Guidance for the Collection of Fish Tissue and/or Water Column Data for Implementation of Indiana's Selenium Chronic Aquatic Life Criteria

Version 1.0 Date: November 2024

IDEM Contact for this guidance document:
Gabby Ghreichi, Surface Water Quality Standards Coordinator
gghreich@idem.in.gov or at 317/234-1191

Guidance for the Collection of Fish Tissue and/or Water Column Data for Implementation of Indiana's Selenium Chronic Aquatic Life Criteria

Indiana Department of Environmental Management
Office of Water Quality November 2024

Purpose of this Guidance

Indiana Department of Environmental Management (IDEM) Office of Water Quality (OWQ) developed this document to provide appropriate and acceptable methodologies for the collection of selenium fish tissue and water column data in support of IDEM's February 1, 2022 updated aquatic life chronic water quality criterion for selenium. Entities may elect to acquire these data to supplement a reasonable potential analysis outside of the Great Lakes system, or to support derivation of site-specific water column criterion elements using a bioaccumulation factor (BAF) empirical modeling approach.

This guidance document does not entail IDEM's 303(d) listing strategy for selenium or any other parameter. Please reference IDEM's Consolidated Assessment and Listing Methodology (CALM) document for Indiana 303(d) listing information. Information about Indiana's 303(d) list can be found here: Indiana's Impaired Waters 303(d) List.

Note: This document may be updated and revised in the future to reflect changes, developments, and any new information.

Overview of Indiana Selenium Aquatic Life Criterion

In 2016, the United States Environmental Protection Agency (U.S. EPA) published *Aquatic Life Ambient Water Quality Criterion for Selenium - Freshwater*, a National Recommended Water Quality Criterion (NRWQC) pursuant to Section 304(a) of the Clean Water Act. This document was revised in 2021 and EPA's technical support documents for selenium were finalized in 2024. The criterion recognizes that although selenium can cause acute toxicity at high concentrations, its most harmful effects result from chronic toxicity due to selenium's bioaccumulative properties. The latest scientific research shows that the reproductive life-stages of egg-laying vertebrates are the most sensitive to the toxic effects of selenium, particularly fish. Aquatic organisms are primarily exposed to selenium through their diets rather than directly from water. In aquatic communities, fish are the most sensitive to selenium effects and toxicity occurs when the selenium is transferred to eggs, reducing reproductive success and survival (USEPA 2021). The latest scientific information indicates that a criterion element derived from fish is expected to be protective of the aquatic community, since other taxa appear to be less sensitive to selenium than fish.

The NRWQC aquatic life criterion (ALC) for selenium is composed of four elements, all of which are protective against chronic selenium effects. The recommended elements are: (1) a fish egg-ovary element; (2) a fish whole-body and/or muscle element; (3) a water column element which includes one value for lentic (still water) and one value for lotic (flowing water) aquatic systems; and (4) a water column intermittent element to account for potential chronic effects from short-term exposures. The egg-ovary element takes precedence over fish whole-body or muscle tissue elements, which in turn take precedence over the water column elements (USEPA 2024a).

Each of the four elements consists of a magnitude, duration and frequency of

exceedance. The duration component of the fish tissue elements is stated as an "instantaneous measurement", because "Fish tissue data provide instantaneous point measurements that reflect the integrative accumulation of selenium over time and space in fish populations at a site" (Appendix A). Therefore, the hierarchy of the fish tissue criterion elements (i.e., egg-ovary data supersedes muscle/whole-body) applies within each fish species to protect populations of species within a fish community and not across populations of different species. The fish tissue elements of the NRWQC aquatic life selenium criterion were derived using species-specific conversion factors to calculate a toxicity value. Therefore, egg/ovary data from one species cannot be directly compared to whole-body or muscle fillet tissue concentrations from a different species of fish. This is inconsistent with the procedure used to develop the criterion (USEPA 2024d).

The NRWQC recognized that selenium bioaccumulation potential depends on the structure of the food web and several biogeochemical factors that characterize a particular aquatic system. In Appendix K of the NRWQC document, U.S. EPA provided two methodologies, a mechanistic modeling approach and a BAF empirical modeling approach, to translate a fish tissue criterion element (egg-ovary, whole-body, or muscle) into a site-specific water-column concentration to more precisely manage selenium in specific aquatic systems (USEPA 2021).

On February 1, 2022, Indiana revised its Water Quality Standards (WQS) rules to include the NRWQC aquatic life criterion for selenium for waters both inside and outside the Great Lakes system. In addition, for waters outside of the Great Lakes system, Indiana included site-specific criterion elements for portions of the state where fishes in the Order Acipenseriformes (sturgeon and paddlefish) do not occur at the site. Indiana also included a provision for deriving site-specific water column criterion elements using the mechanistic and BAF empirical modeling approaches. Indiana's selenium aquatic life criteria are included in Appendix A. A summary of the BAF empirical modeling approach is attached in Appendix B.

Implementation of the Selenium Criterion in National Pollutant Discharge Elimination System (NPDES) Permitting

For NPDES-permitted facilities that continuously or intermittently discharge effluent containing selenium, IDEM will use the water column elements of the four-part criterion to conduct a reasonable potential determination and to develop water quality-based effluent limitations (WQBELs) for NPDES permit limits[1]. However, for a discharge to waters outside the Great Lakes system, fish tissue concentrations in the receiving water can be used to conduct a reasonable potential determination instead of the water column elements. If effluent concentrations are elevated and show reasonable potential

[1] NPDES permit writers use a reasonable potential to exceed analysis (RPE) to determine whether a water quality-based effluent limitation (WQBEL) or monitoring is required for a pollutant in an NPDES permit. They use this process to determine whether a discharge causes, has the reasonable potential to cause, or contributes to an excursion above an applicable water quality criterion. When conducting this analysis based solely on effluent data, permit writers use calculations and procedures outlined by U.S. EPA (USEPA 1991).

to exceed the chronic selenium criterion, a permittee may submit a fish sampling plan to the agency, to determine if the fish tissue elements of the criterion also show reasonable potential to exceed the criterion. The fish sampling plan is subject to IDEM review and approval. To use fish tissue concentrations to conduct a reasonable potential to exceed determination: (1) sources of selenium must have been present and are not expected to increase; (2) the population of fish sampled has been exposed to the existing levels of selenium and the system is determined to be in steady state; and (3) collection of fish tissue data demonstrate that the fish tissue concentrations are below the applicable fish tissue criterion.

NPDES-permitted facilities may also pursue the derivation of site-specific water column criterion elements that may be used by IDEM to conduct a reasonable potential determination and to develop WQBELs for NPDES permits. Site-specific water column criterion elements must be derived using either the empirical BAF or mechanistic modeling method, as described in Appendix K of the NRWQC for selenium (USEPA 2021). Any proposal to derive a site-specific water column criterion element must be submitted to IDEM's Office of Water Quality for review and approval of the methodology and sampling plan prior to initiation of sampling. A site-specific water column element will require the "site" or waterbody segment to be identified and defined. Site-specific factors such as the waterbody flow regime, the presence of dams, point source discharges, major tributaries, and other waterbody features such as oxbows or more lentic type features can influence the "site" identification process. Any proposal to derive a site-specific water column criterion element must be protective of downstream designated uses for aquatic life and human health. More than one "site" and applicable water column criterion may be necessary, to adequately protect designated uses and account for the spatial and temporal effects of selenium. As an example, if selenium concentrations are significantly different between several locations within a site, it may be appropriate to separate the site into multiple sites.

This document contains the guidance necessary for collecting fish tissue and water column samples to investigate selenium in fish tissue and in the water column, conduct a reasonable potential to exceed determination and/or to derive a site-specific water column criterion element using a BAF empirical modeling approach. This document also provides information on how a waterbody would be considered "fishless". Only the water column element of the selenium criterion will apply to "fishless" waterbodies. IDEM may approve, approve with modification, or deny any alternative site-specific selenium water column criterion element proposed by an entity. Any site-specific criterion element approved by IDEM must be submitted to and approved by U.S. EPA before it can be incorporated into a final NPDES permit or used for other Clean Water Act purposes (327 IAC 2-1-8.9 and 2-1.5-16). IDEM may also, at its discretion, request additional information if needed in accordance with 327 IAC 2-1, 2-1.5 and 327 IAC 5.

The procedures described in this document are applicable to aquatic systems that are in steady state with selenium (existing discharges), and do not apply when new or increasing inputs of selenium are added to a waterbody. For new selenium inputs, selenium in fish tissue must be allowed to come into equilibrium with the water column

before fish tissue concentration criterion elements would supersede water column concentration criterion elements (USEPA 2021). When selenium inputs change, causing the concentration in the water column to increase or decrease, the fish tissue will not immediately reflect the change in water chemistry. U.S. EPA estimates that the concentration of selenium in fish tissue will not reach steady state for several months in lotic systems and longer time periods (e.g., as long as 2 to 3 years) in lentic systems. Generally, when any major changes to water column selenium concentrations occur for current and new discharges, IDEM will require a minimum duration of 12 months of steady state before fish tissue collection to assess bioaccumulation in the resident fish population. However, IDEM will consider site-specific factors that could shorten or lengthen the timeframe to achieve steady state.

To characterize the contribution of selenium from NPDES-permitted facilities, IDEM will consider downstream sampling reaches that are large enough to include samples collected within and downstream of areas of incomplete mixing. This will help to characterize the range of bioaccumulation potential in the tissue samples as the water column concentrations decrease. This could include collecting fish and water samples from multiple stream reaches downstream of the discharge. These data would be used for both the reasonable potential to exceed determinations and the derivation of site-specific water column criterion elements.

