UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION III
1650 Arch Street
Philadelphia, Pennsylvania 19103-2029

February 1, 2022

Mr. Brian I. Bridgewater, Assistant Director
Water Quality Standards
Division of Water and Waste Management
West Virginia Department of Environmental Protection
601 57th Street, S.E.
Charleston, West Virginia 25304

Dear Mr. Bridgewater:

In accordance with the Clean Water Act (CWA) Section 303(c) and its implementing regulations at 40 CFR 131.20(a), a state shall from time to time, but at least once every 3 years, hold public hearings for the purpose of reviewing applicable water quality standards (WQS), and, as appropriate, modify and adopt WQS regulations (i.e., triennial review of state WQS regulations). The West Virginia Department of Environmental Protection (WVDEP) has indicated that it intends to initiate a triennial review in 2022. The purpose of this letter is to provide advice from the United States Environmental Protection Agency (EPA) on revisions to §47 CSR 2 that WVDEP should consider in its triennial review. The advice provided is strictly for WVDEP's consideration and does not constitute approval or disapproval decisions under CWA §303(c) and 40 CFR §131.21. Neither are these comments a determination by the EPA Administrator under CWA §303(c)(4)(B) and 40 CFR §131.22(b) that revised or new standards are necessary to meet the requirements of the Act.

EPA 304(a) Criteria Recommendations

A triennial review of a state's WQS regulations provides an excellent opportunity for WVDEP and its stakeholders to review West Virginia's current numerical criteria for toxic substances in surface waters and determine whether those criteria continue to be protective of designated uses and are based on sound scientific rationale, as required by 40 CFR 131.11(a)(1). In addition, federal regulation at 40 CFR 131.20(a) requires that states provide an explanation if not adopting new or revised criteria for parameters for which EPA has published new or updated CWA Section 304(a) criteria recommendations. This regulation is meant to foster meaningful and transparent involvement of the public and intergovernmental coordination with local, state, and federal entities in light of recent science provided by EPA through its criteria recommendations. EPA will not approve or disapprove this explanation. For West Virginia's triennial review, the state will need to provide explanations for each instance where new or revised criteria are not adopted for parameters where EPA has published new or updated CWA Section 304(a) criteria recommendations since May 30, 2000, as indicated in the preamble to the final rule Water Quality Standards Regulatory Revisions dated August 21, 2015 (80 FR 51020, 51028-29).

Aquatic Life Criteria – Recommended Revisions

In its next triennial review, EPA encourages WVDEP to propose revised criteria for the protection of aquatic life in order to be consistent with EPA recommendations for the following:

- Aluminum (*Final Aquatic Life Ambient Water Quality Criteria for Aluminum – 2018,* EPA 822-F-18-003)
- Ammonia (*Aquatic Life Ambient Water Quality Criteria for Ammonia – Freshwater 2013,* EPA 822-R-18-002)
- Cadmium (*Aquatic Life Ambient Water Quality Criteria for Cadmium – 2016,* EPA 820-R-16-002)
- Copper, statewide application (*Aquatic Life Ambient Freshwater Quality Criteria – Copper 2007 Revision*, EPA-822-R-07-001)
- Selenium (*2021 Revision to Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016,* EPA 822-R-21-006)

Aluminum

EPA issued revised recommended freshwater aluminum criteria for the protection of aquatic life on December 21, 2018. The updated recommended criteria reflect the latest science showing that three water chemistry parameters (pH, total hardness, and dissolved organic carbon (DOC)) can affect the toxicity of aluminum by affecting the bioavailability to aquatic species. These revised recommended criteria are not fixed acute and chronic values but depend on a site's water chemistry. These criteria use a Multiple Linear Regression (MLR) model to normalize the toxicity data and provide a range of acceptable values. The criteria, which are expressed as total recoverable, are calculated based on a site's pH, total hardness, and DOC. You can find more information about the 2018 recommended aluminum water quality criteria online at https://www.epa.gov/wqc/aquatic-life-criteria-aluminum.

West Virginia had previously adopted hardness-based aquatic life aluminum criteria in 47CSR2, Appendix E, Table 1, sections 8.1.1 and 8.1.2. Those criteria were submitted to EPA for review and approval under CWA Section 303(c), but EPA never approved these criteria and they are not effective for CWA purposes. By letter dated March 2, 2021, West Virginia withdrew the criteria from EPA's consideration. Since these criteria are not effective for purposes of the CWA, their continued presence in Appendix E, Table 1 is likely to lead to confusion. WVDEP should consider taking steps necessary to remove them from West Virginia's WQS regulation in order to increase transparency and prevent confusion among stakeholders.

Ammonia

EPA issued revised ammonia criteria on August 22, 2013. In updating the 1999 ammonia criteria, EPA conducted an extensive literature review of the latest scientific evidence that incorporated new toxicity data from 69 studies, including new data on freshwater mussels and gill-bearing snails, which are both sensitive to ammonia toxicity. In particular, the freshwater mussels are more sensitive to ammonia than organisms included in the 1999 dataset. You can find more information about the 2013 ammonia water quality criteria online at https://www.epa.gov/wqc/aquatic-life-criteria-ammonia.

Cadmium

EPA issued revised recommended cadmium criteria on April 4, 2016 (*Aquatic Life Ambient Water Quality Criteria for Cadmium - 2016*, EPA-820-R-16-002). EPA prepared an update of the chronic aquatic life criteria document for cadmium based on the latest scientific information and current EPA policies and methods. The 2016 updated criteria include new data for 75 species and 49 genera not previously represented. The freshwater acute criterion was derived to be protective of aquatic species and further lowered to protect the commercially and recreationally important rainbow trout, consistent with procedures described in EPA's current aquatic life criteria guidelines; it is slightly lower (i.e., more stringent) than the 2001 acute criterion for dissolved cadmium. The freshwater chronic criterion is slightly higher (i.e., less stringent) compared to the 2001 chronic criterion for dissolved cadmium; this modest increase is primarily due to the inclusion of four new genera, and the reanalysis of other data. You can find more information about the recommended 2016 cadmium water quality criteria online at https://www.epa.gov/wqc/aquatic-life-criteria-cadmium.

Copper, statewide application

EPA issued revised recommended copper criteria on February 22, 2007 (*Aquatic Life Ambient Freshwater Quality Criteria – Copper 2007 Revision*, EPA 822-R-07-001). These revised recommended criteria accurately reflect the latest scientific knowledge and are based on the application of the biotic ligand model (BLM), a metal 'bioavailability' model that uses receiving water body characteristics to develop site-specific water quality criteria. In WV's WQS regulations at 47 CSR 2.8.5.a, WVDEP allows for the use of the BLM for the development of site-specific copper criteria and EPA is recommending that WVDEP consider adopting the copper BLM as the aquatic life criteria approach for freshwaters statewide. You can find more information about the 2007 copper BLM water quality criteria online at https://www.epa.gov/wqc/aquatic-life-criteria-copper.

Selenium Criterion

EPA issued a revised recommended selenium criterion on June 30, 2016 (*Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016*, EPA-822-R-16-006). The 2016 criterion reflects the latest scientific knowledge, which indicates that selenium toxicity to aquatic life is primarily based on organisms consuming selenium-contaminated food rather than by being exposed only to selenium dissolved in water. The final criterion is expressed both in terms of fish tissue concentration (egg/ovary, whole body, muscle) and concentration in water (lentic, lotic). EPA acknowledges that West Virginia's fish tissue-based criterion elements are approximate to that of EPA's but is recommending the adoption of the water column elements of EPA's recommended criterion (lentic, lotic and intermittent exposure) and revision to the fish tissue elements and footnotes to be wholly consistent with EPA recommendations. You can find more information about the 2016 recommended selenium water quality criterion online at https://www.epa.gov/wqc/aquatic-life-criterion-selenium.

In order to facilitate implementation of West Virginia's selenium criterion, WVDEP should consider adopting a performance-based approach for establishing site-specific water column elements of the selenium criterion. A performance-based approach relies on West Virginia adopting a process (i.e., a criterion derivation methodology, with associated implementation procedures) rather than a specific outcome (e.g., numeric criterion or concentration of a pollutant) in its WQS regulation. West Virginia could accomplish this by adding a footnote in Appendix E, Table 1 that indicates that translated water column values for selenium will be based on dissolved total selenium in water and will be derived using the methodology described in Appendix K *Translation of a Selenium Fish Tissue Criterion Element to a Site-Specific Water Column Value* to EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater.*

Aquatic Life Criteria – Recommended Adoptions

WVDEP should also propose to adopt the following for which West Virginia has no aquatic life criteria at this time:

- Acrolein (*Ambient Aquatic Life Water Quality Criteria for Acrolein*)
- Carbaryl (*Aquatic Life Ambient Water Quality Criteria for Carbaryl - 2012*, EPA-820-R-12-007)
- Diazinon (*Aquatic Life Ambient Water Quality Criteria - Diazinon*, EPA-822-R-05-006)
- Nonylphenol (*Aquatic Life Ambient Water Quality Criteria* – Nonylphenol, EPA-822-R-05-005)
- Tributyltin (TBT) (*Ambient Aquatic Life Water Quality Criteria for Tributyltin (TBT) – Final,* EPA 822-R-03-031)
- Nutrient Criteria (total phosphorus, total nitrogen, chlorophyll a and water clarity) for Rivers and Streams (*Ambient Water Quality Criteria Recommendations – Information Supporting the Development of State and Tribal Nutrient Criteria – Rivers and Streams in Nutrient Ecoregion XI*, EPA 822-B-00-020).

Acrolein

EPA issued recommended acrolein criteria in July 2009 for the protection of aquatic life from the potential effects of acrolein. Acrolein is used as a biocide for aquatic weed control and is primarily used for irrigation canals. Calculated using EPA's 1985 *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses,* EPA used toxicity data and other information on the effects of acrolein that were subjected to both internal and external peer review. You can find more information about EPA's recommended acrolein water quality criteria online at https://www.epa.gov/sites/default/files/2018-12/documents/ambient-wqc-acrolein.pdf.

Carbaryl

EPA issued recommended carbaryl criteria in April 2012 for the protection of aquatic life from the potential effects of carbaryl. Calculated using EPA's 1985 *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses,* toxicity data for developing the water quality criteria were obtained from peer-reviewed open literature studies and from studies submitted to EPA for the registration and reregistration of carbaryl. To ensure the quality of the information, EPA's Office of Water worked closely with the Office of Pesticide Programs and subjected the toxicity data and other information on the effects of carbaryl to both internal and external peer review. You can find more information about the 2012 carbaryl recommended water quality criteria online at https://www.epa.gov/wqc/aquatic-life-criteria-carbaryl.

Diazinon

EPA issued recommended diazinon criteria in December 2005 for the protection of aquatic life from the potential effects of diazinon. Diazinon is a pesticide traditionally used to control insects. After December 31, 2004, it became unlawful to sell diazinon for outdoor, non-agricultural uses in the United States. However, it is lawful to use diazinon for non-residential or agricultural uses that are consistent with product labeling and precautions. Calculated using EPA's 1985 *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses,* toxicity data was obtained through the literature search and evaluation of EPA's Office of Pesticides Program data. You can find more information about the 2005 diazinon recommended water quality criteria online at https://www.epa.gov/sites/default/files/2019-03/documents/ambient-wqc-diazinon-final.pdf.

Nonylphenol

West Virginia does not currently have nonylphenol criteria in place, therefore EPA recommends the adoption of EPA's recommended nonylphenol criteria for the protection of aquatic life. EPA issued nonylphenol criteria in December 2005. Most nonylphenol is used in the production of other chemicals, including detergents, lubricants, and emulsifiers for agrichemicals. They are persistent in the environment, moderately bioaccumulate, and can have estrogenic effects in aquatic organisms. Calculated using EPA's 1985 *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses,* EPA's recommended criteria for freshwater are based on 18 freshwater species and 2 subspecies from 15 genera. You can find more information about the 2005 nonylphenol criteria online at https://www.epa.gov/sites/default/files/2019-03/documents/ambient-wqc-nonylphenol-final.pdf.

Tributyltin

EPA issued recommended tributyltin (TBT) criteria in 2003 for the protection of aquatic life. TBT is an organotin compound used primarily as a biocide in antifouling paints. It is extremely toxic to aquatic life and is an endocrine-disrupting chemical that causes severe reproductive effects in aquatic organisms. TBT is extremely stable and resistant to natural degradation in water. Because of its chemical properties and widespread use as an antifouling agent, concerns have been raised over the risks it poses to both freshwater and saltwater organisms. Calculated using EPA's 1985 *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*, the final ambient water quality criteria document for TBT contains ambient water quality criteria designed to protect aquatic organisms and their uses. The recommended national criteria for freshwater aquatic life states that, except possibly where a locally important species is very sensitive, freshwater aquatic life and their uses should not be affected unacceptably if the criteria are not exceeded. You can find more information about the 2003 TBT recommended water quality criteria online at https://www.epa.gov/sites/default/files/2019-02/documents/ambient-wqc-tributyltin-final.pdf.

Nutrient Criteria

EPA issued recommended ecoregional nutrient criteria for rivers and streams in 2001. These numeric nutrient criteria provide recommended values for total nitrogen, total phosphorus, chlorophyll-a, and water clarity (turbidity) by ecoregion. You can find more information about the recommended Rivers and Streams nutrient criteria online at https://www.epa.gov/nutrient-policy-data/ecoregional-nutrient-criteria-rivers-and-streams.

West Virginia could consider this and other EPA guidance as it develops nutrient criteria protective of West Virginia waterbodies, as anticipated by federal regulation at 40 CFR 131.11(b)(1)(ii) and (iii). Those provisions note that in establishing numeric criteria, that criteria can be based on EPA's 304(a) guidance modified to reflect site-specific conditions (40 CFR 131.11(b)(1)(ii)) or other scientifically defensible methods (40 CFR 131.11(b)(1)(iii)).

Note that EPA is aware that WVDEP's has developed Total Maximum Daily loads which rely on fecal coliform as a surrogate for addressing aquatic life impairments in surface waters due to organic enrichment. EPA continues to recommend that WVDEP analyze available data and where necessary collect additional data to confirm that fecal coliform is an appropriate surrogate. Nutrient criteria would also be helpful in addressing impairments of surface waters due to organic enrichment.

