# 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

<table>
<thead>
<tr>
<th>Action By</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garrett Stillings, Prepared, SOP Author</td>
<td>Garrett Stillings</td>
<td>5-9-17</td>
</tr>
<tr>
<td>Melanie Arnold, Reviewed, Monitoring Section Supervisor</td>
<td>Melanie Arnold</td>
<td>5-9-17</td>
</tr>
<tr>
<td>Andrea Keatley, Approved, Water Quality Branch Manager</td>
<td>Andrea Keatley</td>
<td>5/9/17</td>
</tr>
<tr>
<td>Andrea Keatley, Acting Reviewed and Approved, Water Quality Branch QA Coordinator</td>
<td>Andrea Keatley</td>
<td>5/9/17</td>
</tr>
<tr>
<td>Lisa Hicks, Approved, Division of Water, Quality Assurance Officer</td>
<td>Lisa Hicks</td>
<td>5/11/2017</td>
</tr>
<tr>
<td>Peter Goodmann, Approved, Division of Water, Director</td>
<td>Peter Goodmann</td>
<td>5/11/2017</td>
</tr>
</tbody>
</table>
## Revision History

<table>
<thead>
<tr>
<th>Date of Revision</th>
<th>Page(s) Revised</th>
<th>Revision Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 1, 2017</td>
<td>All pages</td>
<td>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.</td>
</tr>
<tr>
<td>July 1, 2014</td>
<td>All pages</td>
<td>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.</td>
</tr>
<tr>
<td>March 13, 2008</td>
<td>All pages</td>
<td><strong>Standard Methods for Assessing Biological Integrity of Surface Waters in Kentucky</strong> 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.</td>
</tr>
<tr>
<td>July, 2002</td>
<td>All pages</td>
<td>Methods for Assessing Biological Integrity of Surface Waters in Kentucky original document.</td>
</tr>
</tbody>
</table>

**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.
# Table of Contents

Procedures ........................................................................................................................................ 4
Scope and Applicability.................................................................................................................... 4
Definitions.......................................................................................................................................... 5
Health & Safety Policy/Section ......................................................................................................... 5
Cautions ............................................................................................................................................... 5
Personnel Qualifications / Responsibilities .................................................................................... 6
Equipment and Supplies .................................................................................................................. 6
Methods ............................................................................................................................................. 7
  Initial Sample Processing ................................................................................................................ 7
  Cleaning of Work Utensils ............................................................................................................... 7
  Tissue Preparation ......................................................................................................................... 8
  Resection of Fish Fillets ............................................................................................................... 8
  Dissection of Whole Body Samples ............................................................................................... 9
  Qualifying Composite Samples ................................................................................................... 9
  Preservation ..................................................................................................................................... 10
  Lyophilization and Homogenization ............................................................................................. 10
  Transferring Homogenized Sample to Receiving Vessel and Storage ......................................... 11
  Dry Weight to Wet Weight Conversion ......................................................................................... 11
Quality Control and Quality Assurance .......................................................................................... 11
  Delivery to the Analytical Laboratory ......................................................................................... 11
  Balance Calibration Checks ........................................................................................................ 12
  Replicate (Splits) and Rinsate Blanks .......................................................................................... 12
  Data Storage, Entry and Verification ............................................................................................ 12
Appendix A. Suggested Taxonomic References ............................................................................. 14
Appendix B. Fish Tissue Data Sheet .............................................................................................. 15
Appendix C. Lyophilization Data Sheet .......................................................................................... 16
Appendix D. Lyophilization Procedures .......................................................................................... 17
Appendix E. Wet/Dry Weight Conversion Information .................................................................. 18
Appendix F: Chain of Custody ......................................................................................................... 19
Appendix G. Scale Check Log ......................................................................................................... 20
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 (<=0.01g)
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 KDOH 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 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 ≤-20 °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: 
\[
\frac{(\text{Wet Weight} - \text{Dry Weight})}{\text{Wet Weight}}
\]

**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 recalibration, 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


Kentucky Division of Water (KDO). 2015. KWADE Monitoring Station Creation. Kentucky Department for Environmental Protection, Frankfort, Kentucky.


Appendix A. Suggested Taxonomic References


Cicerello, R.R. and R.S. Butler. 2007. Distribution and status of Etheostoma tecumsehi, the Shawnee darter, a species endemic to the Pond River, Green River drainage, Kentucky. SFC Proceedings No. 49.