Personnel Qualifications and Responsibilities

Individuals conducting fish tissue collections must possess a valid Indiana Department of Natural Resources (IDNR) Scientific Purposes License (State Form 21945 (R7/7-15)). A Scientific Purposes License is required by State law in Indiana Code 14-22-22 for the activities pertaining to the capture/handling/collection of wild animals for scientific purposes (IDNR 2019). IDNR will not issue a Scientific Purposes License to an individual who does not have the requisite educational qualifications and/or professional experience to conduct this work. Field personnel conducting fish tissue and water chemistry collections should comply with Indiana boating safety requirements (USGS 2016). For electrofishing collection methods, all sampling crew members involved with electrofishing activities should have at least one year of experience in sampling methodology and taxonomy of fish communities in the region. IDNR must be contacted prior to any kind of fish tissue collection.

Work Plan

Monitoring plans must be submitted to IDEM for review and approval prior to commencing any sampling for the purposes of collecting fish to conduct a reasonable potential to exceed determination for a discharge to waters outside the Great Lakes system or for deriving a site-specific bioaccumulation factor. The draft work plan must also be submitted to IDNR with the application for the Scientific Purposes License and should include a provision for reporting to IDNR on the fish collected/sacrificed/wasted during completion of the approved work plan. Collection and analyses described in the monitoring plan should meet data quality assessment (DQA) Level 3 requirements as described in the *Technical Guidance for the Office of Water Quality External Data Framework (EDF)* (IDEM 2024, Section 8).

Fish Tissue Data Collection

Fish Tissue Data Collection: Overview

Selenium is an essential nutrient for fish reproduction, but excessive levels can cause reproductive impairments (e.g., larval deformity or mortality). Selenium tends to accumulate in the eggs and ovaries of fish due to its importance in the reproductive process. As female fish develop their eggs, selenium is selectively transferred from the muscle tissue and other body stores into the eggs and ovaries. The concentration of selenium in the eggs and ovaries can therefore serve as a sensitive indicator of the female's exposure and potential impact on fish reproduction. However, IDEM does not encourage egg-ovary collection due to practical difficulties, such as reproductive failures that already exist in a waterbody, limited spawning timeframes within a year, asynchronous spawning, small fish body sizes that hinder egg collection, hazards in the field (e.g., high flows), and the high rate of mortality associated with egg collection. Instead, IDEM encourages the collection of whole-body and muscle tissues for a reasonable potential analysis or site-specific water column criterion. Due to the transfer of selenium to eggs and ovaries, collection of fish for whole-body and muscle tissue analysis must be collected no earlier than two months after the end of spawning season, preferably in October or later. This will avoid collecting fish tissue that is depurated of selenium. U.S. EPA recommends, if possible, to identify and sample male fish rather than female fish for whole fish and muscle tissue analysis (USEPA 2024b).

See Table 1 for detailed guidance on target species groups, spawning season, preparation type, number of fish per composite sample, and minimum size requirements. Species in each group are listed in order of preferred sampling priority, meaning the first listed genus/species in a group should be collected, but if not present, move to the next listed species. If a permittee is interested in eggs and/or ovary collection, please see the section below on egg/ovary collection as well as Appendix C for more details.

When selecting target fish species for selenium criterion monitoring, the focus should be on species that may potentially accumulate high concentrations of selenium (e.g., molluscivorous species), are sensitive to selenium, and that are easy to identify (USEPA 2024b). EPA recommends that States develop a priority list of target species in order to protect species that are sensitive to selenium and/or have a high bioaccumulation potential. Selenium bioaccumulation potential and sensitivity in species do not always overlap. To collect fish representing both scenarios, Table 1 lists three groups of species that are required to be analyzed for selenium concentrations when fish tissue is collected for a reasonable potential analysis or site-specific water column criterion.

Do not collect fish species that are absent from Table 1. For the purposes of this guidance document, IDEM will not consider or accept data collected for species not included in this table, unless IDEM has pre-approved a different set of target fish species depending on the geographic location of sampling (e.g., open waters of Lake Michigan). IDEM will evaluate each proposal to sample fish tissue on a case-by-case

basis. IDEM will consider the type of waterbody, drainage area, the home range of species, and site-specific conditions during the review of the fish sampling plan. IDEM must approve any permittee's plan to sample fish tissue for either a reasonable potential analysis and/or for a site-specific BAF study.

Table 1. List of Target Species to be analyzed for Selenium Listed in Order of Preference

Species	Spawning Season	Media Type	Number of Fish per Composite Sample	Size of Fish (inches)
Group 1 - Target Molluscivorous Species				
Freshwater Drum [5] (*Aplodinotus grunniens*)	May-July	Muscle/Fillet	3-5	≥ 15
Common Carp [3] (*Cyprinus carpio*)	May-Aug	Muscle /Fillet	3-5	≥ 13
White Sucker [4] (*Catostomus commersonii*)	April-May	Muscle/Fillet	3-5	≥ 10
Temperate Bass Genus [5] (*Morone species)*)	April-June	Muscle/Fillet	3-5	≥ 10
Group 2 - Target Sensitive Species				
Bluegill [1], [7] (*Lepomis macrochirus*)	May-Aug	Whole-Body	3-5	≥ 4
Sunfish Genus [1], [7] *(Lepomis species)*	May-Aug	Whole-Body	3-5	≥ 4
Black Bass [2], [7] (*Micropterus species)*	April-July	Muscle/Fillet	3-5	≥ 10
Rock Bass [7] (*Ambloplites rupestris*)	May-July	Muscle/Fillet	3-5	≥ 5
Redhorse Genus [4] (*Moxostoma species)*	April-May	Muscle/Fillet	3-5	≥ 13
Carpsucker Genus [4] (*Carpiodes species)*	June-Sep	Muscle/Fillet	3-5	≥ 10
Buffalo Genus [4] (*Ictiobus species)*	April-May	Muscle/Fillet	3-5	≥ 17
Group 3 - Target Whole Body Composite Species				
Sunfish Genus [1], [7] *(Lepomis species)*	May-Aug	Whole-Body	3-5	≥ 4
Most Prominent *Cyprinidae species* [6] (excluding Common Carp)	April-Aug	Whole-Body	≥12	≥ 2

[1] Spotte 2007, [2] Heidinger 1976, [3] Kottelat *et al*. 2007, [4] Kay *et al*. 1994, [5] Wallus *et al.* 2006, [6] Trautman 1981, [7] Wallus *et al*. 2008

The frequency, timing, and location of sampling activities will be reviewed to identify and address any bias that may may impact IDEM's decision making (IDEM 2024).

In lotic (flowing) systems, the upper boundary of the first sample reach for fish collection should begin immediately downstream of the effluent outfall, with additional downstream sub-reaches extending to a maximum distance based on stream size (see Table 2) Sampling should begin in the sub-reach closest to the outfall and may require multiple passes to collect enough fish from each group listed in Table 1.

If the target fish tissue samples are not collected in the first sub-reach, proceed to the next downstream sub-reach. Continue sampling only until the target fish tissue samples are collected. If no acceptable fish are found within the maximum stream reach distance, the stream reach will be considered fishless, requiring the application of only the water column element.

Record the coordinates of the upstream and downstream limits of each sampling sub-reach and note the sub-reach where the fish tissue samples were collected. Sample all available habitat where the target species are likely to reside. For streams less than 25 meters wide, collect from both banks; for wider streams, collect from the same bank as the outfall. Avoid confluence areas when possible.

Selenium bioaccumulation is typically a far-field, not a near-field issue (USEPA 2024c). Downstream sampling is required to characterize the range of bioaccumulation as water concentrations decrease (USEPA 2024b). In addition, different fish species have different home ranges and can move in and outside of that range. IDEM will require at least two reaches - one near the outfall and one in the well-mixed portion of the stream.

For lentic (standing) systems, the fish sampling reach should include shoreline littoral habitats within 500 meters of either side of the outfall, shoreline littoral habitats located near the mid-point in the lake, and shoreline littoral habitats at the opposite end of the lake. Lentic systems require a site-specific approach to develop an acceptable reach and sub-reach sampling strategy, and IDEM will determine whether additional sub-reaches are appropriate on a site-specific basis. Record coordinates that bracket the sampling area.

Table 2. Recommended maximum sampling reach and designated sub-reach lengths for collecting fish tissue samples for lotic waterbody categories. See footnote [2] below for site-specific considerations on sub-reach length and maximum sample reach length.

Waterbody Category	Drainage Area (mile^2)	Maximum[2] Sample Reach Length (meters)	Sub-reach Length (meters)
Headwater Stream	<20 mi^2 wadeable	400	100
Wadeable Stream	>20-1000 mi^2 wadeable	500	100
Large River	1000 – 2000 mi^2 not wadeable	1000	500
Great River	>2000 mi^2 not wadeable	1000	500

Fish sampling will be conducted using standard electrofishing methods, gill nets or other appropriate catch method in accordance with *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1, Fish Sampling and Analysis, Third Edition* (USEPA 2000a, Section 6.2.1), or other appropriate catch method, per American Fisheries Society *Fisheries Techniques* (Zale *et al.* 2013). See Appendix E for recommended electrofishing equipment for fish collection. It is highly desirable to collect live, intact fish that have not been mutilated by the collection gear and that do not have any skin lacerations or fin deterioration that would allow body fluids to leak out of the specimen or contaminants to pass into the specimen after collection (USEPA 2000a).