Human Health Criteria – Recommended Revisions

WVDEP should consider updates to its human health criteria for the following:

Bacterial Pathogen Indicator

It would be appropriate during this triennial review for WVDEP to reconsider its use of fecal coliform as an indicator of illness to swimmers. EPA has discouraged the use of fecal coliforms as indicators of fecal contamination since the 1986 publication of its Ambient Water

Quality Criteria for Bacteria because fecal coliforms are not reliable indicators of illness to swimmers. In that document, EPA recommends that for the protection of water contact recreation in freshwater, the better indicator is *E. coli*. In 2012, EPA updated its recommended water quality criteria (RWQC; EPA 820-F-12-058) and considered the latest research and most recent science. Based upon that review, EPA determined that *E. coli* is still the best indicator to use in freshwater to protect the public from exposure to harmful levels of pathogens while participating in primary contact recreation activities such as swimming. WVDEP can find more information on the 2012 recreational water quality criteria online at https://www.epa.gov/wqc/recreational-water-quality-criteria-and-methods.

Microcystins and Cylindrospermopsin

WVDEP should consider the adoption of microcystins and cylindrospermopsin criteria for the protection of human health while recreating issued by the EPA in 2019 (*Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin*, EPA-822-R-19-001). These recommended values may also be implemented as swimming advisories. Microcystins and cylindrospermopsin are groups of toxins produced by cyanobacteria, previously called blue-green algae, which are ubiquitous in nature and are found in surface waters. Environmental conditions that promote excessive growth of cyanobacteria in surface waters can lead to situations in which cyanobacterial cell density is high, known as blooms. Many factors can play a role in the development of blooms and their production of cyanotoxins. The recommended criteria values are based on the human health risks associated with incidental ingestion while recreating in freshwaters containing these cyanotoxins. You can find more information about the 2019 microcystins and cylindrospermopsin criteria and implementation materials online at: https://www.epa.gov/wqc/recreational-water-quality-criteria-and-methods.

Revisions to WV's Current Human Health Criteria

On August 8, 2021, EPA approved West Virginia's revisions to 24 of its criteria for the protection of human health. In June 2021, WVDEP proposed the revision to 31 additional human health criteria and the adoption of 4 new human health criteria. If the criteria proposed for revision in 2021 are finalized as anticipated in 2022, the majority of WV's human health criteria will be consistent with EPA recommendations. All of the proposed criteria are consistent with EPA recommendations and it is anticipated that the criteria will be finalized and submitted to EPA for its CWA 303(c) review in 2022. Once the second set of revisions are finalized, there will still be several of West Virginia's current criteria for the protection of human health that are inconsistent with EPA's CWA Section 304(a) recommendations. These include Antimony, 2,4,6-Trichlorophenol, Dioxin (2,3,7,8-TCDD), and Selenium. WVDEP can refer to EPA's Human Health Criteria Table to review EPA's most recent recommendations and access additional information on each of the parameters. The table can be found online at https://www.epa.gov/wqc/national-recommended-water-quality-criteria-human-health-criteria-table

Human Health Criteria –Recommended Adoption

There are several parameters for which EPA makes recommendations under CWA Section 304(a) for which West Virginia has no criteria. EPA recommends that WVDEP propose to adopt criteria for the following:

- 1,1,2-Trichloroethane
- 1,2,4,5-Tetrachlorobenzene
- 1,2,4-Trichlorobenzene
- 1,2-Dichloropropane
- 1,2-Diphenylhydrazine
- 1,3-Dichloropropene
- 2,4,5-Trichlorophenol
- 3,3′-Dichlorobenzidine
- 3-Methyl-4-Chlorophenol
- Acrolein
- alpha-Endosulfan
- Benzidine
- beta-Endosulfan
- Bis(2-Chloro-1-Methylethyl) Ether
- Bis(2-Chloroethyl) Ether
- Bis(2-Ethylhexyl) Phthalate
- Bis(Chloromethyl) Ether
- Chlorodibromomethane
- Chlorophenoxy Herbicide (2,4-D)
- Chlorophenoxy Herbicide (2,4,5-TP) [Silvex]
- Dinitrophenols
- Endosulfan Sulfate
- Endrin Aldehyde
- Heptachlor Epoxide
- Hexachlorobutadiene
- Hexachlorocyclohexane (HCH)
- Hexachlorocyclopentadiene
- Hexachloroethane
- Isophorone
- Nitrobenzene
- Nitrosodibutylamine (2002)
- Nitrosodiethylamine (2002)
- Nitrosopyrrolidine (2002)
- N-Nitrosodimethylamine (2002)
- N-Nitrosodi-n-Propylamine (2002)
- N-Nitrosodiphenylamine (2002)
- Pentachlorobenzene
- p,p′-Dichlorodiphenyldichloroethane (DDD)

- p,p′-Dichlorodiphenyldichloroethylene (DDE)
- trans-1,2-Dichloroethylene (DCE)
- Zinc (2002)

WVDEP can refer to EPA's Human Health Criteria Table to review EPA's most recent recommendations and access additional information on each of the parameters. The table can be found online at https://www.epa.gov/wqc/national-recommended-water-quality-criteria-human-health-criteria-table

Additional Recommendations

Compliance Schedule Authority

In 2015 when EPA revised the federal WQS regulations at 40 CFR 131, 40 CFR 131.15 was added. That provision states "If a State intends to authorize the use of schedule of compliance for water quality-based effluent limits in NPDES permits, the State must adopt a permit compliance schedule authorizing provision. Such authorizing provision is a water quality standard subject to EPA review and approval under section 303 of the Act and must be consistent with sections 502(17) and 301(b)(1)(C) of the Act."

EPA has confirmed that West Virginia has two compliance schedule authorizing provisions, both of which are located in the state's NPDES permitting regulations. One pertains to all NPDES permits (Title 47 Legislative Rule Department of Environmental Protection, Water Resources, Series 10 NPDES Program) which the other pertains to Coal Mining Facilities specifically (Series 30 WV/NPDES Rule for Coal Mining). EPA has no record of having approved either of these compliance schedule authorizing provisions pursuant to CWA Section 303(c).

The upcoming triennial review provides WVDEP with an opportunity to align its regulations with 40 C.F.R. 131.15. WVDEP could do so by including the NPDES compliance schedule provisions as part of its triennial review, including them within the public hearing (40 CFR 131.20(b)) and providing an Attorney General certification that the NPDES provisions were adopted pursuant to state law. Alternatively, West Virginia could adopt a new provision in its WQS regulation authorizing the use of compliance schedules with a cross reference to the existing NPDES-related compliance schedule language in permitting regulations.

§47-2: Appendix A, Category B-2 – Trout Waters

It has come to EPA's attention that the list of Trout Waters (Category B2) in Appendix A may not be comprehensive. WV's WQS does indicate that the list contains known trout waters and is not intended to exclude any waters which meet the definition of "Trout waters" in Section 2.19. However, in order to be completely transparent to all stakeholders, WV should have a process in place for keeping the list current and adding trout waters that meet the definition at 47-2.19.

Note that in accordance with federal regulation at 40 CFR 131.10(j), if streams are removed from Appendix A, and presumably assigned the "Warm water fishery streams" designated use, as that use requires criteria less stringent than previously applicable a use attainability analysis must be conducted.

§47-2-7 West Virginia Waters

In 2015, West Virginia adopted variances at §47-2-7.2.d.8.2 (Martin Creek of Preston County and its tributaries, including Glade Run, Fickey Run, and their unnamed tributaries and §47-2-7.2.d.11 (Maple Run, Left Fork Little Sandy Creek and their unnamed tributaries). Both variances indicate that conditions in the watersheds will be evaluated and reported upon during each triennial review throughout the variance period. As part of this triennial review, WVDEP should evaluate the relevant data from those watersheds. Should that data indicate that a higher condition is achievable during the term of the variance, the variance should be revised to reflect that condition. WVDEP should note revisions need to be done in accordance with federal regulations at 40 CFR §131.14.

West Virginia regulations at §47-2-7.2.d.16 & §47-2-7.2.d.20.2 establish a site specific selenium criterion for Connors Run and Little Scary Creek of 62 ug/l. Based upon West Virginia's revision to its selenium criterion for the protection of aquatic life and EPA 2016 revised selenium criterion (EPA 822-R-16-006), WVDEP should review the site-specific criteria for Connors Run and Little Scary Creek and determine if they are scientifically defensible and protective of designated uses as required by 40 CFR §131.11.

§47-2-8.5.a. Specific Water Quality Criteria

WVDEP established at § 47-2-8.5 a process by which site-specific criteria can be established through the NPDES permitting process. One of the methods for establishing site-specific criteria identified in § 47-2-8.5.a is the Streamlined Water-Effect Ratio (WER) Procedures for Discharges of Copper (March 2001). EPA's A*quatic Life Ambient Freshwater Quality Criteria – Copper* (EPA-822-R-07-001, February 2007) provides for calculation of site-specific numeric criteria for copper that is more scientifically defensible than older procedures, such as the WER guidance. For site-specific criteria to be effective for CWA purposes, they must be approved by EPA under its CWA 303(c) authority. In evaluating site-specific criteria to determine whether a site-specific criterion is protective of designated uses and based on sound scientific rationale as required by 40 CFR 131.11, EPA would use the science provided in the 2007 copper criteria document. Therefore, we are recommending that WVDEP delete the Streamlined WER guidance from the provision and rely on the Biotic Ligand Model guidance to establish site-specific copper criteria.

Thank you for considering EPA's recommendations in the preparation of West Virginia's review of its WQS regulation. EPA would be happy to assist the State as necessary to complete this review. If you have any questions regarding this letter, please contact me at (215)814-5737 or have your staff contact Charlie Brown at (215)814-2782.

Sincerely,

Voigt, Gregory

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Gregory Voigt, Chief
Standards and TMDLs Section
Water Division

March 16, 2022

Brian Bridgewater
Environmental Resources Program Manager
West Virginia Department of Environmental Protection Division of Water
601 57th Street SE
Charleston, WV 25304

RE: Proposed Catch and Release Regulations on Elkhorn Creek in McDowell County

Dear Brian,

I am writing to inquire concerning the proposed implementation of Catch and Release regulations upon the watershed of Elkhorn Creek by the Department of Natural Resources which includes 171 miles of water which contains and reproduces wild brown and rainbow trout. First, I wholeheartedly support the designation. In fact, I support stricter regulations than those proposed. In the general Catch and Release regulations you may fish with artificial lures that have treble hooks but contain no barbs. Single hooked lures may contain barbs. I would support the additional regulation of no treble hooks of either configuration.

I have fished the Elkhorn for several years and been fortunate to locate and successfully land many fish that most true fisherman would consider once-in-a-lifetime catches. I consider it one of if not the premier trout fisheries on the east coast. My interest is how to sustain this precious resource well beyond my lifetime for future generations to enjoy.

I have reviewed the Elkhorn's current Department of Environmental Protection designation as a water body and cannot determine its status. I cannot find it listed as a Trout Water under your B2 designation. On the DEP's webpage under Water Quality Standards, it depicts the current rule as 47CSR2-Requirements Governing Water Quality Standards. In it the following can be found under the definition of "Trout Waters" and I quote *"2.19 Trout Waters are waters which sustain year-round trout populations. Excluded are those waters which receive annual stockings of trout but which do not support year-round trout populations."*

Elkhorn Creek does not receive annual stocking and does support year-round trout population in as much by definition it meets the stated definition of "Trout Waters". The Department of Natural Resources at the request of the McDowell County Commission is considering the much stricter designation of Catch and Release. It's interesting to me that this is occurring.

My question is simple and is this. Why is the Department of Natural Resources considering this designation of Catch and Release when the Department of Environmental Protection not have it listed even as "Trout Waters?" I wholeheartedly support the inclusion of the Elkhorn and it's 171 miles of watershed into DEP's such designation of Trout Waters (B2) which would provide much more protection of this incredible resource.

Certainly, much more focus and effort need to be exerted to aid the waterway in returning to a more aesthetically pleasing state. By changing the designation of the water to B2 the Elkhorn watershed will begin the healing process.

I appreciate the consideration of the Department of Environmental Protection to change the watershed's designation and would ask the following question "Why would the Department of Natural Resources consider designating this watershed as a Catch and Release and the Department of Environmental Quality not recognize its status as a "Trout Water" under B2?"

Thank you for taking the time to read and consider my communication and I look forward to your response.

Respectfully Submitted,

Kevin Belcher

(Wild Rainbow Trout Landed and Released in the Elkhorn Watershed)

Giles County

VIRGINIA'S MOUNTAIN PLAYGROUND™

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25304-000199

April 15, 2022

Brian Bridgewater
Water Quality Standards, DWWM
West Virginia Department of Environmental Protection
601 57th Street SE
Charleston, WV 25304

Re: Proposed revisions to Legislative Rule §47 CSR 2 "Requirements Governing Water Quality Standards"

Dear Mr. Bridgewater,

Trout Unlimited is the largest coldwater conservation organization in the nation, representing thousands of members throughout West Virginia. TU's mission is to protect and restore America's coldwater trout and salmon fisheries for the enjoyment of our children. TU would like to present our comments and concerns related to proposed revisions to Legislative Rule §47 CSR 2 "Requirements Governing Water Quality Standards". The triennial review is the right time for WVDEP to undertake a thorough and comprehensive assessment of the state's trout waters and make sure that West Virginia water quality standards are fully protective of all trout waters. Our comments follow:

1) The current B2 trout waters list in Appendix A of the proposed water quality standards underrepresents the state's exceptional trout resources and has not been updated for over ten years. TU and its partners are aware that the West Virginia Department of Environmental Protection (WVDEP) is currently assessing the state's trout stream list and associated GIS data. Likewise, the reference in §47CSR2-4.1.b.2.B to streams as listed in the West Virginia High Quality Streams, Sixth Edition, prepared by the Wildlife Resources Section of the Division of Natural Resources (2011) should be removed. Reliance on a list that is over ten years old does not provide appropriate protection of existing trout communities.

