stream length.

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SOP – Standard Operating Procedure

WQB – Water Quality Branch

Wadeable or Wadeable Stream – Stream that is equal to or greater than 6 square miles in catchment area.

1.C. Personnel Qualifications / Responsibilities

Individuals conducting fish tissue collections shall possess a valid KDFWR Scientific Wildlife Collecting Permit, if applicable. Field personnel conducting fish tissue collections must also have basic knowledge of aquatic organisms and their habitats, stream geomorphology and stream physical processes. Most importantly, field personnel must be able to properly identify the target species.

1.D. Recommended Equipment and Supplies

- Backpack Electrofishing Unit (including Probe, Ring and Rat Tail)
- Backpack Electrofishing Unit Battery
- Dip Nets (at least 3)
- Seine (Wadeable Streams)
- 5 Gallon Bucket
- Measuring Board (in mm)
- Sterile Whirl-pack Bags
- Gallon of De-ionized Water
- Waders and Boots
- First Aid Kit
- Polarized Sunglasses
- Waterproof Pen
- Permanent Marker
- Powderless Latex or Nitrile Gloves
- Chain-of-Custody Documents
- Cooler
- Ice

1.E. Methods

1.E.1. Purpose

In order to protect the aquatic life use from the bioaccumulative effects of selenium, KDOW has promulgated a chronic selenium water quality standard based on whole-body

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fish tissue DW concentration. Information obtained from the fish tissue survey will be used to determine compliance with the KPDES permit. The collection of fish tissue is required when the average effluent selenium concentration discharged from a permitted outfall exceeds 5.0 µg/L (KDOW 2013). Results of selenium residue in fish tissue samples will be used to determine compliance with the KPDES permit.

1.E.2. Precautions Before Sampling

While following the sampling methods outlined herein, it is important to keep the sampling reach intact and undisturbed. Field personnel shall not walk through the reach until sampling has occurred. If the sampling reach has been disturbed by other activities, sufficient time shall be allowed for the water to clear and fish to settle back into normal habitats. Electrofishing in turbid water can result in less effective sampling results. Polarized sunglasses are recommended when electrofishing, since they will cut down on the glare of the water. Optimal sampling conditions, such as high water clarity, normal ambient flow conditions and high ambient sunlight conditions, will enhance sampling efficiency. If sampling conditions are not adequate or practical, the survey should be postponed until conducive sampling conditions exist.

Electrofishing unit settings shall be set based on the conductivity of the water. To minimize stress and mortality, it is important to use the minimum amount of electrical energy needed to stun fish. Select initial voltage setting at 150-400 V for high conductivity conditions (>300 µS/cm), 500-800 V for medium conductivity (100 to 300 µS/cm), and 900-1100 V for low conductivity (<100 µS/cm). Set the pulse width between 2-6 ms and pulse frequency between 40-60 Hz. Adjust the voltage, pulse width and pulse frequency to efficiently capture fish without inducing excessive stress and mortality.

1.E.3. Headwater Streams

To determine selenium residue in fish tissue, a target species composite sample and one duplicate/replicate sample are required at each station. Two to five individuals of the target species shall be used to establish an individual whole-body composite or duplicate/replicate sample.

1.E.3.A. Target Species Composite Sample

A composite, whole-body sample shall consist of two to five (2-5) individuals selected from the taxa listed in Table 1.E.3.A. The composite sample may be of any taxa listed, but a composite sample shall consist of individuals of the same taxon. The individuals of a composite sample shall be, at a minimum, the size listed in Table 1.E.3.A and shall be within 75% of the length of the longest individual. These fish lengths represent reproductive maturity for each of these target species. A duplicate/replicate sample shall be collected at each sampling station following the same guidelines as stated for the composite sample of the target species.

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Table 1.E.3.A. Common fishes of headwater streams.

Fish Taxa	Minimum Length at Reproductive Maturity
<i>Campostoma spp.</i> (Stonerollers)	80 mm
<i>Catostomus commersonii</i> (White Sucker)	150 mm
<i>Chrosomus erythrogaster</i> (Southern Redbelly Dace)	50 mm
<i>Hypentelium nigricans</i> (Northern Hogsucker)	125 mm
<i>Rhinichthys atratulus</i> (Blacknose Dace)	60 mm
<i>Semotilus atromaculatus</i> (Creek Chub)	100 mm

1.E.3.B. Sample Reach

The first sample reach shall begin 5 meters below the outfall that exceeded the monthly average effluent selenium concentration of 5.0 µg/L and extend 100 meters downstream from that point in the receiving stream. Where the effluent receiving stream is a drainage ditch and not part of the upper-most channel-defined reaches (i.e., ephemeral or intermittent channels) of a watershed, the sample collection effort will commence in the uppermost receiving stream at the discharge point of the effluent ditch.

Field personnel shall measure out this sample reach before conducting the survey. Sampling shall begin at the downstream end of the reach (and, if needed, all subsequent reaches) and continue upstream until the most upstream end of the reach has been sampled. Every effort shall be made to obtain the composite and duplicate/replicate samples of the target species within the first sample reach. If a composite sample and the duplicate/replicate sample of the target species cannot be obtained within the first sample reach, field personnel shall proceed to sample the next downstream 100 meter reach. Every effort shall be made to obtain the composite sample and the duplicate/replicate sample of the target species within the second sample reach.

Field personnel shall continue downstream using successive 100-meter reaches until adequate target species composite and duplicate/replicate samples are obtained or the stream receiving the effluent empties into its receiving stream. In the event the effluent receiving stream is less than 100 meters in length every effort shall be made to collect fish from the available habitat of that stream, but when fish are not present in such streams the collection effort is extended into the next receiving stream. That collection effort will continue at the point the stream empties into its receiving stream with sampling conducted in successive downstream 100-meter reaches.

However, no more than a total of four 100-meter reaches shall be sampled; this is inclusive of all sampled reaches. Should the stream receiving the effluent discharge empty into its receiving stream less than four successive 100-meter reaches from the

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point of effluent discharge, then sampling shall continue in the receiving stream from that confluence until one has sampled linear reaches totaling no more than four successive (inclusive of all reaches sampled) 100-meter reaches.

Once two composite samples have been collected sampling may cease. If adequate composite and duplicate/replicate samples of the target species cannot be obtained then the 5.0 µg/L water column limit shall apply.

1.E.3.C. Target Species Composite Sample Collection

All members of the fish tissue collection crew shall don powderless latex or nitrile gloves. The sampling crew shall consist of a minimum of two members. Dipnets, seine and backpack electrofishing units are all instruments used in the collection of fish; the hydrological and physical characteristics of the stream to be sampled will determine what equipment is appropriate. If a backpack electrofishing unit is utilized, one individual operates the backpack electrofishing unit while the other(s) work the seine (if used) and dip nets, and carry the bucket used to transport captured fish. The backpack electrofishing unit operator shall also carry a dip net (Barbour et al. 1999) if using one probe and rat tail configuration. Backpack electrofishing sampling consists of working in an upstream direction in a side-to-side/bank-to-bank sweeping technique. Crew members with dip nets walk alongside and behind the electrofishing unit operator to collect stunned fish. If necessary, a seine can be used to sample deep pool habitat more efficiently after electrofishing. The seine can also be used to block off the width of stream while the electrofishing unit operator shocks fish downstream into the seine. This technique is especially useful when the water is slightly turbid. In shallow headwater streams, use of seine or dip nets may be the appropriate equipment utilized in procurement of fishes.