Composite samples of a species from each group listed in Table 1 will be collected in the order listed. For example, target molluscivorous species such as Freshwater Drum are always a primary target species, but if Freshwater Drum are not collected from a site, then Common Carp becomes a primary target, etc. A composite sample is comprised of multiple fish of the same species, and of similar size whose percent in total length between the smallest and largest individuals is ≥ 75 percent (smallest individual total length divided by the largest individual length x 100 = greater than or

[2] The distances listed in Table 2 are general guidelines based on the overall waterbody category. However, the maximum reach distance as well as the sub-reach distances will be a site-specific determination. The mobility and home range of the target species may impact the reach sampling distance. Selenium bioaccumulation is known to be a far-field problem. For example, sampling may need to occur at sites further downstream to capture the spatial extent of selenium bioaccumulation. IDEM will work with the permittee on designing the sampling plan to account for the aforementioned factors.

equal to 75 percent). Fish collected for composite samples for a given sampling site should be collected within no more than one week of each other (USEPA 2024b). Muscle plugs or muscle tissue biopsy samples are not acceptable. Composite samples must be comprised of whole-body or skin-off boneless fish fillets, which require sacrificing fish.

In certain cases, sampling individual fish as opposed to collecting composite samples may be appropriate. It may be beneficial to collect individual fish samples if the site of interest is known to be impacted by elevated selenium concentrations or if conducting a site-specific study. Individual samples can help describe the range of variability within a population. Individual samples may also demonstrate which fish are more migratory within a population (USEPA 2024b). A measure of central tendency will be applied to individual fish species. IDEM requires a minimum of 5 individuals for each target species sampled.

Once fish are obtained using the collection methods described above, they should be identified to species. Record and report any diseases and deformities noted in collected fish. Identification and enumeration of non-target species collected in each sub-reach should be recorded and returned to the water. Fish selected as composite samples should be brought to the sample processing station from the field on wet ice. Sample processing should be conducted within 24 hours of sample collection. The type of ice to be used for shipping should be determined by the length of time the samples will be in transit to the analytical laboratory. Wet ice may be used if the time of collection to delivery at the analytical laboratory is less than 24 hours. Storage and shipment of samples on dry ice is recommended if the time of collection to delivery at the analytical laboratory exceeds 24 hours. Sample processing of fish should be conducted in accordance with Section 7.2.2 of *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1, Fish Sampling and Analysis, Third Edition* (USEPA 2000a). Data record keeping for fish collections should follow Section 6.2.3.1 of this Guidance. In addition to the information listed in the U.S. EPA Guidance, weight (grams) and total length (millimeters) of each individual fish and preparation type (i.e., whole-body, skinless boneless fillet) of each composite sample should also be recorded on the data sheet. See Appendix D for examples of fish data reports.

EPA recommends laboratory analytical methods to measure total selenium in egg-ovary, whole fish and fish muscle tissue samples, summarized in Table 3, below.

Table 3. List of Test Procedures for Total Selenium in Fish Tissue

Method[1]	Technique	Method Detection Limit
EPA Method 6020B	Inductively Coupled Plasma - Mass Spectrometry (ICP-MS)	0.2 mg/kg
EPA Method 7742	Atomic Absorption, Borohydride Reduction	0.05 mg/kg
USGS I-9020-05	Collision/Reaction Cell ICP-MS	0.008 µg/g
NOAA 140.1	Graphite Furnace-Atomic Absorption	
EPA Method 200.8, Rev 5.4	Inductively Coupled Plasma - Mass Spectrometry	

[1]See Table 5 in the Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2021 Revision to its 2016 Selenium Criterion (USEPA 2024b)

Egg/Ovary Collection

Due to the safety, timing and difficulty in obtaining egg/ovary data from target species listed in Table 1, IDEM does not recommend egg/ovary collection. Females are typically gravid for a small window of time for most synchronous species. The timing of the spawning season is unique to the species, geography, and environmental cues such as temperature, flow, and photoperiod (USEPA 2024b). If a permittee is interested in obtaining egg/ovary data, the permittee must submit a robust sampling plan and explain how the challenges will be addressed. IDEM must have the opportunity to review and provide feedback on the egg/ovary sampling plan. At a minimum, IDEM requires the following information within a sampling plan to use egg/ovary data for reasonable potential determination or to develop a BAF to derive a site-specific water column criterion:

- Procedures for collecting gravid females.
- Procedures for the timing and collection of target species prior to the first spawning event of the year.
- Concurrent collection of fish tissue (fillet and whole-body) samples for each species which was sampled for eggs/ovaries.
- Requirement for replicate egg/ovary samples (similar to the requirements for whole-body and fillet data collection).
- Water temperature monitoring will be required for Spring sampling events to ensure the samples are taken prior to spawning.

IDEM does not have regular experience in collecting egg/ovary samples. IDEM recommends that any permittee interested in collecting egg/ovary data work with an experienced fisheries biologist to develop an approvable sampling plan that addresses the critical timing piece. Please reference Appendix C for EPA's guidance on egg/ovary collection and preservation techniques. The Appendix comes directly from EPA's technical support document on fish

tissue (USEPA 2024b).

Fish Tissue Data Collection: Sampling Design

U.S. EPA recommends that when implementing the selenium criterion, the fish tissue elements take precedence over the water column elements, except in certain circumstances (e.g., new selenium inputs; Appendix A). Fish tissue may be collected and analyzed to obtain data for a reasonable potential to exceed determination for a discharge to waters outside of the Great Lakes system, or to derive a site-specific water column criterion element using the BAF approach described in Appendix K of the NRWQC for selenium (USEPA 2021). Appendix B contains select pages from Appendix K of the NRWQC document that covers the empirical BAF approach.

Two fish tissue data collection sampling designs are included below. The "Fish Tissue Data Collection: Reasonable Potential Determination" includes the minimum fish tissue data requirements to allow IDEM to conduct a reasonable potential to exceed determination using fish tissue data. The "Fish Tissue Data Collection: Site-Specific BAF" includes the minimum fish tissue data requirements for deriving a site-specific selenium water column criterion element.

Fish Tissue Data Collection: Reasonable Potential Determination

For the use of fish tissue data in conducting a reasonable potential to exceed determination for a discharge to waters outside the Great Lakes system, IDEM requires collection of a fish tissue composite sample from each of the three target species groups in Table 1 and the collection of a replicate composite sample for each of the selected target species (or the appropriate number of individual fish, as applicable). Fish collection must occur at a minimum of two locations downstream of the permitted entity. The first sampling reach must be located immediately downgradient of the outfall and the second sampling reach should be selected at a location downstream of the facility's outfall and be representative of a well-mixed area of the receiving stream.

Replicate analyses provide statistical power to estimate the variability of contaminant levels within a species. Replicate composite samples should be as similar to each other as possible. In addition to being members of the same species, individuals within each composite should be of similar average total length, as described in Section 6.1.1.6 of *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1, Fish Sampling and Analysis, Third Edition* (USEPA 2000a). The requirements for determining whether fish are placed in acceptable replicate samples are described in Section 6.1.2.7 of this Guidance.

Fish collection should follow the procedures described in the *Fish Tissue Sampling: Overview* section, above, with sampling for the first reach beginning at the outfall and proceeding downstream in designated sub-reaches, only until the required target fish

tissue samples are collected. Ideally, composite samples and their replicates should be collected in the same sub-reach, or in adjoining sub-reaches. All fish should be collected within no more than one week of each other so that temporal changes in target analyte concentrations associated with the reproductive cycle of the target species are minimized (USEPA 2000a). Based on this requirement a total of six (6) composite samples will be collected for analysis from the first sampling reach downstream of the outfall and six (6) composite samples will be collected from the second sampling reach located outside of the mixing zone, for the reasonable potential to exceed determination.

In smaller streams where Target Molluscivorous Species and/or Target Sensitive Species may not occur in the sampling reach, IDEM may apply protective factors for the aquatic life assemblage to compensate for the missing targeted group(s) of fish when evaluating the fish tissue data for the reasonable potential to exceed determination. For example, if only fish sensitive to selenium are collected in the reach, a protective factor might be warranted to protect downstream fish that bioaccumulate selenium at a faster rate than sensitive fish. If acceptable fish are not present within the maximum distance identified in Table 2 for the first sampling reach, IDEM will use the water column element to conduct a reasonable potential to exceed determination for the discharge.

While not required, IDEM recommends collecting fish tissue samples at an appropriate upstream location, distant enough from the first downstream reach so that fish are not intermingled. The fish species and sizes from the upgradient location should match those of the downstream samples as best as possible to assess selenium in fish tissue populations under ambient conditions.

Fish Tissue Data Collection: Site-Specific BAF

An entity may elect to collect paired fish tissue and water column samples to support a site-specific water column criterion that reflects local surface water conditions and is protective of the downstream fish community. Per Indiana's WQS, EPA must also approve any site-specific BAF. Therefore, any proposal to collect paired fish tissue and water column samples for a BAF study will need to be reviewed by both IDEM and U.S. EPA Region 5. At a minimum, IDEM will require fish sampling at a reach just downstream of the outfall location as well as in a second reach at a point further downstream that is representative of the well-mixed portion of the stream. Depending on the site, more than two sampling reaches may be required for fish sampling for a site-specific BAF study. To understand the dynamics of selenium at the site and the impact on the fish community, IDEM may require fish sampling at several locations downstream of the outfall location.

