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Ohio Environmental Protection Agency

Division of Surface Water

Fish Tissue Collection Manual

Cooperative Fish Tissue Monitoring Program

State of Ohio

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Acronyms

COC	Chain of Custody
DES	Division of Environmental Services
DSW	Division of Surface Water
EAS	Ecological Assessment Section
EPA	Environmental Protection Agency
FEG	Fish Evaluation Group
FTBMP	Fish Tissue Baseline Monitoring Program
FTCMP	Fish Tissue Consumption Monitoring Program
IAFTMC	Inter-Agency Fish Tissue Monitoring Committee
MSWBC	Mixed Species Whole Body Composite
ODH	Ohio Department of Health
ODNR	Ohio Department of Natural Resources
ORSANCO	Ohio River Valley Water Sanitation Commission
QA/QC	Quality Assurance/Quality Control
QAPP	Quality Assurance Project Plan
RM	River Mile
SFF	Skin-off Fillet
SFFC	Skin-off Fillet Composite
SOF	Skin-on Fillet
SOFC	Skin-on Fillet Composite
SOP	Standard Operating Procedure
WB	Whole Body
WBC	Whole Body Composite

1.0 Objectives

The objective of the Fish Tissue Monitoring Program is to protect and enhance public health by giving technically sound, practical advice about the risks and benefits of consuming Ohio's Sport Fish. Specific goals include:

1) The analyses of fish fillet and whole body samples to determine the potential for human health and environmental effects associated with elevated levels of chemical contaminants,

2) To establish a comprehensive, historical database to evaluate contaminant concentrations, which affect the issuance or removal of human health fish consumption advisories and/or environmental impact assessments,

3) To identify the extent and magnitude of chemical contaminants in fish and to enable anglers to make informed decisions about where to fish and safely consume their catch, and

4) To prioritize water bodies based on impaired fish consumption use as determined by the water quality standards for the purposes of making TMDL determinations.

This guidance is designed to ensure that all fish tissue sample analytical results are of consistent, high quality, and that the public receives the best information possible about fisheries within the State, while the State obtains the best data possible to evaluate and protect fishery resources.

2.0 Field Preparations and Collections

2.1 Program Descriptions

The Ad-Hoc Inter-Agency Fish Tissue Monitoring Committee (IAFTMC) is composed of members from the following State of Ohio Agencies:

Department of Health (ODH)
Department of Natural Resources (ODNR)
Environmental Protection Agency (Ohio EPA)

The members of the IAFTMC meet to identify water bodies, select appropriate sampling locations, species, sample types and sample number for each location in Ohio's jurisdictional waters. The Ohio EPA, Division of Surface Water, and ODNR, Division of Wildlife, (the Agencies which do most of the field collecting) identify and assign the necessary resources to implement the plan. Fish tissue samples are collected and analyzed primarily for three major programs.

The largest fish tissue monitoring program, the Fish Tissue Consumption Monitoring Program (FTCMP), is overseen by the IAFTMC. Fillet composite samples are collected and analyzed to determine contaminant concentrations in edible portions of sport fish. For Lake Erie and Ohio River samples, fillet composite samples are either scaleless, skin-on fillet composites (SOFC) taken from scaled fishes (*i.e.,* black basses, crappies, trout, walleye, white bass, etc.), or skin-off fillet composites (SFFC) taken from scaleless fishes (*i.e.,* catfishes and bullheads). Common carp samples may be either skin-on or skin-off depending upon sampling objectives. For all other State Waters (i.e. rivers, streams and lakes) all samples are composite skin-off fillet (SFFC) samples.

Both Ohio EPA and ODNR collect FTCMP samples. Ohio EPA is responsible for collecting samples from rivers and streams. ODNR, Division of Wildlife, is responsible for collecting samples from state managed reservoirs and lakes, the Ohio River, and Lake Erie. The Ohio River Valley Water Sanitation Commission

(ORSANCO) also collects samples from the Ohio River. Both State Agencies follow Ohio EPA Standard Operating Procedures (SOPs). Ohio EPA utilizes the fish tissue data generated from this program to conduct fish consumption risk assessments. Risk assessments may result in the issuance of new fish consumption advisories, updates (modifications) of existing fish consumption advisories, or the rescission of old fish consumption advisories. Ohio EPA oversees the release and dissemination of fish advisory information to the public. ODH has statutory authority to oversee the advisory program. Ohio EPA, Division of Surface Water, is responsible for generating a fish tissue monitoring section in Ohio's Biennial Integrated Water Quality Report to Congress (the historical 305(b) Biennial Report) highlighting the findings of this Program.

A second, smaller program is called the Fish Tissue Baseline Monitoring Program (FTBMP). This Program is not officially part of the Inter-Agency cooperative effort, and is solely administered by the Ohio EPA. However, the FTBMP is voluntarily integrated into the planning and implementation of the FTCMP to maximize resources. Samples taken for the FTBMP include fillet samples (as mentioned previously) and whole body composite (WBC) samples of bottom feeding fish such as common carp and suckers. Whole body composite samples include the entire fish in the sample rather than just the fillet. Whole body composite samples can be used as environmental screening tools to detect potential contaminant hot-spots. Whole body samples can also be used to assess the risk of fish consumption to wildlife.

Ohio EPA's remediation programs sometimes conduct fish tissue sampling to determine the magnitude and extent of known or suspected contaminated areas. Samples for this program may be collected by Ohio EPA, private consultants, or both. A study plan (Quality Assurance Project Plan - QAPP) must be developed and reviewed by appropriate Ohio EPA staff prior to the collection of any samples to ensure adequate spatial coverage, number of samples, types of samples, appropriate species, parameter coverage, detection limits, analytical protocols, and Quality Assurance/Quality Control (QA/QC) procedures. Typically, when evaluating an entity, samples will need to be taken from a minimum of three locations; one site upstream from the entity to establish background conditions, one site immediately downstream to determine the severity of the contamination and another site at a distance to determine if the contamination has migrated downstream and might need further sampling. More sites may be required if a more complex site or problem is being evaluated. The length of reach sampled should, in many cases, follow the sampling distances established by the Ohio EPA Biomonitoring Program (i.e., ~300 to 500 meters in boatable streams or ~100 to 200 meters for wadeable and headwater streams), but may be altered to ensure that adequate samples are collected.