Collected fish shall be frequently transferred from dip nets to a bucket of water to lessen stress and mortality. In addition, water in the bucket shall be changed periodically (warmer water temperatures require more frequent water changes) to reduced stress and mortality of fish.

1.E.3.D. Target Species Composite Sample Processing

Once adequate composite and duplicate/replicate samples of the target species are collected, the processing procedure can begin. A sterile Whirl-pack bag shall be used to contain the samples. On the outside of the bags, the collectors shall write the following information with a permanent marker: station #, permit #, stream name, location, latitude and longitude (resolve to seconds or to five decimal places), county, date, time, species collected, number of individuals collected, the parameter or analyte to be tested and whether it is the composite sample or the duplicate/replicate sample of the target species. The longest individual in the bucket shall be measured in millimeters and placed in a sterile Whirl-pack bag. The length of the first individual shall be recorded on the COC

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sheet and the 75th percentile of that individual's length shall be calculated. One to four other individuals within the 75th percentile shall be measured and placed in the Whirl-pack bag with the longest individual. These lengths are recorded on the COC sheet along with the first. The duplicate/replicate sample shall be processed in the same manner as the first sample. All other fish that are being held in the bucket can be released once the duplicate/replicate sample has been processed. The bucket and measuring board shall be triple rinsed with de-ionized water after processing the samples.

The samples shall be kept on ice in a cooler until transported to a freezer for long-term storage. Maximum holding time on ice in a cooler is 12 hours. Samples shall be processed and analyzed in the lab within 30 days of collection.

1.E.4. Wadeable Streams

To determine selenium residue in fish tissue, a composite sample and one duplicate/replicate sample of the target species are required at each station. Two to five individuals of the target species shall be used to establish an individual whole-body composite or duplicate/replicate sample.

1.E.4.A. Target Species Composite Sample

A composite, whole-body sample shall consist of two to five (2-5) individuals from the taxa listed in Table 1.E.4.A. The composite sample may be of any taxa listed, but a composite sample shall consist of individuals of the same taxon. The individuals of a composite sample shall be, at a minimum, the size listed in Table 1.E.4.A and within 75% of the length of the longest individual of that species. These fish lengths represent reproductive maturity for each of these target species. A duplicate/replicate sample shall be collected at each sampling station following the same guidelines as stated for the target species composite sample.

1.E.4.B. Sample Reach

The first sample reach shall begin 5 meters below the outfall(s) that exceeded the monthly average effluent selenium concentration of 5.0 µg/L and extend 100 meters downstream from that point. If the discharge is into a drainage ditch, sampling should begin at the point the ditch discharges into the wadeable stream.

Field personnel shall measure out this sample reach before conducting the survey. Sampling shall begin at the downstream end of the reach (and, if needed, all subsequent reaches) and continue upstream until the most upstream end of the reach has been sampled. Every effort shall be made to obtain the composite and duplicate/replicate samples of the target species within the first sample reach. If a composite sample and the

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Table 1.E.4.A. Common fishes of wadeable streams.

Fish Taxa	Minimum Length at Reproductive Maturity
<i>Campostoma sp.</i> (Stonerollers)	80 mm
<i>Catostomus commersonii</i> (White Sucker)	150 mm
<i>Chrosomus erythrogaster</i> (Southern Redbelly Dace)	50 mm
<i>Hypentelium nigricans</i> (Northern Hogsucker)	125 mm
<i>Rhinichthys atratulus</i> (Blacknose Dace)	60 mm
<i>Semotilus atromaculatus</i> (Creek Chub)	100 mm
<i>Ambloplites rupestris</i> (Rock Bass)	100 mm
<i>Cyprinella spp.</i> (Shiners)	75 mm
<i>Etheostoma caeruleum</i> (Rainbow Darter)	45 mm
<i>Etheostoma flabellare</i> (Fantail Darter)	45 mm
<i>Lepomis spp.</i> (Sunfish)	70 mm
<i>Luxilus chrysocephalus</i> (Striped Shiner)	80 mm
<i>Lythrurus spp.</i> (Finescale Shiners)	45 mm
<i>Pimephales notatus</i> (Bluntnose Minnow)	60 mm

duplicate/replicate sample of the target species cannot be obtained within the first sample reach, field personnel shall proceed to sample the next downstream 100-meter reach. Every effort shall be made to obtain the composite sample and the duplicate/replicate sample of the target species within the second sample reach.

Field personnel shall continue downstream using successive 100-meter reaches until adequate target species composite and duplicate/replicate samples are obtained or the stream receiving the effluent empties into its receiving stream. In the event the effluent receiving stream is less than 100 meters in length every effort shall be made to collect fish from the available habitat of that stream, but when fish are not present in such streams the collection effort is extended into the next receiving stream. That collection effort will continue at the point the stream empties into its receiving stream with sampling conducted in successive downstream 100-meter reaches.

However, no more than a total of four 100-meter reaches shall be sampled; this is inclusive of all sampled reaches. Should the stream receiving the effluent discharge empty into its receiving stream less than four successive 100-meter reaches from the point of effluent discharge, then sampling shall continue in the receiving stream from that confluence until one has sampled linear reaches totaling no more than four successive (inclusive of all reaches sampled) 100-meter reaches.

Once two composite samples have been collected sampling may cease. If adequate composite and duplicate/replicate samples of the target species cannot be obtained then the 5.0 µg/L water column limit shall apply.

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1.E.4.C. Target Species Composite Sample Collection

All members of the fish-tissue collection crew shall don powderless latex or nitrile gloves. The sampling crew shall consist of a minimum of two members. One individual operates the backpack electrofishing unit while the other(s) work the seine (if used) and dip nets, and carry the bucket used to transport captured fish. The backpack electrofishing unit operator shall also carry a dip net (Barbour et al. 1999) if using one probe and rat tail configuration. Sampling consists of using a backpack electrofishing unit working in an upstream direction in a side-to-side/bank-to-bank sweeping technique. Crew members with dip nets walk alongside and behind the electrofishing unit operator to collect stunned fish. If necessary, a seine can be used to sample deep pool habitat more efficiently after electrofishing. The seine can also be used to block off the width of stream while the electrofishing unit operator shocks fish downstream into the seine. This technique is especially useful when the water is slightly turbid.

Collected fish shall be frequently transferred from dip nets to a bucket of water to lessen stress and mortality. In addition, water in the bucket shall be changed periodically (warmer water temperatures require more frequent water changes) to reduce stress and mortality of fish.