TU, in conjunction with the West Virginia Rivers Coalition, has embarked on a process to produce a wild trout atlas that compiles WVDEP and the West Virginia Department of Natural Resources (WVDNR) trout stream lists and identified gaps in B2 trout water designations. The WV Wild Trout Collaborative, a workgroup composed of conservation organizations and state and federal agencies tasked with managing trout resources in WV, has been formed to assure trout resources are provided adequate protections. To date, through this collaboration 53 streams have been identified that contain trout DNA that were not previously included on the WVDEP or WVDNR trout stream list. This is one example of the inadequacies of the current trout list that illustrates the need to reassess how trout streams are defined and identified in the state. A streamlined process is also needed to add additional trout streams to the list in a timely manner rather than once every ten years.

2) TU questions whether incorporation of waters identified by other entities (in this case WVDNR) is an adequate method to use for granting corresponding water quality protections to West Virginia waters. In its reliance on the work and judgement of another entity, this removes WVDEP from the process of conducting its own scientific investigations of fishery communities in these waters. These analyses must be science-based and not subject to outside pressures that are inherent in processes related to fisheries management, such as revenues from license sales, preference for management as a stocked versus wild fisheries, and other localized, but not science-based, issues. WVDEP should maintain its own list of these waters and allow WVDNR-listed streams to be added to the WVDEP list upon evaluation. In cases of conflict, the more stringent of the uses must be protected.

3) TU and its partners request the WVDEP form a workgroup to reassess the state's B2 trout waters in Appendix A, develop a process to update the state's trout stream list, create additional transparency in the listing process by making the lists publicly available, and recommend revisions to 47CSR2 related to trout waters as part of this triennial review. We recommend this workgroup be comprised of representatives from the WV Wild Trout Collaborative, as well as other scientists with specialized expertise in trout.

4) TU disputes the approach/criteria as outlined in §47CSR2-6.1.b related to removal of a designated use if an existing use is not being met because of infeasibility for a number of reasons, especially 6.1.b.4 allowing for lesser protections based on "human-caused conditions or sources of pollution [that] prevent the attainment of the use and cannot be remedied or would cause more environmental damage to correct than to leave in place". While TU acknowledges that circumstances exist, such as the removal of a wetland to install a system to treat abandoned mine drainage, where these types of exceptions are appropriate, we are concerned that the section is sufficiently ambiguous and could lead to removal of a designated use for a human-caused reason, such as point/nonpoint sources of pollution that are difficult/costly to address. The Clean Water Act requires that a use attainability analyses (UAA) and/or total maximum daily loads (TMDL) are the tools to address these situations. It is not allowable to remove a designated use when existing uses are not being met without the analyses provided by these tools, a public comment period and subsequent inclusion in the next triennial review process. The circular logic of "trout would not be able to exist in that water if not for ______ [insert anthropogenic influence here] and, therefore, if we treat the water, it will destroy the fishery" is not an appropriate reason to continue activities that allow for ongoing water quality impairment.

5) The relation of designated and existing uses in the water quality standards is not protective under the current regulations. As commented above, the correct tools must be used to remove a designated use from a water or address non-attainment of a designated use; these decisions cannot be made without these critical analyses. In addition, where existing uses are documented, such as in the case of many streams identified using the analyses completed by TU and partners previously in this document, WVDEP has the requirement to evaluate these data and, when appropriate, protect uses based on these data. Conversely, relying on and/or allowing the WVDNR to add to or remove from the list of trout waters throughout West Virginia can allow streams with documented existing uses of propagation of trout to be removed via methods that often incorporate and, in some cases, may incorporate or rely on human desires rather than (or in addition to) scientific data, resulting in the downgrades in uses and thus protections. Again, WVDEP must develop and rely on its own scientifically based trout stream list and evaluate recommendations from the WVDNR for inclusion on the list.

6) The text contained in §47CSR2-4.1.b.2 should be changed from "high quality waters <u>may</u>…" to "high quality waters <u>shall</u>…." [underlining used for emphasis]. The language currently contained in this section allows for discretion on the part of WVDEP (and/or its secretary) to exclude waters as described in sections 4.1.b.2.A-C from being included in the definition of high quality waters. This can create the situation where certain waters that otherwise meet these qualifications are not included as high quality and thus, not provided adequate resource protections.

7) The text contained in §47CSR2-7.2.c.4.C should be changed from "the secretary <u>may</u>…" to "the secretary <u>shall</u>…" [underlining used for emphasis]. The language currently contained in this section allows for the secretary to waive the need for additional studies in cases where adequate data for decision-making as part of a petition process are not available. It is critical that all applications for a site-specific determination contain current and adequate data to demonstrate the need for alternative criteria based on the circumstances of the site to justify a different criterion from other waters of similar uses/characteristics.

Trout Unlimited appreciates the opportunity to provide comment to WVDEP as it considers changes to its water quality standards. Should you have questions, please contact Brad Riffee at bradriffee@gmail.com; Randy Kesling at rkesling@ma.rr.com; or Dustin Wichterman at Dustin.Wichterman@tu.org. Thank you.

Sincerely,

Brad Riffee
Brad Riffee, President
West Virginia Council Trout Unlimited

Randy Kesling
Randy Kesling, Conservation Committee Chair
West Virginia Council Trout Unlimited

Dustin Wichterman

Dustin Wichterman, Mid Atlantic Coldwater Habitat Program Associate Director
Trout Unlimited

Jennifer Orr-Greene

Jennifer Orr-Greene, Eastern Policy Director
Trout Unlimited

CC: Jake Lemon, Monitoring and Community Science Manager, Trout Unlimited

April 15, 2022

WV Department of Environmental Protection
601 57th Street SE
Charleston, WV 25304

Attn: Brian Bridgewater

Re: 2022 Triennial Review of Water Quality Standards

Mr. Bridgewater:

West Virginia Rivers Coalition, on behalf of our members and the organizations signed below, respectfully submits the following comments on the WV Department of Environmental Protection's (WVDEP's) Rules Governing Water Quality Standards (47CSR2) as part of the 2022 Triennial Review.

Human Health Criteria

In 2015, EPA updated its National Recommended Water Quality Criteria for human health for 94 chemical pollutants to reflect the latest scientific information and EPA policies, including updated fish consumption rate, body weight, drinking water intake, health toxicity values, bioaccumulation factors, and relative source contributions. During the 2019 triennial review, WVDEP chose to only update the criteria for pollutants for which the WVDEP already had existing standards.

Our concern is that our citizens remain vulnerable to health risks posed by toxins and carcinogens in EPA's 2015 recommended criteria updates that remain unregulated in West Virginia. Through our participation in WVDEP's Human Health Criteria Workgroup, WVDEP stated that its intention was to first address the recommended updates to criteria that WV currently had existing standards for, and then it would consider the adoption of the remaining recommended updates f which WV has yet to establish standards. Now is the time to consider adopting the remaining 2015 recommended criteria; there should be no further delay.

We request that WVDEP adopt the remaining human health criteria included in EPA's 2015 recommended updates so that West Virginia has more comprehensive standards in place to adequately protect public health.

B2 Trout Waters

The current B2 trout waters list in Appendix A of the state's water quality standards underrepresents the state's exceptional trout resources and has not been updated for many years. We are aware that WVDEP is currently assessing the state's trout stream list as it relates to documenting designated uses of state waters prescribed by the Clean Water Act. This triennial review is the right time for WVDEP to undertake a thorough and comprehensive assessment of the state's trout waters and make sure that our water quality standards are fully protective of all trout waters.

In partnership with Trout Unlimited, WV Rivers embarked on a process to produce a wild trout atlas that compiled WVDEP and WVDNR trout stream lists and identified gaps in B2 trout water designations. We also formed the WV Wild Trout Collaborative, a workgroup composed of conservation organizations and state and federal agencies tasked with managing trout resources in WV. Through this process, we have so far identified 53 streams containing trout DNA that were not previously included on the WVDEP or WVDNR trout stream list. This is one example of the inadequacies of the current trout list that illustrates the need to reassess how trout streams are defined and identified in the state. A streamlined process is needed to add additional trout streams to the list.

At the same time, we recognize that there may be a hesitancy to publicly list every trout water due to concerns about protecting these sensitive resources. For this reason, any change in the water quality standards to explicitly list more trout waters should continue to allow WVDEP to enforce B2 trout water quality standards for all waters in which trout presence is demonstrated – whether or not those waters are on the list.

WVDEP must ensure that all trout waters in the state have adequate protections. We understand this process deserves careful scientific review and urge the WVDEP to immediately begin research and consultation with stakeholders and the scientific community.

We request the WVDEP form a workgroup to reassess the state's B2 trout waters in Appendix A, develop a process to update the state's trout stream list, and recommend revisions to 47CSR2 related to trout waters as part of this triennial review. We recommend this workgroup be comprised of representatives from the WV Wild Trout Collaborative, as well as other scientists with specialized expertise in trout.

Conductivity Standard

WVDEP needs to implement a water quality standard based on ionic toxicity from surface mining operations. The scientific literature has demonstrated for more than 10 years that such a standard is necessary to protect aquatic life. Indeed, in 2011, EPA scientists Susan Cormier and Glenn Suter established a benchmark for conductivity to be used for just such a purpose. (EPA, 2011). The methodology was peer-reviewed and published in 2013 (Cormier and Suter, 2013).

The scientific justification has only become more robust since then. Appendix A includes a literature review that summarizes the support for a conductivity threshold of 300 µS/cm for protection of aquatic life from ionic pollution from surface mines. The causal link between highly conductive ionic runoff and a violation of the existing water quality standard for aquatic life has been recognized by federal judges in West Virginia and the Fourth Circuit Court of Appeals, which are also summarized in Appendix A.

We request that WVDEP implement a water quality standard for ionic pollution from surface mines at a threshold of 300µS/cm. Failure to do so would be a gross disregard of the science and the law.

Selenium Standard

Since the implementation of fish-tissue based selenium standards, coal mining operations across the state have abandoned planned, or even active, selenium treatment systems because of the reduced stringency of that standard. Though the WVDEP guidance was written in 2016 and updated in 2018, it fails to take account of the findings of a 2015 paper by USGS scientist Nathaniel Hitt.

In that paper, Dr. Hitt raised serious concerns about the implementation of the tissue standard without specific limitations on sample sizes that would be used to assess attainment. Dr. Hitt cautioned that sample sizes of at least 8 fish would be required to detect increases of 1.2 mg/kg Se above the EPA threshold with 80% confidence (at a type-1 error threshold of 0.20). Further, samples of fewer than 5 fish would be unable to detect increased mean Se concentrations of 1.7 mg/kg with even an 80% confidence.

Currently WV's guidance on fish tissue does not necessitate sample sizes of greater than even 5 fish. *See* WV Selenium Aquatic Life Standard Implementation at 4-5 (rev.4 2018). For example, a composite sample with 3 fish of the same species and one of a different minnow group could be combined for analysis resulting in a 4-fish sample. *Id.* Given the strong motivation for companies to avoid costly selenium treatment, the standard should be revised to eliminate Type-II errors (in other words, errors that fail to recognize selenium concentrations above the threshold).

We request that a requirement for an 8-fish minimum composite sample be adopted to determine compliance with the fish-tissue based selenium standard.

Dr. Hitt's paper is attached in Appendix B for reference.

Transitioning from fecal coliform to E. coli for bacteria standard

In 2012, EPA recommended using Escherichia coli (E. coli) as an indicator of fecal contamination for fresh water. The neighboring states of Virginia, Pennsylvania, Ohio and Kentucky all use E. coli to protect primary contact recreational uses in surface waters. For the past 10 years, WV has resisted making the transition from fecal coliform to E. coli. In 2016, the transition was attempted but later abandoned. Other states, such as Colorado, adopted dual fecal coliform and E. coli criteria in anticipation of the transition.

E. coli reflects stronger scientific links between potential sources of bacteria and likelihood of human illness following contact with water contaminated by fecal material. The most recently recommended indicators represent types of bacteria that are more specifically linked to pollution sources related to fecal material from warm blooded animals. This means that measurement of E. coli provides a more defensible link to pollution sources like malfunctioning wastewater treatment plants or failing home septic systems. Because of this and the more defensible correlation with human illness, E. coli is a better indicator and a more modern and protective standard for water quality criteria.

We request that DEP develop a process to make the transition from fecal coliform to E.coli as the contact recreation standard.

Thank you for your careful consideration of these comments. We look forward to discussing our recommendations at the public meeting on June 28, 2022.

Signed,

Angie Rosser
West Virginia Rivers Coalition

Mike Becher
Appalachian Mountain Advocates

Linda Frame
West Virginia Environmental Council

Amanda Pitzer
Friends of the Cheat

Larry Thomas
West Virginia Highlands Conservancy

Charles Marsh
Sleepy Creek Watershed Association

Dave Bassage
New River Conservancy

Robin Blakeman
West Virginia Interfaith Power and Light

Tim Reese
Friends of Cacapon River

Jennifer Orr-Greene
Trout Unlimited

Brad Riffee & Randy Kesling
WV Council of Trout Unlimited

Brent Walls
Upper Potomac Riverkeeper

Appendix A Scientific and Judicial Justification for Conductivity Standard

Literature Review

Surface coal mining in the Appalachian region of relevance here requires disturbing and removing overburden rock layers to access coal-rich deposits, which exposes pyrite-rich spoils to rainwater, accelerating the dissolution of naturally occurring metals and other cations (e.g., HCO3, SO4, Ca, Mg, Fe, Mn, Se, K, NO3/NO2) in the process. In fact, the accelerated chemical weathering measured below mountaintop mines are the highest ever recorded (Ross et al. 2018). The ionic soup and elevated conductance produced by the above processes is a characteristic signal of surface coal mining in southern West Virginia, southwestern Virginia, and eastern Kentucky (Pond et al. 2008, US EPA 2011, Lindberg et al. 2011, Griffith et al. 2012, Cormier et al. 2013a, Kunz et al., 2013; Merriam et al. 2015).