Laboratory analyses of fish tissue samples for the first two programs are the responsibility of Ohio EPA, Division of Environmental Services (DES), which follows U.S. EPA approved sample preparation and analytical protocols for heavy metals and organic contaminants. Laboratory analyses for the remediation program may be conducted by a laboratory contracted by the entity being evaluated, by Ohio EPA, Division of Environmental Services, or both. This may entail analyses of split field samples and/or sample duplicates. In all cases, sampling and analyses follow Ohio EPA and U.S. EPA approved protocols. Sample collection and analytical responsibilities are determined prior to sample collection and analyses.

2.2 Field Sampling Equipment

Ohio EPA and ODNR are the main agencies responsible for the collection of State fish tissue field samples. A variety of different methods are suitable for use in the collection of fish. Ohio EPA relies primarily on electrofishing methods as documented in Biological Criteria for the Protection of Aquatic Life: Volume III: Standardized Biological Field Sampling and Laboratory Methods for Assessing Fish and Macroinvertebrate Communities (Ohio EPA 1989). ODNR uses a variety of active collection techniques including electrofishing, angling, trawling (in Lake Erie), and passive capture methods including netting (trap nets, fyke nets, etc.). These fish sampling methods are well proven and widely used. Representatives of entities being evaluated for contaminant problems may choose from any of these methods depending upon local conditions, with the goal being to secure adequate samples.

The field crew must prepare the fish tissue sample once fish have been obtained. A variety of equipment is required to ensure that a proper and valid sample is obtained. The following checklist should be used to ensure possession of the necessary equipment and supplies to prepare a sample without contaminating it:

Extra Heavy Duty Aluminum foil, 24" by 500' roll (available at restaurant supply stores)	Sharp, decontaminated fillet knives with plastic handles
Waterproof labels for individual fish tissue samples	Fillet board
Waterproof ink pen for recording information	Knife sharpening equipment
Coolers for cold storage of fish tissue samples	Teflon plastic squirt-bottles for dispensing acetone
Water (wet) ice	Pesticide grade acetone for decontaminating knives, scalers, pliers, etc.
Dry ice	Metric measuring board for measuring fish total length in millimeters
Fiberglass fish club for sacrificing fish	Metric weighing scales for weighing fish in grams
Powder free latex gloves for handling the fish	Zip closure heavy duty plastic bags
DES fish tissue submission form + chain of custody	Scalers and spoons for descaling fish
Field notebook	Sample location maps

The purposes for each item will be discussed in succeeding sections.

2.3 Field Preparations

Several pre-fieldwork activities should be undertaken before beginning fieldwork in order to reduce the amount of time and effort spent in the field. Foremost of these activities is to complete a field reconnaissance of all proposed sampling locations to determine the easiest and safest access locations. For stream work, the best access location may require "motoring" or otherwise navigating downstream/upstream to the actual sampling location. However, situations do arise where stream access may become unsuitable (road construction activities, as example) or extremely difficult. In these cases, best professional judgment should be used with regard to obtaining a sample from the chosen location. In some cases a site may be moved and still provide adequate data (**Be sure to exhaust all options for stream access before abandoning a sampling location**).

Another suggestion to speed field processing time is to clean the knives prior to going into the field to collect samples. First wash the fillet knives in a regular solution of soapy water taking care to avoid injuring oneself on the sharp blade. After thoroughly rinsing the knife with water and then deionized water, let air dry. Once the knives are dry, rinse with acetone making sure to decontaminate both sides of the blade and the plastic handle. Let air dry and then wrap the blade in a piece of clean aluminum foil. Store clean, foil wrapped knives in zip closure plastic bags. Use a clean knife with each sample. This procedure should also be used when cleaning scalers, spoons and pliers.

A notebook is an essential field item. The notebook is useful for writing notes to remember to obtain fresh supplies, to fix a piece of equipment, or to make a minor change in the location of a sampling site on the study plan to conform with field conditions. General field observations for each sampling location should also be recorded.

3.0 Fish Collection Methodology

As mentioned previously, the methodology for capturing fish will be dependent upon the type of water resource to be sampled (lake, reservoir, stream, river). The sampling procedures for each water resource are detailed below. However, if it is ever unclear as to how or whether to take a fish tissue sample, always err on the side of taking the sample. If there is doubt about the size, species of fish, or any other parameter, collect the sample, wrap each fish or pair of fillets individually and label the sample properly. It is always easier to sort out the details of the appropriateness of a sample back at the laboratory than it is to return to the field to recollect a sample.

3.1 Lake Erie

The Ohio Department of Natural Resources (ODNR) typically is responsible for collecting Lake Erie fish samples for Ohio's Fish Tissue Monitoring Program. For Lake Erie, station locations are identified by Lake Erie grid number (Smith *et al.* 1961). The latitude and longitude of the Lake Erie grid number's center where the fish are caught is used to identify the Lake Erie fish collection station location. A station where the grid's center latitude and longitude are located inland of the Lake Erie shore is moved lakeward within the grid so that the station latitude and longitude are located over the Lake. GPS units should be set to collect waypoints in NAD 83 and provide the latitude and longitude in decimal degrees to at least 6 decimal places. In Lake Erie, all scaled fishes except for common carp should be collected as skin-on fillets. Common carp and catfish should be collected as skin-off fillets.

3.2 Ohio River

ODNR is responsible for collecting Ohio's Fish Tissue Monitoring Program samples in the Ohio River. ODNR follows the same fish sampling and sample preparation procedures that are used by Ohio EPA in large Ohio rivers when collecting Ohio River samples. The ODNR/Ohio EPA collection and analyses of Ohio River fish samples are done independent of the Ohio River Valley Water Sanitation Commission's (ORSANCO's) Ohio River Fish Tissue Monitoring Program. ORSANCO and ODNR/Ohio EPA exchange and share Ohio River fish tissue data.

