

Standard Operating Procedure for Resection of Fish Fillets and Homogenization of Tissue Samples

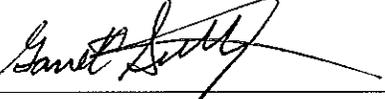
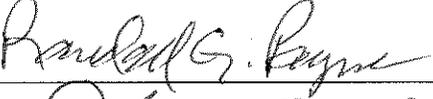
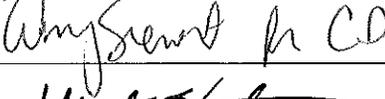
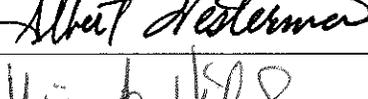
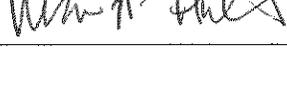
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Revision History

Date of Revision	Page(s) Revised	Revision Explanation
July 1, 2014	Section 9. Fish and macroinvertebrate contaminant analysis	Standard Methods for Assessing Biological Integrity of Surface Waters in Kentucky Standard Operating Procedures for Resection of Fish Fillets and Homogenization of Tissue Samples was separated from preceding document and revised/updated for general content regarding methods following recommendations in USEPA (2000).
March 13, 2008		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		Methods for Assessing Biological Integrity of Surface Waters in Kentucky original document

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Procedures

Scope and Applicability

Fish consumption advisories are jointly issued by 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. A human health risk-based methodology based on previously developed protocols by the Great Lakes Sport Fish Advisory Task Force (GLSFATF 1993) and the U.S. Environmental Protection Agency are used to determine if fish consumption advisories should be issued and what restriction level the advisories should recommend. This system provides 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.

On December 6, 2004, representatives from the Division of Water, Department of Fish and Wildlife Resources and the Department for Public Health signed an Inter-Agency Agreement to work together on the issuance of fish consumption advisories. This document outlines the roles of each agency, but does not detail the standard operating procedures concerning how fish consumption advisories should or will be issued. The purpose of this document is to provide all involved agencies with detailed laboratory procedures for resection of fish fillets and homogenization of tissue samples.

This manual has been developed by the Division of Water as guidance for the uniform and accurate procedures for resection of fish fillets and homogenization of tissue samples for Tier 1 and Tier 2 studies. Tier 1 studies are used for assessments and screening studies and Tier 2 studies are designed to address specific issues related to fish tissue contamination. The procedures defined herein are required for all procedures for resection of fish fillets and homogenization of tissue samples and QA/QC activities resulting in information used for issuing fish consumption advisories. Resultant information can be used in the Integrated Report to Congress on Water Quality in Kentucky (305[b] and 303[d] Reports) and other data needs of the KDOW from the receipt of tissue samples in the KDOW biological laboratory to the point where homogenized samples are submitted to the analytical laboratory for analysis. 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 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

EDAS – Ecological Data Application System

KDOW – Kentucky Division of Water

MSDS – Materials Safety Data Sheet

PPE – Personal Protective Equipment

PTFE – Polytetrafluoroethylene (Teflon®)

QA/QC – Quality Assurance/Quality Control

Health & Safety Policy/Section

Laboratory safety procedures outlined in the Kentucky Safety Manual (<http://eppcintra.ky.gov/EEC/dep/depsafety/>) should be followed by staff employees while working in the laboratories.

Proper PPE shall be worn by all personnel while processing samples and handling chemicals. Refer to the appropriate MSDS sheet 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 exist for particulate, vapor, liquid or foreign objects to become lodged in the eye. Forceps should be used to remove specimens from collecting jars to reduce the risk of exposure from chemical preservatives.

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. A fume hood shall be used when utilizing any carcinogenic compound (e.g., formalin). Fume hoods will also be used when changing preservatives in collection jars to reduce the potential for adverse effects to the researcher or others.

Toxic or caustic materials must be stored in a chemical storage cabinet. Excess toxic or caustic materials must be stored in the hazardous materials building. Two or more personnel should be involved in retrieving materials from the hazardous materials building. It is recommended that the door of the hazardous materials building be propped open when entering so that ventilation can occur.

