DRAFT Guidance for the Collection of Fish Tissue and/or Water Column Data for Implementation of Indiana's Selenium Chronic Aquatic Life Criteria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

IDEM Office of Water Quality 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 [INSERT DATE UPON ADOPTION] updated aquatic life chronic water quality criterion for selenium. Entities may elect to acquire these data to permit limits are needed, or to support derivation of site-specific water column criterion elements using a bioaccumulation factor (BAF) empirical modeling approach.

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. 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. 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 (U.S. EPA 2016).

The 2016 NRWQC selenium criterion 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.

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, 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 (U.S. EPA 2016).

On [DATE OF ADOPTION TO BE INSERTED], Indiana revised its Water Quality Standards (WQS) to include the 2016 NRWQC chronic criterion for selenium. Indiana's selenium aquatic life criterion (ALC) for waters outside of the Great Lakes system include two sets of criterion elements: the 2016 NRWQC criterion elements, and 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's selenium

ALC for waters within the Great Lakes system include the 2016 NRWQC criterion elements, and do not include site-specific criterion elements.

Indiana's selenium ALC also includes a provision for deriving site-specific water column criterion elements using the BAF approach, as described in Appendix K of the NRWQC for selenium (U.S. EPA, 2016). U.S. EPA proposed guidance for translating selenium fish tissue criterion elements to a site-specific water column criterion element, including for the BAF empirical modeling approach (U.S. EPA 2018b) concurrent with their proposed rule to revise the current Clean Water Act selenium water quality criterion applicable to certain fresh waters of California (U.S. EPA 2018a). A summary of the BAF empirical modeling approach is attached in Appendix A. Indiana's WQS ALC are included in Appendix B.

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 limits ("WQBELs") for NPDES permit limits.[1] However, in some cases 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. 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 levels are below the applicable fish tissue criterion element.

This document contains the guidance necessary for collecting fish tissue and water column samples to investigate selenium in fish tissue and 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. 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 purpose. 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 dischargers), and do not apply when new or

[1] NPDES permit writers use a reasonable potential analysis to determine whether a Water Quality-Based Effluent Limit (WQBEL) or monitoring is required for a pollutant in a NPDES permit. They use this process to determine whether a discharge has the potential to cause or contribute to an excursion above an applicable water quality standard, using calculations and procedures outlined by U.S. EPA (U.S. EPA 2001).

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 (U.S. EPA 2016). 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 and for new discharges, IDEM will require a minimum duration of 12 months before fish tissue may be sampled to assess bioaccumulation in the resident fish population. IDEM will consider site-specific factors that could shorten or lengthen this estimated time frame.

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 (U.S. PS 2016). For electrofishing collection methods, all sampling crew members involved with electrofishing activities should review *Principles and Techniques of Electrofishing* (U.S. FWS 2018) and have at least one year of experience in sampling methodology and taxonomy of fish communities in the region.

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 outside the Great Lakes basin 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 2015, Section 7, IDEM 2015a).

Fish Tissue Data Collection

Fish Tissue Data Collection: Overview

Selenium bioaccumulates through the aquatic food chain and chronic exposure in fish can cause reproductive impairments (e.g., larval deformity or mortality). Fish egg-ovary concentration is the best indicator of potential adverse effects on fish reproduction, however, due to the potential reproductive failures that already exist in a waterbody, limited timeframes within a year, asynchronous spawning, insufficient sizes, and hazards in the field (e.g., high flows), IDEM allows the collection of whole body and muscle tissues for use in conducting a reasonable potential to exceed determination for a discharge outside the Great Lakes system or to develop a BAF to derive a site-specific water column criterion element. Since selenium concentrates in 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, since female fish transfer selenium to eggs during egg development. For whole fish and muscle tissue samples, U.S. EPA recommends, if possible, to identify in the field, sampling male fish rather than female fish (U.S. EPA 2018b).

See Table 1 for 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, and so on. In the case that Common Carp are collected as a target species, the eggs and/or ovaries may be collected for analysis.

When selecting target fish species for selenium criterion monitoring, entities must focus on species that may potentially accumulate high concentrations of selenium (molluscivorous species), are sensitive to selenium, and that are easy to identify. Selenium bioaccumulation potential and sensitivity in species does 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 used to conduct a reasonable potential to exceed determination or developing a BAF to derive a site-specific water column criterion element.

Do not collect fish species that are not listed in Table 1. IDEM will not consider or accept data collected for species not included in this table.