The reported river mile (RM) and the latitude and longitude for a sample location should be the beginning point of the sampled reach. GPS units should be set to collect waypoints in NAD 83 and provide the latitude and longitude in decimal degrees to at least 6 decimal places. In the Ohio River, all scaled fishes except for common carp should be collected as skin-on fillets. Common carp and catfish should be collected as skin-off fillets.

3.3 Rivers and Streams

Ohio EPA is typically responsible for collecting Ohio river and stream fish samples for Ohio's Fish Tissue Monitoring Program. The number of fish tissue sampling stations in a river or stream is dependent upon river/stream size. In large rivers (greater than 500 square mile drainage area) like the Scioto River or Muskingum River, sampling stations typically are located every 7 or 8 miles. In smaller rivers/streams (200 – 500 square mile drainage areas) like the Olentangy River or Big Darby Creek, sampling stations might be chosen every 5 to 6 miles. In small streams like Scippo Creek which has a drainage area of 51.7 square miles, three (3) sampling locations, one at an upstream control location or upstream from an entity/location of concern, a near downstream location and a far downstream location are needed. The final number of stations may be adjusted based upon data requirements.

Areas with known sources of contamination or that are heavily recreated may require additional sites. In the case of Scippo Creek, which had a known PCB source, additional sites might be warranted to more precisely delineate the extent and magnitude of the contamination. Human health assessments may require four stations, the fourth location being a second far downstream location. When an area of concern is located close to a river confluence, the confluence is bracketed with sampling locations situated upstream and downstream from the confluence. Heavily fished streams like the Mad River and the Stillwater River should have fish tissue sampling stations chosen every 5 miles. A stream location

that is visually impacted, or that is known to be receiving chemicals of concern, may be bracketed by additional station locations to evaluate fish tissue chemical uptake.

Fish sampling zone distances vary. If all the priority fish species, numbers and sizes can be collected in a short reach, the crew processes the samples and then proceeds to the next station. Within a reach, crews generally move from spot to spot sampling the proper habitat for the larger game species. In larger rivers, the sampling zone distance required to secure an adequate sample is approximately 500 meters. At wading sites, crews generally sample approximately 200 meters. Longer distances may be required if the priority fish cannot be found within the standard distances. Usually, standard distance zone sampling requires 45 minutes to one hour to either collect the required specimens, or to make the decision to move to the next sampling location.

Samples generally are collected within a reach by moving from downstream to upstream in wading sized streams. Rivers and streams that are sampled with boat electrofishing equipment are typically sampled proceeding from upstream to downstream going with the current. Local conditions may permit or require that sampling be conducted in a different pattern. Regardless the goal should be to collect an adequate sample.

The reported river mile (RM) and the latitude and longitude for a sample location should be the beginning point of the sampled reach. GPS units should be set to collect waypoints in NAD 83 and provide the latitude and longitude in decimal degrees to at least 6 decimal places.

3.4 Inland Lakes and Reservoirs

ODNR is responsible for collecting inland lake and reservoir fish tissue samples for Ohio's Fish Tissue Monitoring Program. The number of fish sample locations in inland lakes and reservoirs varies. A small lake/reservoir (249 acres or less) will have one fish sampling location, most lakes/reservoirs (250 to 999 acres) will have two sampling locations and large lakes/reservoirs (1000 acres or greater) will have three or more sampling locations. At least three priority species-specific composite samples are collected at each sampling location in a screening evaluation. At least 3 samples from each trophic level, preferably of the same species, are collected at each location in follow-up lake evaluations for verifying that there is indeed a contaminant problem. Specific species collected and analyzed at a lake/reservoir may be selected based upon ODNR creel data and/or stocking and management activities. This evaluation procedure is considered a screening step to determine if there are any fish tissue contaminant problems. If a possible problem is identified, follow-up sample collections and analyses are scheduled.

The lake sampling station location should be identified by latitude and longitude coordinates on each fish tissue sample label and sample submission form. The midpoint of small lakes may by chosen. GPS units should be set to collect waypoints in NAD 83 and provide the latitude and longitude in decimal degrees to at least 6 decimal places.

4.0 Fish Tissue Sample Type

Sample collections may involve seven different types of fish tissue samples. Composite samples are preferred.

- Skin-off Fillet (SFF)
- Skin-off Fillet Composite (SFFC)
- Skin-on Fillet (SOF)
- Skin-on Fillet Composite (SOFC)
- Whole Body (WB) Generally not used for the Fish Tissue Consumption Monitoring Program
- Whole Body Composite (WBC) Generally not used with Fish Tissue Consumption Monitoring Program
- Mixed Species Whole Body Composite (MSWBC) (used in special situations, approved by Ohio EPA)

4.1 Collection of Fish Species, Length Categories and Sample Preparation

For all sample types, the laboratory requires a minimum **150 grams** of sample to perform the required analyses. Make sure to obtain enough fish for a properly sized sample if possible. Supplying an inadequate amount of sample may result in the laboratory only being able to analyze for metals or for organic contaminants, but not for both sets of parameters. If the sampler is unable to supply an adequate amount of tissue due to the scarcity of fish, and knows that there is a greater interest in one of the parameter groups, the preferred parameter group should be indicated on the Fish Tissue Sample Submission Sheet. Otherwise the laboratory will make the decision.

4.2 Whole Body (WB) and Whole Body Composites (WBC)

Whole body and whole body composite samples are not typically collected for the Fish Tissue Consumption Monitoring Program and are normally reserved for the other two programs: the Fish Tissue Baseline Program and the Fish Tissue Targeted Assessment Program. Whole body composite samples (preferred sample type) and whole body samples are the easiest samples to prepare, because they do not involve cutting the fish. The fish sample is preserved whole for later analyses.

