

**COLORADO DEPARTMENT OF PUBLIC HEALTH AND ENVIRONMENT  
WATER QUALITY CONTROL DIVISION  
MONITORING UNIT**

STANDARD OPERATING PROCEDURES  
FOR THE COLLECTION AND  
PROCESSING OF FISH TISSUE SAMPLES

Prepared by: \_\_\_\_\_ Date: \_\_\_\_\_  
Physical Research Scientist

Reviewed by: \_\_\_\_\_ Date: \_\_\_\_\_  
Monitoring Unit Manager

Approved by: \_\_\_\_\_ Date: \_\_\_\_\_  
Quality Assurance Officer

## Table of Contents

	Page
1. Scope and Application .....	3
2. Summary of Methods.....	3
3. Interferences.....	4
4. Equipment and Supplies .....	5
5. Fish Collection.....	5
6. Fish Processing .....	6
7. Compositing Scheme Table .....	6
8. Sample Handling and Preservation.....	7
9. References.....	8

## **1. Scope and Application**

The State of Colorado monitors potential contaminants in fish tissue because the consumption of contaminated fish can pose a hazard to human health and also because they can be an indication of a potential contamination in a waterbody.

This Standard Operating Procedure is applicable to the collection, filleting and sample preparation of fish tissue from streams, rivers, lakes and reservoirs within the State of Colorado. It is applicable only to the investigation of mercury, arsenic and selenium in fish tissue. If the Division makes the decision to investigate for other contaminants of concern, this SOP will be modified accordingly.

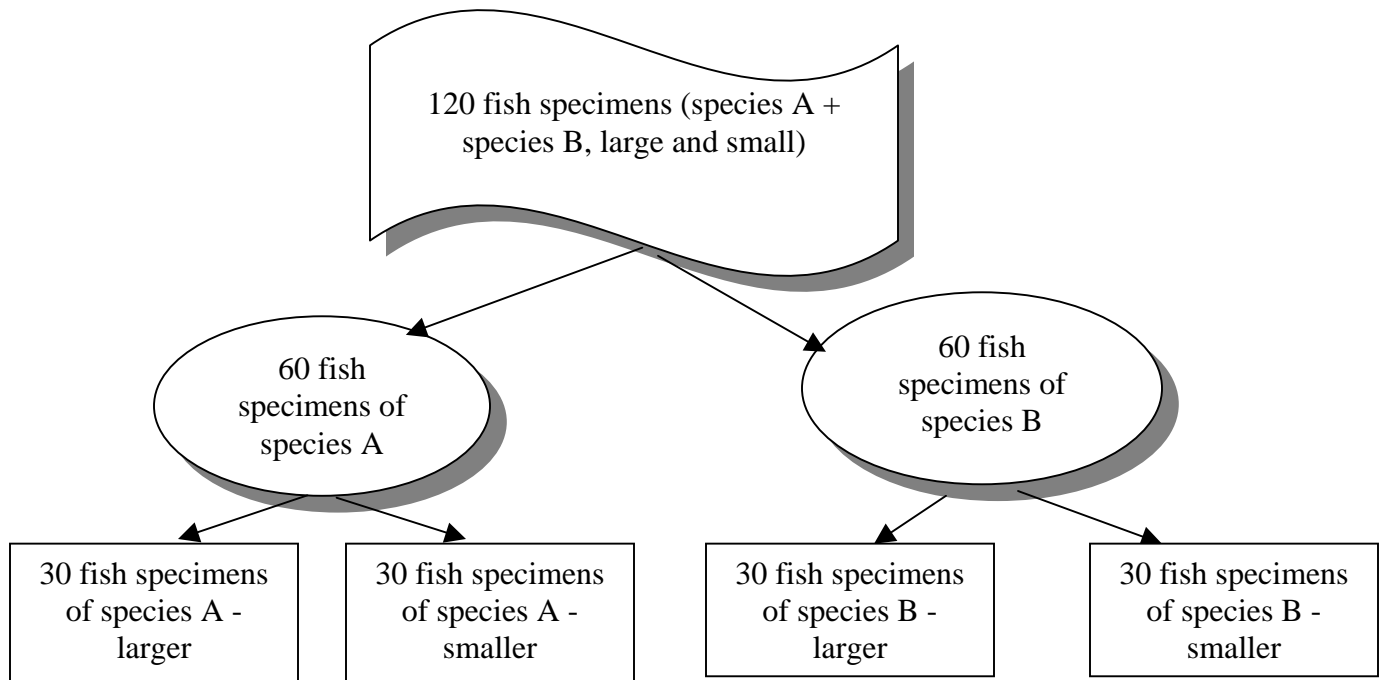
## **2. Summary of Methods**

The Monitoring Unit of the Water Quality Control Division conducts fish tissue studies with the cooperation of the Colorado Division of Wildlife. The Division of Wildlife collects the fish, using gillnets or electrofishing. Skinless fillets are collected from freshly killed fish, in the same way that anglers fillet fish. Composites of fish fillets from the same species and size classes are analyzed for total mercury, arsenic and selenium by the Colorado Department of Public Health and Environment laboratory. In some cases, depending on available resources, individual samples may be submitted for analysis. The state might also analyze fish fillets for potential organic compounds, depending on available resources. Data are reported as  $\mu\text{g/g}$  on a wet-weight basis.

