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

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

Action By	Signature	Date
John Brumley Author, Environmental Scientist III	<i>Randall G. Payne</i>	4-9-14
Randall Payne Reviewed, Environmental Scientist III	<i>Randall G. Payne</i>	4-9-14
Clark Dorman, Approved, Manager Water Quality Branch	<i>Clark Dorman</i> for Clark Dorman	8/09/2014
Lisa Hicks Approved, Quality Assurance Officer, Division of Water	<i>Lisa A. Hicks</i>	4/9/14
Peter Goodmann, Director, Division of Water	<i>Peter Goodmann</i>	8/29/2014
Larry Taylor Approved, Quality Assurance Manager, Department for Environmental Protection	<i>Larry Taylor</i>	4/9/14

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1. Procedures

1.A. Scope and Applicability

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

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

1.B. Definitions

CFR – Code of Federal Regulations

COC – Chain-of-Custody

DNR – Department for Natural Resources

DW – Dry Weight

EPA – U.S. Environmental Protection Agency

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

KDFWR – Kentucky Department of Fish & Wildlife Resources

KDOW – Kentucky Division of Water

KPDES – Kentucky Pollutant Discharge Elimination System

QA – Quality Assurance

QC – Quality Control

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

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SOP – Standard Operating Procedure

WQB – Water Quality Branch

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

1.C. Personnel Qualifications / Responsibilities

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

1.D. Recommended Equipment and Supplies

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

1.E. Methods

1.E.1. Purpose

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

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

1.E.2. Precautions Before Sampling

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

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

1.E.3. Headwater Streams

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

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

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

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

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

1.E.3.B. Sample Reach

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

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

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

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

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

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

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

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

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

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

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

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

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

1.E.4. Wadeable Streams

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

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

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

1.E.4.B. Sample Reach

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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2. Quality Assurance/Quality Control

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

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

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

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

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

Fish Tissue Processing SOP (KDOW 2008)

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

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

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

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

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

3. References

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

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Appendix A1

Selenium Fish Tissue Chain-of-Custody Sheet

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

Station #: _____ **Date:** _____

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

KPDES Permit#: _____

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

Lat/Long Downstream Reach: _____

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

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

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

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

Total # Fish Collected in Sample: _____

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

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Appendix A2

Example of a Filled Out Chain-of-Custody Sheet

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

Station #: UTHF-001-Dup

Date: 5/23/13

Stream / Location: UT Horse Fork – Downstream Outfall

Time: 1234 CST

DNR Permit#: 745-2525

KPDES Permit#: KY0100000

County: Hancock Lat/Long Upstream Reach: 37.770/-86.803

Lat/Long Downstream Reach: 37.771/-86.803

Outfall #: 003 Duplicate/Replicate (circle one): yes no

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

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

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

Total # Fish Collected in Sample: 5

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