The primary fish species of interest for WB and WBC sample collecting include (in order of preference):

WB and WBC SAMPLES
Common carp
Channel catfish
Bullheads (*Ameiurus* spp.)
Buffalo (*Ictiobus* spp.)
Sunfish *(Lepomis spp.)*
Creek chub
Other species as required

The ideal WBC sample consists of five (5) fish of the same species, with the smallest fish in the sample within 90% of the total length of the largest fish chosen for the sample. If the smallest fish is not 90% of the largest under no circumstances should a composite be made up of fish with a size difference (largest to smallest) greater than 75%. Total length of the fish is determined by measuring the greatest distance in a straight line (not following body curves) from the anterior most projecting parts of the head to the farthest tip of the caudal fin when its rays are squeezed together (Trautman 1981). In situations where there are few fish or the fish are very large, a minimum sample of two (2) fish should be used. In situations where the goal is to determine food chain risks, species of smaller size are sometimes selected. Based on the purpose of the tissue collection, common ubiquitous species such as creek chub, gizzard shad, or shiner species may be tested. In these cases, more than 5 individuals may be needed to ensure an adequate sample weight. The smallest fish in the sample again should be at least 90% of the total length of the largest fish chosen for the sample. If the smallest fish is not 90% of the largest under no circumstances should a composite be made up of fish with a size difference (largest to smallest) greater than 75%.

An attempt should be made to collect the same species of fish within an entire sub-basin to permit comparability and facilitate the risk assessments. It is also important to try to collect fish of the same

length grouping(s) in order to reduce sample variability. To accomplish this, each of the fish should be pre-measured to determine their appropriateness for the sample. For example:

> *On a sampling run in Mack Creek, 8 common carp are collected with the following lengths (in millimeters): 325, 330, 340, 350, 360, 600, 650, and 660. Which fish should be selected for the WBC sample? There are two obvious length groups to choose from. The 325, 330, 340, 350, and 360 length carp (325 mm is within 36 mm - 10% - of 360 mm), or the 600, 650 and 660 length common carp (600 mm is within 66 mm of 660). The larger fish are probably older and therefore have been exposed to pollutants longer (although this is not always true) and may provide a better sample than the smaller fish.*

Since length usually correlates well with age, fish of a similar length are usually from a similar age class and have, therefore, been exposed to contamination in their surroundings for approximately the same amount of time. If possible, it is also preferable to collect fish from the same size range throughout the entire sub-basin. This also applies to the two types of fillet composite samples (SOFC and SFFC). In the previous example, if adequate sample analytical resources are available, samples from both size classes may be analyzed.

To prepare a whole body composite, obtain a bucket of water from the stream from which the fish were removed. Put on latex gloves prior to handling fish for tissue processing. Latex gloves are changed between each sample (latex gloves do not need to be changed between each fish processed within a composite sample). Dip the fish into the water to rinse any residue off the outside of the fish. Sacrifice the fish by administering a moderate blow to the nape using the fiberglass fish club. Take care to strike the fish with some restraint as you do not want to cause bleeding or other unnecessary fluid losses. It does not take a heavy blow to sacrifice most fish, except common carp and catfish. Try lighter blows at first to experiment with the right amount of force.

After sacrificing the fish, weigh the entire fish on the 1000 gram (to the nearest gram) or 10 kg scale (to the nearest 50 grams) and measure the total length again, to the nearest millimeter using the measuring board. Record the weights and lengths on the sample label and on the Ohio EPA Division of Environmental Services Tissue Sample Submission Form using waterproofing ink.

Following this, wrap the fish in clean aluminum foil. Place the packet in an appropriate sized zip closure heavy duty plastic bag and include the sample label ensuring that the label is securely inside the bag. (NOTE: If the fish happen to be large, you may need to label each individual fish in the composite, since it may be impossible to bag all the fish together and store them properly.) Quickly place the fish in the sample cooler after including the label.

The cooler should be filled with about 40 pounds of dry ice (enough for about 4 days in the field) which will serve to freeze the fish. Dry ice should be placed on top of the fish initially to provide for quicker freezing, since cold carbon dioxide is heavier than air and will flow over the wrapped fish to the bottom of the cooler. Check the dry ice often to ensure that it does not run too low, thus causing the fish to thaw. (Safety Note: Dry ice is very cold! Do not handle it with your bare hands; severe burns may result. Ask the dry ice vendor to wrap each 20 pound block in paper. This will facilitate safe handling.)

4.3 Multiple Species Whole Body Composite Samples (MSWBC)

The Multiple Species Whole Body Composite Sample is used only in special situations, primarily wildlife exposure assessments. Multiple-species whole body composite samples of small fish (minnows, shiners, small suckers) can be used to evaluate wildlife exposure. This is the only occasion when a multiple-species sample is collected and analyzed for an exposure assessment. All of the species in the multi-species sample must be identified. The smallest fish length in the sample must be 90% of the largest fish length in the sample. If the smallest fish is not 90% of the largest under no circumstances should a composite be made up of fish with a size difference (largest to smallest) greater than 75%.

4.4 Fillet Preparation (covers skin-on and skin-off fillets and fillet composites)

A skin-on "Standard Fillet" is prepared using the following procedure. Put on latex gloves prior to handling fish for tissue processing. The fish is rinsed with water taken from the stream from which the fish was collected to remove any sediment and/or organic matter present on the fish. All scales are then removed. A shallow cut is made through the skin on either side of the dorsal fin from the base of the head to the tail. A second cut is made along the entire length of the gill cover cutting through skin and flesh to the bone. A third cut is made along the belly (includes the belly flap) from the base of the pectoral fin to the tail and along the side of the anus and the fin directly behind. The skin-on fillet is removed and major bones are removed. Both fillets are prepared as described. Skin-off fillets are prepared using the same procedure as skin-on fillets, except that the skin is removed from the fillet without scaling. Fillets from both sides of the fish are typically taken. Fish composite samples may be subsampled in the following manner: if a minimum of 3 fish are collected and each fish is 400 millimeters or longer, than a fillet from only one side of each fish is required.

4.5 Skin-on Fillet Composite (SOFC)

The skin-on fillet composite (SOFC) sample is prepared by scaling and filleting the fish and leaving the skin on the fillet. ODNR obtains SOFC samples in the Lake Erie and Ohio River components of the Fish Tissue Consumption Monitoring Program.