1.E.4.D. Target Species Composite Sample Processing

Once adequate composite and duplicate/replicate samples of the target species are collected, the processing procedure can begin. A sterile Whirl-pack bag shall be used to contain the samples. On the outside of the bags, the collectors shall write the following information with a permanent marker: station #, permit #, stream name, location, latitude and longitude (resolve to seconds or to five decimal places), county, date, time, species collected, number of individuals collected, the parameter or analyte to be tested and whether it is the composite sample or the duplicate/replicate sample of the target species. The longest individual in the bucket shall be measured in millimeters and placed in a sterile Whirl-pack bag. The length of the first individual shall be recorded on the COC sheet and the 75th percentile of that individual's length shall be calculated. One to four other individuals within the 75th percentile shall be measured and placed in the Whirl-pack bag with the longest individual. These lengths are recorded on the COC sheet along with the first. The duplicate/replicate sample shall be processed in the same manner as the first sample. All other fish that are being held in the bucket can be released once the duplicate/replicate sample has been processed. The bucket and measuring board shall be triple rinsed with de-ionized water after processing the samples.

The samples shall be kept on ice in a cooler until transported to a freezer for long-term storage. The maximum holding time on ice in a cooler is 12 hours. Samples shall be processed and analyzed in the lab within 30 days of collection.

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2. Quality Assurance/Quality Control

A field crew will consist of at least one person who is knowledgeable of the identification and nomenclature of Kentucky fishes. All members of the sampling crew will don powderless latex or nitrile gloves during collection and processing of the sample. After any sampling has been completed, all sampling gear will be thoroughly cleaned to remove all fish so that no fish are carried to the next site. The equipment shall be examined prior to sampling at the next site to ensure that no fish are present.

Field data must be complete and legible and entered on COC sheets and on the Whirl-pack bag. While in the field, the field team should possess sufficient copies of COC sheets for all anticipated sampling sites, as well as copies of all applicable SOPs. The following information shall be written on the COC sheet: station #, permit #, stream name, location, latitude and longitude (resolve to seconds or to five decimal places), county, date, time, species collected, number of individuals collected, collectors, parameter to be tested and whether it is the target species composite sample or the duplicate/replicate sample. Each collector will also sign and date the Whirl-pack bag as well as the COC sheets.

When delivering a target species composite sample to the laboratory for processing, the proper COC sheet that corresponds with the sample must be delivered to the laboratory at the same time. When the collector relinquishes the sample to the sample lab custodian, the collector will sign and date the COC in the "Relinquished By" space and the lab sample custodian will sign and date the COC in the "Received By" space. All lab data submitted to KDOW for selenium compliance must be accompanied with corresponding COC sheets.

2.A. Procedures for the Preparation of Fish Tissue and Methods for the Determination of Selenium in Fish Tissue

For fish tissue preparation for the determination of total selenium, the following procedures shall be used by the laboratory.

Fish Tissue Processing SOP (KDOW 2008)

Processing will be conducted in a certified "clean laboratory environment" with pre-cleaned stainless steel countertops and pre-cleaned stainless steel equipment:

1. Place composite samples in freezer when delivered from the field and allow to freeze.
2. Weigh composite sample to determine amount of dry ice to use during homogenization.
3. Remove frozen sample from freezer.

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4. Remove frozen individual fish from plastic freezer bag using nitrile gloves.
5. Place each individual fish from one composite into a stainless steel industrial blender.
6. Place the equivalent amount of dry ice in the blender that was determined prior to freezing for the composite sample (Ex. If the composite sample weighed 110 grams, then you would add 110 grams of dry ice to the blender for homogenization).
7. Homogenize sample in blender.
8. Remove homogenized sample with stainless steel utensil and place in pre-cleaned glass jar with Teflon-lined lid.
9. Label jar with all of the composite sample information from the sample bag.
10. Place jar with homogenized sample into freezer until ready for analysis.
11. Clean all equipment and countertops between composite samples with the following cleaning process:
 - a. Wash with mild detergent
 - b. Rinse with hot tap water
 - c. Rinse with distilled water
 - d. Rinse with 10% nitric acid
 - e. Rinse with acetone
 - f. Allow to air dry

Analytical test methods and procedures shall be selected from those approved by the U. S. Environmental Protection Agency (EPA) for the detection of total selenium. Those methods may be found in Title 40 Code of Federal Regulations (CFR) § 136.3 and on the internet at: <http://www.ecfr.gov/cgi-bin/retrieveECFR?gp=1&SID=13d0f1bbace392bf04234338940d5712&ty=HTML&h=L&n=pt40.23.136&r=PART> Table IB (accessed April 4, 2014).

An EPA link to specific CWA methods referenced in Title 40 CFR § 136 may be found at: http://water.epa.gov/scitech/methods/cwa/methods_index.cfm (accessed April 4, 2014).

3. References

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish, second edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water, Washington, D.C.

Kentucky Division of Water (KDOW). 2013. 401 KAR 10:031. Kentucky Department for Environmental Protection. Division of Water. Frankfort, Kentucky.

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Kentucky Division of Water (KDOW). 2008. Standard Methods for Assessing Biological Integrity of Surface Waters in Kentucky. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky.

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Appendix A1

Selenium Fish Tissue Chain-of-Custody Sheet

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**SELENIUM FISH TISSUE
CHAIN-OF-CUSTODY**

Station #: _____ **Date:** _____

Stream / Location: _____ **Time:** _____

KPDES Permit#: _____

County: _____ **Lat/Long Upstream Reach:** _____

Lat/Long Downstream Reach: _____

Outfall #: _____ **Duplicate/Replicate (circle one):** yes no

Flow status (circle one): runoff event high flow low flow normal other

Fish #	Genus	Species	Length (mm)	Comments
001				
002				
003				
004				
005				
006				
007				

Length (mm) of 75%tile of Longest Fish: _____

Total # Fish Collected in Sample: _____

Collected by: _____	Date: _____	Time: _____
Relinquished by: _____	Date: _____	Time: _____
Received by: _____	Date: _____	Time: _____

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Appendix A2

Example of a Filled Out Chain-of-Custody Sheet

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**SELENIUM FISH TISSUE
CHAIN-OF-CUSTODY**

Station #: UTHF-001-Dup

Date: 5/23/13

Stream / Location: UT Horse Fork – Downstream Outfall

Time: 1234 CST

DNR Permit#: 745-2525

KPDES Permit#: KY0100000

County: Hancock

Lat/Long Upstream Reach: 37.770/-86.803

Lat/Long Downstream Reach: 37.771/-86.803

Outfall #: 003

Duplicate/Replicate (circle one): yes no

Flow status (circle one): runoff event high flow low flow normal other

Fish #	Genus	Species	Length (mm)	Comments
001	Semotilus	atromaculatus	120 mm	
002	Semotilus	atromaculatus	112 mm	
003	Semotilus	atromaculus	104 mm	
004	Semotilus	atromaculatus	123 mm	Longest
005	Semotilus	atromaculatus	98 mm	
006				
007				