For deriving a site-specific BAF, IDEM requires triplicate composite fish tissue samples (or the appropriate number of individual fish, as applicable) from the Target Molluscivorous Species, the Target Sensitive Species, and the Target Whole-Body Composite Species, listed in Table 1, at each location designated as

a site-specific BAF fish sampling site. Triplicate analyses provide additional statistical power to estimate the variability of contaminant levels within a species. Triplicate composite samples should be as similar to each other as possible. In addition to being members of the same species, individuals within each composite should be of similar average total length, as described in Section 6.1.1.6 of *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1, Fish Sampling and Analysis, Third Edition* (USEPA 2000a). The requirements for determining whether fish are placed in acceptable replicate samples are described in Section 6.1.2.7 of this Guidance.

Fish collection should follow the procedures described in the *Fish Tissue Sampling: Overview* section, above, with sampling beginning at the outfall and proceeding downstream in designated sub-reaches, only until the required target fish tissue samples are collected. Ideally, composite samples and their replicates should be collected in the same sub-reach, or in adjoining sub-reaches. All fish should be collected within no more than one week of each other so that temporal changes in target analyte concentrations associated with the reproductive cycle of the target species are minimized (USEPA 2000a). Based on this requirement a total of nine (9) composite samples will be collected from the first sampling reach downstream of the outfall and nine (9) composite samples will be collected from the second sampling reach located outside of the mixing zone. If it is not possible to collect any target fish tissue samples within the maximum distance identified in Table 2 and specified time frame for the first sampling reach, it will not be possible to derive a site-specific BAF. The entity could elect to use the mechanistic modeling methodology described in Appendix K of the NRWQC document to derive a site-specific water column criterion element.

In smaller streams where Target Molluscivorous Species and/or Target Sensitive Species may not occur, site-specific sampling approaches may be warranted. IDEM may apply protective factors for the aquatic life assemblage to compensate for the missing targeted group(s) of fish when evaluating the fish tissue data for the BAF and site-specific water column criterion calculation. For example, if only fish sensitive to selenium are collected from the reach, a protective factor might be warranted to protect downstream fish that bioaccumulate selenium at a faster rate than sensitive fish.

While not required, background samples may be collected at an appropriate upstream location, distant enough from the first downstream reach so that fish are not intermingled. For the upstream location, only one composite sample from each category is necessary. However, the fish sizes for these composites should match those of the downstream samples of the same species as best as possible. The need for upstream samples for deriving a BAF will be assessed on a case-by-case basis.

Water Chemistry Data Collection

Water Chemistry Data Collection: Reasonable Potential Determination

Water chemistry monitoring is not required when collecting fish tissue to be used for a reasonable potential to exceed determination. However, water chemistry data collected concurrent with fish tissue data collection can provide important information about selenium in the water column upgradient of the site and in the receiving waters. To characterize selenium in the receiving waters, IDEM recommends entities collect a mid-stream grab water sample upgradient of the site, grab water samples along a transect perpendicular to the stream flow at two representative locations within the first fish sampling reach, and a mid-stream grab water sample within the second fish sampling reach. IDEM recommends collecting the upgradient sample at least 50 meters upstream of the outfall. IDEM recommends collection of grab samples at the first reach downstream of the outfall at 25%, 50% and 75% distance across the stream width of each transect. Collect grab samples at mid-depth using a Van Dorn sampler or comparable sampling device. Measure and record temperature, pH, specific conductance, dissolved oxygen and oxidation reduction potential when collecting grab surface water samples. Samples collected for water quality analysis should be analyzed for total recoverable selenium and total dissolved selenium. Collecting water column samples for three months surrounding and during fish tissue data collection is advised.

Water Chemistry Data Collection: Site-Specific BAF

Water chemistry monitoring is required for deriving a site-specific water column criterion element using the BAF approach. Entities must collect semimonthly samples for 12 months (a minimum of 24 sampling events) to assess spatial and temporal selenium concentrations in the receiving water across a broad range of flow conditions.

General guidance for collecting water samples in support of a site-specific BAF for lotic and lentic systems is described below; however, IDEM may modify these requirements based on site-specific conditions (e.g., headwater streams, large reservoirs).

For lotic systems, collect individual grab surface water samples as described below:

For headwater and wadeable stream sites (Table 2)
Collect an individual grab sample midstream at least 50 meters upstream of the outfall. Collect grab samples along a transect at two representative locations of the first fish sampling reach for the study up to the downstream limit of the sample reach designated for the waterbody (i.e., 400 meters or 500 meters, Table 2). Collect the grab samples at 25%, 50% and 75% of the distance across the stream width of each transect. In addition, collect an individual grab sample midstream within the second fish sampling reach that is representative of the well-mixed portion of the stream. Collect all grab samples at mid-depth using a Van Dorn sampler or comparable sampling device. Measure and record temperature, pH, specific conductance, dissolved oxygen and oxidation reduction potential when collecting grab surface

water samples and record the coordinates of sampling locations. Samples collected for water quality analysis should be analyzed for total recoverable selenium and total dissolved selenium. Eight (8) grab samples per bimonthly sampling event will be collected under this format for sites in headwater streams or wadeable streams.

For large river and great river sites (Table 2)
Collect an individual grab sample midstream at least 50 meters upstream of the outfall. Collect grab samples at 25% of the stream width on the outfall stream bank side of the waterbody, at two representative locations of the first fish sampling reach up to 1000 meters downstream of the outfall. In addition, collect an individual grab sample midstream within the second fish sampling reach that is representative of the well-mixed portion of the stream. Additional water chemistry sampling locations may be required depending on the specific site. Collect all grab samples at mid-depth using a Van Dorn sampler or comparable sampling device. Measure and record temperature, pH, specific conductance, dissolved oxygen and oxidation reduction potential when collecting grab surface water samples and record the coordinates of sampling locations. Samples collected for water quality analysis should be analyzed for total recoverable selenium and total dissolved selenium. Four (4) grab samples per bimonthly sampling event will be collected under this format for sites in large or great rivers.

For lentic systems, collect individual grab surface water samples as described below:

For lentic systems
Water column sampling requirements for lentic systems will be determined on a site-specific basis. Generally, monitoring along three transects may be required: within 50 meters of the outfall, the opposite end of the lake or reservoir and the mid-point in between the two transects. Collect all grab samples at mid-depth using a Van Dorn sampler or comparable sampling device. Measure and record temperature, pH, specific conductance, dissolved oxygen and oxidation reduction potential when collecting grab surface water samples and record the coordinates of sampling locations. Samples collected for water quality analysis should be analyzed for total recoverable selenium and total dissolved selenium.

The appropriate preservation and pretreatment steps should be taken for the types of data required (i.e., total dissolved selenium and total recoverable selenium), and holding time from sample collection to first use in the laboratory must be no more than six months.

Water Chemistry Data Collection: Laboratory Analytical Methods

Appendix L of the NRWQC document provides several U.S. EPA approved analytical methods under 40 CFR § 136 specifically for measuring total recoverable selenium in water. Three U.S. EPA-approved analytical methods that may be sufficiently sensitive for the purposes of implementing a selenium water quality criterion are listed below (Table 4). Samples collected for water quality analysis should be analyzed for total

recoverable selenium and total dissolved selenium using the below specified methods. Alternate methods may be used after obtaining approval from IDEM.

The selenium results obtained from the sampling must be quantifiable to be used in the BAF calculation. If they are not, a more sensitive analytical method must be used to analyze for selenium.

Table 4. Suggested U.S. EPA-Approved Methods for Selenium in Water

Method	Technique	Method Detection Limit
American Public Health Standard Method 3114 B (2009) or 3114 C (2009)	Hydride generation atomic absorption spectrometry (HG_AAS)	2 µg/L
EPA Method 200.8 Rev 5.4 (1998)	Inductively coupled plasma mass spectrometry (ICP-MS) Ion Monitoring Mode	2.1 µg/L
EPA Method 200.9, Rev 2.2 (1994)	Stabilized temperature graphite furnace atomic absorption (STGF-AA)	0.6 µg/L

Data Quality

Analytical determinations must be conducted by a qualified analytical service laboratory with National Environmental Laboratory Accreditation Program (NELAP) certification for metals determination on biological tissues and water samples. To ensure comparability of data for IDEM's DQA 3 uses, organizations are encouraged to use the same analytical methods that the Office of Water Quality (OWQ) uses for selenium in fish tissue (EPA Method 6020A), moisture content in fish tissue (ASTM Method 1995) and selenium in water column (EPA Method 200.9). Information on these methods, including their associated quantitation limits, are provided in OWQ's Watershed Assessment and Planning Branch QAPP (IDEM 2023a). Laboratory analytical procedures should consist of quality control checks to ensure the quality of data meets DQA Level 3 as described in Section 6 of the *Technical Guidance for the Office of Water Quality External Data Framework (EDF)* (IDEM 2024).

Data Reporting

Field data measurements should be recorded on a field data sheet (Appendix D) and submitted with the final report.

All data submitted will be required to fulfill DQA Level 3 criteria as outlined in the *Technical Guidance for the Office of Water Quality External Data Framework (EDF)* (IDEM 2024). Data will be submitted to the EDF through a secondary portal which allows submissions from external sources. The portal facilitates external sources to utilize

either online entry or templates via Microsoft (MS) Excel which allow users to structure the collected data prior to submittal. Data submittals to the EDF should be submitted on an annual basis for projects extending through multiple years. Once data is submitted, it will follow the same data quality assessment process used to validate data collected within any of the OWQ programs. This process will ensure that all necessary documentation has been provided and data meets criteria for a specific data quality assessment level assigned.