The primary sport fish species of interest for SOFC samples (and skin-off fillet composite/SFFC for several species noted) include in general descending order of preference:

LAKE ERIE/OHIO RIVER SPORT FISH PREFERENCE – SOFC and SFFC	
Walleye (SOFC)	Freshwater drum (SOFC)
Saugeye (SOFC)	Smallmouth buffalo (SOFC)
Sauger (SOFC)	White crappie (SOFC)
Yellow perch (SOFC)	Black crappie (SOFC)
White bass (SOFC)	Sunfish (*Lepomis spp*.) (SOFC)
Smallmouth bass (SOFC)	Steelhead trout (SOFC)
Largemouth bass (SOFC)	Northern pike (SOFC)
Spotted bass (SOFC)	Whitefish (SOFC)
Channel catfish (SFFC)	Striped x white bass (wiper) (SOFC)
Flathead catfish (SFFC)	* Other species based upon creel survey results
Rock bass (SOFC)	

The selection of priority species for a specific location may be based upon creel survey results for the collection site, or fish stocking and management activities at inland lakes and reservoirs.

The type of fish collected may be dependent on the area sampled (*e.g.,* Lake Erie samples may include walleye, white bass, and steelhead; reservoirs and streams may include crappie, rock bass, saugeye, and black basses). Sunfish or other species should be collected if other species cannot be collected, or if they are a significant portion of the creel survey consumption results. In addition, an attempt should be made to collect the same species within a sub-basin as with WBC samples. It is also preferable to have fish from the same size range throughout the sub-basin, if possible.

The SOFC sample consists of both fillets from each fish for up to five (5) fish of the same species with a minimum of two (2) fish for a sample. (NOTE: In some circumstances, more than 5 fish fillets may be needed to have the minimum 150 grams necessary for the laboratory to analyze the sample.) Sample weights greater than 150 grams are preferred to ensure adequate material to analyze all parameters. In some cases, a single fish may be used for a sample if it is large enough to meet the 150 grams limit and no other fish are available. However, multiple fish should be the norm for a good sample. In order to reduce variability in the sample, it is important to collect fish of the same length grouping (see the example for WBC samples).

Once the appropriate fish have been collected and size groupings have been selected, the SOFC sample may be prepared. Latex gloves are worn when handling fish for tissue processing and must be changed between each sample (latex gloves do not need to be changed between each fish processed within a composite sample). Fish are first rinsed in a bucket of water from the stream/lake where the fish were collected. The fish are then sacrificed using the same procedure described in the WBC sample section. The fish are then weighed and measured with the results recorded on the sample label and the Tissue Sample Submission Form.

Before scaling and filleting the fish, clean aluminum foil from the roll should cover the filleting surface. Scale the fish taking care to remove all the scales. Wash off all the loose scales with water from the stream/waterbody that the fish was collected. Fillet the fish (both sides including the belly flesh) as illustrated in the Appendix diagrams leaving the skin attached. After completing the filleting, set aside both of the fillets on a sheet of clean aluminum foil. Follow this procedure for all fish in the sample. Care must be taken when filleting the fish in the field so that the sample material is not contaminated during the filleting procedure. After you finish filleting all of the fish in the sample, wrap the fillets (both fillets from all of the fish) together in one clean foil packet and secure the wrapping. Place the sample in a zip closure plastic bag and include the sample label with the wrapped fillets. (NOTE: If the fish fillets happen to be large (e.g., steelhead trout, common carp), you may need to include a sample label with each individual pair of fish fillets in the composite; number each as follows: 1 of 5, 2 of 5...5 of 5.) When wrapping the fillets, the foil should be folded over at least 1/2 inch away from the fillets. This will diminish the likelihood of the foil becoming trapped in the frozen tissue, which causes problems when processing the samples at the lab.

Quickly place the fish in the sample cooler after affixing the labels. The cooler may be filled with wet ice if the samples will be transferred to a freezer by the end of the day. Care should be taken to ensure that the fish fillets do not become submerged in water. Placing the fish fillets in a zip closure plastic bag should prevent this. If you are on an extended field trip, the cooler should contain about 20 pounds of dry ice for one overnight stay and 40 pounds of dry ice when out for 2-3 nights, to properly freeze the fish fillets. Dry ice should be placed on the top of the fish initially to provide for quicker freezing. Carbon dioxide is heavier than air and will flow over the fish to the bottom of the cooler. Monitor the amount of the dry ice remaining in the cooler to ensure that it does not run too low causing the fish to thaw.

4.6 Skin-off Fillet Composite (SFFC)

Fillet samples for all Ohio inland waters (i.e. rivers, streams, lake and reservoirs) are skin-off fillet composites to reflect what is found on the fish consumer's table. The skin-off fillet composite sample involves filleting the fish and removing the skin from the fillet. Both Ohio EPA and ODNR obtain these types of composite samples as part of the Fish Tissue Consumption Monitoring Program and Fish Tissue Baseline Monitoring Program. Skin-off fillet composite samples are obtained in the same way as skin-on fillet composite samples except that the skin is removed from the fillet. Please see the description of the SOFC sample procedure for details. The following table provides the list of fish species preferred for collecting SFFC samples in Ohio rivers, streams, inland lakes and reservoirs.

RIVER/ STREAM/ RESERVOIR FISH PREFERENCE - SFFC	
Common carp	Walleye
Smallmouth bass	Saugeye
Largemouth bass	Sauger
Spotted bass	Striped x white bass (wiper)
Channel catfish	White bass
Rock bass	White crappie
Freshwater drum	Black crappie
Flathead catfish	Smallmouth buffalo
Northern pike	Black buffalo
Yellow perch	Bigmouth buffalo
Sunfish (Lepomis spp.)	White sucker
Bullhead spp.	

4.7 Collection Problems

In some situations, particularly in very degraded streams, it may be difficult to collect the proper numbers and types of fish necessary to complete a sample. In these cases, it may be necessary to substitute other species of fish, or abandon the sample altogether. Substitute species of fish will depend upon the species of fish present at the sampling location and the purpose of the sampling. Substitute fish for SFFC composite samples might include members of the sucker family (e.g., golden redhorse -*Moxostoma erythrurum*). Sucker species should also be collected where a consumption advisory for that species is currently in place.