Length (mm) 75%tile of Longest Fish: 92 mm

Total # Fish Collected in Sample: 5

Collected by: John Johnson ABC Consulting	Date: 5/23/13	Time: 1234 CST
Relinquished by: John Johnson ABC Consulting	Date: 5/23/13	Time: 1536 CST
Received by: William Williamson DEF Laboratory	Date: 5/23/13	Time: 1536 CST

Appendix D-3

Standard Operating Procedure for Preparation and Homogenization of Fish Tissue Samples

Commonwealth of Kentucky
Energy and Environment Cabinet
Department for Environmental Protection
Division of Water

Effective Date: May 11, 2017

Revision Date: May 1, 2017

Revision No: 2.0

Document Control No: DOWSOP0300032

Action By:	Signature	Date
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Melanie Arnold Reviewed, Monitoring Section Supervisor		5-9-17
Andrea Keatley Approved, Water Quality Branch Manager		5/9/17
Andrea Keatley, Acting Reviewed and Approved, Water Quality Branch QA Coordinator		5/9/17
Lisa Hicks Approved, Division of Water, Quality Assurance Officer		5/11/2017
Peter Goodmann Approved, Division of Water, Director		5/11/2017

Revision History

Date of Revision	Page(s) Revised	Revision Explanation
May 1, 2017	All pages	Sections added include Lyophilization homogenization, dissection of whole bodies and dry weight to wet weight calculations. Revised Fish Tissue Data Sheet and created Lyophilization Data Sheet and Scale Check Log.
July 1, 2014	All pages	Laboratory Procedures for Resection of Fish Fillets and Homogenization of Tissue Samples was separated from preceding document and revised/updated for general content regarding laboratory methods.
March 13, 2008	All pages	Standard Methods for Assessing Biological Integrity of Surface Waters in Kentucky General Content-Document was re- formatted for maintaining headers, section titles, etc in a consistent style. All references to detailed water chemistry sampling were removed, and a reference inserted directing the reader to the 'Standard Operating Procedures for Sampling and Monitoring Surface Waters for Kentucky', in draft.
July, 2002	All pages	Methods for Assessing Biological Integrity of Surface Waters in Kentucky original document.

Suggested Citation: Kentucky Division of Water (KDOW). 2017. Standard Operating Procedure for Preparation and Homogenization of Fish Tissue Samples, Version 2.0. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky.

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Procedures

Scope and Applicability

This manual has been developed by the Division of Water as guidance for the uniform and accurate procedures for the preparation and homogenization of tissue samples. The procedures defined herein are required for the preparation and homogenization of tissue samples and QA/QC activities resulting in information used for issuing fish consumption advisories and the biennial Integrated Report to Congress on Water Quality in Kentucky (305[b] and 303[d] Reports). Any data submitted to KDOW for review will undergo QA/QC review and those identified as not following the methods set forth in this document will be flagged as not suitable for issuing fish consumption advisories or for the Integrated Report. These data may be retained in KDOW files for other data purposes.

Fish consumption advisories are jointly issued by the representatives from the Division of Water, Department of Fish and Wildlife Resources and Department for Public Health when contaminants in fish tissue exceed the level considered safe for unlimited human consumption. On December 6, 2004 each agency signed an Interagency Agreement to work together on the issuance of fish consumption advisories. The Interagency Agreement was updated June 24, 2015. The Interagency Agreement outlines the roles of each agency, but does not detail the standard operating procedures concerning how fish consumption advisories should or will be issued. Human health risk-based methodologies, based on previously developed protocols by the Great Lakes Sport Fish Advisory Task Force (GLSFATF 1993) and the U.S. Environmental Protection Agency (EPA 2000a), are used to determine if fish consumption advisories should be issued and what restriction level the advisories recommend. The protocols provide information in greater detail to target populations such as women of childbearing age and children, and recommends the number of fish meals a person may consume to minimize health risks.

Section 305(b) of the Federal Clean Water Act requires Kentucky to monitor, assess and report on the quality of its waters in accordance with Kentucky Water Quality standards. Federal fish tissue based water quality criterion for methylmercury (USEPA 2001) and selenium (USEPA 2016a) have been created and reported in wet weight and dry weight units, respectively. Kentucky has promulgated state specific selenium criterion in 401 KAR 10:031. Surface Water Standards. Kentucky fish tissue resultant information will be used to assess waterbodies for impairment based on Kentucky surface water standards.

Definitions

ESB-Environmental Services Branch

GLSFATF-Great Lakes Sport Fish Advisory Taskforce

KDOW – Kentucky Division of Water

KWADE – Kentucky Water Assessment Data for Environmental Monitoring

SDS –Safety Data Sheet

PPE – Personal Protective Equipment

PTFE – Polytetrafluoroethylene (Teflon)

USEPA-United States Environmental Protection Agency

Health & Safety Policy/Section

Proper PPE shall be worn by all personnel while processing samples and handling chemicals. Refer to the appropriate SDS for the correct PPE while handling chemicals. It is recommended that lab coats also be worn to protect clothing from spillage. Protective eyewear should be worn when the potential exists for particulate, vapor, liquid or foreign objects to become lodged in the eye. When working with chemicals that cause harmful fumes, personnel shall use a fume hood to reduce the threat of inhalation exposure to them and their fellow coworkers.

Toxic or caustic materials must be stored in a chemical storage cabinet. When a chemical spill (e.g. a broken mercury thermometer, broken large containers of acids or preservatives) occurs, the first line supervisor will be notified. The first line supervisor will notify the second line supervisor and the division safety officer. The division safety officer will then notify the department safety officer. Do not attempt to clean-up a chemical spill, if inhalation exposure or skin, throat or eye irritation is a threat. Extreme care shall be taken when processing tissue samples. When cutting frozen fish samples with a band saw or knife, fingers should be kept away from the blade at all times. Care shall be taken when handling and cleaning blenders to reduce the chance of cutting fingers by the blades. Ear protection shall be used when loud machinery will be in use (i.e. processing fish tissue samples using saws or other electronic machinery).

If injury or exposure occurs within the laboratory facilities, then proper first aid attention will be administered by other lab personnel as soon as possible. If the condition is serious, the victim should be transported to a medical facility as soon as possible. For chemical exposures refer to the appropriate SDS for first aid treatment. Safety Data Sheets shall be maintained in a readily accessible location in the lab for each chemical stored or used in the lab. If any exposure occurs while in the laboratory, a 1A1 exposure or injury form needs to be submitted to the Division of Workman's Compensation within 24 hours of exposure or injury.

