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Attached is the Final Selenium Implementation required by the West Virginia Legislature. The West Virginia Legislature during its 2013 Regular Session with an effective date of July 12, 2013 adopted House Bill 2579. The Bill does not change the existing water quality criteria for selenium however, calls for the DEP to propose a new selenium criterion for legislative approval within two years. The Bill also requires the DEP to establish an implementation plan within six months that includes, at a minimum: (1) implementing the criteria as a threshold standard; (2) a monitoring plan that will include chemical speciation of any selenium discharge; (3) a fish population survey and monitoring plan to be implemented at a representative location to assess any possible impacts from selenium discharges if the criteria are exceeded; and, (4) reporting the results of the monitoring to DEP for use in development of a state-specific criteria.

The Statewide Monitoring Plan for Assessment of Potential Selenium Impact to Fish Populations will be implemented as required under House Bill 2579 to achieve the goal Legislature.

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STATEWIDE MONITORING PLAN FOR ASSESSMENT OF POTENTIAL SELENIUM IMPACTS TO FISH POPULATIONS

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SUMMARY

Selenium toxicity poses a potential threat to aquatic life exposed to excessive inputs. Yet, selenium also serves as an essential biological micronutrient and provides antioxidant properties to tissues. Variability in the literature regarding a suggested tissue-based criterion for freshwater fishes and subsequent protective water quality concentrations indicates the complexity of the bioaccumulation processes surrounding selenium. Uptake and subsequent impacts of selenium exposure may be largely dictated by the type of environmental conditions prevailing at influenced locations (i.e., lotic versus lentic environs) and the species, or valence state, of the selenium present. Monitoring of the potential deleterious effects to fish populations, which are among the most selenium-sensitive forms of aquatic life, will serve as the best indicator of problematic levels of selenium exposure and facilitate the establishment of appropriate tissue-based and/or water quality thresholds.

Pursuant to the mandates of HB 2579, the WVDEP is implementing the following as a methodology for the assessment of fish populations and an overall monitoring plan to be used in the development of state-specific criteria for aquatic life exposure to waterborne selenium. The WVDEP is implementing a tiered approach to the assessment of aquatic life resources or fish populations, initiated by the presence of elevated waterborne selenium (i.e., concentrations exceeding 5µg/L), and the subsequent implementation of criteria derived from the assessment results. Major components of the monitoring plan include chemical speciation analysis of selenium discharges, measurements of fish tissue concentrations to determine the extent of selenium uptake, and larval fish (early life stage) deformity evaluations to determine any potential population-level effects. The monitoring plan prescribes the utilization of these data gathered at locations exposed to increased selenium inputs and the incorporation of these findings, along with information from similar, relevant studies, in the development of state-specific (tissue-based and/or waterborne) aquatic life criteria for selenium.
METHODODOLOGY

INITIATION OF MONITORING—THRESHOLD TRIGGER AND MONITORING PLAN

The initiation of the monitoring plan to assess the potential impacts of selenium exposure on fish populations, which includes water chemistry, biological tissue, and early life stage deformities analyses, commences after the current national recommended chronic waterborne criterion for selenium is exceeded (i.e., concentrations > 5µg/L) in certain effluents and/or in-stream monitoring locations. Although this current criterion may be shown to be overly protective via the study results, the low-level waterborne concentration trigger will facilitate evaluation of potential impacts to fish populations at more subtle, sub-lethal exposures and ensure the detection of all potential selenium-related impacts. An Order will be issued to holders of NPDES permits per the Implementation Plan for House Bill 2579 regarding representative sites for monitoring and data collection. Following issuance of an Order, a fish population survey and monitoring plan including the following major components will be submitted to the WVDEP biologists for review and concurrence within 30 days: (1.), detailed description(s) of all proposed monitoring locations, including accurate geographic coordinates, maps, and proximities to selenium-source discharges; (2.), proposed methods for collection and analysis of water chemistry at monitoring locations, including chemical analytes, monitoring frequencies, and analytical laboratories; (3.), an approach to chemical speciation of affected waters, including proposed analytical laboratories; (4.), proposed methods for collection and evaluation of selenium concentrations in fish tissues, including collection/preservation techniques, species and tissues targeted for collection, and analytical laboratories; (5.), proposed methods for early life stage (larval fish) deformity analyses, including species targeted for collection, techniques used to obtain fertilized eggs, and methods for evaluation of potential selenium-induced developmental deformities; (6.), proposed data reporting procedures, including format(s) and frequencies; and (7.), documentation of the qualifications of the scientists/personnel conducting the studies.