Sampling events are coordinated with the Division of Wildlife and occur either when the Division of Wildlife will also conduct a regularly scheduled fish population survey or when they have resources to conduct a special sampling event for the fish tissue study. Every effort is made to conduct sampling when the target species are most frequently harvested by anglers; in most cases, the most desirable sampling period is from late summer to early fall. If possible, sampling will not occur during the spawning period of the particular target species.

Whenever possible, two target species are collected at each waterbody, one being a predator species and the other either a forage or a bottom-feeder species. In many cases, this is not possible due to the diversity of species found at each lake, so other combinations of fish species, independent of trophic level classification may be sampled. The goal is to collect 120 fish specimens from each waterbody, 60 specimens per species, divided in two size classes (30 larger and 30 smaller fish) (see figure 1). The outcome of field sampling efforts will depend on the natural diversity, abundance and availability of fish in each lake. Division of Wildlife personnel, with their knowledge of site-specific fisheries and human consumption patterns, will aid in the determination of the availability of target species.

Figure 1. Number and Distribution of Fish Collection Effort.



Every effort should be made to sample when water and weather conditions are conducive to safe and efficient sampling.

The Monitoring Unit keeps binders with all the raw data generated by the Fish Tissue Study. The binders contain the following information, for all the waterbodies sampled: the field data form, the compositing scheme table, the laboratory sample preparation form, a copy of the chain of custody documents as submitted to the laboratory, the laboratory data analyses results, and any laboratory QA/QC reports, whenever available. The binders are organized per sampling year (state fiscal year) and labeled. The data are also entered in an Access database, which is located in the Monitoring Unit. Data will be uploaded into STORET at some time in the future.

### 3. Interferences

Contamination of fish fillets during fish capture and field processing is a major possible source of error. Contact of the fillets with fish mucus slime coat, sampling gear and work area can also cause contamination. Failure to process fresh fish quickly and failure to immediately freeze fillets can lead to potential loss of contaminants.

In order to minimize the opportunities for contamination, personnel are trained to use the SOPs in the field and in the laboratory. Collected fish are kept in live wells or buckets filled with water until they can be either filleted or frozen for transport to the laboratory. If fish are being filleted at the waterbody, all surfaces are kept clean at all times and covered with plastic sheeting. Cutting boards and knives are kept clean between fish specimens of the same species and washed and rinsed thoroughly every time there is a change in species being processed. If fillets are being extracted in the field, they are immediately labeled and wrapped in aluminum foil or placed in Ziploc bags.

If fillets are being extracted at the laboratory, they are immediately placed inside a new Nalgene 50ml capped vial. All surfaces, cutting boards, measuring boards and knives are kept rinsed and clean. Laboratory analytical error is also possible and is addressed in the referenced EPA method. Consult Appendices D and E for Laboratory Standard Operating Procedures for the Analyses of Metals in Fish Tissue.

#### **4. Equipment and Supplies**

- a. Fish capture gear and boat. Typical gear includes gillnets and boat-mounted electrofishing equipment.
- b. Buckets, tubs and live wells made of plastic or aluminum for holding live fish after capture.
- c. Weighing scales of sufficient capacity to weigh fish collected, with 1% full scale resolution; balance pan.
- d. Measuring board calibrated in centimeters or inches.
- e. Stainless steel fillet knives; plastic kitchen cutting board.
- f. Plastic bags; Ziploc-brand heavy-duty freezer bags or equivalent; plastic wrap, Saran wrap or equivalent; heavy-duty aluminum foil; paper towels.
- g. Vinyl or polyethylene disposal, non-powered gloves.
- h. Field data forms (Attachment A); labels for fish specimens, "write-in-rain" paper.
- i. Ice chests; ice.

#### **5. Fish Collection**

Only freshly killed or live fish are processed for fish tissue analysis. Fish are collected by the Division of Wildlife using gillnets or by electrofishing. Gillnets are pulled from the waterbodies, placed in buckets on the boat and transported to the shore. Once on shore, fish are removed from the gillnets as soon as possible, rinsed and placed on ice. Fish that are collected using electrofishing are kept in live wells or buckets filled with water while on the boat, until they are transported to the shore. Once on shore, they are killed, rinsed and placed in ice. After collection, depending on the sampling circumstances for each waterbody, fish can either be processed in the field or transported to the Department of Public Health and Environment's laboratory.