Early Studies on Water Quality Impacts of Surface Mining. Many studies since 2005 have reported that streams below surface mines and valley fills which exhibit elevated conductivity (e.g., >300 µS/cm, but often >> 1000 µS/cm) also show signs of biological impairment, and these studies have implicated ions contributing to stream conductivity as the most likely drivers (Cormier et al. 2013a, b). These articles cumulatively have more than fifty authors and have been peer-reviewed by dozens of eminent scientists.

The first article documenting a relationship between loss of biota and elevated conductivity in alkaline streams of Appalachia was Hartman et al.'s 2005 paper in *Hydrobiologia*. Using a paired study design, they showed substantial degradation of the benthic macroinvertebrate community associated with elevated conductivity. Pond et al.'s 2008 peer-reviewed study in the *Journal of the North American Benthological Society* stated in the abstract:

> We characterized macroinvertebrate communities from riffles in 37 small West Virginia streams (10 unmined and 27 mined sites with valley fills) sampled in the spring index period (March–May) and compared the assessment results using family- and genus-level taxonomic data. Specific conductance was used to categorize levels of mining disturbance in mined watersheds as low (500 µS/cm), medium (500–1000 µS/cm), or high (1000 µS/cm). Four lines of evidence indicate that mining activities impair biological condition of streams: shift in species assemblages, loss of Ephemeroptera taxa, changes in individual metrics and indices, and differences in water chemistry.

Pond et al. (2008) showed that macroinvertebrates associated with low-conductivity, low-sulfate reference streams decline sharply with increasing conductivity associated with alkaline mine drainage (i.e., sites with elevated sulfate, low chloride, and basic pH). Pond et al. (2008) further found that "[o]ur results confirm that MTM impact to aquatic life is strongly correlated with ionic strength in the Central Appalachians, but habitat quality did explain some variance in MMIs and other metrics." *Id.* at 725.

In 2010, Pond published a peer-reviewed paper in *Hydrobiologia* which found that in eastern Kentucky "[m]ean mayfly richness and relative abundance were significantly higher at REF [reference] sites compared to all other categories; MINED sites had significantly lower metric values compared to RESID [residential] and MINED/RESID sites." He further stated that "[a]nalyses from WV mining areas . . . indicated that the decline of mayflies from mountaintop mining correlates most strongly to specific conductance." *Id.* at lines 603-607. Thus, Pond found that mayflies declined or were eliminated from mined areas and that the abundance of mayflies was more closely related to conductivity than to habitat.

In linking surface mining to biota, Gerritsen et al. (2010) demonstrated that surface coal mining had a distinct biotic signal distinguishing chemical stress from habitat alteration and altered food resources in particular (temperature was not a significant factor in this case). This study illustrates that it is possible to decompose lowered multi-metric benthic condition scores (e.g., the West Virginia Stream Condition Index—WVSCI; Virginia Stream Condition Index—VSCI) scores using assemblage composition to distinguish the characteristic biotic signals of surface mining.

In a geographic study of mining extent, Petty et al. (2010) found that streams in catchments with very low (i.e., 1-5%) mining intensity were nonetheless biologically degraded, and these streams consistently had elevated conductivity and sulfate. Similarly, Merriam et al. (2010) found that in streams degraded by moderate amounts of either human development or mining, mining further degraded developed streams, highlighting the distinctively harmful effect of mining impacts.

Palmer et al. (2010) published a peer-reviewed study in *Science*, a premier scientific journal. They found that as mining increased, conductivity and sulfate increased, and biological condition (as measured by WVSCI) declined, including a decline in mayflies.

Merriam et al. published a peer-reviewed paper in early 2011 in the *Journal of the North American Benthological Society* on the effects of mining and residential development in Central Appalachia. The paper found that "mining (% of total subwatershed area) caused acute changes in water chemistry," that sites affected by mining and development "had lower Ephemeroptera, Plecoptera, Trichoptera richness than sites affected by either stressor alone," and that the biological impairment threshold was breached when mining activities covered about 25% of the cumulative subwatershed area. (abstract & p. 411). The study's authors "observed biological impairment when conductance reached 250 μS/cm." (pp. 413-14). More recently, Merriam et al. (2015) showed consistently positive effects of surface mining on conductivity and consistently negative effects on biotic integrity throughout southern West Virginia. These effects, though variable in degree across local watersheds, were exacerbated by residential development and the presence of underground mines.

The EPA Benchmark Study. In 2011, EPA scientists issued a report called "A Field-Based Aquatic Life Benchmark for Conductivity in Central Appalachian Streams." The Benchmark was authored by scientists like Cormier and Suter, who had published important papers in the area of ecological causation. (p. ix). Pond was also a contributor to the report. (p. x). Before publication, the Benchmark was reviewed by a scientific advisory board, which itself was composed of top scientists who possessed expertise in the area. (pp. xi-xii). The Benchmark used EPA's standard method for deriving water-quality criteria to derive a conductivity benchmark of 300 µS/cm. (p. xiv). This method was itself later published and subjected to independent peer review by the authors (Cormier et al., 2013c, Cormier and Suter, 2013). Under that method, EPA sets the benchmark at the level needed to protect 95% of macroinvertebrate taxa. (p. xiv). Figure 8 in the benchmark graphs the species sensitivity distribution and shows that extirpation increases as conductivity increases. (p. 18). Five percent of taxa are lost when conductivity rises to 295 µS/cm, over 50% are lost at 2000 µS/cm, and close to 60% are lost at 3000 µS/cm. (p. 18).

EPA conducted a detailed causal assessment and concluded that there is a causal relationship between conductivity and stream impairment in West Virginia. (pp. 40, A-40 ("This causal assessment presents clear evidence that the deleterious effects to benthic invertebrates are caused by, not just associated with, the ionic strength of the water. . . . When [other potential] causes are absent or removed, a relationship between conductivity and ephemeropteran [, i.e. mayfly,] richness is still evident.") Again, the authors later subjected their conclusions to independent peer review in demonstrating that alkaline mine drainage was a likely cause of biological degradation in Appalachian streams (Cormier et al., 2013a).

EPA considered potential confounding factors using an unusually large sample of sites across the region, including "habitat, organic enrichment, nutrients, deposited sediments, pH, selenium, temperature, lack of headwaters, catchment area, settling ponds, dissolved oxygen, and metals." (p. 41). EPA found that only pH was a confounder and controlled it by removing sites with low pH. (p. 41). EPA concluded that "[t]he signal from conductivity was strong so that other potential confounders that were not strongly influential could be ignored with reasonable or greater confidence." (p. 41). Once again, later independent reviews corroborated the interpretation that this observed relationship persisted even after confounding factors including habitat quality, deposited sediment, pH, selenium, catchment area, settling ponds and metals were considered and their effects addressed analytically (Cormier and Suter, 2013).

EPA's benchmark report also analyzed the relationship between the WVSCI biological impairment threshold and conductivity levels, and found that a WVSCI score of 64 (well below the current impairment threshold of 72) corresponds to streams with conductivity of about 300. (p. A-36). Since the WVSCI was developed independent of the benchmark, this is a separate method that validates the relationship between impairment and conductivity.

Post-Benchmark Studies. After the EPA benchmark was issued, a relevant corroborating study came from the same physiographic region but across state lines in Virginia. Here the authors used a selection of 22 sites where temperature and habitat conditions were of reference quality to test for the effect of total dissolved solids and conductance on macroinvertbrate indices (Timpano et al. 2011). The authors found that elevated conductance above 332 µS/cm associated with surface mining caused degradation for the VSCI and that levels below 465 uS/cm led to the occurrence of only 95% of reference genera. The results are important because they demonstrated that high conductivity levels alone are sufficient to generate degradation of biotic integrity in the absence of other stressors, and because those results are qualitatively very similar to those of US EPA (2011).

Also after the Benchmark, Palmer and Bernhardt published a peer-reviewed study in 2011 in the *Annals of the New York Academy of Sciences*. The report stated that surface mining in Central Appalachia has caused greatly increased sulfate concentrations and electrical conductivity in downstream waters, and that analysis of the West Virginia database of small streams "found that sulfate concentrations were highly correlated with conductivity, Ca, Cl, Fe, Mg, and Hardness—all of which contribute to heightened ionic stress in these impacted streams." (pp. 47-48). The report further found that this elevated conductivity leads to the loss of sensitive macroinvertebrate taxa, such as mayflies in Central Appalachian streams below coal mines. (p. 48)

Lindberg and Bernhardt published a peer-reviewed study in 2011 in the *Proceedings of the National Academy of Sciences*. The study found that all tributaries draining mountaintop-mining-impacted catchments in a portion of the Upper Mud River watershed in West Virginia were characterized by high conductivity and increased sulfate concentration. Sulfate concentration "was significantly positively correlated with constituents typically derived from rock and coal weathering (SO4, Ca, Mg, Li, Rb, and U) in the mainstem as well as the MTM-affected tributaries." (p. 2) The study "conclusively demonstrates that the observed increases in conductivity and Se concentration can be attributed directly to the area extent of surface coal mining occurring in the watershed." (p. 5) The study also stated that "the constituent weathering-derived salts that contribute to conductivity are not ameliorated nearly two decades after reclamation." *Id*.

Northington et al. (2011) published a paper in *Hydrobiologia* that examined the impacts of habitat restoration on streams draining alkaline mines in southeastern Virginia. They found no impact of restoration on biotic impairment; in many cases, habitat scores were further degraded by restoration attempts and showed no impact on high levels of specific conductivity.

In 2012, Pond published a peer-reviewed paper in *Hydrobiologia* that showed species composition changed dramatically as a function of land use and that conductivity was an excellent indicator of how many individuals of certain types of macroinvertebrate taxa normally abundant in Appalachian streams would be found at a disturbed site. Pond compared types of land disturbance at 94 sites in Kentucky, including mining sites, and stated in the abstract that "[c]ore caddisfly genera were extirpated from most disturbed sites." He found that "no habitat factors were significantly correlated with relative abundance metrics," while "major ion concentrations (measured as specific conductance) were also highly correlated with Plecoptera and Trichoptera richness . . . but not abundance." *Id*. at 11-12. Average site tolerance value "was most strongly correlated with specific conductance." *Id.* He concluded that the predominant naturally occurring stonefly genera in eastern KY headwater streams "serve to indicate 'healthy' Appalachian streams" and his data "revealed high rates of extirpation of many genera and entire families from headwater streams affected by varying levels of mining and residential disturbance." *Id*. at 18.

In 2012, Pond et al. published a peer-reviewed paper in *Environmental Monitoring and Assessment*, the abstract of which "described the development, validation, and application of a geographically- and seasonally partitioned genus-level index of most probable stream status (GLIMPSS) for West Virginia wadeable streams." He found that GLIMPSS detected greater stream impacts to benthic invertebrates than did the WVSCI method because it used a more sensitive genus-level rather than a family-level analysis. The threshold for impairment as measured by GLIMPSS is a score of 55 for the Mountain Summer category and 53 for Mountain Spring Pond, Table 8, p. 1532. (The Mountains category has previously been used by the WVDEP to calculate GLIMPSS Scores for streams at issue in this case.) This paper also underscored the importance of direct interpretation of taxa lists and submetric indices when interpreting benthic responses to mountaintop mining.

In their 2012 peer-reviewed "How Many Mountains" study, Bernhardt and King found that streams receiving water from mining catchments had significantly higher conductivity than streams in unmined areas. They also found that, after screening out potential confounding factors, high conductivity was highly correlated with lower numbers of sensitive taxa and declining WVSCI scores. The study used a different statistical method than the method used in EPA's benchmark and reached the same conclusion that five percent of stream taxa are lost when conductivity reaches about 300 µS/cm. The study stated in its abstract:

> The extent of surface mining within catchments is highly correlated with the ionic strength and sulfate concentrations of receiving streams. Generalized additive models were used to estimate the amount of watershed mining, stream ionic

> strength, or sulfate concentrations beyond which biological impairment (based on state biocriteria) is likely. We find this threshold is reached once surface coal mines occupy >5.4% of their contributing watershed area, ionic strength exceeds 308 µS/cm−1, or sulfate concentrations exceed 50 mg/L−1. Significant losses of many intolerant macroinvertebrate taxa occur when as little as 2.2% of contributing catchments are mined.

The Bernhardt et al. (2012) paper also identified 50 taxa that consistently declined in response to conductivity associated with surface mining. The greatest cumulative taxa declines occurred at 283 uS/cm (95% CI 178-289) and 50 mg/L sulfate (95% CI 27-57). The taxa most sensitive to mining included a wide variety of mayfly, stonefly, caddisfly, and beetle larvae characteristic of Central Appalachian streams. Mayflies in particular appeared especially sensitive to ionic stress from alkaline mine drainage, though stoneflies and caddisflies are also vulnerable (see also Pond 2010, 2012). As expected, a few highly tolerant taxa increased in relative abundance along the mining gradient, primarily genera of highly tolerant midges (*Chironomidae*) and the tolerant caddisflies *Chimarra* and *Hydropsyche*.

As mentioned earlier, Cormier and Suter published six peer-reviewed studies in 2013 based on different sections of EPA's benchmark report in *Environmental Toxicology and Chemistry*, a high-quality scientific journal. In the first study entitled "A Method for Deriving Water-Quality Benchmarks Using Field Data," they described a method for using biological and water-quality parameters to develop a field-based benchmark to protect 95% of the genera from extirpation. The use of field data is helpful where lab-based data is not available, such as where susceptible species and sensitive life stages are difficult to maintain and test in the laboratory.

In the second study entitled "Derivation of a Benchmark for Freshwater Ionic Strength," they developed an aquatic life benchmark in West Virginia for specific conductance as a measure of ionic strength that is expected to prevent the local extirpation of 95% of species from neutral to alkaline waters containing a mixture of dissolved ions in which the mass of $SO2^{-4}$ + HCO^{-3} is greater than or equal to Cl-. Extirpation concentrations of specific conductance were estimated from the presence and absence of benthic invertebrate genera from 2,210 stream samples in West Virginia. The study concluded that the extirpation concentration is 300 µS/cm. One of the reasons for using field data rather lab data is that *Ephemeropterans* (mayflies), which are the most sensitive to the ionic mixture, are not available as cultured animals for toxicity tests.