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. Gloves should be used when handling dry ice. Dry ice should also be used in well-ventilated environments. Care shall be taken when handling and cleaning blenders to reduce the chance of cutting fingers by the blades. Fans and the fume hood shall be on when cleaning fish tissue processing equipment with acetone and nitric acid. 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 MSDS sheet for first aid treatment. MSDS 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 edible tissue, homogenizing, compositing) should be done in an appropriate laboratory facility under clean-room conditions. Clean-rooms or work areas should be free of metals and organic contaminants. Ideally, these areas should be under positive pressure with filtered air (HEPA filter class 100) to reduce airborne contamination from dust particles. 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 rinse 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 in glass jars. Stainless steel instruments are in use by the KDOW, if the above parameters are of concern, additional equipment may need to be obtained before processing of tissue samples. Equipment rinse blanks and blender blanks may be used to evaluate the possibility of contamination (USEPA 2000).

Personnel Qualifications / Responsibilities

All biologists will meet at least the minimum qualifications for their job classification. In addition, fisheries biologist will be trained in the collection and identification of fish by formal academic instruction. Fisheries biologist 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
- Cutting board
- Stainless steel filet knife
- Knife sharpening stone
- Zip lock type bags (various sizes)
- High speed stainless steel blender (various sizes)
- Freezer (≤ -20 °C)
- Disposable gloves (powder free)
- Stainless steel counter tops
- Fume hood
- Level 1 Glass jars with PTFE lined lids (60 and 500mL)
- Bench sheets
- Sample log
- Taxonomic literature (Appendix A)
- Laboratory detergent (Luminox®)
- Liquid nitrogen
- Stainless steel trays
- Cryogenic trays

Methods

The following sections describe the methods that should be followed with the laboratory procedures for resection of fish fillets and homogenization of tissue samples. Samples are collected at designated sites for fish contaminate studies. Individual fish should be placed in a zip lock type bag. Fish that are to be processed as composites should be placed together in one zip lock type bag. Small fish that will be processed as whole body samples may be wrapped in aluminum foil as a group and placed in a zip lock type bag. 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.

Initial Sample Processing

All samples should be logged into the sample log upon returning from the field. Information included in the logbook (or file) includes Station I.D., stream name, latitude and longitude, date collected, type of sample, number of samples, date-to-lab, date of resection of fish fillets, date of homogenization of tissue samples and laboratory personnel.

Fish will be identified, weighed (g), measured (mm) and recorded on the Fish Tissue Data Sheet (Appendix B). The total body length will be determined by measuring from the anterior most part of the fish to the tip of the longest caudle fin ray (when the lobes of the caudle fin are compressed).

Cleaning of Equipment

Equipment will be cleaned following USEPA (2000) for samples for both organic and metals analysis between samples: knives, utensils and containers will be cleaned thoroughly with a detergent solution, rinsed with tap water, soaked in acid solution and then rinsed with metal-free water. Quartz, PTFE, glass or plastic should be soaked in 50 percent HNO₃, for 12 to 24 hours at room temperature. Acids used should be at least reagent grade. Stainless steel parts will be cleaned as stated for quartz, PTFE, glass or plastic, omitting the acid soaking step.

Resection of Fish Fillets

Resection of fillets will be processed within 48 hours of collection. If resection of fillets cannot be performed within 48 hours of collection in the biological laboratory, resection of fillets will be performed in the field. If resection of fillets will be 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 in the field records and on the sample processing record of the location of resection of fillets.

Prior to resection of fillets, hands should be washed and rinsed thoroughly in tap water, followed by distilled water. Powder free gloves are to be worn. A protective glove may be worn under a powder free disposable glove to help prevent cutting injuries while resection of fillets. Specimens should come into contact with non-contaminating surfaces only. Fish will be filleted on cutting boards that are covered with heavy duty aluminum foil that is changed after each sample. The aluminum foil covering on the workstation will be replaced between samples.

Knives with stainless steel blades will be utilized. Only right fillets will be used as part of the qualifying composite sample. If only a small sample will be obtained (consult Table 1 for minimum fillet weight), the right and left fillet will be used. 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 ventral including the belly flap area of the fish. If skin on fillet is required, each fish will be scaled prior to resection of fillet. Fish will be rinsed in de-ionized distilled water after scaling. Any bones should be removed from the fillet if present after resection.