Data Analysis for the Empirical BAF Approach

IDEM will use mean water column and composite (or a measure of central tendency of the appropriate number of individual fish, as applicable) fish tissue sampling data from the Target Molluscivorous Species or from the species known to bioaccumulate selenium more rapidly or are particularly sensitive to the effects of selenium at a site, to calculate BAFs using Equation 1, and the target water column criterion element using Equation 2 (USEPA 2000b). IDEM will use best professional judgement in selecting the fish tissue concentration used in the BAF calculation, in order to be protective of fish most susceptible to the effects of selenium bioaccumulation. The fish tissue criterion element used in Equation 2 will be the same tissue type that was collected to calculate the BAF.

Molluscivorous fish bioaccumulate selenium more rapidly than other assemblages, often including fish more sensitive to the effects of selenium. If molluscivorous species are not present at the site, IDEM will calculate the BAF using species present at the site. As a protective factor for the aquatic life assemblage at the site, IDEM may apply a ten percent uncertainty factor to the target water column concentration. If target molluscivorous or target sensitive species are not at the site, application of an additional protective factor(s) may be warranted. IDEM needs to approve a fish sampling plan before fish tissue sampling occurs. IDEM will use best professional judgement in determining which target fish species and/or protective factor needs to be used if molluscivorous or target sensitive species are not at the site.

Equation 1

Where:

$$BAF = \frac{C_{tissue}}{C_{water}}$$

BAF = bioaccumulation factor derived from site-specific field-collected samples of tissue and water (L/kg)
C tissue = concentration of selenium in field collected fish tissue (mg/kg dw)
C water = ambient concentration of selenium in water (mg/L)

Equation 2

$$C_{target} = \frac{C_{tissue\ criterion\ element}}{BAF}$$

Where:

C_{target} = translated site-specific water column criterion element (mg/l)
$C_{tissue\ criterion\ element}$ = tissue criterion element (mg Se/kg dw)
BAF = bioaccumulation factor derived from site-specific field-collected samples of tissue and water (L/kg)

Below is an example of the derivation of a site-specific water criterion element for a waterbody impacted by selenium using fish tissue data from the target molluscivorous species, Freshwater drum (*Aplodinotus grunniens).*

Mean site-specific selenium fish muscle tissue concentration (Freshwater drum, mg/kg, dw)	9.0
Selenium fish muscle tissue criterion (mg/kg, dw)	11.3
Mean ambient selenium water column concentration (mg/L)	0.002
Site-specific target water column criterion element concentration (mg/L)	X

Equation 1

Where:

$$\boldsymbol{BAF = \frac{C_{tissue}}{C_{water}}}$$

$$\boldsymbol{= \frac{9.0\,\frac{mg}{kg}\,dw}{0.002\,\frac{mg}{L}} = 4,500\,\frac{L}{kg}}$$

Equation 2

$$\boldsymbol{C_{target} = \frac{C_{tissue\ criterion\ element}}{BAF} = \frac{11.3\,\frac{mg}{kg}\,dw}{4500\,\frac{L}{kg}} = 0.002511}$$

Site-specific water column criterion element concentration is **0.0025 mg/L (2.5 µg/L).**

Below is an example of the derivation of a site-specific water column criterion for a waterbody impacted by selenium when target molluscivorous species are not present, but are expected to be present, at the site, using fish tissue data from Bluegill (*Lepomis macrochirus),* a species that is sensitive to selenium.

Mean site-specific selenium whole fish tissue concentration (Bluegill, mg/kg, dw)	6.0
Selenium whole fish tissue criterion (mg/kg, dw)	8.5
Mean ambient selenium water column concentration (mg/L)	0.003
Site-specific target water column criterion element concentration (mg/L)	X

Equation 1

$$\boldsymbol{BAF = \frac{C_{tissue}}{C_{water}}}$$

$$= \frac{6.0\ \frac{mg}{kg}dw}{0.003\ \frac{mg}{L}} = 2{,}000\ \frac{L}{kg}$$

Equation 2

$$C_{target} = \frac{C_{tissue\ criterion\ element}}{BAF} = \frac{8.5\ \frac{mg}{kg}dw}{2{,}000\ \frac{L}{kg}} = 0.00425\frac{mg}{l}$$

Since the fish tissue data are not representative of target molluscivorous species, applying a protective factor of 10 percent to the site-specific water column criterion element (C target) is warranted.

Equation 3

$$C_{target\ (protective)} = \left[C_{target} - \left(C_{target}\ X\ 0.1\right)\right] =$$

$$\left(0.00425\frac{mg}{l} - 0.000425\frac{mg}{l}\right) = 0.003825\frac{mg}{l}$$

Applying a protective factor of 10 percent will result in a site-specific protective target water column criterion concentration for this paired data of **0.0038 mg/l or 3.8 µg/L**

References

ASTM. 1995. ASTM D 2974-87: Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Other Organic Soils. American Society for Testing and Materials, Philadelphia, PA. http://gsrpdf.lib.msu.edu/ticpdf.py?file=/1990s/1993/930331.pdf

Blatchley, W.S. 1938. The Fishes of Indiana. The Nature Publishing Co. Indianapolis, IN. USA. 121pp.

Heidinger, R.C. 1976. FAO Fish. Synop.No.115. Synopsis of biological data on the largemouth bass *Micropterus salmoides* (Lacepédes) 1802. 85pp.

IDEM. 2023a. Quality Assurance Project Plan (QAPP) for Indiana Surface Waters, Revision 5. B-001-OWQ-WAP-XX-23-Q-R5. Indiana Department of Environmental Management, Office of Water Quality,2 Assessment Branch, Indianapolis, Indiana.

IDEM. 2023b. Fish Community Field Collection Procedures. B-009-OWQ-WAP-XXX-23-T-R1. Office of Water Quality, Watershed Assessment and Planning Branch. Indianapolis, Indiana.

IDEM. 2024. Technical Guidance for the IDEM Office of Water Quality External Data Framework. Indianapolis, Indiana. https://ecm.idem.in.gov/cs/idcplg?IdcService=GET_FILE&dID=83720107&dDocName=83724150&Rendition=web&allowInterrupt=1&noSaveAs=1

IDNR. 2019. Scientific Collectors/Purposes License. https://in.gov/dnr/fishwild/7671.htm

Kay, L. K., R. Wallus, and B.L. Yeager. 1994. Reproductive biology and early life history if the fishes in the Ohio River drainage. Volume 2: Catostomidae. Tennessee Valley Authority, Chattanooga, Tennessee, USA.

Kottelat, M. and J. Freyhof, 2007. Handbook of European freshwater fishes. Publications Kottelat, Cornol and Freyhof, Berlin. 646 pp.

Spotte, S. 2007. Bluegills: biology and behavior. American Fisheries Society. Bethesda, Maryland.

Trautman, M.B. 1981. The Fishes of Ohio. 2nd ed. Ohio State University Press. xxvi + 782pp.

United States Power Squadron (U.S. PS). State of Indiana: State-Specific Boating

Safety Requirements 2017. https://www.usps.org/e_stuff/documents/in_boating_safety_requirements.pdf.

USEPA. 1990. Method 200.8: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma – Mass Spectrometry, Revision 5.4. Washington, DC. https://www.epa.gov/sites/production/files/2015-06/documents/epa-200.8.pdf

USEPA. 1991. Technical Support Document for Water Quality Based Toxics Control. EPA/505/2-90-001 March 1991. http://www.epa.gov/npdes/pubs/owm0264.pdf.

USEPA. 1993. Method 6020A: Inductively Coupled Plasma-Mass Spectrometry. U.S. Environmental Protection Agency. February 2007, Washington, DC. http://www.epa.gov/wastes/hazard/testmethods/sw846/pdfs/6020a.pdf

USEPA. 2000a. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1, Fish Sampling and Analysis Third Edition. U.S. Environmental Protection Agency. Washington, DC. https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=20003OMP.txt

USEPA. 2000b. Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health. Technical Support Document Volume 3: Development of Site-Specific Bioaccumulation Factors. EPA 822-R-09-008. Office of Water. Washington, D.C.

USEPA. 2007a. Method 6020A: Inductively Coupled Plasma-Mass Spectrometry. U.S. Environmental Protection Agency. February 2007, Washington, DC. http://www.epa.gov/wastes/hazard/testmethods/sw846/pdfs/6020a.pdf

USEPA. 2021. 2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium- Freshwater 2016. EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington DC.

USEPA. 2024a. Technical Support for Adopting and Implementing the EPA's 2016 Selenium criterion in Water Quality Standards. U.S. Environmental Protection Agency, Office of Water, Washington DC.

USEPA. 2024b. Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion. U.S. Environmental Protection Agency, Office of Water, Washington DC.

USEPA. 2024c. Frequently Asked Questions: Implementing Water Quality Standards Based on the EPA's 2016 Selenium Criterion in Clean Water Act Section 402 NPDES Permits. U.S. Environmental Protection Agency, Office of Water, Washington DC.

USEPA. 2024d. Frequently Asked Questions: Implementing the EPA's 2016 Selenium Criterion in Clean Water Act Sections 303(d) and 305(b) Assessment, Listing, and Total Maximum Daily Load Programs. EPA-820-R-24-004. U.S. Environmental Protection Agency, Office of Water, Washington DC.