Cautions

Several cautions exist with regard to activities and negligence that could possibly cause equipment damage, degradation of the sample and possible invalidation of the results. Potential sources of

contamination include dust, instruments, utensils, work surfaces and containers that may contact the samples. All sample processing (i.e., filleting, removal of other tissue, homogenizing, compositing) will be processed in a clean environment set away from sources of potential contamination. All instruments, work surfaces, and containers used to process samples must be of materials that can be cleaned easily and that are not themselves potential sources of contamination. The predominant metal contaminants from stainless steel are chromium and nickel. If these metals are a concern, then use of stainless steel in processing should be limited and/or appropriate equipment and rinsate blanks procured. If chromium and nickel are not a concern, the use of high-quality, corrosion resistant stainless steel for sample processing equipment is acceptable. If aluminum is of concern, the tissue samples should be placed on glass cutting boards and in glass or plastic containers. Stainless steel instruments and heavy duty aluminum foil are in use by the KDOW; therefore, if the above parameters are of concern, additional equipment may need to be obtained before processing of tissue samples. Equipment rinsate blanks may be used to evaluate the possibility of contamination (USEPA 2000b).

Personnel Qualifications / Responsibilities

All biologists will meet at least the minimum qualifications for their job classification. In addition, fisheries biologists will be trained in the collection and identification of fish by formal academic instruction. Fisheries biologists that have not had formal academic instruction in collection and identification of fish will be deemed technically competent based on their knowledge, skills and abilities by KDOW management. Taxonomic education will continue with on-the-job training, interaction with experienced taxonomists and continued outside training when education opportunities become available. All laboratory personnel performing sample processing procedures should be trained or supervised by an experienced fisheries biologist in the laboratory procedures for resection of fish fillets and homogenization of tissue samples.

Equipment and Supplies

The following is a list of common equipment and supplies typically employed:

- Heavy duty aluminum foil
- Glass cutting board
- Stainless steel filet knife
- Knife sharpening stone
- High speed stainless steel blender (various sizes)
- Freezer (≤ -20 °C)
- Disposable gloves (powder free)
- Whirl-pack® (24 oz., 6" W x 9" L)
- Fish Tissue Data Sheets
- Lyophilization Data Sheets
- Taxonomic literature (Appendix A)
- Laboratory detergent (Liquinox®)
- Stainless steel trays
- Freeze dryer
- Precision balance (≤ 0.01 g)

Methods

The following sections describe the laboratory procedures for the preparation and homogenization of fish tissue samples. Samples are collected at designated sites for fish contaminant studies and put on ice in clean coolers for tissue preservation. Individual samples can be laid directly on ice in contact with other samples as long as they are rinsed before resection and there is no risk of puncturing the skin. Small fish that will be processed as whole body samples may be placed in a zip top type bag as a group. Composite samples should only contain fish of the same species. Taxonomic references are listed in Appendix A. All samples will be delivered to the KDOW biological laboratory on ice.

Fish fillets and/or whole body samples will be the tissue types covered in these procedures. To assess methylmercury Kentucky water quality standards and consumption limits, fillets will be used. To assess selenium Kentucky water quality standards, whole body samples will be used.

Initial Sample Processing

All samples must be recorded into the Fish Tissue Data Sheet (Appendix B) upon returning from the field. Information included in the Fish Tissue Data Sheet includes waterbody sampled, collection date and time (EST), location on waterbody sampled, basin where the waterbody is located, SiteID of location sampled, county of locations sampled, coordinates of location sampled, collection method, and collector's names and any notes that should be included with the sample. Data fields such as date and time of resection of fish fillets (if applicable) and laboratory personnel who performed each task should also be included. Other fish tissue data sheet field definitions are described below.

- **Sample ID:** assigned by current year and in consecutive order.
- **Sample Method:** Individual or a composite sample.
- **Tissue Type:** Use abbreviations RF=Right Fillet; LF=Left Fillet; BF=Both Fillets; WB=Whole Body.
- **Species:** Species of the sample collected.
- **Length:** Length of the sample collected in millimeters.
- **Weight:** Weight of the sample collected in grams.
- **Sex:** Sex of sample collected.
- **Age:** Age of sample collected.
- **Aging Method:** **O**=Otolith; **S**=Scale; **F**=Fin; **S**=Spine

Cleaning of Work Utensils

Equipment will be cleaned following USEPA (2000b) for both organic and metals analysis between the processing of each sample. Glass and stainless steel knives/utensils/parts should be cleaned thoroughly with a detergent solution, rinsed with tap water, rinsed with pesticide grade acetone or isopropanol and then rinsed with metal/organic- free de-ionized water.

Tissue Preparation

All samples will remain on ice until tissue preparation can begin. Tissue preparation should occur within 48 hours of collection (USEPA 2000b). If tissue preparation cannot be performed within 48 hours of collection in the biological laboratory, tissue preparation will be performed in the field. If tissue preparations are performed in the field, a clean area will be set up away from sources of exhaust and areas where gasoline or grease are used to help reduce the potential for surface and airborne contamination of the samples. A notation will be made on the Fish Tissue Data Sheet of the location of tissue preparation. Care must be taken to avoid contaminating tissues with material released from inadvertent puncture of internal organs. If the tissue is contaminated by materials released from the inadvertent puncture of the internal organs during resection, the tissue will be rinsed in deionized water.

Prior to tissue preparation, hands will be washed and rinsed thoroughly in tap water, followed by deionized water. Powder-free gloves are to be worn when handling the samples. A protective glove may be worn under a powder-free disposable glove to help prevent cutting injuries while resecting fillets. Knives with stainless steel blades will be used in the resection of fillets. Specimens will be prepared on glass cutting boards or on cutting boards covered with heavy duty aluminum foil that is changed after each sample. Only parts of the specimen that will not be sent to the analytical laboratory for analysis should come in contact with aluminum foil.

NOTE: Changing cutting boards with heavy duty aluminum foil after each use does not require the cutting board to be cleaned between each sample.

Resection of Fish Fillets

Target fillet (or composite) weight is >50 g wet weight. Only fillets from the right side of each fish will be used as part of the qualifying individual sample. If the target weight is not met, the left fillet(s) should be removed and added to the sample. If the sample still does not meet target weight after combining both right and left fillets, personal communication with the qualified analytical laboratory that will be analyzing the tissue samples should commence to identify if the sample can be sufficiently analyzed. Qualifying composite samples are described below and will only be composed of right fillets or both fillets from each sample. Fillets will be processed as boneless skin-on/off (per study plan) and will include all flesh and fatty deposits from the nape to the caudal fin and from the dorsal fin of the back down to venter including the belly flap area of the fish. If skin-on fillet is required, each fish will be scaled prior to the resection of the fillet and rinsed in de-ionized water after scaling. Any bones should be removed from the fillet if present after resection.

NOTE: KDOW has observed that a skin-on fillet often is difficult to homogenize and skin-off fillets are preferred.