MONITORING SITE LOCATIONS

Site description(s) and location(s) shall be included in the selenium fish population survey and monitoring plan for review and verification by WVDEP biologists prior to initiation of study. Proposed locations will be selected from watersheds in which streams have demonstrated elevated selenium concentrations and may include, but are not limited to, the following candidate watersheds/sub-watersheds: Elk River watershed (Birch River and affected tributaries); Coal River watershed (Pond Fork, Spruce Fork, and affected tributaries); Kanawha River watershed (Cabin Creek, Campbells Creek and affected tributaries); Gauley River watershed (Twentymile Creek and affected tributaries); Twelvepole Creek watershed (Kiah Creek and affected tributaries); Tug Fork watershed (Pigeon Creek and affected tributaries); and Guyandotte River watershed (Island Creek, Pine Creek, and affected tributaries). In general, survey stations should be situated at the suitable locations downstream of continuous discharge(s) that have exceeded or are currently exceeding the selenium trigger value of 5µg/L. All study locations must be perennial, wadeable, and contain adequate habitat and flow levels to
support sufficient fish populations to allow for a comprehensive analysis of fish tissues and facilitate the collection of spawning adults and/or mature gametes. Locations where multiple discharges contribute to elevated waterborne selenium concentrations may be monitored to characterize impacts of selenium exposure at broader watershed levels, so long as the discharges are reasonably proximate to one another and adequately mixed in the receiving waters. Sites must allow for complete mixing of receiving stream water and discharge(s), and, where possible, avoid major confluences before establishment of a study reach.

**WATER CHEMISTRY MONITORING—SURFACE WATER**

Surface water chemistry monitoring will occur at locations with known elevated levels of waterborne selenium (i.e., locations where concentrations have exceeded 5µg/L). Waterborne selenium concentrations will be monitored at these locations twice monthly for one year, and will be analyzed at minimum detection limits ≤ 0.6 µg/L. Methods for the collection, preservation, and chain-of-custody requirements for water samples will closely adhere to those specified in WVDEP (2013), and EPA Laboratory Analysis Method 200.8, or equivalent, will be followed for the derivation of selenium concentrations in water matrices (USEPA, 1994a). In waters where elevated sulfates may produce interference with analytical results, gaseous hydride atomic absorption (EPA method 7741A) analyses are recommended (USEPA, 1994b). Analytical results will be reported for both total and dissolved selenium. Chemical analyses (total and dissolved concentrations) for other metals/ions will also be performed concurrently on the collected water samples, with particular focus on the possible antagonistic relationship between selenium and certain constituents (copper, mercury, lead, zinc, cadmium, arsenic, and sulfates). Physicochemical information regarding dissolved oxygen, pH, temperature, and specific conductance will also be obtained during each monitoring event. At lotic sites, stream flow will measured during each sampling event, or when assessable, in cubic feet per second (cfs) using a current-velocity meter; these methods are described in WVDEP (2013). At lentic stations, depth stratified water samples will collected monthly to identify any layering of waterborne constituents within the water column. The results of these chemical analyses will be reported to WVDEP and utilized in exposure/transport considerations in the development of state-specific selenium criteria.

**WATER CHEMISTRY MONITORING—SPECIATION ANALYSES**

Bioaccumulative differences between selenium species are reported from the literature (USEPA, 2004) along with their potential to occur as a result of certain seleniferous inputs. In order to determine the presence of selenium in the form that is more conducive to bioaccumulation in organisms, analyses of the valence state of selenium must be performed. Analysis of waterborne selenium in regard to the proportion of selenite, selenium in the IV (Se\(^{+4}\)) valence state, to selenate, selenium in the VI (Se\(^{+6}\)) valence state, as well as the proportionality of organic and elemental selenium (Se\(^{0}\)) will be performed once at monitoring locations during low flow conditions. Methods for the collection, preservation, and chain-of-custody requirements for water samples will adhere to those specified for grab samples in WVDEP (2013). Due to the technical nature of the work, few entities are capable of performing these analyses; therefore, water samples
may require shipment to distant analytical laboratories. Careful attention should be given to adherence to sample hold times and chain-of-custody procedures in these instances. The results of the selenium speciation analyses will be reported to WVDEP and considered in the development of state-specific selenium criteria.