USGS. 2016. Techniques of Water-Resources Investigations of the United States Geological Survey. U.S. Geological Survey. December 2016, Washington, DC. https://pubs.usgs.gov/twri/index090905.html

Wallus, R. and T.P. Simon. 2004. Reproductive biology and early life history of fishes in the Ohio River drainage. Volume 3: Ictaluridae – Catfish and Madtoms. CRC Press, Boca Raton, Florida, USA.

Wallus, R. and T.P. Simon, T. P. 2006. Reproductive biology and early life history if the fishes in the Ohio River drainage. Volume 5: Aphredoderidae through Cottidae, Moronidae, and Sciaenidae. CRC Press, Boca Raton, Florida, USA.

Wallus, R. and T.P. Simon, T. P. 2008. Reproductive biology and early life history if the fishes in the Ohio River drainage. Volume 6: Elassomatidae and Centrarchidae. CRC Press, Boca Raton, Florida, USA.

Zale, A., D. Parrish and T. M. Sutton, editors. 2013. Fisheries Techniques, 3rd edition. American Fisheries Society. ISBN: 978-1-934874-29-5, 1,009 pages.

Appendix A

Indiana Surface Water Quality Aquatic Life Criteria for Selenium

Guidance for the Collection of Fish Tissue and/or Water Column Data for Implementation of Indiana's Selenium Chronic Aquatic Life Criteria

Indiana Department of Environmental Management
Office of Water Quality November 2024

Surface Water Quality Aquatic Life Criterion for Selenium for Acipenseriformes (Sturgeon and Paddlesfish) Waters in Indiana

Media Type	Fish Tissue[1]		Water Column[4]	
Criterion Element	Egg-ovary[2]	Fish Whole-body or Muscle[3]	Monthly Average Exposure	Intermittent Exposure[5]
Magnitude	15.1 mg/kg dry weight	8.5 mg/kg dry weight whole-body or 11.3 mg/kg dry weight muscle (skinless, boneless fillet)	1.5 µg/l in lentic aquatic systems 3.1 µg/l in lotic aquatic systems	$WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1 - f_{int})}{f_{int}}$
Duration	Instantaneous measurement[6]	Instantaneous measurement[6]	30 days	Number of days/month with an elevated concentration
Frequency	Not to be exceeded	Not to be exceeded	Not more than once in three years on average	Not more than once in three years on average

1. Fish tissue elements are expressed as steady-state.
2. Egg-ovary supersedes any whole-body, muscle, or water column element when fish egg-ovary concentrations are measured, except as noted in footnote 4 below.
3. Fish whole-body or muscle tissue supersedes water column element when both fish tissue and water concentrations are measured, except as noted in footnote 4 below.
4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. When selenium inputs are increasing, water column values are the applicable criterion element in the absence of steady-state condition fish tissue data.
5. Where $WQC_{30\text{-}day}$ is the water column monthly element for either lentic or lotic waters; C_{bkgrnd} is the average background selenium concentration; and f_{int} is the fraction of any 30-dya period during which elevated selenium concentrations occur, with f_{int} assigned a value ≥ 0.033 (corresponding to 1 day).
6. Fish tissue data provide instantaneous point measurements that reflect integrative accumulation of selenium over time and space in fish population(s) at a given site.

Guidance for the Collection of Fish Tissue and/or Water Column Data for Implementation of Indiana's Selenium Chronic Aquatic Life Criteria

Indiana Department of Environmental Management
Office of Water Quality November 2024

Surface Water Quality Aquatic Life Criterion for Selenium in Non-Acipenseriformes (No Sturgeon or Paddlefish) Waters Outside of the Great Lakes System

327 IAC 2-1-6

Media Type	Fish Tissue[1]		Water Column[4]	
Criterion Element	Egg-ovary[2]	Fish Whole-body or Muscle[3]	Monthly Average Exposure	Intermittent Exposure[5]
Magnitude	19.0 mg/kg dry weight	9.5 mg/kg dry weight whole-body or 13.1 mg/kg dry weight muscle (skinless, boneless fillet)	2.7 µg/l in lentic aquatic systems 5.5 µg/l in lotic aquatic systems	$WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1 - f_{int})}{f_{int}}$
Duration	Instantaneous measurement[6]	Instantaneous measurement[6]	30 days	Number of days/month with an elevated concentration
Frequency	Not to be exceeded	Not to be exceeded	Not more than once in three years on average	Not more than once in three years on average

1. Fish tissue elements are expressed as steady-state.
2. Egg-ovary supersedes any whole-body, muscle, or water column element when fish egg-ovary concentrations are measured, except as noted in footnote 4 below.
3. Fish whole-body or muscle tissue supersedes water column element when both fish tissue and water concentrations are measured, except as noted in footnote 4 below.
4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. When selenium inputs are increasing, water column values are the applicable criterion element in the absence of steady-state condition fish tissue data.
5. Where $WQC_{30\text{-}day}$ is the water column monthly element for either lentic or lotic waters; C_{bkgrnd} is the average background selenium concentration; and f_{int} is the fraction of any 30-dya period during which elevated selenium concentrations occur, with f_{int} assigned a value ≥ 0.033 (corresponding to 1 day).
6. Fish tissue data provide instantaneous point measurements that reflect integrative accumulation of selenium over time and space in fish population(s) at a given site.

Appendix B

Translating the Concentration of Selenium in Tissue to a Concentration in Water Using Bioaccumulation Factors (BAF) from Appendix K of the 2016 U.S. EPA NRWQC for Selenium in Freshwater

2.0 Translating the Concentration of Selenium in Tissue to a Concentration in Water using Bioaccumulation Factors (BAF)

2.1 Summary of the BAF Approach

A bioaccumulation factor (BAF) is the ratio (in milligrams/kilogram per milligrams/liter, or liters per kilogram) of the concentration of a chemical in the tissue of an aquatic organism to the concentration of the chemical dissolved in ambient water at the site of sampling (U.S. EPA 2001c). BAFs are used to relate chemical concentrations in aquatic organisms to concentrations in the ambient media of aquatic ecosystems where both the organism and its food are exposed and the ratio does not change substantially over time. The BAF is expressed mathematically as:

$$BAF = \frac{C_{tissue}}{C_{water}} \qquad \textit{(Equation K-8)}$$

Where:

BAF	=	bioaccumulation factor derived from site-specific field-collected samples of tissue and water (L/kg)
C_{tissue}	=	concentration of chemical in fish tissue (mg/kg)
C_{water}	=	ambient concentration of chemical in water (mg/L)

The site-specific BAF can then be applied to the tissue criterion to solve for a target site-specific water column criterion (C_{target}):

$$C_{target} \times \frac{C_{egg-ovary\ criterion}}{BAF} \qquad \textit{(Equation K-9)}$$

Where:

C_{target}	=	site-specific water criterion concentration (mg/L)
$C_{egg\text{-}ovary\ criterion}$	=	national egg-ovary tissue criterion (15.1 mg Se/kg dw)
BAF	=	bioaccumulation factor derived from site-specific field-collected samples of tissue and water (L/kg)

To translate a fish tissue criterion to a water concentration value, a site-specific, field-measured BAF for the waterbody could be developed, and then a water concentration criterion could be calculated using Equation K-9. Detailed information about how to derive a site-specific, field-measured BAF is provided in *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000) Technical Support Document Volume 3: Development of Site-specific Bioaccumulation*

Factors (U.S. EPA 2009). Although this guidance was developed for deriving human health criteria, the methodological approach is also applicable to the derivation of aquatic life criteria. The following example illustrates the calculation of a site specific water column criterion using the BAF approach.

2.1.1 Example: Derivation of a site specific water column criterion for a waterbody impacted by selenium

Available data for a hypothetical site indicate that the average egg/ovary tissue concentration of selenium for the bluegill (*Lepomis macrochirus*) is 22 mg/kg (dw). This concentration exceeds the USEPA proposed egg/ovary criterion of 15.1 mg/kg (dw). The ambient selenium water column concentration at that hypothetical site is 4.0 µg/L. The following calculation shows how to derive a target water column that would achieve a site-specific criterion using the bioaccumulation factor (BAF) approach.

Site specific selenium egg/ovary concentration (bluegill; mg/kg dw)	22.0
Selenium egg/ovary criterion (mg/kg, dw)	15.1
Ambient selenium water column concentration (µg/L)	4.0
Target water column concentration (µg/L)	X

Set up proportional equation to solve for allowable water column concentration:

$$\frac{Site\ specific\ egg/ovary\ conc.\ (\frac{mg\ Se}{kg\ dw})}{Site\ specific\ water\ concentration\ (\frac{\mu g\ Se}{L})} = \frac{Criterion\ egg\ ovary\ conc.\ (\frac{mg\ Se}{kg\ dw})}{Target\ water\ concentration\ (\frac{\mu g\ Se}{L})}$$

Solve for the target water concentration that will achieve a site-specific criterion:

$$\frac{22.0\ (\frac{mg\ Se}{kg\ dw})}{4.0\ (\frac{\mu g\ Se}{L})} = \frac{15.1\ (\frac{mg\ Se}{kg\ dw})}{Target\ water\ concentration\ (\frac{\mu g\ Se}{L})}$$

Target water concentration = 2.75 µg/L.