In the third study entitled "A Method for Assessing Causation of Field Exposure-Response Relationships," Cormier and Suter developed a weight-of-evidence method to determine how an association in the field is causal. They identified six characteristics of causation: co-occurrence, preceding causation, interaction, alteration, sufficiency, and time order.

In the fourth study entitled "Assessing Causation of the Extirpation of Stream Macroinvertebrates by a Mixture of Ions," they applied that method to determine that the relationship between conductivity and extirpation of benthic macroinvertebrates was causal. They stated in their abstract that "a mixture containing the ions Ca^{+}, Mg^{+}, HCO^{-3}, and SO^{-4}, as measured by conductivity, is a common cause of extirpation of aquatic macroinvertebrates in Appalachia where surface coal mining is prevalent."

In the fifth study entitled "A Method For Assessing The Potential For Confounding Applied To Ionic Strength In Central Appalachian Streams," they evaluated twelve potential confounders: habitat, organic enrichment, nutrients, deposited sediments, pH, selenium, temperature, lack of headwaters, catchment area, settling ponds, dissolved oxygen, and metals. They concluded that pH, temperature, habitat, and deposited sediments were not confounding factors.

In the sixth study entitled, "Relationship of Land Use and Elevated Ionic Strength in Appalachian Watersheds," they found that, based on a 10th quantile regression analysis, 300 µS/cm was exceeded when 3.3% or more of an area was covered by valley fills. They also confirmed that coal-mining activities are the primary source of high conductivity waters.

In 2014, Pond et al. published a study in *Environmental Management* that sampled fifteen headwater streams with valley fills in Central Appalachia that had been reclaimed from eleven to thirty-three years earlier. The study found that nearly 90% of these streams exhibited biological impairment, and that valley fill sites with higher WVSCI scores were located near undisturbed tributaries that could be the sources of sensitive taxa as drifting colonists. This result could explain why there are occasional passing stream condition index scores at sites when water chemistry and upstream land use would predict impairment. Pond stated in this article:

> Although these VFs were constructed pursuant to permits and regulatory programs that have as their stated goals that (1) mined land be reclaimed and restored to its original use or a use of higher value, and (2) mining does not cause or contribute to violations of water quality standards, we found sustained ecological damage in headwaters streams draining VFs long after reclamation was completed.

His three main conclusions were that "(1) temporal ecological impacts persist downstream of VFs, given 11-33 years post-reclamation; (2) many expected taxa were missing from VF streams (suggesting local extirpations) and the scraper feeding group was significantly reduced; and (3) water quality is most likely the primary barrier to recovery but proximity to clean sources (intervening tributaries) may contribute some sensitive taxa that increase the biological indices used to measure condition." *Id.* at 11. Elaborating on these three points, he further explained that conductivity was persistent and habitat was not a confounding factor for the observed stream impairment:

> our data indicated that highly elevated ionic concentrations may persist for over 30 years post-reclamation and that these chemical signatures result in damaged

> aquatic communities. Habitat can be a limiting factor, but by design, we removed significant habitat degradation factors by selecting sample reaches with relatively good habitat and intact riparian vegetation at reference and VF sites . . .
>
> after 11-33 years post-reclamation, bioassessment indices indicated persistent temporal effects; almost 90% of our streams draining old VFs scored below impairment thresholds using GLIMPSS and O/E [observed/expected predictive model]. . . .
>
> Overall, biological variation was strongly correlated with water chemistry and less with reach-scale habitat and landscape conditions. Since ion concentrations explained the greatest amount of biological impacts and were the most altered (compared to reference), this suggests that recovery is potentially hindered by ions, even in forested reaches long after reclamation. Causal analyses by Suter and Cormier (2013) provided evidence that ions (measured as specific conductance) negatively affected invertebrates despite other stressors present. . .
>
> Cormier et al. (2013b) and Suter and Cormier (2013) provided strong causal evidence that Appalachian macroinvertebrate extirpation is linked to increasing ions (as specific conductance), a finding supported by our study.

Id. at 12-13.

In 2014, USGS scientists Nathaniel Hitt and Douglas Chambers published a peer-reviewed paper looking at the effects of mountaintop mining on fish assemblages. Among other findings they noted that most obligate invertivores were extirpated at MTM sites, indicating that conductivity effects on macro-invertebrates resulted in impacts higher up the food chain, on fish. They also found that the effects of MTM were not related to physical-habitat conditions but were associated with water-quality variables, which may limit quality and availability of benthic macroinvertebrate prey.

More recently, several other authors have examined linkages between surface mining and biotic response using geographically extensive datasets. Voss et al. (2015) expanded on the work of Bernhardt et al. (2012) with their dataset and used Bayesian models of invertebrate abundance to generate a risk landscape of impairment at various levels of dissolved solids. They showed results that corroborated all earlier studies. Merriam et al. (2015a&b) used an independent sample of some 170 West Virginia streams that accounted for effects of surface mining, residential development, and underground mines on stream chemistry and biotic index scores. They showed that while all land uses produced increases in stream conductance, the chemical signature of each was relatively distinct, their impact on stream invertebrates was largely additive in streams with surface mining, and co-occurrence of residential development or underground mines with surface mines could exacerbate the apparent effect of mining activity on conductivity and stream biota.

Cook et al. (2015) used a smaller set of nested sites from Virginia to assess the relative impact of conductivity and habitat degradation downstream of surface mines. They found a strong habitat degradation effect when habitat scores were quite low (i.e., “poor”) and stream conductivity did not vary greatly. Bier et al. (2014) studied bacterial community responses to mountaintop mining below valley fills. In an article published in *The International Society for Microbial Ecology Journal*, they described marked changes in bacterial composition and, in some cases, functional capacity in streams with alkaline mine drainage.

In 2016, Ross et al. published a paper in *Environmental Science & Technology* that concluded the scale of surface mining impacts penetrates deep into bedrock and persists over longer time-scales than two-dimensional land use assessments would suggest. Rather than deforestation, the authors associated the effects of surface mining with impacts more closely associated with volcanic eruptions. Nippgen et al. (2017) published a paper in the same journal showing that the result of such deep impacts was an extension of perennial baseflow, shortening of elevated flow periods, and increases in flow yield from landscapes with valley fills when compared to those without valley fills. Such findings imply that in addition to elevated ionic concentrations, tributaries draining mined watersheds also contend with more constant levels of these concentrations through time, thus increasing the potential stress on stream biota. Such findings are stunning when combined with the temporal and spatial extent of surface mining mapped across Central Appalachia through time by Perciak et al. (2018) in the *Public Library of Science* (PLoS One).

In addition to their 2011 report, Timpano and his colleagues have conducted additional relevant work. In 2015, they published an article in the *Journal of the American Water Resources Association* that was a peer-reviewed recapitulation of their 2011 report. Boehme et al (2016) described seasonal patterns of impairment across a gradient of mining impacts in the peer-reviewed journal *Ecological Indicators*. They found altered benthic communities in medium to high specific conductivity streams marked by increases in conductivity-tolerant taxa and decreases in sensitive taxa. They found richness metrics to be more sensitive than aggregate indices or those based on relative abundance because some tolerant taxa occurred in most major groups. They proposed an alternate EPT formulation excluding relatively tolerant *Hydropsychids, Baetids,* and *Leuctrids*. They also showed that temporal biotic variability increased within medium conductivity streams as compared to reference or high conductivity streams. Timpano et al. (2018) published a follow-up paper, also in *Ecological Indicators,* in which they demonstrated seasonality in benthic response to alkaline mine drainage over a 4.5 year period. The authors identified groups of conductivity-sensitive and tolerant taxa and critical conductivity levels at which biotic metrics experienced significant changes in response to increasing conductivity.

Giam et al. (2018) found that streams affected by coal mining averaged one-third (32%) lower taxonomic richness and one-half (53%) lower total abundance than unmined streams, with these impacts occurring across all taxa investigated thus far (invertebrates, fish, and salamanders). Even after post-mining reclamation, biodiversity impact persisted. Giam identified the Elk River watershed in West Virginia as one of the watersheds of highest conservation concern because of reduced biodiversity from coal mining. The Seven Pines Mine discharges into tributaries of the Elk River.

Importantly, the analytical techniques employed by Bernhardt et al. (2012), Voss et al. (2015), Cook et al. (2015), and Merriam et al. (2015) differed markedly from those employed by US EPA (2011), Cormier et al. (2013c), Timpano et al. (2011; 2015; 2018), and Pond et al. (2008). Moreover, Pond collected samples from West Virginia and Kentucky; US EPA (2011), Bernhardt (2012), and Voss (2015) relied on the WVDEP sampling database; Merriam (2015) collected their own West Virginia data; whereas Cook (2015) and Timpano (2011, 2015, 2018) sampled in southwestern Virginia. Nonetheless, all groups arrived at qualitatively similar conclusions regarding the potential of degradation from stream conductance downstream of Appalachian surface mines. Many noted that when habitat is highly degraded, it too contributes to poor biotic condition. However, all agreed that conductivity is sufficient *by itself* to produce biotic impairment. Such agreement is rather rare in stream ecology, and supports interpretation of the general role of elevated conductivity as a causal agent in stream impairment.

Experimental Studies. Laboratory studies are also largely consistent with the tolerance thresholds of organisms derived from field observation. Kennedy et al. published a peer-reviewed paper in 2004 in *Environmental Monitoring and Assessment* which tested simulated coal mine discharge waters in Ohio. Seven-day lethality tests on *Isonychia bicolor*, a mayfly, found that lowest observed effect in mine effluent dominated by sulfates, bicarbonates, and sodium were 1582 µS/cm, 966 µS/cm, and 987 µS/cm in three tests. These values bracket the field-derived extirpation value of 1180 µS/cm.

In 2013, Kunz et al. published a peer-reviewed paper in *Environmental Toxicology and Chemistry*. Here, researchers exposed amphipods, mussels, and a species of mayfly to reconstituted mine water from 3 surface mines with an ionic composition characteristic of mountaintop-mining-impacted streams in Central Appalachia. Toxicity to the mayfly was determined to be between 800 and 1300 µS/cm, which is consistent with the field-derived extirpation value of 1092 µS/cm.

Clements & Kotalik (2016) were the first to be able to rear a healthy native stream assemblage complete with mayflies in a mesocosm experiment. They exposed stream assemblages to three types of effluent at various concentrations, and their results suggest that mining effluent similar to that in the Central Appalachian surface mines in southwestern Virginia is not immediately toxic to many invertebrates, but has its strongest impact on the survival of early instars while inducing changes in foraging behavior in older larvae. The authors concluded that the benchmark standards of the EPA (2011) were reasonable for protecting aquatic life.

Moving from mesocosms to natural experiments, Voss and Bernhardt (2017) published a study in *Limnology and Oceanography* that examined macroinvertebrate populations in an unimpacted stream as it conflued with mine-impacted tributaries. They found that not only were mining impacts associated with elevated levels of conductivity and sulfate as well as loss of sensitive taxa, the losses translated directly into depressed biomass throughout the year that were most apparent when pollutant concentrations rise with summer baseflow. They concluded that elevated ionic strength depresses insect production by preventing sensitive taxa from completing their life cycles in mining-impacted streams. Due to the domination of surface coal mining in Appalachia (recently documented by Perciak et al. 2018), altered production patterns are likely impacting regional food webs.

Summary of Scientific Research to Date. Together with the Benchmark, dozens of scientists in the field of ecology and ecological causation have reviewed the evidence establishing that conductivity in mine drainage is a cause of biological degradation in Appalachian streams. All of the science has passed peer review or the EPA's Scientific Advisory Board. The studies used a scientifically valid method of causal assessment. The primary data source used by EPA and Cormier et al. for evidence of confounding is West Virginia's watershed analysis database, which means that it is highly relevant to streams in southern West Virginia and neighboring states within the same region. The weight of evidence indicates that habitat, temperature, and sedimentation are not confounding factors in Central Appalachian mine sites generally or in this case specifically. There are no peer-reviewed studies with reliable study designs that contradict any of these findings.

The studies clearly show that levels of conductivity above ~300 uS/cm and elevated sulfate levels are common below Appalachian mine sites and lead to extirpation of invertebrate genera (EPA 2011; Cormier and Suter 2013; Cormier et al. 2013a; Timpano et al. 2015, 2018) and that the ions found coming out of the outlets at the Seven Pines Mine are consistent with those associated with coal mining pollution in this region (Pond et al. 2008; Palmer et al. 2010; Bernhardt and Palmer 2011; Lindberg et al. 2012;Pond et al. 2012; Pond et al. 2013; Pond et al. 2014; Timpano et al. 2015, 2018). The ionic mixture of calcium, magnesium, sulfate, and bicarbonate in circum-neutral mine water causes the loss of aquatic macroinvertebrates in Appalachian areas where surface coal mining is prevalent; it is the mixture of ions that causes the biological impairment (Cormier et al. 2013b; Comier and Suter 2013). These ions also lead to reductions in fish assemblages in the affected streams (Hitt et al. 2014).

Altogether, *nine* different scientific methods have been used in these different studies by different scientists to reach the same conclusion about the causal link between conductivity and downstream impairment. First, the Benchmark used a species sensitivity distribution to model the conductivity level at which different genera are extirpated, and determined that 5% of taxa are lost at 300 µS/cm (pp. 18-19). Second, the Benchmark modeled conductivity against WVSCI scores, and determined that 300 µS/cm corresponded to a failing WVSCI score of 64 (p. A-36). Third, the Benchmark used a logistic regression, and found that the probability of impairment, as measured by WVSCI, was 59% at 300 µS/cm and 72% at 500 µS/cm (p. A-36). Fourth, Timpano et al. (2011; 2015) used a controlled study design to isolate the effects of elevated conductivity from habitat and temperature. Fifth, King and Bernhardt in their paper used Generalized Additive Models (GAMs) and a different statistical method called Threshold Indicator Taxa Analysis (TITAN) to corroborate earlier results. Sixth, Voss used a Bayesian estimation procedure on the WVDEP data. Seventh, Timpano et al. (2018) used a multivariate approach (NMS) and GAMs fit to submetrics from independent samples in Virginia to reach qualitatively similar conclusions. Eighth, Clements & Kotalik (2016) used a mesocosm to demonstrate experimental effects of conductivity on aquatic insects. Finally, Voss and Bernhardt (2017) used an *in situ* natural experiment to demonstrate the impacts of high-conductivity mine drainage on mayfly production and survival.