5.0 Sample Documentation

5.1 Analytical Submission Forms

Sample labels and tissue sample submission forms must be filled out in waterproof blue or black indelible pen only. Forms must not be filled out with pencil. Penciled information can be altered and therefore will not conform to the chain-of-custody procedure.

The space “Collected By” must be filled out and must be a person or list of persons. The “Sample Location” box should match the “Water body, River Mile and Location” spaces on the fish tissue labels. The reported river mile (RM) and the latitude and longitude for a sample location should be the beginning point of the sampled reach. Similarly the midpoint of a small lake or reservoir has also been adopted as the sample location and latitude/longitude. Conventions for larger lakes and Lake Erie have been described in Section 3.4 Inland Lakes and Reservoirs. The latitude and longitude for the collection site must be reported and must be provided as decimal degrees (ex. 38.26351,-84.67584) Map Datum NAD 83. Percent (%) lipids must always be marked on the organic analytical form.

The standard fish tissue analytes (SIMA 1 metals, mercury, percent lipids, pesticides, and PCBs) should be indicated on the sample request form - individual chemical parameters are listed in Table 1. Additional analytes must be identified on the analytical sample form as needed. If a large volume of tissue is collected for a sample and must be stored as multiple packages this should be denoted on the packages and on the top of the tissue sample submission forms in the manner of 1 of 3, 2 of 3, etc.

All copies of the form must stay together until the samples are delivered to the Division of Environmental Services Laboratory (DES). If a copy is desired prior to DES delivery (e.g., when ODNR staff transfer fish to Ohio EPA EAS staff) a photocopy should be made and retained. Copies of the form can be supplied to the sampler after sample submittal to the laboratory and the assignment of Fish Evaluation Group (FEG) numbers and the laboratory metals and organics numbers.

5.2 Chain of Custody Form (COC)

The purpose of the Chain-of-Custody Form is to document that there has been no tampering with the collected samples. The field "Collected By" must be the name of the person or persons who collected the sample; at least one of these individual's signature must match the first line of the "Relinquished by" field towards the bottom of the form. As mentioned above for the Tissue Sample Submission Form, this form must be signed (the name can be printed in addition to provide clarity) in waterproof indelible blue or black ink. The person receiving the samples must then countersign in the following "Received by" slot. Any subsequent transfer of samples must proceed in the same fashion with the person transferring the responsibility of the samples signing the "Relinquished by" slot and the person accepting the samples and responsibility of the samples security countersigning the "Received by" slot and so on until they are deposited in the laboratory. All four sheets of the COC must stay together until the samples are submitted to the laboratory. If a copy is desired prior to DES delivery (e.g., when ODNR staff transfer fish to Ohio EPA EAS staff) a photocopy should be made and retained. Copies of the form can be supplied to the sampler after the assignment of FEG numbers and sample submittal to the laboratory and subsequent assignment of the laboratory metals and organics numbers.

Data from samples collected during the field season must be entered into the Excel spreadsheet Fish Tissue Log Book in the EAS Office, with backup copies on the EAS shared directory. Each sample (whether collected by EAS personnel or ODNR personnel) should be assigned a FEG log number. The FEG log number must be added to the sample label and the sample returned to the freezer.

When submitting a COC you must provide a separate COC for each sampling event. Considerable confusion exists about when additional COCs are required. Each discrete sampling event requires a new COC form. A general rule of thumb is if in doubt create an additional COC form – you can't have too many, but you can have too few! A few examples may clarify the situation. Sampling of a large watershed or along the shoreline of Lake Erie might require several weeks of sampling. All of the sampling conducted within a given week without a break within the same watershed or general area of Lake Erie could be included on the same general COC form. However, if the sampling straddled a weekend or another couple of days where the sampling was suspended that would require splitting the samples amongst two COC forms. Similarly, if sampling jumped around between different watersheds within the same time frame additional forms would be required. If there are any questions as to the need for additional COC forms or how to fill them out direct those questions to the DES Sample Receiving Coordinator at (614) 644-4243.

Who should fill out the COC form also appears to be a point of confusion. The person who collects the sample must be the person who signs for the sample or who transfers custody of the sample to the person transporting the sample to the Groveport Field Office or the analytical laboratory. All members of the sampling crew can appear on the "Collected By" field, as long as the samplers were together during that sampling event. If a sampler collected fish on different days, or at another location, they must submit another COC.

6.0 Laboratory Storage

Immediately place all fish tissue samples in the freezer upon returning from the field or upon transfer from samplers. Check to see that all labels are still attached to the samples. If possible, box or bag samples having the same COC together to facilitate future handling. Be sure to keep samples frozen at all times including when transferring them to the analytical laboratory, or at an interim storage facility.

Samples are kept frozen at minus twenty-one degrees Celsius at the field storage facility and at the DES laboratory, with up to a one year holding time according to Ohio EPA described methods and standard operating procedures (SOPs).

6.1 Transfer of Samples to the Ohio EPA, Ecological Assessment Section

ODNR and Ohio EPA have agreed to route all fish tissue samples through Ohio EPA prior to delivering them to a laboratory for analyses. A representative of the Ohio EPA, EAS will coordinate the transfer of samples from ODNR district offices to Ohio EPA, EAS in Groveport. Prior to each field season, ODNR will be provided with appropriate labels, Tissue Sample Submission Forms, and Chain-of-Custody Forms to ensure proper sample delivery. All forms should be properly completed by ODNR personnel prior to delivery to EAS. Details of sample submissions from outside sources will be negotiated prior to sampling with EAS staff.

Transfer of fish tissue samples collected by ODNR to Ohio EPA should commence as soon as possible after sample collection to ensure that samples will be processed well within the analytical laboratory sample holding time (i.e., one year from the time of sample collection). ODNR is responsible for transporting the samples to the Ohio EPA, EAS Office located at 4675 Homer Ohio Lane, Groveport, Ohio 43125, at times agreed to by both parties. Although deliveries of large batches of samples are permissible, Chain-of Custody Forms should be filled out for no more than 30 to 35 samples, which is the maximum number of samples that the DES laboratory will accept at a given time (25 samples is the current preferred number of samples to receive in a batch). ODNR samples will be received at the EAS Groveport Field Office and checked using proper Chain-of-Custody Procedures (*i.e.,* each sample will be cross checked with the data sheets and the Chain-of-Custody Form to ensure that all samples are accounted for). EAS staff will then assign a FEG sample number to each sample which is marked on the sample label, the Tissue Sample Submission Form and the electronic Fish Tissue Logbook.