2.2 Managing Uncertainty using the BAF Approach

Uncertainty can be introduced when using the BAF approach to derive a water concentration value from a fish tissue criterion concentration. Inaccurate water concentration values can result when BAFs are derived from water and fish tissue concentration measurements that are obtained from sources that do not closely represent site characteristics, or from field data collected from large-scale sites that encompass multiple water bodies or ecosystems. Most of this uncertainty results from differences in the

bioavailability of selenium between the study sites where measurements are made to derive the BAF, and the site(s) to which the BAF is used to derive needed water concentration values.

Because of uncertainties associated with the BAF approach, EPA does not recommend developing BAFs from data extrapolated from different sites or across large spatial scales. EPA's Framework for Metals Risk Assessment (U.S. EPA 2007) outlines key principles about metals and describes how they should be considered in conducting human health and ecological risk assessments due the the effects of water chemistry on bioavilability of such chemicals. The current science does not support the use of a single, generic threshold BAF value as an indicator of metal bioaccumulation. The use of BAFs are appropriate only for site-specific applications where sufficient measurements have been taken from the site of interest and there is little or no extrapolation of BAF values across differing exposure conditions and species.

The preferred approach for using a BAF to implement the selenium fish tissue criterion is to calculate a site-specific, field-measured BAF from data gathered at the site of interest, and to apply that BAF to that site. A site-specific, field-measured BAF is a direct measure of bioaccumulation in an aquatic system because the data are collected from the aquatic ecosystem itself and thus reflects real-world exposure through all relevant exposure routes. A site-specific, field-measured BAF also reflects biotic and abiotic factors that influence the bioavailability, biomagnification, metabolism, and biogeochemical cycling of selenium that might affect bioaccumulation in the aquatic organism or its food web. Appropriately developed site-specific, field-measured BAFs are appropriate for all bioaccumulative chemicals, regardless of the extent of chemical metabolism in biota from a site (U.S. EPA 2000).

Although a site-specific, field-measured BAF is a direct measure of bioaccumulation, its predictive power depends on a number of important factors being properly addressed in the design of the field sampling effort. For example, sampling in areas with relatively long water residence times should be a priority because selenium bioaccumulation occurs more readily in aquatic systems with longer residence times (such as wetlands, oxbows, and estuaries) and with fine particulate sediments high in organic carbon. In addition, migratory species should generally not be used because their exposure to selenium could reflect selenium concentrations in areas other than where the fish were caught. Fish may also need to be sampled and BAF values recalculated if selenium levels significantly change over time because BAFs are known to be affected by the ambient concentration of the metals in the aquatic environment (McGeer et al. 2003; Borgman et al. 2004; DeForest et al. 2007). States and tribes should refer to *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000) Technical Support Document Volume* (U.S. EPA 2009) for guidance on appropriate methods for developing a site-specific, field-derive BAF.

The advantage of using the BAF approach is its relative simplicity, especially when the data necessary to derive the BAF is already available. Furthermore, the BAF approach is completely empirical and does not require any specific knowledge about the physical, chemical, or biological characteristics of the waterbody. The relationship between the concentration of selenium in fish tissue and water is directly determined by direct measurements in these media. This may be advantageous when there are uncertainties with how to collect a particulate sample that is representative of the base of the food web, or dilution concerns (e.g., sandy streams with little surface area for algae sampling and high potential for sand contamination of a benthic sediment sample).

Limitations of the BAF approach should be considered before deciding if this method is appropriate for translating the selenium FCV to a water concentration value. One disadvantage of the BAF approach is the considerable effort and resources necessary to collect sufficient data to establish the relationship between tissue and water concentrations. Resource use increases as the spatial scale and complexity of the aquatic system increases. Furthermore, the BAF approach does not allow extrapolation across species, space, and large time scales because the site-specific factors that might influence bioaccumulation are integrated within the tissue concentration measurements and thus cannot be individually adjusted to extrapolate to other conditions. Thus, site-specific, field-measured BAFs only provide an accounting of the uptake and accumulation of selenium for an organism at a specific site and point in time. This is more important in lotic habitats, since the kinetics of selenium bioaccumulation may be very different at a site upstream or downstream from the site of interest.

As noted previously, NPDES permitting regulations at 40 CFR § 122.45(c) require WQBELs for metals be expressed as total recoverable metal unless an exception is met under 40 CFR § 122.45(c)(1)-(3). Guidance for converting expression of metals in water from dissolved to total recoverable can be found in *Technical Support Document for Water Quality-based Toxics Control* (U.S. EPA 1991) and *The Metals Translator: Guidance for Calculating a Total Recoverable Permit Limit from a Dissolved Criterion* (U.S. EPA 1996). Whether or not a water concentration value derived from a site-specific, field-derived BAF requires conversion from dissolved to total recoverable selenium depends on how the BAF is developed. Generally, conversion would not be necessary if the BAF is derived from water concentration values that measure total selenium; however, conversion would be necessary if the BAF was derived from water concentration values that measured dissolved selenium. Table K-4 compares some of the principle characteristics of the mechanistic bioaccumulation modeling approach or the BAF approach for translating the selenium FCV to a water concentration.

Appendix C
Egg and Ovary Sample Preparation Guidance from EPA's Technical Support Document on Fish Tissue Collection April 2024

Appendix C: Egg and Ovary Sample Preparation

Scope

This guidance is for egg and ovary collection from freshwater fish. The egg extraction method is excerpted and adapted from a more comprehensive guidance, *Standard operating procedure for evaluating selenium-induced deformities in early life stages of freshwater fish* (Janz and Muscatello 2008), that includes gamete collection, embryo incubations and evaluation of selenium-induced deformities in freshwater fish. The ovary dissection method was compiled from peer-reviewed literature.

1) Field collection and handling of adult fish

> "Spawning adults can be collected in the field using a wide variety of techniques, including fish traps (e.g., hoop or trap nets), electrofishing or angling in areas close to spawning areas. Gillnets are also effective in capturing fish during spawning migrations, but it is essential to monitor these nets constantly to remove fish immediately after capture. If possible, the use of passive capture methods (e.g., hoop or trap nets) is recommended since this is the least stressful capture technique of those listed above. Trap nets are usually set up in creeks, streams or narrows in lakes, although successful fish capture can also occur when these nets are set perpendicular to shore in lentic habitats. Trap or hoop nets can be purchased from fisheries suppliers, or even constructed in creeks and streams using chicken wire, baling wire and reinforcing bar." (Janz and Muscatello 2008)

Fish should be held in livewells until adult female fish are selected for egg collection.

2) Egg collection procedures

Fish should be carefully observed for signs of physical damage, mortality, or other sources of stress. Since any handling of the fish will remove the protective body layer of slime, fish should be handled as little as possible using dip nets and soft material gloves. Adult fish for egg collection should be randomly selected from livewells.

> "Eggs should not be in contact with water; thus, it is imperative to dry the area surrounding the urogenital opening with paper towels. All the material used for egg collection should be carefully cleaned and dried. Precautions to avoid fecal, blood or urine contamination should be taken. [Eggs] must be kept covered to avoid direct sun exposure. [Egg collection] should proceed after recording weight and length [of the gravid female]. Gentle pressure from behind the pectoral fins towards the anus is applied to

> be repeated several times. Check that eggs are released 'clean' (e.g., without

> feces) before starting collection to avoid contamination of the entire egg batch. Eggs are individually collected into pre-cleaned stainless steel bowls and kept covered in a cool place until use. Collected eggs should be closely inspected and eggs with adhered feces, urine or blood discarded by using a clean plastic pipette." (Janz and Muscatello 2008)

Eggs are then weighed to the nearest gram using a top-loading digital scale, frozen for storage, and shipped for laboratory analysis when appropriate. An individual or composite homogenate tissue sample of 20 grams ww should be collected for analysis of selenium.

3) Ovary dissection procedures

Fish designated for ovary collection should be humanely euthanized, and necropsy procedures should commence immediately following euthanasia (Wolf et al. 2004). The fish should be placed in right lateral recumbency on a piece of aluminum foil. The left body wall should be removed by using fine dissecting instruments (Wolf et al. 2004). To identify female specimens for ovary collection, sex is determined by macroscopic inspection when the body cavity is opened. The ovaries are paired organs suspended from the dorsal wall, with color ranging from clear to white to yellow-orange. A yellow-orange color is indicative of a ripening or ripe adult specimen. Further, increased blood flow during the reproductive season causes the ovaries to become highly vascularized and appear reddish. In cross-section, the ovaries are round to elliptical and contain a central cavity (lumen). In young fish, the texture of the ovaries varies from smooth to slightly granular. The ovarian texture in a ripe fish will be highly granular (Fisheries Information Network 2006). If inspection of the ovaries reveals that the specimen is immature or developing, it is not recommended that the eggs/ovarian tissue be used for tissue monitoring for selenium.

After confirmation that the specimen is a ripe female, the ovaries should be excised by severing the oviducts and mesenteric attachments. All gonads are dissected in a caudal to cranial direction (Wolf et al. 2004). Ovaries are then weighed to the nearest gram using a top-loading digital scale, frozen for storage, and shipped for laboratory analysis when appropriate (Orr et al. 2012). An individual or composite homogenate sample of 20 grams ww of tissue should be collected for analysis of selenium.

4) Storing fish eggs and ovaries

"Eggs and ovaries should be kept frozen until analysis. After collection, samples should be kept in a container with ice or freezer packs until transfer to a freezer (–20°C) for storage" (Janz and Muscatello 2008). It is recommended to transfer the samples collected from each individual female into sealed resealable plastic storage bags to "prevent water (from ice melting) entering the sample" (Janz and Muscatello 2008). Recommendations for the storage, preservation and holding time for egg and ovary samples are equivalent to other tissue samples. Samples should be frozen at –20°C in plastic, borosilicate glass, quartz, or PTFE bottles. The recommended maximum holding time is six months but can be up to two years for most trace metals, including selenium (USEPA 2000).