When the fillet is removed it should be placed on a clean glass cutting board and not on aluminum foil. In order to facilitate homogenization, fillet(s) should be cut into small pieces (≤ 1 in) and placed inside a certified clean container. The recommended sample container is a sterile whirlpak®, but can be any certified clean container that can be freeze dried without damage or weight loss. The certified clean container should be weighed to the nearest 0.01g before storing the fillet inside. The certified clean container with the fillet should then be weighed (nearest 0.01g wet weight). If the samples volume is too large to be placed in one whirlpak® or approved clean container, the sample can be divided and placed in multiple containers creating a subsample. These data (subsample number and container weights) will be recorded on the Lyophilization Data Sheet (Appendix C).

Sample information will be written on the outside of the container with a waterproof marker. If aging structures or organism sex is required for a project, collect this information after resection of fillets has occurred.

Dissection of Whole Body Samples

Dissection of whole body samples will be processed using of all body parts, bones, body liquids and scales. It should also include all stomach contents. Whole body samples should be diced into small pieces (≤ 1 inch or as small as it can be safely dissected) with stainless steel saw blades and/or knives on clean glass cutting boards. Care must be taken when dissecting large specimens because it can be difficult to slice through bones and scales. The recommended sample container is a sterile whirlpak®, but can be any certified clean container that can be freeze dried without damage or weight loss. The certified clean container should be weighed to the nearest 0.01g before storing the whole body sample inside. The certified clean container with the whole body sample should then be weighed (nearest 0.01g wet weight). If the samples volume is too large to be placed in one whirlpak® or approved clean container, the sample can be divided and placed in multiple containers creating a subsample. These data (subsample number and container weights) will be recorded on the Lyophilization Data Sheet (Appendix C). Sample information will be written on the outside of the container with a waterproof marker.

Qualifying Composite Samples

Individual samples are preferred over composite samples. Individual samples provide a direct measure of the range and variability of contaminants in the target fish population. Composite samples can be utilized when both the right and left fillets of an individual sample weighs <50 g wet weight, whole body samples or when it is cost-prohibitive to analyze individual samples. Qualifying composite samples must adhere to a set of guidelines:

- 1.) All tissue in the composite must be the same species.
- 2.) Right fillets or both fillets should only be used unless it's a whole body sample.
- 3.) All tissue in the composite must be of similar size so that the smallest individual in a composite is no less than 75 percent of the total length of the largest individual (USEPA 2000b).

It should be noted in the Fish Tissue Data Sheet which individuals make up the composite sample. Sample details such as the length and weight can be averaged to describe the composite sample.

Preservation

Once samples are received from the field and resection of fillets has occurred, samples are placed in the laboratory freezer and stored at $\leq -20^{\circ}\text{C}$ until samples can be lyophilized.

Lyophilization and Homogenization

Lyophilization should occur in an appropriate amount of time to allow the analytical laboratory to analyze the samples within six months of collection. A detailed illustration of lyophilization procedures are shown in Appendix D. Personnel should use powder-free gloves when handling samples. Frozen samples inside the certified clean containers (i.e. whirlpak®) will be placed inside the freeze dryer for lyophilization. The certified clean containers will be left slightly open for the evacuation of moisture. Care must be taken when opening the containers as to not allow any contamination or sample loss, including frozen moisture to occur. The freeze dry cycle will consist of a nine hour freeze and seven hour drying time. The process may be changed based on the amount of moisture in each sample cycle. If reporting in wet weight, the percent moisture will be recorded before removing the sample from the lyophilized container. See the “Dry Weight to Wet Weight Conversion” section for the percent moisture calculation. After the percent moisture is calculated and recorded, the sample should be transferred into a stainless steel blender for homogenization. A fillet sample with >75% moisture loss and a whole body sample with >70% moisture loss is normally sufficient for homogenization.

NOTE: Wet weight samples should not be lyophilized after blender homogenization to ensure the correct percent moisture calculation.

Depending on the samples volume, the appropriate blender cup size should be used. Most fillets can be homogenized in the small blender cups (32 ounces) while whole body samples will likely require the large blender cups (1 gallon). If subsamples were created, they should all be combined for homogenization. Wet weight samples should not be lyophilized after blender homogenization to ensure correct percent moisture calculation. The sample should be blended until no obvious non-homogenized masses are visible stirring with a clean stainless steel spoon for verification. After homogenization, the sample will be placed back into the original container for processing and storage. Excess sample tissue can be discarded after homogenization if a sufficient sample weight has been attained.

When reporting in dry weight (i.e. selenium analysis), percent moisture is not needed; therefore samples need to be measured to a constant weight. It may take several lyophilization cycles including a cycle(s) AFTER homogenization. When there is no weight loss and the sample weights remain constant, the sample is completely dry.

NOTE: It is imperative that as much moisture be evacuated from the sample as possible.

To ensure that a record of the sample weights for the calculation of moisture loss is recorded, a Lyophilization Data Sheet (Appendix C) should be used. The Lyophilization Data Sheet field definitions are described below.

- **Sample ID:** ID number designated for each sample (individual or composite) and is assigned by year and in consecutive order.
- **Subsample Number:** Should read # of specified subsample of # of containers used for the sample.
- **Container Weight:** Weight of empty sample container with seal removed in grams.
- **Sample Wet Weight:** Wet weight of the sample subtracting the container weight in grams.
- **Reporting Type Goal:** Wet weight (i.e. mercury) or Dry weight (i.e. selenium).
- **Cycle Weights:** Sample weight + container weight after lyophilization cycle.
- **% Moisture:** The results of the calculation: $[(\text{Wet Weight} - \text{Dry Weight}) / \text{Wet Weight}]$.

Transferring Homogenized Sample to Receiving Vessel and Storage

Homogenized samples can be sealed in the original container. An additional zip-top freezer bag will be added around the original container to ensure no moisture is lost or added during storage. At this point, all homogenates will be stored at -20°C until processed for analysis in the analytical laboratory.

Dry Weight to Wet Weight Conversion

When the Reports of Analysis are delivered from the Environmental Services Branch laboratory, all samples that have been lyophilized will be reported in dry weight and converted to wet weight for the Integrated Report and consumption advisories unless dry weight is recommended (i.e. selenium). The conversion from dry weight to wet weight requires obtaining the percent moisture of the sample being analyzed (USEPA 2016b). To calculate percent moisture, samples must be weighed before and after freeze drying. Samples will be weighed on a scale to the nearest 0.01g. Percent moisture should be included on the Chain of Custody and presented with the official lab Report of Analysis. The conversion formulas and additional information, which includes justification are found in Appendix E.

Quality Control and Quality Assurance

Delivery to the Analytical Laboratory

Samples will be delivered to the appropriate analytical laboratory following KDOW (2009). A chain of custody will be assigned with the samples (Appendix C). Analysis of samples should occur within six months of sample collection.