## 6. Fish Processing

- a) **Field:** Fish processed in the field are kept in ice until the fillets can be extracted. A processing center is set up by the shore, in a suitable place. The working table is covered with plastic sheeting which can be changed if necessary, so that surfaces are always kept clean. Fish species, lengths and weights are taken for all fish specimens to be included in the study and written on the field data form (Attachment A). All pertinent information about the waterbody being investigated and the personnel conducting the study is also captured in the same field data form. A unique identifier number is assigned to each fish specimen in the field data form; the same unique identifier number follows each fish specimen through the whole sampling and investigation process. Each fish is filleted, the fillets are skinned and each skinless fillet is either wrapped in aluminum foil or placed inside a Ziploc bag. A sample label containing information about the waterbody and the unique fish specimen identifier is placed either inside the aluminum foil wrap or the Ziploc bag. The fillets, wrapped and labeled, are placed in ice chests containing enough ice to keep all samples preserved until they can be transported to the laboratory and placed in the designated laboratory freezers.
- b) **Laboratory:** Fish processed at the laboratory are placed in ice in the field as soon as possible. Soon after being captured, they are killed, rinsed and placed in heavy-duty plastic bags, grouped by species in one or more bags per species; a field data form is placed inside each bag and the plastic bags are placed inside ice chest containing enough ice to keep all the fish preserved until they can be transported to the laboratory. The field data form contains the information about the waterbody, the crew conducting the sampling and the common name for the fish species in each plastic bag. At the laboratory, the fish are either filleted soon after arriving from the field or placed in the laboratory freezer. The filleting done at the laboratory is similar to the field – fish are taken out of the freezer the previous afternoon, rinsed and cleaned as well as possible, the lengths are taken (no weights) and the same field data form is completed, with all the pertinent information, minus the weights. The skinless fillets (or as much fish tissue as can fit) are placed inside new 50ml Nalgene capped and labeled vials; the vials are kept archived for a minimum 6-month period.

## 7. Compositing Scheme Table

Whenever a certain waterbody will be analyzed using composite samples, the compositing scheme table is prepared before the samples are prepared. The fish specimens are grouped by species and ranked within each species by size, starting with the largest specimen. Each composite sample is made up of a certain number of fish specimens, depending on how many fish are available. (for a detail explanation of the sample design, consult the Quality Assurance Project Plan). For example, if each composite sample is made up of four fish specimens, composite sample number one is

made up of fish specimens numbers 1, 2, 3 and 4; composite sample number two is made up of fish specimens numbers 5, 6, 7 and 8, etc. Composite samples are made up this way until all fish specimens are included in a composite sample. Sometimes an odd number of fish is left over and they are not used in the compositing scheme. The compositing scheme table is also kept for the records, in the binder with all the rest of the raw data.

## **8. Sample Preparation**

Samples to be submitted to the laboratory for analyzes are prepared at the Department's laboratory. If composite samples are being submitted, the compositing scheme table has to be prepared ahead of the sample preparation. In some cases, depending on available resources, individual samples are submitted.

Fish tissue that will be used in the sample preparation can be extracted either from the archived vials, in the cases when the fish specimens have been filleted and archived, or from the fillets, at the same time that fish specimens are being filleted and archived, depending on logistics. Currently, the Monitoring Unit is analyzing fish tissue for mercury, arsenic and selenium. In the future, depending on resources, other inorganic and organic contaminants might be added.

For mercury analysis, a labeled, 50 ml Nalgene vial with a blue cap is used. A small amount of fish tissue is added from each fish that makes up the composite; a record is kept of the amounts extracted from each fish and of the total amount in the vial in the Laboratory Sheet. (Attachment B) The total amount should be between 1.5 and 2.0 grams, and the amount from each fish should be very similar, not varying from each other more than about 0.05 grams.

For arsenic and selenium, a labeled, 30 ml centrifuge vial with cap is used. The same process of compositing as described above is used. The total amount submitted in each vial is kept as close to 1.00 gram as possible.

Whenever an individual sample is submitted for analysis, the processes described above are used, but all the amount submitted comes from only one fish specimen.

Chain of custody documentation is prepared and submitted with the samples. (Attachment C) One copy of the documentation is kept with the Monitoring Unit binders containing the raw data and records.

## 9. References

1. American Public Health Association; American Water Works Association; and Water Environment Federation. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20<sup>th</sup> Edition.
2. U.S.EPA 2000. *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories*. EPA-823-B-00-007.

## **APPENDIX A**

### **Field Data Form**



## **APPENDIX B**

### **Laboratory Sheet**



## **APPENDIX C**

### **Chain of Custody**