Collectively, these papers show remarkably consistent results across researchers and analytical methods, when the enterprise of scientific publication and peer review is set up to reward dissent or critical interpretations. This pattern of consistent support and corroboration without any substantive contradiction from independent investigators constitutes extremely strong empirical evidence that ionic stress produced by surface coal mining in West Virginia is a general cause of biological impairment.

Judicial Decisions

In multiple opinions since June 2014, Judge Robert C. Chambers of the U.S. District Court for the Southern District of West Virginia (Huntington Division) has found as fact that high levels of ionic pollution, measured as conductivity, causes biological impairment in Appalachian streams. Further, Judge Chambers has recognized that conductivity is the result of mining operations such as the one proposed by the applicant. His findings have been upheld by the United States Court of Appeals for the Fourth Judicial Circuit.

In a June 2014 decision Judge Chambers wrote:

> The Court will now assess the evidence presented at trial to determine whether Plaintiffs have proven this aspect of their case by a preponderance of the evidence. First, it is important to note that the EPA has spoken to both general causation theories 1) through its October 2005 "Mountaintop Mining/Valley Fills in Appalachia Final Programmatic Environmental Impact Statement" ("EIS") and, most importantly, 2) through its March 2011 Benchmark, entitled "A Field–Based Aquatic Life Benchmark for Conductivity in Central Appalachian Streams." Pls.' Ex. 9; *see* Tr. at 61–62. In its EIS, the EPA identified two downstream impacts from mountaintop mining valley fills: 1) increases in conductivity and 2) decreases in the number of invertebrate taxa. *See* Tr. at 62.

In its nearly three-hundred page scientific Benchmark—after considering and then ruling out the potential confounding effects of habitat, organic enrichment, nutrients, deposited sediments, pH, selenium, temperature, lack of headwaters, catchment areas, settling ponds, dissolved oxygen, and metals—the EPA found that "salts, as measured by conductivity, are a common cause of impairment of aquatic macroinvertebrates" in Central Appalachian streams. EPA's Benchmark at A–1, B–1; *see also id.* at A–40 ("This causal assessment presents clear evidence that the deleterious effects to benthic invertebrates are *caused by, not just associated with,* the ionic strength[, i.e., conductivity,] of the water.... When [other potential] causes are absent or removed, a relationship between conductivity and ephemeropteran [, i.e. mayfly,] richness is still evident." (emphasis added)); *id.* at A–37 ("As conductivity increases, the occurrence and capture probability decreases for many genera in West Virginia ... at the conductivity levels predicted to cause effects. The loss of these genera is a severe and clear effect."). The Benchmark also found that "of the [nine] land uses ... analyzed, only mining especially associated with valley fills[, i.e., mountaintop mining with valley fills,] is a substantial source of the salts that are measured as conductivity." *Id.* at A–18.

The Benchmark ultimately concluded that the "chronic aquatic life benchmark value for conductivity" in West Virginia streams is 300 µS/cm. *Id.* at xv. To derive this recommended high-end threshold value, the EPA used the 5th percentile of a species sensitivity distribution, based on the standard methodology for deriving water-quality criteria, meaning that this 300 µS/cm benchmark value for conductivity is "expected to avoid the local extirpation [due to the salts measured as conductivity] of 95% of native species." *Id.* at xiv. In support of both the specific 300 µS/cm benchmark value and the general causal linkage between conductivity and impairment to aquatic macroinvertebrates, the Benchmark contains a graph which charts, for 163 genera, the level of salt exposure above which a genus is effectively absent from water bodies in a region, with conductivity readings on the x axis and proportion of genera extirpated on the y axis. *Id.* at xiv, 18 fig. 8. A fairly consistent line is formed as conductivity and extirpation both increase, illustrating the causal connection between conductivity and significant biological impairment which Plaintiffs seek to prove. *See id.* at 18 fig. 8. . . .

Second, two of the authors of the Benchmark, Dr. Susan Cormier and Dr. Glenn Suter, subsequently published four different peer-reviewed journal-article versions of several sections of the EPA's Benchmark—including the section regarding the causal link between conductivity and biological impairment and the section ruling out potential confounding factors—in the scientific journal *Environmental Toxicology and Chemistry*. *See* Tr. at 84. Plaintiffs' expert Dr. Palmer testified that this is a quality journal which focuses specifically on topics such as biological response to pollutants. *Id.* at 84–86. Plaintiffs' expert Dr. King testified that "the list of the number of people who commented on [these journal articles] in the acknowledgments [section and] the peer reviews ... [is] impressive." *Id.* at 258. He also testified that, in his own professional opinion, he found the articles "rigorous and very defensible." *Id.*

Third, numerous other scientific articles published in peer-reviewed journals-both before and after publication of the Benchmark—lead to the same conclusions. In 2008, Dr. Gregory Pond-who would later be one of the contributors to the EPA's Benchmark—published a peer-reviewed scientific article in the *Journal of the North American Benthological Society,* based upon a field study he conducted which found that, as surface coal mining with valley fills—and its associated conductivity—increased, benthic macroinvertebrate taxa decreased. *See* Gregory J. Pond et al., *Downstream Effects of Mountaintop Coal Mining: Comparing Biological Conditions Using Family— and Genus–Level Macroinvertebrate Bioassessment Tools*, 27 J.N. Am. Benthological Soc'y 717 (2008), Pls.' Ex. 15; Tr. at 64–65; Pls.' Ex. 9. Dr. Palmer testified that the *Journal of the North American Benthological Society* is the highest impact freshwater journal in existence. Tr. at 65. . . .

Fourth, multiple different scientific methods were used at different times by different scientists to come to the same conclusions regarding the causal link between surface mining, conductivity, and biological impairment, which, Dr. Palmer testified, is the "strongest form of evidence" possible. Tr. at 83, 89–90, 248–52, 272, 274. For example, in its Benchmark, the EPA created a species sensitivity distribution—modeling the conductivity level at which each of 163 different genera are extirpated—which revealed that about five percent of taxa are lost at about 300 μS/cm. *See* EPA's Benchmark at 18–19. The Benchmark also used another method: modeling conductivity against WVSCI scores. *Id.* at A–35, –36. That modeled relationship revealed that the benchmark threshold of 300 μS/cm corresponded with a failing WVSCI score of 64. *Id.* at A–36. Using logistic regression, the probability of impairment—as measured by WVSCI—at 300 μS/cm was calculated to be 59%. *Id.* At 500 μS/cm, the probability of impairment was 72%. *Id.;* Tr. at 324. In the 2012 Bernhardt and King paper, two different methods were used to determine the biological impairment effects of conductivity: generalized additive regression models for three different biological response variables—including the number of intolerant taxa and WVSCI scores—and the Threshold Indicator Taxa Analysis ("TITAN") method, which Dr. King developed. *How Many Mountains* at C–D, P022–23; Tr. at 272, 274. Each of these different methods, conducted by different scientists at different times and subjected to the rigorous peer-review process required by scientific journals, resulted in the same conclusion: conductivity associated with surface mining causes biological impairment, such that about five

> percent of taxa are lost at about 300 µS/cm. EPA's Benchmark at 18, A–36; *How Many Mountains* at F; Tr. at 274.
>
> Fifth, the Court finds the expert testimony of Dr. Palmer and Dr. King to be very persuasive. . .
>
> Finally, even though the WVDEP's Guidance purports to find that there is no causative effect between conductivity and low WVSCI scores, two portions of the Guidance seriously undermine this assertion and, ironically, support Plaintiffs' case. First, the Guidance includes a scatterplot graph of conductivity and associated WVSCI scores which reveals a clear reduction in WVSCI scores as conductivity increases; in fact, above 1500 µS/cm, only 2 scores out of approximately 100 fall above the passing WVSCI score threshold of 68 and the vast majority fall under 60.6. WVDEP's Guidance at 6. This strong association supports, rather than contradicts, a causal connection. Second, Figure 2 in the Guidance concludes that conductivity measurements that fall within the range of 1075–1532.9 µS/cm are “likely stressor[s]” and that measurements above 1533 µS/cm are “definite stressor[s].” *Id.* at 7. Almost all of the recent conductivity measurements at the sites at issue in this case fall within these two categories; many are firmly within the “definite stressor” category. Thus, the WVDEP's Guidance is additional evidence that high levels of conductivity cause biological impairment.
>
> In the face of such overwhelming scientific evidence, this Court **FINDS** that Plaintiffs have proven, by a preponderance of the evidence, that, 1) controlling for other potential confounding factors, high conductivity in streams causes or at least materially contributes to a significant adverse impact to the chemical and biological components of aquatic ecosystems—proof of which can be shown through low WVSCI scores—and 2) surface mining causes—or at least materially contributes to—high conductivity in adjacent streams.[1]

Judge Chambers built upon his findings in a subsequent decision in a similar case issued in January 2015. There the Judge concluded:

> In multiple ways, the chemical and the biological components of the aquatic ecosystems found in Stillhouse Branch have been significantly adversely affected by Defendant's discharges. The water chemistry of this stream has been dramatically altered, containing levels of ionic salts—measured as conductivity—which are scientifically proven to be seriously detrimental to aquatic life. The biological characteristics of the stream have also been significantly injured, in that species diversity—and, in some areas, overall aquatic life abundance—is profoundly reduced. Stillhouse Branch is unquestionably biologically impaired, in violation of West Virginia's narrative water quality standards, with current WVSCI scores falling well below the threshold score of 68.

> Losing diversity in aquatic life, as sensitive species are extirpated and only pollution-tolerant species survive, is akin to the canary in a coal mine. This West Virginia stream, like the reference streams used to formulate WVSCI, was once a thriving aquatic ecosystem. As key ingredients to West Virginia's once abundant clean water, the upper reaches of West Virginia's complex network of flowing streams provide critical attributes—“functions,” in ecological science—that support the downstream water quality relied upon by West Virginians for drinking water, fishing and recreation, and important economic uses. Protecting these uses is the overriding purpose of West Virginia's water quality standards and the goal of the state's permit requirements.[2]

Judge Chamber’s decision in that case was ultimately upheld in full, by the United States Court of Appeals for the Fourth Judicial Circuit. After examining his review of the evidence and the applicable law, that court found:

> The court noted that peer-reviewed scientific articles first recognized the relationship of mining, conductivity, and decreased Index scores in 2008, a year before issuance of [the permittee’s] renewal permit. See [Chambers Decision] (citing Pond et al., supra n.1). Other articles strengthened these findings. *Id.* (citing, among others, M.A. Palmer et al., Mountaintop Mining Consequences, 327 Sci. 148 (2010) (finding that as conductivity increased, Index scores decreased)). In rebuttal, [the permittee] offered an expert whom the district court found unqualified—an assessment [the permittee] does not challenge on appeal. . . . In sum, [the permitte’s] arguments as to why the district court erred in finding that [it] violated its permit, like [the permitee’s] arguments as to the permit’s reach, uniformly fail.[3]

Judge Chambers issued another decision in August 2015, finding:

> On the basis of this outstanding collection of peer-reviewed studies, the Court finds that the link between surface mining and biological impairment of downstream waters has been sufficiently—if not definitively—established in the scientific literature. “There's field data. There's lab data. There's observational data. There's field experimental data. There's toxicity testing.” Tr. 2 at 141, ECF No. 100. Through myriad lines of evidence, researchers have reached the same general causation conclusion, without a single peer-reviewed publication reporting contrary findings. In Dr. Palmer's expert opinion, there is no remaining doubt on the question of general causation, leaving only surprise that researchers are continuing to study the question. *Id.* at 141 (“I would say there's no doubt. What surprised me is that the studies continue to go on.... because it's been so well-established.”).[4]

Subsequent cases have continued to result in findings that ionic toxicity measured as conductivity *causes* biological impairment in streams beneath surface mines.[5]

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Integrated Environmental Assessment and Management — Volume 11, Number 1—pp. 143–149

Threshold-Dependent Sample Sizes for Selenium Assessment with Stream Fish Tissue

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(Submitted 13 March 2012; Returned for Revision 12 February 2013; Accepted 21 June 2013)

ABSTRACT

Natural resource managers are developing assessments of selenium (Se) contamination in freshwater ecosystems based on fish tissue concentrations. We evaluated the effects of sample size (i.e., number of fish per site) on the probability of correctly detecting mean whole-body Se values above a range of potential management thresholds. We modeled Se concentrations as gamma distributions with shape and scale parameters fitting an empirical mean-to-variance relationship in data from southwestern West Virginia, USA (63 collections, 382 individuals). We used parametric bootstrapping techniques to calculate statistical power as the probability of detecting true mean concentrations up to 3 mg Se/kg above management thresholds ranging from 4 to 8 mg Se/kg. Sample sizes required to achieve 80% power varied as a function of management thresholds and Type I error tolerance (α). Higher thresholds required more samples than lower thresholds because populations were more heterogeneous at higher mean Se levels. For instance, to assess a management threshold of 4 mg Se/kg, a sample of eight fish could detect an increase of approximately 1 mg Se/kg with 80% power (given $\alpha = 0.05$), but this sample size would be unable to detect such an increase from a management threshold of 8 mg Se/kg with more than a coin-flip probability. Increasing α decreased sample size requirements to detect above-threshold mean Se concentrations with 80% power. For instance, at an α-level of 0.05, an 8-fish sample could detect an increase of approximately 2 units above a threshold of 8 mg Se/kg with 80% power, but when α was relaxed to 0.2, this sample size was more sensitive to increasing mean Se concentrations, allowing detection of an increase of approximately 1.2 units with equivalent power. Combining individuals into 2- and 4-fish composite samples for laboratory analysis did not decrease power because the reduced number of laboratory samples was compensated for by increased precision of composites for estimating mean conditions. However, low sample sizes (<5 fish) did not achieve 80% power to detect near-threshold values (i.e., <1 mg Se/kg) under any scenario we evaluated. This analysis can assist the sampling design and interpretation of Se assessments from fish tissue by accounting for natural variation in stream fish populations. *Integr Environ Assess Manag* 2015;11:143--149. Published 2014 SETAC[#]

Keywords: Selenium Bioaccumulation Fish Simulation Power Analysis Parametric Bootstrapping

Health and Ecological Risk Assessment

INTRODUCTION

Selenium (Se) contamination of aquatic ecosystems is a global concern because of its mobilization from anthropogenic activities and the narrow margin between nutritional optimality and toxicity (Young et al. 2010). For oviparous animals, a primary mechanism of toxicity involves recruitment failure as the result of Se transfer into eggs and associated developmental abnormalities and increased juvenile mortality rates (Janz et al. 2010). Ecological risk assessments for Se therefore are expected to be most precise when Se concentrations in organismal tissues are known (Hodson et al. 2010). In contrast, aqueous concentrations of Se may or may not indicate exposure risks for wildlife, depending on Se redox states, the kinetics of particulate formation from dissolved Se, and trophic transfer into dietary sources for target populations (Stewart et al. 2010; Janz et al. 2014).