6.2 Transfer of Samples to the Analytical Laboratory

Samples must be stored in a cooler for transport to the laboratory. Samples should be accompanied by the Tissue Sample Submission Form and the Chain of Custody Form. Sample delivery must be coordinated with the Sample Receiving Coordinator at the Ohio EPA, Division of Environmental Services Laboratory, located on the Ohio Department of Agriculture Campus, 8955 East Main Street, Reynoldsburg, Ohio 43068.

Once at the laboratory, each sample should be placed on the laboratory table and the corresponding Tissue Sample Submission Form placed with the sample itself. A DES staff member will then assign a DES log number to each sample. EAS personnel must then copy each DES log number by the corresponding FEG log number in the file folder that holds the lab forms. The Sample Receiving Coordinator will then complete the next portions of the Chain of Custody Form and give the EAS representative a copy.

7.0 Conclusion

By following this set of guidelines for obtaining fish tissue samples, we can be assured of high quality samples that will provide the State with useful and defensible information. If you have any questions about this guidance or would like to incorporate a time or effort-saving idea into the guidance, please contact Ben Rich, Ohio EPA, Division of Surface Water, Ecological Assessment Section, 4675 Homer Ohio Lane, Groveport, Ohio 43125 by email at Ben.Rich@epa.state.oh.us or by phone at 614-836-8772.

Table 1: Ohio EPA Division of Environmental Services (2010) Fish Tissue Method Reporting Limits (MRLs). Fish tissue MRLs and Total Metals are reported in ug/kg wet weight. Fish tissue analytical results include percent lipid for each sample. * Parameters analyzed historically, but now by special request only.

LOW LEVEL			CONSUMPTION LEVEL		
Parameter	MDL	Unit	Parameter	MDL	Unit
Aldrin	4	ug/kg	Aldrin	10	ug/kg
a-BHC	4	ug/kg	a-BHC	10	ug/kg
b-BHC	4	ug/kg	b-BHC	10	ug/kg
d-BHC	4	ug/kg	d-BHC	10	ug/kg
y-BHC	4	ug/kg	y-BHC	10	ug/kg
4,4'-DDD	4	ug/kg	4,4'-DDD	10	ug/kg
4,4'-DDE	4	ug/kg	4,4'-DDE	10	ug/kg
4,4'-DDT	4	ug/kg	4,4'-DDT	10	ug/kg
Dieldrin	4	ug/kg	Dieldrin	10	ug/kg
Endosulfan I	4	ug/kg	Endosulfan I	10	ug/kg
Endosulfan II	4	ug/kg	Endosulfan II	10	ug/kg
Endosulfan sulfate	4	ug/kg	Endosulfan sulfate	10	ug/kg
Endrin	4	ug/kg	Endrin	10	ug/kg
Heptachlor	4	ug/kg	Heptachlor	10	ug/kg
Heptachlor epoxide	4	ug/kg	Heptachlor epoxide	10	ug/kg
Methoxychlor	4	ug/kg	Methoxychlor	10	ug/kg
Mirex	4	ug/kg	Mirex	10	ug/kg
Hexachlorobenzene	4	ug/kg	Hexachlorobenzene	10	ug/kg
Alpha-Chlordane*	4	ug/kg	Alpha-Chlordane*	10	ug/kg
Gamma-Chlordane*	4	ug/kg	Gamma-Chlordane*	10	ug/kg
Oxychlordane*	4	ug/kg	Oxychlordane*	10	ug/kg
cis-Nonachlor*	4	ug/kg	cis-Nonachlor*	10	ug/kg
trans-Nonachlor*	4	ug/kg	trans-Nonachlor*	10	ug/kg
Toxaphene*	20	ug/kg	Toxaphene*	20	ug/kg
PCB-1016	20	ug/kg	PCB-1016	50	ug/kg
PCB-1221	20	ug/kg	PCB-1221	50	ug/kg
PCB-1232	20	ug/kg	PCB-1232	50	ug/kg
PCB-1242	20	ug/kg	PCB-1242	50	ug/kg
PCB-1248	20	ug/kg	PCB-1248	50	ug/kg
PCB-1254	20	ug/kg	PCB-1254	50	ug/kg
PCB-1260	20	ug/kg	PCB-1260	50	ug/kg
			Total Arsenic	40	ug/kg
			Total Cadmium	4	ug/kg
			Total Lead	40	ug/kg
			Total Mercury	24	ug/kg
			Total Selenium	40	ug/kg

References

Division of Environmental Services (DES). 2003. E-mail from Roman Khidekel to John F. Estenik (DSW). 04/25/2003.

Ohio EPA. 1989. Biological Criteria for the Protection of Aquatic Life: Volume III: Standardized Biological Field Sampling and Laboratory Methods for Assessing Fish and Macroinvertebrate Communities. Division of Surface Water.

Smith, S., H. Buettner and R. Hile. 1961. Fishery Statistical Districts of the Great Lakes. Great Lakes Fish. Comm. Tech. Rept. No. 2.