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

Species	Spawning Season	Media Type	Number of Fish/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	Egg/Ovary and/or Muscle /Fillet	3-5	≥ 13
White Sucker [4] (*Catostomus commersonii*)	April-May	Muscle/Fillet	3-5	≥ 10
Bullhead Genus [8] (*Ameiurus spp.*)	May-June	Muscle/Fillet	3-5	≥ 6
White Bass [5] (*Morone chrysops*)	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 spp.)*	May-Aug	Whole Body	3-5	≥ 4
Black Bass [2], [7] (*Micropterus spp.)*	April-July	Muscle/Fillet	3	≥ 10
Rock Bass [7] (*Ambloplites rupestris*)	May-July	Muscle/Fillet	3-5	≥ 5
Redhorse Genus [4] (*Moxostoma spp.)*	April-May	Muscle/Fillet	3-5	≥ 13
Carpsucker Genus [4] (*Carpiodes spp.)*	June-Sep	Muscle/Fillet	3-5	≥ 10
Buffalo Genus [4] (*Ictiobus spp.)*	April-May	Muscle/Fillet	3-5	≥ 17
Group 3 - Target Whole Body Composite Species				
Sunfish Genus [1], [7] *(Lepomis spp.)*	May-Aug	Whole Body	3-5	≥ 4
Most Prominent *Cyprinidae spp.* [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, [8] Wallus *et al*. 2008

The frequency and timing of sampling activities, and the location of sampling sites, will be reviewed to identify any bias that may exist and to evaluate the potential effect of said bias on OWQ decision-making (IDEM 2015).

In lotic systems the upper limit of the sample reach for fish collection should begin immediately below the effluent outfall. The sample reach extends downstream in designated sampling sub-reaches to a maximum distance; this distance is based on the drainage-based category of the lotic system (Table 2). Begin collecting fish from the sub-reach closest to the outfall. For a reasonable potential to exceed determination, collect fish only from the sub-reach closest to the outfall. When collecting fish tissue for a site-specific BAF, 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. While sampling, record the location coordinates for the upstream and downstream limits of each sampling sub-reach. For fish tissue samples, record the sub-reach where target fish tissue samples are collected.

Sampling should encompass all available habitat where the target species are likely to reside. For lotic systems less than 25 meters wide, collect samples of target species from both sides of the stream. For lotic systems greater than 25 meters wide, collect samples of target species on the same bank of the stream as the outfall. Confluence areas should be avoided whenever possible.

In lentic systems, sample the shoreline littoral habitats within 100 meters of either side of the outfall. 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. For lentic systems, record coordinates that bracket the sampling area.

Table 2. Maximum sampling reach and designated sub-reach lengths for collecting fish tissue samples for lotic waterbody categories.

Waterbody Category	Drainage Area (mile^2)	Maximum 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	250
Great River	>2000 mi^2 not wadeable	1000	250

Fish sampling will be conducted using standard electrofishing methods, gill nets or other appropriate catch method in accordance with *Fish Collection Sampling Methods,* the *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1, Fish Sampling and Analysis Third Edition* (U.S. EPA 2000, Section 6.2.1), or other appropriate catch method, per American Fisheries Society *Fisheries Techniques* (Zale *et al.* 2013). See Appendix D 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 (U.S. EPA 2000).

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 equal to 75 percent). The 75 percent rule does not apply to fish for egg-ovary samples. Fish collected for composite samples for a given sampling site should be collected within no more than one week of each other. Muscle plug or muscle tissue biopsy samples are not acceptable. Composite samples must be comprised of whole body or skin-off boneless fish fillets, which requires sacrificing fish.

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 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 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* (U.S. EPA 2000). Data record keeping for fish collections should follow Section 6.2.3.1 of *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1, Fish Sampling and Analysis Third Edition* (U.S. EPA 2000). 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.

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	Technique	Method Detection Limit
EPA Method 6010 C	Inductively Coupled Plasma – Atomic Emission Mass Spectroscopy	5 mg/kg
EPA Method 6020A	Inductively Coupled Plasma - Mass Spectrometry	0.2 mg/kg
EPA Method 7742	Atomic Absorption, Borohydride Reduction	0.05 mg/kg
USGS I-9020-05	Inductively Coupled Plasma - Mass Spectrometry	0.008 µg/g

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 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 (U.S. EPA 2016).

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 outside the Great Lakes basin, 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. Fish collection must occur within the first sub-reach immediately downgradient of the outfall for the permitted entity.

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 sub-reach, a protective factor might be warranted to protect downstream fish that bioaccumulate selenium at a faster rate than sensitive fish. If fish are not present in the receiving waterbody in the first sub-reach below the outfall, 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 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.

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 (U.S. EPA 2000a, Section 6.1.2.7). The requirements for determining whether fish are placed in acceptable replicate samples are described in Section 6.1.2.7 of *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories* (U.S. EPA 2000a).

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 (U.S. EPA 2000a). Based on this requirement, a total of six (6) composite samples will be collected for analysis from the closest sub-reach downstream of the outfall for the reasonable potential to exceed determination.

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.