Regulatory agencies in the United States, Canada, New Zealand, and Australia implement guidelines and standards for Se primarily based on aqueous Se concentrations (Hodson et al. 2010). In the United States, the Environmental Protection Agency uses a national recommended water quality standard of 5 μg Se/L for freshwater ecosystems (USEPA 1999). In 2004, the USEPA issued a draft Se criterion based on a mean whole-body fish tissue concentration of 7.9 mg Se/kg (USEPA 2004), and the state of Kentucky recently issued a chronic water quality criterion of 8.6 mg Se/kg for whole-body samples (Payne 2013). The USEPA approved the use of fish tissue for Se assessment in Kentucky but did not define implementation guidance (USEPA 2013). The USEPA subsequently issued a draft water quality criterion for whole-body freshwater fish of 8.1 mg Se/kg (USEPA 2014). The development of a fish tissue standard has been controversial, in part because of the unknown effects of fish species, age, sex, and season on the precision and accuracy of Se assessments (Lemly and Skorupa 2007).

Fishes may exhibit substantial variation in Se body burdens because of temporal change in Se bioavailability within a site, interspecific differences in diet, ontogenetic changes in diet, or movements across Se exposure gradients. Temporal variation in Se bioavailability may be caused by Se immobilization in sediments (Long et al. 1990), changes in seasonal weathering from upstream sources (Lindberg et al. 2011), or dilution of aqueous Se concentrations over time (Ruhl et al. 2010). Interspecific differences in fish diets are well known and are

Additional Supporting Information may be found in the online version of this article.

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Published online 10 September 2014 in Wiley Online Library (wileyonlinelibrary.com).

DOI: 10.1002/ieam.1579

manifest in the diversity of species traits related to gape size, gill raker morphology, pharyngeal teeth, and body form (Gerking 1994). Nearly all species of freshwater fish undergo ontogenetic shifts in feeding (Gerking 1994), and seasonal variation in resource availability affects stream fish diets and competitive interactions (Angermeier 1982). Fish movements for resource tracking or dispersal (Schlosser 1991) also may affect fish exposure to Se across space and time. For instance, Palace et al. (2007) documented patterns of Se assimilation in Rainbow Trout (*Oncorhynchus mykiss*) otoliths suggestive of fish movements between low- and high-Se streams. Variation in Se exposure and assimilation dynamics highlights the importance of an effective sampling design to assess contamination in stream fish populations and communities.

We investigated the sample size requirements (i.e., number of fish per site) to determine whether Se whole-body concentrations in stream fish populations exceed potential management thresholds. We used modeling and simulation techniques to estimate the Type II error rate (β) and, conversely, 1-β, which indicates the statistical power of a sampling design. We evaluated sample size requirements for freshwater whole-body fish tissue standards ranging from 4 to 8 mg Se/kg dw, encompassing a range of management thresholds proposed previously (Lemly 1993; DeForest et al. 1999; Lemly 2002; Hamilton 2003; USEPA 2014). Our purpose is not to evaluate the validity of potential management thresholds but to inform sampling protocols given observed variation within stream fish populations.

MATERIALS AND METHODS

We evaluated Se tissue concentrations in stream fish populations from 20 locations in southwestern West Virginia, USA (Figure 1; Supplemental Data Table 1). The dataset consisted of 382 individual whole-body Se concentrations from three common fish species (creek chub [*Semotilus atromaculatus*], green sunfish [*Lepomis cyanellus*], and central stoneroller [*Campostoma anomalum*]) collected between 2006 and 2011 by the West Virginia Department of Environmental Protection (WVDEP 2009) and Presser (2013). These species are commonly used for Se assessments in the study area because of their local abundance, widespread distribution, and resiliency to environmental degradation (Leonard and Orth 1986; Hitt and Chambers 2014). Fish were collected using standard backpack electrofishing techniques, euthanized with tricaine methanesulfonate, frozen at field sites, and shipped to analytical laboratories for chemical analysis. The WVDEP (2009) samples were analyzed by Bio-Chem Testing (Hurricane, WV, USA) and REI Consultants (Beaver, WV, USA). Samples from Presser (2013) were analyzed by the US Geological Service (USGS) Columbia Environmental Research Center (Columbia, MO). All laboratories used flow injection hydride generation atomic absorption spectrophotometry to quantify Se concentrations in whole-body fish samples (Brumbaugh and Walther 1989). We limited our analysis to population mean Se concentrations within the modeled range (<11 mg Se/kg).

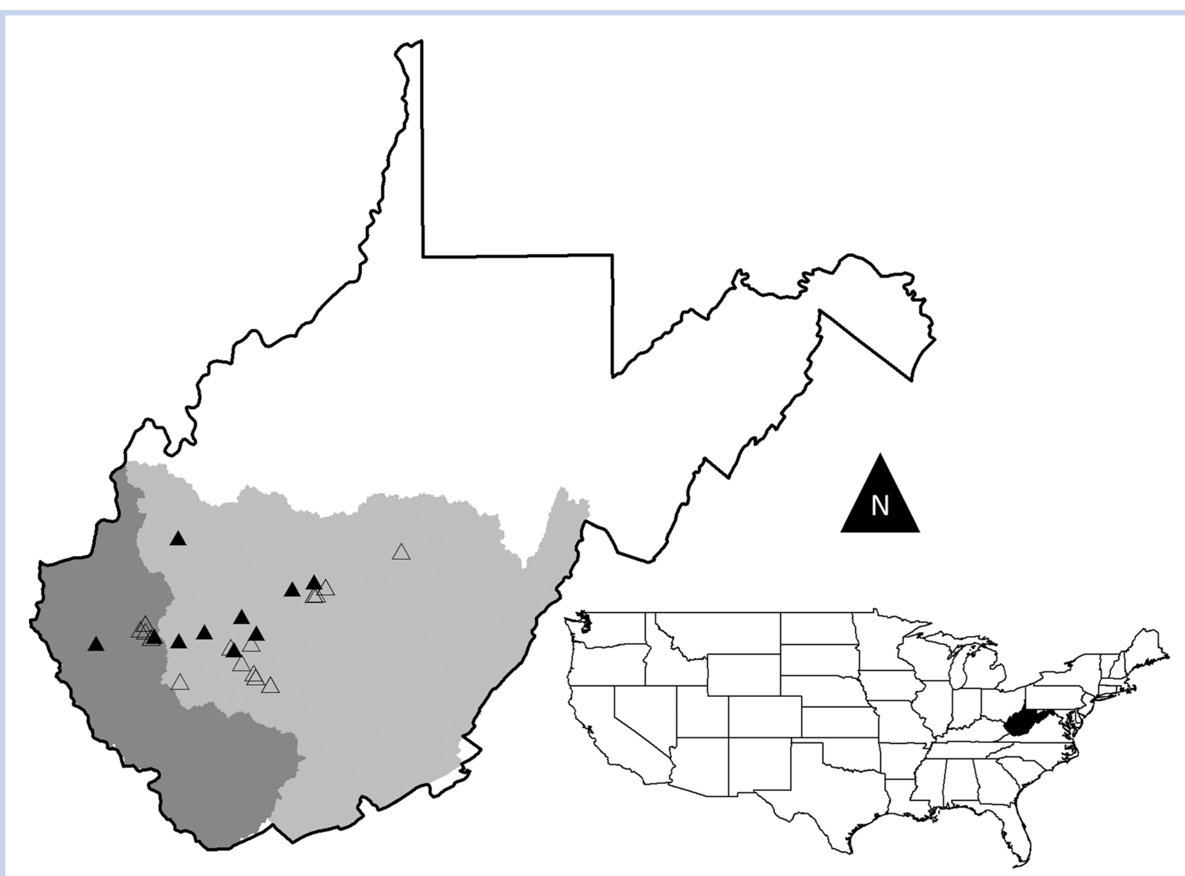

Figure 1. Map of sample sites in West Virginia, USA. Open triangles indicate sample sites from Presser (2014); filled triangles indicate sites from WVDEP (2009). The light gray polygon indicates the New-Kanawha River basin, and the dark gray polygon indicates the Guyandotte-Twelvepole River basins. Site data are provided in Supplemental Data Table 1.

We used parametric bootstrapping techniques (Good 2005) to assess sample size requirements for Se assessments of stream fish populations. Our approach involved 1) characterization of the mean-to-variance relationship for sampled populations, 2) simulation of increasing mean Se concentrations from theoretical distributions where the true mean and variance are known, 3) sampling from the modeled Se distributions with alternative sample sizes and Type I error rate (α) scenarios, and 4) calculation of statistical power from the probability that sampled mean concentrations were below a given management threshold when the true mean value was above it (i.e., Type II error rate).

We used gamma probability distributions to model whole-body Se concentrations. We chose gamma distributions because exploratory analyses indicated the observed data were not normally distributed, and gamma distributions provided good fits to observed whole-body Se data. Gamma distributions represent continuous variation in real numbers as defined by a shape parameter (k) and a scale parameter (θ). Properties of the gamma distribution are useful for simulation because combinations of the shape and scale parameter define the distribution mean ($k^*\theta$), variance ($k^*\theta^2$), and coefficient of variation (CV) $\left(\frac{1}{\sqrt{k}}\right)$. To fit the observed mean CV (0.24), we fixed k at 16.7 for all simulations. For each threshold, θ values were parameterized to minimize the difference between the threshold value and the mean of the distribution. For instance, at a threshold of 4 mg Se/kg, the calculated baseline θ was 0.24 (given k of 16.7 and baseline mean [$k^*\theta$] of 4.0).

We modeled increasing mean Se concentrations by increments of 0.1 mg Se/kg above management thresholds by adjusting θ in gamma distributions. Field sampling was simulated by drawing random samples from the modeled distributions using different numbers of draws (i.e., sample sizes) and compositing scenarios of 2 and 4 fish (i.e., multiple individuals combined for laboratory analysis; Rohde 1976). If the sampled mean was less than the calculated critical value for a given sample size and threshold, this represented a Type II error whereby the sampled mean Se concentration failed to detect that the true mean was above the threshold. For each management threshold scenario, we calculated power for sample sizes of 2, 4, 8, 16, and 32 individuals and for α-levels of 0.05, 0.1, and 0.2. We repeated the sampling process 10^4 times for each threshold, sample size, and α-level combination and calculated Type II error rates as the number of errors divided by 10^4 in each case.

RESULTS

Observed mean Se concentrations ranged from approximately 2 to 10 mg Se/kg in population samples (Figure 2). Creek chub, central stoneroller, and green sunfish showed a positive relationship between mean Se concentrations and the standard deviation of individual Se values (Figure 2) as indicated by positive linear regression coefficients and R^2 values above 0.75 for each species (Table 1). Green sunfish supported the highest rate of increase in standard deviation per unit mean Se concentration, and central stoneroller supported the lowest rate of increase (model coefficients = 0.420 and 0.187, respectively; Table 1), and differences in model slopes among species were marginally significant (analysis of covariance interaction term $p < 0.1$). Our modeled constant CV across all species (0.24) tracked well with the observed CV values (Figure 2).

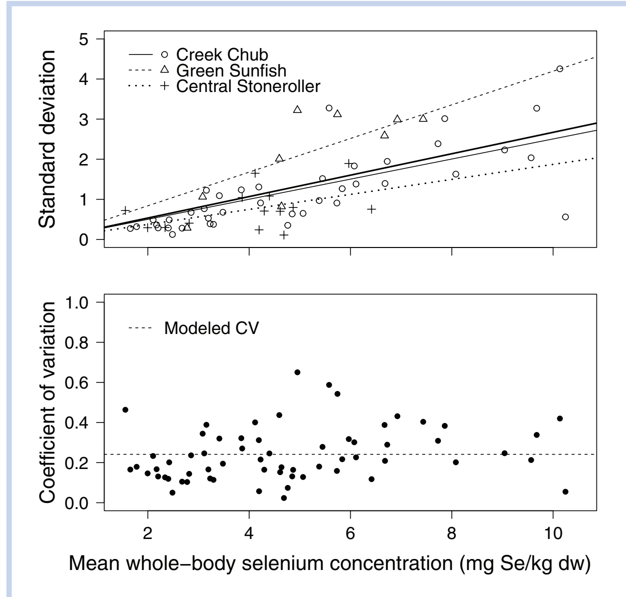

Figure 2. Relationship between selenium concentration mean and standard deviation in stream fish populations. Linear regression coefficients are in Table 1. We used the mean coefficient of variation (0.24) as a constant for simulating sampling across mean values.

The relationship between sample size and statistical power varied across true mean Se concentrations, management thresholds, and Type I error rate tolerance. As expected, power increased as true mean Se concentrations diverged from management thresholds and as sample sizes increased (Figure 3). The shape of power curves varied among management thresholds for given sample sizes. For instance, at a threshold of 4 mg Se/kg, a composite sample of 8 fish could achieve 80% power to detect an increase in mean concentrations of approximately 1 mg Se/kg (i.e., >5 mg Se/kg), whereas this sample size would not be expected to detect this level of change for a threshold of 8 mg Se/kg (i.e., 9 mg Se/kg). Instead, a sample of 8 fish at the higher threshold would reach 80% power when mean Se concentrations exceeded the threshold by approximately 2 mg Se/kg (given $\alpha = 0.05$) (Figure 3).