**Appendix B. Fish Tissue Data Sheet**

**FISH TISSUE DATA SHEET-example**

<table>
<thead>
<tr>
<th>Waterbody: Cave Run Lake</th>
<th>Collection Date: 05/29/2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location: Near Banger Ramp</td>
<td>Start Time: 1200</td>
</tr>
<tr>
<td>Site ID: DOW05036025</td>
<td>Basin: Licking</td>
</tr>
<tr>
<td>County: Rowan</td>
<td></td>
</tr>
<tr>
<td>Coordinates (Latitude/Longitude): 38.04375 -83.43882</td>
<td>Collection Method: Large Boat Electrofisher</td>
</tr>
<tr>
<td>Tissue Preparation Location:</td>
<td>Collectors: Garrett Stillings, Rodney Pierce and Robert Johnson</td>
</tr>
<tr>
<td>Notes: Lesions were found on Field Sample ID: 17-001</td>
<td></td>
</tr>
</tbody>
</table>

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

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=Right Fillet; BF=Both Fillets; WB=Whole Body; NR=Not Recorded
### Appendix C. Lyophilization Data Sheet

#### Lyophilization Data Sheet – example

<table>
<thead>
<tr>
<th>Sample Details</th>
<th>Sample Weight + Container Weight After Lyophilization Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample ID</strong></td>
<td><strong>A</strong></td>
</tr>
<tr>
<td>16-010</td>
<td>4/15/16 1015</td>
</tr>
<tr>
<td>16-011</td>
<td>4/15/16 1030</td>
</tr>
<tr>
<td>16-011</td>
<td>4/15/16 1045</td>
</tr>
<tr>
<td>16-012</td>
<td>4/15/16 1100</td>
</tr>
<tr>
<td>16-012</td>
<td>4/15/16 1115</td>
</tr>
<tr>
<td>16-012</td>
<td>4/15/16 1130</td>
</tr>
</tbody>
</table>

**WW**=Wet Weight; **DW**=Dry Weight

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

<table>
<thead>
<tr>
<th>Use This Section Only if Reporting Type is WW.</th>
<th>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.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample ID</strong></td>
<td><strong>W</strong></td>
</tr>
<tr>
<td>----------------</td>
<td>------</td>
</tr>
<tr>
<td>16-010</td>
<td>24.87</td>
</tr>
<tr>
<td>16-011</td>
<td>126.24</td>
</tr>
</tbody>
</table>

*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. ≥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**

\[
\text{Wet Weight} = \text{Total sample weight before lyophilization} \\
\text{Dry Weight} = \text{Total sample weight after lyophilization} \\
\% \text{ Moisture} = [(\text{Wet Weight} - \text{Dry Weight})/\text{Wet Weight}] \times 100 \\
\text{Wet Weight Concentration} = \text{Dry Weight Concentration} \times [1 - (% \text{ Moisture}/100)] \\
\text{Dry Weight Concentration} = \text{Wet Weight Concentration} / [1 - (% \text{ Moisture}/100)]
\]

**Examples:**

\[
\begin{align*}
14\text{-}112 & \quad (35.32 - 6.58)/35.32 = 81.37 = % \text{ Moisture} \\
1.870 \times [1 - (81.37/100)] & = 0.348 = \text{WW Concentration}
\end{align*}
\]

\[
\begin{align*}
14\text{-}125 & \quad (28.73 - 6.24)/28.73 = 78.28 = % \text{ Moisture} \\
0.262 \times [1 - (78.28/100)] & = 0.059 = \text{WW Concentration}
\end{align*}
\]

<table>
<thead>
<tr>
<th>Field ID</th>
<th>Dry Weight (g)</th>
<th>Wet Weight (g)</th>
<th>% Moisture</th>
<th>DW Hg Concentration (mg/kg)</th>
<th>WW Hg Concentration (mg/kg)</th>
<th>WW Hg (mg/kg) from past runs in 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-112</td>
<td>6.58</td>
<td>35.32</td>
<td>81.37</td>
<td>1.87</td>
<td>0.35</td>
<td>0.36</td>
</tr>
<tr>
<td>14-125</td>
<td>6.24</td>
<td>28.73</td>
<td>78.28</td>
<td>0.26</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>
### Appendix F: Chain of Custody

#### Chain of Custody Record

**Program Code:** A20  
**Coordinator:**

<table>
<thead>
<tr>
<th>County</th>
<th>Field ID</th>
<th>Sample Identification</th>
<th>% Moisture Removed</th>
<th>Collection Method</th>
<th>Date</th>
<th>Container 1</th>
<th>Container 2</th>
<th>Container 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Composite</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grab</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**LAB Report #**

**Analysis Requested:** Program Code: A20  
Sample Matrix: [ ] Tissue-Fillet [ ] Tissue-Whole Body  
Sample Type: [ ] Dry Weight [ ] Wet Weight

**Container 1:**  
**Shipment Temp:**

**Samples Collected By:**

**Relinquished by:** __________________________  **Date:** __________________________  **Received by:** __________________________  **Date:** __________________________

**Representing:** __________________________  **Time:** __________________________  **Representing:** __________________________  **Time:** __________________________

---

Standard Operating Procedure for  
Preparation and Homogenization  
of Fish Tissue Samples  

DOWSOP03000032  
Revision: 2.0  
REVISION DATE – 5/1/2017
## Appendix G. Scale Check Log

### Balance Check Log

<table>
<thead>
<tr>
<th>Weighing Session Date/Time</th>
<th>Reference Weight Balance Readings</th>
<th>Check if Recalibrated</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 g</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Weighing Session Date/Time

Reference Weight Balance Readings

Check if Recalibrated

Revision: 2.0

REVISION DATE – 5/1/2017

DOWSOP0300032

Preparation and Homogenization of Fish Tissue Samples