For deriving a site-specific BAF, IDEM requires triplicate composite fish tissue samples from the Target Molluscivorous Species, triplicate composite fish tissue samples from Target Sensitive Species, and triplicate composite fish tissue samples from Target Whole Body Composite Species listed in Table 1. 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 length, as described in Section 6.1.1.6 of the Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories (U.S. EPA 2000).

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, 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 (U.S. EPA 2000a). Based on this requirement a total of nine (9) composite samples will be collected from the sampling reach downstream of the outfall and analyzed. If it is not possible to collect any target fish tissue samples within the specified sample reach and time frame, it will not be possible to derive a site-specific BAF.

While not required, background samples may be collected at an appropriate upstream location, distant enough from the 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.

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.

If fish are not present in the reach, a site-specific water column criterion element cannot be calculated using the BAF methodology. The entity could elect to use the mechanistic modeling methodology described in Appendix K to derive a site-specific water column criterion element.

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 the conduct of 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, and along a transect perpendicular to the stream flow within the fish collection zone. IDEM recommends collecting the upgradient sample at an appropriate upstream location distant enough from the downstream reach so that fish are not intermingled, IDEM recommends collection of grab samples 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. 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, at an appropriate upstream location distant enough from the downstream reach so that fish are not intermingled. In addition, collect grab samples along a transect at the point of compliance (POC) and at 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 and record the coordinates of sampling locations. 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. Seven (7)

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, at an appropriate upstream location distant enough from the downstream reach so that fish are not intermingled. Collect grab samples at 25% of the stream width on the outfall stream bank side of the waterbody at the POC, and then 250 meters, 500 meters, 750 meters and 1000 meters downstream of the outfall. 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 and record the coordinates of sampling locations. Six grab samples per bimonthly sampling event will be collected under this format for sites in large or great rivers.

For lentic systems, water column sampling requirements 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 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 dissolved selenium.

The appropriate preservation and pretreatment steps should be taken for the types of data required (i.e., 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 2016 NRWQC 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 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) Scanning Mode	7.9 µg/L
	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

*IDEM will only approve the use of EPA Method 200.8 Scanning Mode for investigations in waters known to contain selenium concentrations higher than 7.9 ug/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 2017a, Table B4-1). 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*.

Data Reporting

Field data measurements should be recorded on a field data sheet (Appendix C) 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 2015). Data will be submitted to the EDF through a secondary portal which allow 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 individual fish tissue sampling data from the Target Molluscivorous Species to calculate BAFs using Equation 1, and the target water column criterion element using Equation 2 (U.S. EPA 2000b). The fish tissue criterion element used in Equation 2 will be the same tissue type that was collected to calculate the BAF.

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.

Equation 1

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

Where

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)	14.0
Selenium fish muscle tissue criterion (mg/kg, dw)	11.3
Mean ambient selenium water column concentration (mg/L)	0.005
Site-specific target water column criterion element concentration (mg/L)	X

Equation 1

$$BAF = \frac{C\ tissue}{C\ water} = \frac{14.0\frac{mg}{kg}dw}{0.005\frac{mg}{L}} = 2{,}800\frac{L}{kg}$$

Equation 2

$$C\ target = \frac{C\ tissue\ criterion\ element}{BAF} = \frac{11.3\frac{mg}{kg}dw}{2{,}800\ \frac{L}{kg}} = 0.004\ mg/L$$

Site-specific water column criterion element concentration is 0.004 mg/L (4 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)	9.0
Selenium whole fish tissue criterion (mg/kg, dw)	8.5
Mean ambient selenium water column concentration (mg/L)	0.005
Site-specific target water column criterion element concentration (mg/L)	X

Equation 1

$$BAF = \frac{C\ tissue}{C\ water} = \frac{9.0\frac{mg}{kg}dw}{0.005\frac{mg}{L}} = 1{,}800\frac{L}{kg}$$

Equation 2

$$C\ target = \frac{C\ tissue\ criterion\ element}{BAF} = \frac{8.5\frac{mg}{kg}\,dw}{1{,}800\frac{L}{kg}} = 0.004722\frac{mg}{L}$$

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

Equation 3

$$C\ target\ (protective) = [C\ target - (C\ target\ X\ 0.1)]$$

$$= (0.004722\frac{mg}{L} - 0.0004722\frac{mg}{L}) = 0.00425\frac{mg}{L}$$

Applying a protective factor of ten percent will result in a site-specific protective target water column criterion concentration for this paired data of 0.00425 mg/L (4.25 µg/L).

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Appendix A

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

Appendix B

Indiana Surface Water Quality Aquatic Life Criteria for Selenium

Appendix C

IDEM Data Reporting Forms

Appendix D

Fish Collection Sampling Methods Characteristics

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. 2018. Fish Community Field Collection Procedures. B-009-OWQ-WAP-XXX-18-T-R0. Office of Water Quality, Watershed Assessment and Planning Branch. Indianapolis, Indiana.