**FISH TISSUE**

Stream fishes will be collected by standard backpack electroshocking methods; whereas, fishes in lentic waters may be collected by gill nets, hook and line, and electroshocking methods (WVDEP, 2013; USEPA, 1993). Several common species, including bluegill sunfish, green sunfish, creek chub, central stoneroller, rockbass, and white sucker, will be targeted, where available, for whole-body tissue analysis for selenium, and will provide a means for interspecific tissue comparisons among sites. Typically, five to ten individuals of the same species, representing each species present, should be retained for whole-body tissue analyses (USEPA, 1993). Fish collections will occur twice at each proposed location: once in the fall during low-flow conditions (e.g., October); and once in spring, dependent on the maturity/spawning state of resident fishes (e.g., April). Collected specimens will be individually labeled (tagged), double bagged, and iced to 4°C in transit to storage; specimens will be stored at <0°C in preparation for laboratory tissue analysis. Tissue analysis to determine whole-body concentrations of (dry weight) selenium (µg/g, or micrograms of selenium per gram of fish tissue), will be prescribed for all specimens in consistency with EPA’s suggested whole-body chronic exposure tissue criterion of 9.56µg/g (dry weight selenium) for bluegill sunfish (USEPA, 2008). Specifically, EPA Laboratory Analysis Method 3052, or equivalent, will be followed in the preparation of whole-body and other biological tissue matrices for subsequent derivation of selenium concentrations (USEPA, 1994a; USEPA, 1996). Preferably, individual fish will be analyzed for whole-body tissue concentrations, representing as many as ten fish (of the same species) per collection event. However, intraspecific compositing (i.e., combining two or more individuals into a single sample) may be necessary if only smaller species or individuals (<10 cm) are collected in order to achieve a minimum analytical mass of 5g. A thorough inspection of all fishes for anomalies/disease will be completed, following the methods described in USEPA (1993), and findings will be reported to the WVDEP. In order to determine dietary exposure and specific tissue accrual, including selenium transferred maternally to eggs, an additional five to ten individuals, of the same species, will be retained during the collection efforts for more detailed organ/muscle tissue and stomach content analyses. Egg/ovary (gonad) tissue, muscle (fillet) or residual carcass, and stomach contents will also be collected from individual fish, albeit from different individuals than those used in whole body analyses, composited, when necessary to achieve minimum analytical mass, and analyzed for selenium concentrations. Methods for collection, dissection, and preparation of individual (portion) tissues should follow those described in USEPA (1993) and WVDEP (2010) and egg/ovary tissue collections should only be performed during the spring sampling event. A minimal amount (1g-5g) of field-collected fertilized eggs or eggs stripped from females prior to artificial fertilization must be retained for selenium tissue concentration analyses. Of particular importance, these tissues and subsequent analytically-determined selenium concentrations will be directly compared to the developmental deformity rates from larvae representing the same clutch or maternal
Other tissues (e.g., stomach contents, fillet/muscle plugs, and/or residual carcass) will be collected and analyzed during each collection event. All fish tissue selenium concentrations will be reported to the WVDEP and utilized in calculation of state-specific selenium criteria (GEI, 2013).