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 de-ionized distilled water and blotted dry.

After resection of fillets, each fillet will be weighed (nearest 1.0g). Target fillet (or composite) weight is 200 g. If target weight is not met, the left fillet should be removed and added to the sample. In order to facilitate homogenization, fillets should be cut into small pieces (< 1 in²). Pieces of tissue should be arranged individually on aluminum foil so that they can be easily separated during homogenization, wrapped in aluminum foil and stored in a food grade zip lock type bag. Sample information will be written on the outside of the bag with a waterproof marker. If aging structures or organism sex is required for a project, collect after resection of fillets has occurred

Table 1. Recommended minimum individual homogenates weight required for a 200 g composite homogenate (USEPA 2000).

Number of Fish/Sample	Individual weight
3	67
4	50
5	40
6	33
7	29
8	25
9	22
10	20

Preservation of Fish Fillets

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 homogenization is completed.

Homogenization of Fish Fillet Samples

Samples may be homogenized separately, or composited depending on the study objectives and analytical requirements. Large pieces of tissue should be cut into smaller cubes (< 1 in², if not done during resection), with a band saw or stainless steel knife on aluminum foil covered cutting board to facilitate homogenization.

Samples will be homogenized in a stainless steel blender until no chunks or discernable tissue remains in the sample by first freezing tissue samples with liquid nitrogen. Tissue samples are frozen by: 1. place liquid nitrogen in a cryogenic tray, 2. a stainless steel tray is placed in the liquid nitrogen and 3. the tissue sample is placed in the stainless steel tray to freeze. An additional cryogenic tray or other covering may be placed over the sample to help maintain the frozen environment. After tissue samples are completely frozen (this may take a few minutes depending on the size of the tissue sample), place tissue in high speed blender to homogenize. Run blender until sample is reduced to a fine powder. Transfer sample to receiving vessel.

NOTE: By freezing to -196 °C, tissue samples become brittle and allow the mechanical action of the blender to reduce the sample to a fine powder for analysis (Burden 2012).

NOTE: if the sample is not reduced to a fine powder, refreeze tissue and re-homogenize.

NOTE: if large amounts of tissue are to be homogenized at once, small aliquots may be necessary. After small aliquots are homogenized, all aliquots should be combined in one stainless steel tray and mixed thoroughly by quartering and dividing and mixing together several times.

NOTE: some skin-on samples may be difficult to homogenize completely. No chunks of tissue or skin should remain in the homogenate because these may not be extracted or digested efficiently during analysis (USEPA 2000)

Methylmercury (MeHg) Homogenization

MeHg samples must be further homogenized with a mortar and pestle. To grind, hold the pestle with a gloved hand (use a protective glove) and firmly press on the sample while twisting. Continue to grind sample until a very fine paste has been formed (Burden 2012). Transfer sample to MeHg receiving vessel.

Transferring Homogenized Sample to Receiving Vessel and Storage

The homogenized sample is then placed in a certified level 1 glass container with a PTFE lid liner or a qualifying composite is obtained using a stainless steel spoon. After the sample is placed in a certified level 1 glass container with a PTFE lid liner that is appropriately labeled, the sample is placed in a freezer at $\leq -20^{\circ}\text{C}$. At this point, the homogenate will be stored at $\leq -20^{\circ}\text{C}$ until processed for analysis in the analytical laboratory.

Qualifying Composite Samples

Tier 1 – Screening Studies

Composite samples must all be the same species and should 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 2000). Composite samples will be collected by combining all filets in blender and homogenizing. The composite sample (at least 200g) will be placed in a certified level 1 glass container with a PTFE lid liner.

Tier 2 – Intensive Surveys

Composite samples must all be the same species and should 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 2000). Composite samples will be collected by taking equal weights (within 10%) of individual homogenates and placed in a certified level 1 glass container with a PTFE lid liner. Individual fish homogenates may be run as separate samples for Tier 2 studies.