5) Laboratory preparation of egg and tissue samples for metal analysis

"Egg and tissue samples should be thawed, and wet weight recorded for each individual sample. To prevent cross contamination between samples, a plastic foil (e.g., parafilm®) should be placed on the scale and replaced after each weighing. Samples are oven dried at 60°C until constant weight is recorded. It is required to record the moisture content for each individual sample in order to express analytical data on a dry weight basis. Trace element (e.g., selenium) analysis is routinely performed using hydride generation atomic absorption spectrophotometry (HG-AAS) or inductively coupled plasma-mass spectrometry (ICP-MS) and reported on a dry-weight basis." (Janz and Muscatello 2008)

Appendix D

Examples of Data Reporting Forms

Field Record for Biological Tissue Contaminant Monitoring Program

Site ID:_____ Sampling Date and Time:_____
Sample ID:_____ (mm/dd/yyyy) (24hr clock)

SITE LOCATION
Waterbody Name:_____
County_____Fipscode_____Lat./Long.:_____
Location:_____
Waterbody Type: RIVER LAKE RESERVOIR WETLAND
Site Description:_____
_____
Collection Method:_____
Collector's Name(s):_____
Agency:_____Phone:(____) _____

FISH (or other organism) COLLECTED

Composite Sample #:_____Number of Individuals:_____ Lab ID _____
Species Name:_____
Sample Preparation: SKIN-ON SCALELESS SKIN-OFF WHOLE OTHER:_____

Fish#	Length(mm)	Weight(gm)	Sex(M,F)	Fish#	Length(mm)	Weight(gm)	Sex(M,F)
001	_____	_____	_____	007	_____	_____	_____
002	_____	_____	_____	008	_____	_____	_____
003	_____	_____	_____	009	_____	_____	_____
004	_____	_____	_____	010	_____	_____	_____
005	_____	_____	_____	011	_____	_____	_____
006	_____	_____	_____	012	_____	_____	_____

(min length/max length)x 100 = _____% **Composite mean length _____ mm**
(min wt/max wt)x 100= _____% **Composite mean weight_____ gm**
Notes (e.g., DELT anomalies)_____
_____

Composite Sample #:_____Number of Individuals:_____ Lab ID _____
Species Name:_____
Sample Preparation: SKIN-ON SCALELESS SKIN-OFF WHOLE OTHER:_____

Fish#	Length(mm)	Weight(gm)	Sex(M,F)	Fish#	Length(mm)	Weight(gm)	Sex(M,F)
001	_____	_____	_____	007	_____	_____	_____
002	_____	_____	_____	008	_____	_____	_____
003	_____	_____	_____	009	_____	_____	_____
004	_____	_____	_____	010	_____	_____	_____
005	_____	_____	_____	011	_____	_____	_____
006	_____	_____	_____	012	_____	_____	_____

(min length/max length)x 100 = _____% **Composite mean length _____ mm**
(min wt/max wt)x 100= _____% **Composite mean weight_____ gm**
Notes (e.g., DELT anomalies)_____
_____

Composite Sample #:________________________Number of Individuals:__________ Lab ID ________________
Species Name:____
Sample Preparation: SKIN-ON SCALELESS SKIN-OFF WHOLE OTHER:__________________

Fish#	Length(mm)	Weight(gm)	Sex(M,F)	Fish#	Length(mm)	Weight(gm)	Sex(M,F)
001				007			
002				008			
003				009			
004				010			
005				011			
006				012			

(min length/max length)x 100 = _________% **Composite mean length __________ mm**
(min wt/max wt)x 100= _________% **Composite mean weight__________ gm**
Notes (e.g., DELT anomalies)____
____

Composite Sample #:________________________Number of Individuals:__________ Lab ID ________________
Species Name:____
Sample Preparation: SKIN-ON SCALELESS SKIN-OFF WHOLE OTHER:__________________

Fish#	Length(mm)	Weight(gm)	Sex(M,F)	Fish#	Length(mm)	Weight(gm)	Sex(M,F)
001				007			
002				008			
003				009			
004				010			
005				011			
006				012			

(min length/max length)x 100 = _________% **Composite mean length __________ mm**
(min wt/max wt)x 100= _________% **Composite mean weight__________ gm**
Notes (e.g., DELT anomalies)____
____

Composite Sample #:________________________Number of Individuals:__________ Lab ID ________________
Species Name:____
Sample Preparation: SKIN-ON SCALELESS SKIN-OFF WHOLE OTHER:__________________

Fish#	Length(mm)	Weight(gm)	Sex(M,F)	Fish#	Length(mm)	Weight(gm)	Sex(M,F)
001				007			
002				008			
003				009			
004				010			
005				011			
006				012			

(min length/max length)x 100 = _________% **Composite mean length __________ mm**
(min wt/max wt)x 100= _________% **Composite mean weight__________ gm**
Notes (e.g., DELT anomalies)____
____

CATCH SUMMARY **Station #**:

Date:______________ Time:______ County:________________________Collectors:

Waterbody:________________________ Gear Type: BP BT SC Seconds Fished: __________

Shore: RDB LDB RDB LDB RDB LDB RDB LDB

	100 m				**200 m**				**300 m**				**400 m**			
Species/Code	Min	Max	Wt	N	Min	Max	Wt	N	Min	Max	Wt	N	Min	Max	Wt	N

CATCH SUMMARY **Station #:**

Date:______________ Time:______ County:________________________ Collectors:

Waterbody:________________________ Gear Type: BP BT SC Seconds Fished: __________

Shore: RDB LDB RDB LDB RDB LDB RDB LDB

	500 m				600 m				700 m				800 m			
Species/Code	Min	Max	Wt	N	Min	Max	Wt	N	Min	Max	Wt	N	Min	Max	Wt	N

CATCH SUMMARY **Station #**:

Date:______________ Time:_____ County:_______________________Collectors:

Waterbody:______________________ Gear Type: BP BT SC Seconds Fished: __________

Shore

	900 m				1000 m				TOTAL							
Species/Code	Min	Max	Wt	N	Min	Max	Wt	N	Min	Max	Wt	N				

Appendix E

Fish Collection Sampling Methods Characteristics

Guidance for the Collection of Fish Tissue and/or Water Column Data for Implementation of Indiana's Selenium Chronic Aquatic Life Criteria

Indiana Department of Environmental Management
Office of Water Quality November 2024

Fish Collection Sampling Methods Characteristics[1]

	Sampler Type		
	A, B, C	D, E, F	G,H
Gear Used:	A: 17' boat B: 16' boat C: 12' or 14' boat	D: Canoe w/ rattail cathode E: Smith Root Tote Barge System w/ cathode plate F: Longline (150m extension cord)	G: Smith-Root 1.5 KVA w/ Longline (75m extension cord) H: Smith-Root Model LR- 20B or LR-24 backpack
Power Source:	A, B: EG 5000 X Honda Generator with a Smith Root type VI-A (17' or 16' boat) C: Briggs & Stratton 5 HP Generator, Smith Root GPP 2.5 portable electrofisher (RCB-6B Junction Box) in 12' or 14' boat	D, E, and F: Briggs & Stratton 5 HP Generator, Smith Root GPP 2.5 portable electrofisher (RCB-6B Junction Box)	G: Honda EU2000iA generator H: 24V 7Ah battery with will run 40 minutes continuous at 100W
Current Type:	Pulsed DC	Pulsed DC	Pulsed DC
Wattage: (AC Power Source)	A,B: 5000 (17' or 16' boat) C: 2500 (12' or 14' boat)	2500	G:2000
Volts: (DC Output)	A,B: 0-1020, (suggest 340) C: 50-1000 (suggest 300)	50-1000 (suggest 300)	G: 0-560 H: 50-990 (suggest 100-300)
Amperage: (Output)	A,B: 3-6 C: 5	2-4	2-4
Anode Location:	A,B: Electrosphere on boom C: Electrosphere on boom (Large River) or Smith-Root dropper (river with fast current and/or non-wadeable pools)	Smith-Root teardrop, ring, or dropper anode	Smith-Root teardrop or ring anode
Number of Netters & Net Mesh Size:	A,B:2 people netting in the front of the boat with 1/8 inch nets C: 1 person with 1/8 inch net	2 people netting near anode with 1/8 inch nets	1-2 people netting near anode with 1/8 inch net

Sampling Direction:	Downstream and circling around to net fish behind boat (dependent on flow)	Upstream zigzag to collect from all habitats possible	Upstream zigzag to collect from all habitats possible
Stream Size:	A,B: large/great rivers C: Non-wadeable streams	Wadeable streams to headwater tributaries	Headwater tributaries
Sampling Period:	Two months after spawning October-November	Two months after spawning October-November	Two months after spawning October-November

A - Large/Great River
B - Large/Great River
C - Non-Wadeable River/Stream
D - Wadeable Rivers/Streams
E - Wadeable Rivers/Streams
F - Wadeable Rivers/Streams/Headwaters
G - Headwater Streams
H - Headwater Streams (water should be less than knee deep)

[1]Adapted from: IDEM. 2023b. Fish Community Field Collection Procedures. B-009-OWQ-WAP-XXX-23-T- R1. Office of Water Quality, Watershed Assessment and Planning Branch. Indianapolis, Indiana.