**EARLY LIFE STAGE FISH EVALUATION**

Among known toxicological manifestations of exposure to increased selenium in aquatic environs, deformities to larval fish are considered one of the most-sensitive indicators. As such, this monitoring plan will include the collection of information regarding the occurrences of larval fish deformities at study locations. The information will be used to determine the toxicological endpoint(s) for population-level effects, if any, among species exposed to elevated waterborne/dietary selenium concentrations and compared to tissue concentrations (whole-body and egg/ovary) in the development of state-specific criteria. In general, collection of reproductively-mature adults suitable for laboratory spawning studies or gamete collection will occur during the spring survey; however, careful attention must be given to specific locations in regard to the seasonal spawning activities of resident fish. This may require intensive monitoring (e.g., weekly inspection) of fish reproductive condition prior to the spring survey so that spawning activities are not missed. Methods for obtaining and evaluating larval fish should closely adhere to those described in Holm et al. (2005), Janz and Muscatello (2008), and Rottman et al. (1991), where appropriate. In particular, field-collected water utilized in laboratory rearing studies should be initially analyzed for selenium concentration (total and dissolved) and be collected in a quantity sufficient to fulfill all expected laboratory needs (i.e., replenishing of rearing chambers throughout the study). Data collection, including deformity evaluations, will be performed and reported in a manner that clearly identifies incidents of larval fish deformities and allows for comparison of these deformity rates (i.e., number of deformed individuals/total individuals evaluated) to egg tissue concentrations and whole body/gonad (ovary) tissue concentrations. Quality control procedures regarding deformity evaluations/reevaluations must be specifically addressed in the proposed monitoring plan, and all specimens will be retained in preservative (e.g., diluted formalin or glycerol) and be made available for quality control reevaluation upon request by WVDEP.

**DATA REPORTING**

The results of all water chemistry monitoring, including antagonistic constituents, will be reported to WVDEP in a spreadsheet format, which clearly identifies monitoring locations and collection/analytical dates. Data in regard to waterborne selenium will be reported in units of micrograms per liter (µg/L); whereas, other analytes may be reported in units respective of a lowest practical detection limit. Analytical results from chemical speciation will similarly be reported in spreadsheet format that clearly identifies monitoring locations and collection/analytical dates; however, reporting units may be, in certain instances, lower than micrograms per liter (µg/L). All fish tissue analyses for selenium concentrations will be reported in spreadsheet format that identifies the collection location and date, the species of fish, and the type of tissue or matrix (e.g., whole body or ovary). All tissue selenium concentrations will be reported in units of micrograms per gram of dry weight (µg/g dw). Data reporting for deformity evaluations
will be in spreadsheet format that clearly identifies location of larvae origin, collection/fertilization date(s), and incidents of larval fish deformities (i.e., number of deformed individuals/total individuals evaluated). Data coding of larval evaluations must allow for comparison of the deformity rates to egg concentrations from field-collected fertilized eggs and/or stripped eggs (prior to artificial fertilization), which represent eggs from the same clutch or maternal origin. Data regarding waterborne chemical analyses will be reported to WVDEP quarterly with exception of speciation analyses, which may be reported within six months of collection. Tissue concentration data will be reported to WVDEP within two months of collection, and the results of deformity evaluations will be reported within six months of collection. Upon yearly completion of the study, a formal report detailing the monitoring plan and all subsequent analytical results/evaluations will be submitted to WVDEP.

QUALIFICATIONS OF SCIENTISTS

The fish population survey and monitoring plan must be completed by degreed scientists with experience in bioaccumulation or bioassessment studies involving waterborne pollutant exposures and collection/evaluation of impacted biological resources. Water chemistry monitoring, including selenium speciation analyses, may be performed by qualified water monitoring personnel (e.g., chemists or technicians) without specific degrees or experience in aquatic bioaccumulation studies; however, all fish tissue collections and, in particular, early life stage (larval fish) collections and evaluations must be performed by degreed scientists with experience in assessment of aquatic fauna exposed to waterborne toxicants that may result in developmental (teratogenic) deformities. Qualified scientists must also have experience in location and collection of fertilized fish eggs from field locations and have experience in techniques for collection and fertilization of the gametes of fish. Additionally, all individuals taking part in the collection of fishes must have appropriate and valid scientific collecting permits from the WV Division of Natural Resources. The qualifications of all personnel proposed to be involved in the studies will be presented to WVDEP biologists for review and concurrence in the fish population survey and monitoring plan.

LITERATURE CITED


WVDEP—West Virginia Department of Environmental Protection (2010) Selenium-induced developmental effects among fishes in select West Virginia waters. West Virginia Department of Environmental Protection, Charleston, WV.