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Appendix

Field Sampling Checklist

Processing Photos

Filleting Diagrams

Fish Species Collection Tables

FIELD SAMPLING CHECKLIST

A variety of equipment is required to ensure that a proper and valid sample is obtained. The following checklist should be used to ensure possession of the necessary equipment and supplies to prepare a sample without contaminating it:

- Extra heavy duty aluminum foil, 24" by 500' roll (available at restaurant supply stores)
- Sharp, decontaminated fillet knives with plastic handles
- Fillet boards with or without hold down (It is more difficult to wrap foil on a fillet board having a hold down)
- Scalers, pliers, etc. which are properly cleaned
- Knife sharpening equipment (diamond hone, whetstone, etc.) used on clean knives
- Powder free latex gloves for handling the fish, fillets, and knives
- Teflon plastic squirt-bottles for dispensing acetone
- Pesticide grade acetone for decontaminating knives
- Phosphate free liquid detergent for cleaning knives, scalers, and pliers
- Deionized water for rinsing knives, scalers, and pliers
- Metric measuring board for measuring fish total length in millimeters
- Metric weighing scales for weighing fish in grams
- Fish club (fiberglass) for sacrificing fish
- Waterproof labels for labeling individual fish tissue samples
- Waterproof ink pen for recording information
- Clipboard for storing data sheets
- Fishes of Ohio identification book
- Supply of tissue sample submission forms
- Chemistry laboratory chain of custody forms
- Maps to the sampling locations
- GPS Unit for generating lat/longs (NAD 83 – decimal degrees)
- Variety of sizes of zip closure plastic bags
- Coolers for cold storage of fish tissue samples
- Coolant material: dry ice or water (wet) ice

FISH FILLETING PHOTOS

Latex gloves: these are changed after each sample is processed

Large polypropylene board to process fish on and store foil, club,

Decontaminated fillet knife wrapped in aluminum foil

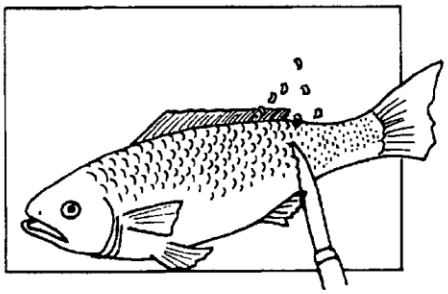
Aluminum foil positioned on board for placing filleted sample

Aluminum foil covering fillet board - use new foil with each sample

FISH FILLETING DIAGRAMS

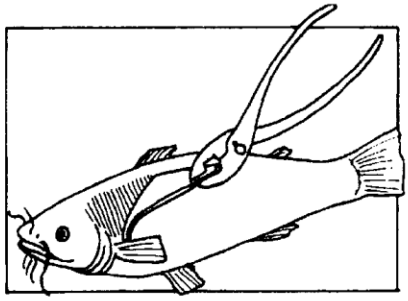
1 **Scaled Fish**

After removing the scales (by scraping with the edge of a knife) and rinsing the fish:



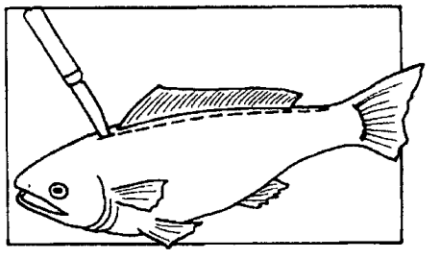
1b **Scaleless Fish**

Grasp the skin at the base of the head (preferably with pliers) and pull toward the tail.



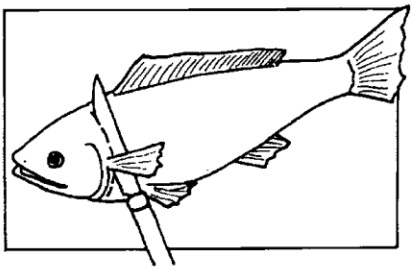
Note: This step applies only for catfish and other scaleless species.

2



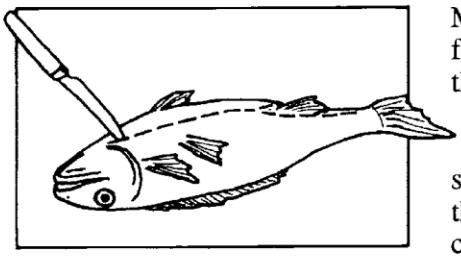
Make a shallow cut through the skin (on either side of the dorsal fin) from the top of the head to the base of the tail.

3



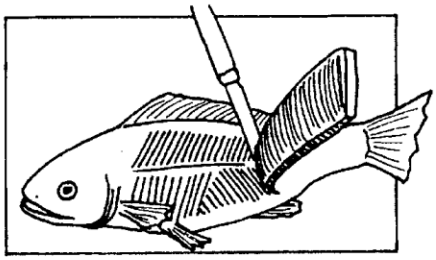
Make a cut behind the entire length of the gill cover, cutting through the skin and flesh to the bone.

4



Make a shallow cut along the belly from the base of the pectoral fin to the tail. A single cut is made from behind the gill cover to the anus and then a cut is made on both sides of the anal fin. Do not cut into the gut cavity as this may contaminate fillet tissues.

5



Remove the fillet.

PREFERRED FISH SPECIES TABLES

RIVER/ STREAM/ RESERVOIR FISH PREFERENCE - SFFC	
Common carp	Walleye
Smallmouth bass	Saugeye
Largemouth bass	Sauger
Spotted bass	Striped x white bass (wiper)
Channel catfish	White bass
Rock bass	White crappie
Freshwater drum	Black crappie
Flathead catfish	Smallmouth buffalo
Northern pike	Black buffalo
Sunfish (Lepomis spp.)	Bigmouth buffalo
Yellow perch	White sucker
Bullhead spp.	

LAKE ERIE/OHIO RIVER SPORT FISH PREFERENCE – SOFC and SFFC	
Walleye (SOFC)	Freshwater drum (SOFC)
Saugeye (SOFC)	Smallmouth buffalo (SOFC)
Sauger (SOFC)	White crappie (SOFC)
Yellow perch (SOFC)	Black crappie (SOFC)
White bass (SOFC)	Sunfish (*Lepomis spp*.) (SOFC)
Smallmouth bass (SOFC)	Steelhead trout (SOFC)
Largemouth bass (SOFC)	Northern pike (SOFC)
Spotted bass (SOFC)	Whitefish (SOFC)
Channel catfish (SFFC)	Striped x white bass (wiper) (SOFC)
Flathead catfish (SFFC)	* Other species based upon creel survey results
Rock bass (SOFC)	

WB and WBC SAMPLES
Common carp
Channel catfish
Bullheads (*Ameiurus* spp.)
Buffalo (*Ictiobus* spp.)
Sunfish *(Lepomis* spp.)
Creek chub
Other species as required