Balance Calibration Checks

All samples should be weighed on a balance that is properly calibrated and of adequate accuracy and precision (USEPA 2000b). Balance checks should be recorded at the beginning of each weighing session using the reference weights 200 g, 100 g and 50 g. For the calculation of percent moisture, the acceptable tolerance between reference weights and the balance readings is ± 0.1 grams. Care must be taken to avoid balance interferences. Reference weight handling and standardization procedures are found in ESB 2015. Balance calibration and corrective actions for out-of-control data will follow procedures outlined in ESB 2015. If the instrument fails to meet accuracy specifications after re-calibration, the balance will be tagged "Out of Service" until repair or replacement of the balance has occurred. A Balance Check Log is available in Appendix G.

Replicate (Splits) and Rinsate Blanks

Replicate (split) samples will be collected by submitting two independent samples of homogenized tissue from the same sample to the analytical laboratory if required by the study plan.

Rinsate blanks are a de-ionized water sample collected by rinsing the equipment that typically comes in contact with the tissue during homogenization. The equipment should be cleaned prior to rinsing using the protocols described in the above section "Cleaning of Work Utensils". The sample will be collected in appropriate bottles and submitted for analysis if required by the study plan.

Data Storage, Entry and Verification

All field and laboratory data will be recorded on the Fish Tissue Data Sheet (Appendix B) and Lyophilization Data Sheet (Appendix F) then digitized to the appropriate project folder. Results from the analytical laboratory should be filed in the project's e-files and recorded into KWADE according to KDOW (2015). The project coordinator will be responsible for reviewing the received data for accuracy and resolve any corrective actions if needed.

References

Environmental Services Branch (ESB). 2015. Standard Operating Procedure for Balance Calibration Checks. Frankfort, Kentucky. DES Doc. 9020.

Great Lakes Sport Fish Advisory Task Force (GLSFATF). 1993. Protocol for a uniform Great Lakes Sport Fish Consumption Advisory. Great Lakes Sport Fish Advisory Task Force, Council of Great Lakes Governors, Chicago, IL. 81p. MESB-FP 9/16/93.

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Appendix A. Suggested Taxonomic References

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Appendix B. Fish Tissue Data Sheet

FISH TISSUE DATA SHEET-example

Waterbody: <i>Cave Run Lake</i>		Collection Date: <i>05/29/2017</i>	
		Start Time: <i>1200</i>	End Time: <i>1400</i>
Location: <i>Near Banger Ramp</i>		Basin: <i>Licking</i>	
Site ID: <i>DOW05036025</i>		County: <i>Rowan</i>	
Coordinates (Latitude/Longitude): <i>38.04375 -83.43882</i>		Collection Method: <i>Large Boat Electrofisher</i>	
Tissue Preparation Location: <input type="checkbox"/> Field <input checked="" type="checkbox"/> Lab		Collectors: <i>Garrett Stillings, Rodney Pierce and Robert Johnson</i>	
Notes: <i>Lesions were found on Field Sample ID: 17-001</i>			

Sample ID (Year- Number; Ex. 17-001; 17-002)	Sample Method (Individual or Composite)	Tissue type (RF,LF, BF,WB)	Species	Length (mm)	Weight (g)	Sex	Aging Method*	Age
17-001	Individual	RF	Largemouth Bass	507	1975	F	O	9
17-002	Individual	BF	Channel Catfish	414	700	M	S	5
17-003	Composite	RF	Bluegill	182	125	NR	NR	NR
17-003	Composite	RF	Bluegill	167	100	NR	NR	NR
17-003	Composite	RF	Bluegill	168	115	NR	NR	NR
17-003	Composite	RF	Bluegill	147	60	NR	NR	NR
17-003	Composite	RF	Bluegill	171	105	NR	NR	NR
17-004	Composite	WB	Creek Chub	50	40	NR	NR	NR
17-004	Composite	WB	Creek Chub	60	50	NR	NR	NR

Resection of fillets or sample dissection by: *Garrett Stillings and Rodney Pierce*

Resection Date: *05/30/2017*

Resection Start Time: *1015*

Resection End Time: *1130*

***Aging Method:** O=Otolith; S=Scale; F=Fin; S=Spine

RF=Right Fillet; LF=Left Fillet; BF=Both Fillets; WB=Whole Body; NR=Not Recorded

Appendix C. Lyophilization Data Sheet

Lyophilization Data Sheet – example

Sample Details							Sample Weight + Container Weight After Lyophilization Cycle					
Sample ID	Date/Time	Subsample Number (ex. 1 of 2; 2 of 2)	A	B	C	Reporting Type Goal (WW or DW)	Cycle #1	Cycle #2	Cycle #3	Cycle #4	Cycle #5	Cycle #6
			Container Weight (g)	Container Weight + Sample WW (g)	Sample WW (g) (A - B)		Weight (g) Date:5/10/16 Time: 1015	Weight (g) Date:5/11/16 Time:1200	Weight (g) Date:5/12/16 Time:1030	Weight (g) Date:5/13/16 Time:1100	Weight (g) Date:5/14/16 Time:1115	Weight (g) Date: Time:
16-010	4/15/16 1015	1 of 1	6.61	72.71	66.10	WW	65.24 <input type="checkbox"/>	54.87 <input type="checkbox"/>	24.87 <input checked="" type="checkbox"/>	- <input type="checkbox"/>	- <input type="checkbox"/>	- <input type="checkbox"/>
16-011	4/15/16 1030	1 of 2	6.59	197.25	190.66	WW	125.65 <input type="checkbox"/>	98.33 <input type="checkbox"/>	57.33 <input type="checkbox"/>	57.01 <input checked="" type="checkbox"/>	- <input type="checkbox"/>	- <input type="checkbox"/>
16-011	4/15/16 1045	2 of 2	6.65	152.91	146.26	WW	100.37 <input type="checkbox"/>	93.35 <input type="checkbox"/>	82.35 <input type="checkbox"/>	70.26 <input type="checkbox"/>	69.23 <input checked="" type="checkbox"/>	- <input type="checkbox"/>
16-012	4/15/16 1100	1 of 3	6.63	40.21	33.58	DW	20.53 <input type="checkbox"/>	15.36 <input type="checkbox"/>	15.30 <input checked="" type="checkbox"/>	- <input type="checkbox"/>	- <input type="checkbox"/>	- <input type="checkbox"/>
16-012	4/15/16 1115	2 of 3	6.65	25.13	18.48	DW	16.35 <input type="checkbox"/>	10.89 <input type="checkbox"/>	8.56 <input checked="" type="checkbox"/>	- <input type="checkbox"/>	- <input type="checkbox"/>	- <input type="checkbox"/>
16-012	4/15/16 1130	3 of 3	6.64	55.23	48.59	DW	35.45 <input type="checkbox"/>	20.79 <input type="checkbox"/>	15.47 <input checked="" type="checkbox"/>	- <input type="checkbox"/>	- <input type="checkbox"/>	- <input type="checkbox"/>

WW=Wet Weight; DW=Dry Weight

=Final Cycle Weight

Check if the cycle was the final lyophilization cycle and the sample was homogenized. Subsamples should be composited before homogenization.

Use This Section Only if Reporting Type is WW.