Whole Body Homogenization

Samples will be homogenized in a stainless steel blender until no chunks or discernable tissue remains in the sample. Whole body fish will be cut into smaller cubes, with a stainless steel knife on an aluminum foil covered cutting board to facilitate homogenization. Whole body composites are homogenized with the same process as described above (*Homogenization of Fish Fillet Samples*).

Upon completion of tissue homogenization, the sample is placed in a certified level 1 glass container with a PTFE lid liner that is appropriately labeled. At this point, the homogenate will be stored at $\leq -20^{\circ}\text{C}$ until processed for analysis in the analytical laboratory.

Quality Control and Quality Assurance

Delivery to the Analytical Laboratory

Samples will be delivered to the appropriate analytical laboratory following KDOW (2009).

Duplicate and Rinsate Blanks

Duplicated samples: two independent samples of homogenized tissue from the same fish or composite sample submitted for analysis if requested by the study plan.

Rinsate Blanks: A de-ionized water sample collected by rinsing the equipment that typically comes in contact with the tissue during homogenization will be collected in appropriate bottles and submitted for analysis if requested by the study plan.

Data Entry

Data will be entered into the EDAS database.

Reference Section

Burden, D.W. 2012. Guide to the Disruption of Biological Samples – 2012 Version 1.1. Random Primers 12:1-25.

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.

Kentucky Division of Water (KDOW). 2009. Sample Control and Management. Kentucky Department for Environmental Protection, Frankfort, Kentucky.

USEPA. 2000. Guidance for assessing chemical contaminant data for use in fish advisories. Vol. 1: Fish sampling and analysis. 3rd Edition. Washington, DC. Office of Water. EPA 823-B-00-007.

Appendix A. Suggested Taxonomic References

- Burr, B.M. and M.L. Warren. 1984. A distribution atlas of Kentucky fishes. Kentucky State Nature Preserves Commission Scientific and Technical Series Number 4:1-398.
- Ceas, P.A. and B.M. Burr. 2002. *Etheostoma lawrencei*, a new species of darter in the *E. spectabile* species complex (Percidae; Subgenus *Oligocephalus*), from Kentucky and Tennessee. *Ichthyological Exploration of Freshwaters* 13(3) 203-216.
- Ceas, P.A. and L.M. Page. 1997. Systematic studies of the *Etheostoma spectabile* complex (Percidae; Subgenus *Oligocephalus*), with descriptions of four new species. *Copeia* (3) 496-522.
- Etnier, D.A. and W.C. Starnes. 1993. The fishes of Tennessee. The University of Tennessee Press. Knoxville, Tennessee.
- 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.
- Comiskey, C.E. and D.A. Etnier. 1972. Fishes of the Big South fork of the Cumberland River. *Journal of the Tennessee Academy of Science* 47(4) 140-145.
- Jenkins, R.E. and N.M. Burkhead. 1993. Freshwater fishes of Virginia. American Fisheries Society, Bethesda, Maryland.
- Kuehne, R.A. and R.W. Barbour. 1983. The American darters. University of Kentucky Press, Lexington, KY.
- Page, L.M. 1983. Handbook of darters. Tropical Fish Hobbyist Publications, Neptune City, NJ.
- Page, L.M., P.A. Ceas, D.L. Swofford and D.G. Buth. Evolutionary relationships within the *Etheostoma squamiceps* complex (Percidae; Subgenus *Catonotus*) with descriptions of five new species. *Copeia* (3) 615-646.
- Page, L.M., M. Hardman, and T.J. Near. 2003. Phylogenetic relationship of barcheck darters (Percidae: *Etheostoma*, Subgenus *Catonotus*) with descriptions of two new species. *Copeia* (3) 512-530.
- Pflieger, W.L. 1997. The fishes of Missouri, revised edition. Missouri Department of Conservation, Jefferson City, Missouri.
- Robison, H.W. and T.M. Buchanan. 1988. Fishes of Arkansas. University Press, Fayetteville, AR.
- Smith, P.W. 1979. The fishes of Illinois. University of Illinois Press, Urbana, IL.
- Trautman, M.B. 1981. The fishes of Ohio with illustrated keys, revised edition. Ohio State University Press, Columbus OH.

Appendix B. Fish Tissue Data Sheet