	W	X	Y	Z	
Sample ID	Sample Weight + Container Weight (g) (Σ Final Cycle weights with the same Sample ID)	Container Weight (g) (Σ A with the same Sample ID)	Sample WW (g) (Σ C with the same Sample ID)	Sample DW (g) (W - X)	% Moisture ((Y-Z)/Y) x 100
16-010	24.87	6.61	66.10	18.26	72.38
16-011	126.24	13.24	336.92	113.00	66.46

Use This Section Only if Reporting Type is DW and Samples with the Same Sample IDs have been Composited and Homogenized.
Reporting Type DW does not require % Moisture.

Sample ID	Sample Weight + Container Weight After Lyophilization Cycle			
	Cycle #1 Weight (g) Date:5/15/16 Time:1030	Cycle #2 Weight (g) Date:5/16/16 Time:1045	Cycle #3 Weight (g) Date:5/17/16 Time:1300	Cycle #4 Weight (g) Date: Time:
16-012	35.32	34.66*	34.66*	-

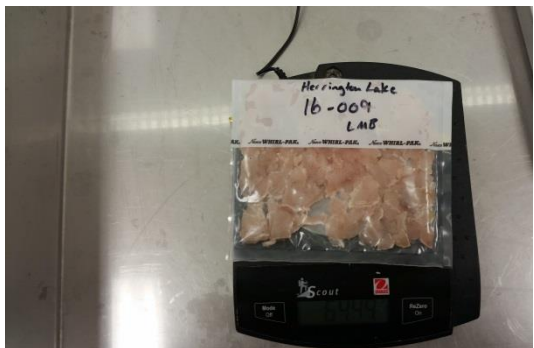
*Samples should be lyophilized until a consistent weight is measured.

Appendix D. Lyophilization Procedures

1. Weigh a clean opened whirlpak® to record **Container Weight (g)**.



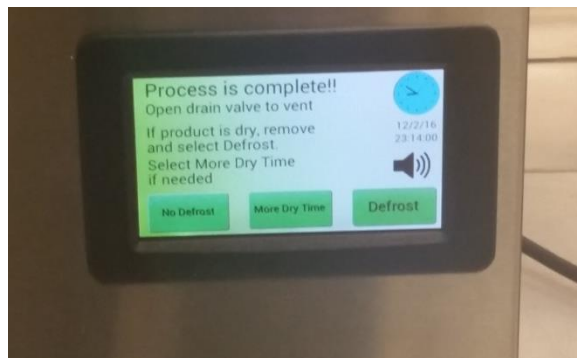
2. Insert diced fish fillet/whole body into the opened whirlpak® and weigh to record **Container Weight + Sample Wet Weight (g)**.



3. Arrange whirlpaks® on trays and place into freeze dryer.



4. Verify if the freeze dryer settings and vacuum pump oil levels are correct for the lyophilization process. Read freeze dryer instruction guide for more information.



5. When cycle is finished, weigh whirlpaks® and calculate percent moisture. Multiple cycles may be needed. $\geq 70\%$ of moisture loss is normally sufficient for homogenization of fish tissue. If reporting in dry weight additional lyophilization cycles are needed after homogenization until weights remain constant.

Appendix E. Wet/Dry Weight Conversion Information

In 2016, the Kentucky Division of Water elected to homogenize fish tissue samples by lyophilization, also known as freeze drying, instead of liquid nitrogen or dry ice homogenization. It was concluded that lyophilization would simplify and accelerate the sample homogenization process and limit chances of contamination. There were specific observations where the process of homogenizing samples that were frozen with liquid nitrogen/dry ice had damaged blender blades, introducing contaminants in the sample. Lyophilized samples can be homogenized easily in a blender with no risk of damaging the blender cups or blades.

From personal communication with the Environmental Services Branch staff, lyophilization aids in the analytical analysis of samples. Dry samples make digestion easier because it pre-concentrates elemental compositions by eliminating water while digestion reagents are not diluted. In an extraction when the sample is not homogenized thoroughly the solvent cannot reach all parts of the sample, which can create inconsistent results. Additionally, there is a significant reduction in solvent usage for primary extraction since the volume/mass of the sample will effectively be reduced by > 1/5th.

Samples homogenized by lyophilization will be reported in dry weight from the Environmental Services Branch and can be converted to wet weight by the user for fish consumption advisories and Integrated Reports. The conversion from dry weight to wet weight requires obtaining the percent moisture of the sample being analyzed (USEPA 2016b). To calculate the percent moisture, samples must be weighed before and after lyophilization. Samples will be weighed on a balance at least to the nearest 0.01 grams. When converting between weights, the least number of significant figures in any number of the conversion determines the number of significant figures in the result. The conversion formulas are described below.

Dry Weight to Wet Weight Conversion Formulas

Wet Weight = Total sample weight before lyophilization

Dry Weight = Total sample weight after lyophilization

% Moisture = [(Wet Weight - Dry Weight)/Wet Weight] x 100

Wet Weight Concentration = Dry Weight Concentration x [1 - (% Moisture/100)]

Dry Weight Concentration = Wet Weight Concentration / [1 - (% Moisture/100)]

Examples:

*14-112 -- [(35.32-6.58)/35.32]x100=81.37=% Moisture 14-125 -- [(28.73-6.24)/28.73]x100=78.28=% Moisture
 1.870 x [1- (81.37/100)]=0.348=WW Concentration 0.262 x [1- (78.28/100)]=0.059=WW Concentration*

Field ID	Dry Weight (g)	Wet Weight (g)	% Moisture	DW Hg Concentration (mg/kg)	WW Hg Concentration (mg/kg)	WW Hg (mg/kg) from past runs in 2014
14-112	6.58	35.32	81.37	1.87	0.35	0.36
14-125	6.24	28.73	78.28	0.26	0.06	0.06

Appendix F: Chain of Custody

Chain of Custody Record

Program Code: A20

Coordinator: _____

County	Field ID	Sample Identification	% Moisture Removed	Collection Method	Date	Container		
						1	2	3
				<input type="checkbox"/> Composite <input type="checkbox"/> Grab		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
						LAB Report #		
				<input type="checkbox"/> Composite <input type="checkbox"/> Grab		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
						LAB Report #		
				<input type="checkbox"/> Composite <input type="checkbox"/> Grab		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
						LAB Report #		

Analysis Requested: Program Code: A20

Sample Matrix: Tissue-Fillet Tissue-Whole Body

Sample Type: Dry Weight Wet Weight

Container 1:

Shipment Temp: _____

Container 2:

Container 3:

Samples Collected By: _____

Relinquished by: _____ Date: _____ Received by: _____ Date: _____

Representing: _____ Time: _____ Representing: _____ Time: _____

Appendix G. Scale Check Log

Balance Check Log

Weighing Session Date/Time	Reference Weight Balance Readings			Check if Recalibrated
	200 g	100 g	50 g	
				<input type="checkbox"/>
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