Increasing tolerance of Type I errors (α) increased power as expected (Figure 3). For example, to assess a management threshold of 6 mg Se/kg with a sample of 8 fish, an α level of 0.05 achieved 80% power at approximately 1.5 mg Se/kg above the threshold, but at an α-level of 0.20 this sample size reached 80% power at approximately 1.0 mg Se/kg. This is because the critical values associated with the threshold-mean sampling distribution decrease as α-levels increase, thus increasing the probability that increasing mean Se concentrations would be correctly detected in simulated sampling distributions. All sample size scenarios exhibited this general pattern, but the magnitude of the effect varied across sample sizes and management thresholds. For example, the sensitivity of a 2-fish sample to assess a management threshold of 6 mg Se/kg improved from approximately 3 mg Se/kg above the threshold where $\alpha = 0.05$ to approximately 2 mg Se/kg above the threshold where $\alpha = 0.20$. Similarly, where $\alpha = 0.05$, a 32-fish sample at this threshold improved from approximately 0.7 mg Se/kg above the threshold to approximately 0.4 mg Se/kg where $\alpha = 0.20$ (Figure 3).

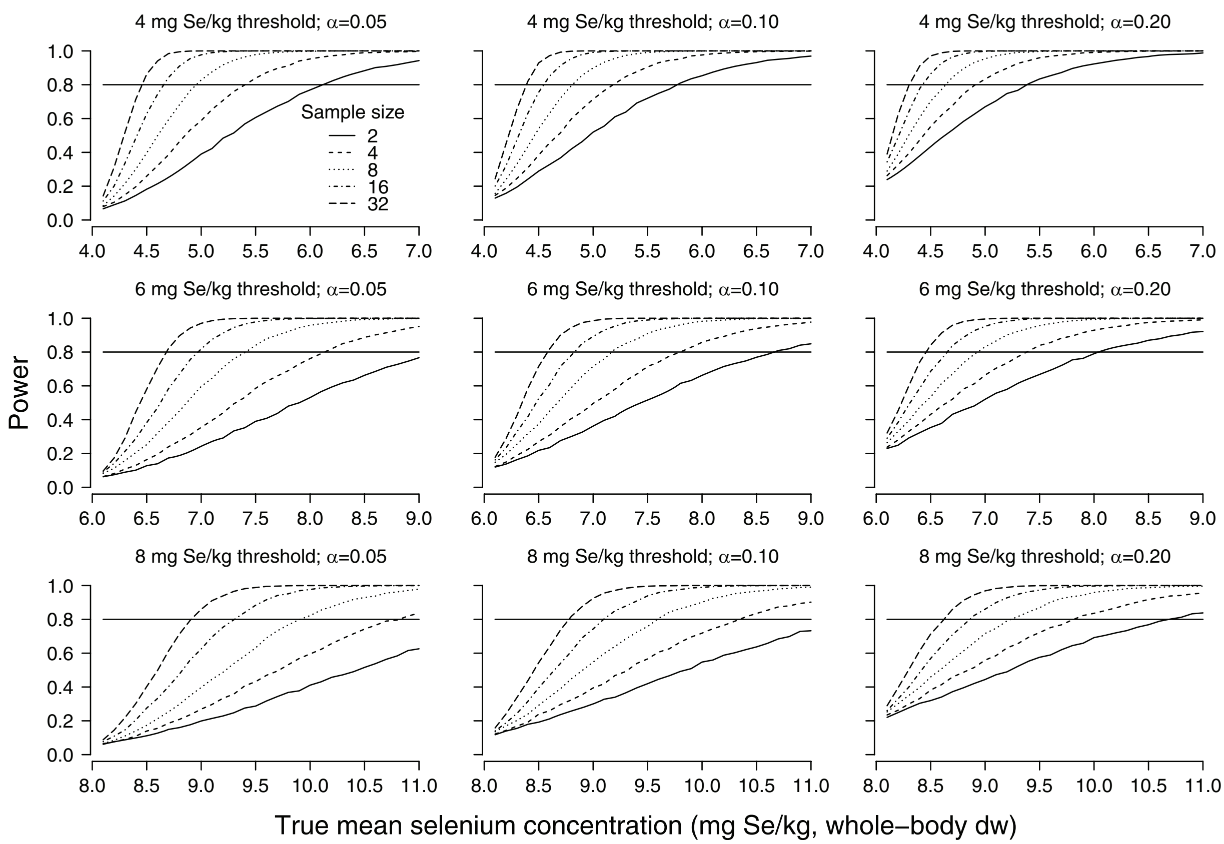

Figure 3. Power curves for modeled sample sizes (plotted lines) across management thresholds (rows) and Type I error rates (columns) to detect true mean selenium concentrations in stream fish tissue whole-body samples. The plotted horizontal lines indicate 80% power for reference.

Higher management thresholds benefited more from relaxed α-levels than lower thresholds. For instance, a sample of 4 fish and threshold of 4 mg Se/kg achieved 80% power at approximately 0.4 units closer to the threshold when α was increased from 0.05 to 0.20 (Figure 3). In contrast, this sample size achieved 80% power at approximately 1.0 units closer to the threshold of 8 mg Se/kg (i.e., detection at ~9.7 mg Se/kg for α = 0.20 vs ~10.7 mg Se/kg for α = 0.05) (Figure 3). Combining individuals into 2- and 4-fish composite samples for laboratory analysis did not decrease power relative to individual samples (results not shown). Low sample sizes (<5 fish) did not achieve 80% power to detect near-threshold values (i.e., ~1 mg Se/kg above thresholds) under any scenario we evaluated (Figure 3).

DISCUSSION

Se concentrations in fish tissue are more closely linked to toxicological endpoints than waterborne Se concentrations (Stewart et al. 2010), and thus fish tissue analysis offers advantages for environmental assessment of Se exposure and risks (Hodson et al. 2010). However, implementation of a Se fish tissue standard introduces important technical questions, including considerations of sample size (Lemly and Skorupa 2007). Our results demonstrate that 1) higher management thresholds required more samples than lower thresholds because populations were more heterogeneous at higher mean Se levels, 2) combining samples into 2- and 4-fish composites did not decrease statistical power relative to individual samples and would decrease analytical costs, 3)

Table 1. Linear models relating mean whole-body selenium concentrations to standard deviation within stream fish population samples[a]

Species	Population samples (individuals)	Linear model coefficient	Standard error of the estimate	R^2
Creek chub	40 (248)	0.251	0.019	0.824
Central stoneroller	14 (78)	0.187	0.030	0.751
Green sunfish	9 (60)	0.420	0.043	0.922
All species	63 (386)	0.267	0.017	0.798

[a]Model coefficients provide the rate of increase in standard deviation per unit increase in mean selenium concentrations assuming a *y*-intercept through the origin. Standard deviations (SD) were calculated among individual samples. Data and linear regression fit lines are plotted in Figure 2.

increasing tolerance for Type I errors can improve the statistical power of a given sample size, and 4) sample sizes of fewer than 5 fish would be expected to require true mean Se concentrations to be greater than approximately 1 mg Se/kg above the threshold for detection with 80% power.

Our simulation model results stemmed from the empirical observation that increasing mean Se concentrations were associated with increasing among-individual variation in fish population samples. We observed this pattern for each species we evaluated (Figure 2; Table 1), and other studies of Se in fish tissue in the study area are consistent with this result (Potesta & Associates 2011; Arnold et al. 2014). The positive relationship between mean and variance was expected because minimum Se concentrations are bounded by 0, so populations with high mean values are more likely to include individuals across a larger range of possible Se concentrations than populations with lower mean Se values. Moreover, stressed populations often show increased variance in cellular and phenotypic endpoints, and this has been observed in many taxonomic groups (i.e., insects, gastropods, fishes, birds, mammals) (Orlando and Guillette 2001). Such intraspecific variation is inherent to mechanisms of natural selection, local coexistence of similar species, and community assembly (Violle et al. 2012) and is expected for Se body burdens in stream fish populations as we observed.

Fish movement patterns also may contribute to the observed mean-to-variance relationship. Stream fish populations typically support resident and mobile individuals (Rodriguez 2002; Radinger and Wolter 2013), so some individuals captured in a stream sampling location may have been exposed to environmental conditions in distant locations as well as those associated with the sampling site. Environmental Se concentrations can exhibit spatial variation within streams as a function of tributary confluences (Lindberg et al. 2011), and mark-recapture studies show intraspecific variation in movement distances for the study species (Storck and Momot 1981; Mundahl and Ingersoll 1989), which could affect fish exposure to Se. Analysis of fish otoliths also suggested that individual fish may move among stream network locations and Se exposure gradients (Palace et al. 2007). Such stream network-scale movements can influence fish community composition (Hitt and Angermeier 2008) and could introduce an important source of variation for Se assessment in stream fishes.

Using fish tissue for Se assessments will require a sufficient number of individuals to be available for sampling in targeted locations. For instance, the USEPA (2013) specified that "if sufficient fish tissue cannot be obtained, the permit holder will be deemed to be in non-compliance…" if aqueous Se concentrations exceed benchmark levels. Our results indicate that sample size requirements will be more achievable at lower management thresholds than higher management thresholds. For instance, 8 individuals were required to detect an increase of 1 mg Se/kg at a threshold of 4 mg Se/kg, whereas between 16 and 32 individuals were required to detect a similar increase from a management threshold of 8 mg Se/kg threshold (Figure 3). A probabilistic survey of stream fish communities in West Virginia (USA) (Detenbeck and Cincotta 2008) detected creek chub at most sample sites (85 of 100), but densities ranged from 0.03 to 22.4 individuals/100 m^2, and most sites (82%) supported fewer than 5 individuals/100 m^2. Assuming a uniform stream width of 5 m, 56% of their sample sites would require electrofishing over 500 m to reach a sample size of 30 creek chub (Detenbeck and Cincotta 2008). Sample sizes necessary to achieve 80% power therefore may not be available within typical electrofishing reach lengths in some Appalachian streams.

Tolerance for Type I error rates (α) provides a tradeoff for consideration in sampling design development. Relaxing α from 0.05 to 0.20 improved the per-sample value for statistical power in all scenarios we evaluated. However, the greatest benefits of increasing α were observed where sample sizes are relatively low (<5 fish) and management thresholds are relatively high (8 mg Se/kg). Accepting a 20% Type I error rate may be unacceptable for assessment purposes, but increases in sample size would be necessary to compensate for the more restrictive α levels (e.g., 0.05). The relative value placed on Type I and II error rates will depend on risk management decisions regarding the consequences of false-positive and false-negative results. Buhl-Mortensen (1996) argues that environmental science should weight the consequences of failing to detect a true effect (Type II error) over the consequences of falsely detecting a noneffect (Type I error).

Combining individuals into 2- and 4-fish composite samples for laboratory analysis did not decrease power relative to individual samples (results not shown) because the reduced number of laboratory samples was compensated for by increased precision of composites for estimating mean conditions in our simulations. Composite sampling is well established as a method for minimizing costs while improving estimates of mean conditions (Boswell et al. 1996). For example, Paasivirta and Paukku (1989) reduced analytical costs by 54% for assessing organochlorine compounds in fish tissues when they switched from individual samples to composite samples. Consistent with our results, their composite sampling approach did not decrease the accuracy of their assessment (Paasivirta and Paukku 1989) because composite samples will tend to estimate mean conditions (Patil 1995). Our results indicate that composite samples would provide cost savings for Se analysis but that low sample sizes (<5 fish) should not be expected to detect near-threshold mean Se concentrations (i.e., within 1 mg Se/kg above thresholds) from either individual or composite samples.

Our simulation models incorporated several assumptions. We assumed an equal probability of sampling all individuals within populations (i.e., no difference in detection rates) and that the sampling distributions were known. An alternative approach would be to conduct sampling in locations where mean conditions are established from prior research to construct empirical sampling distributions for Se assessment. However, this approach would tend to decrease the utility of composite sampling because of diminished degrees of freedom for *t* tests, whereas this was not a factor in our simulations using known sampling distributions. Our approach indicated how characterizing the underlying mean-to-variance relationship can improve the utility of composite sampling in this regard. In addition, although linear model coefficients of determination were high for all species we evaluated (>0.75), further sampling may reveal nonlinear (asymptotic) relationships at higher mean Se concentrations that would need to be incorporated for estimating sample size requirements above the simulation range considered here (4–11 mg Se/kg).

Natural variation in stream fish populations may affect the interpretation of fish tissue-based Se standards based on sample size requirements. Our results indicate that the proposed management threshold of 8.1 mg Se/kg for whole-body fish (USEPA 2014) would require a sample of at least 8 individuals to achieve 80% power for detecting increases of approximately

1.2 mg Se/kg (given $\alpha = 0.20$) or approximately 2 mg Se/kg (given $\alpha = 0.05$) above the threshold. Samples of fewer than 5 fish would be unlikely to detect increased mean Se concentrations within approximately 1.7 mg Se/kg above the proposed threshold (USEPA 2104) with 80% power. Given the rapid transition from nutritional essentiality to toxicity in wildlife populations (Young et al. 2010), study designs capable of detecting near-threshold values may be warranted.

Acknowledgment—Funding for analysis and manuscript preparation was provided by the USGS Leetown Science Center. Sample collection and chemical analysis was supported by the USGS West Virginia Water Science Center. We thank K. Cooper, C. Snyder, J. Daily, S. Faulkner, K. Maloney (USGS Leetown Science Center), D. Chambers, K. Paybins, T. Messinger, J. Wiley (USGS West Virginia Water Science Center), T. Presser (USGS National Research Program), T. May (USGS Columbia Environmental Research Center), and G. Russo (U.S. Environmental Protection Agency) for their assistance.

SUPPLEMENTAL DATA

Table S1. Data sources and sample sizes used for evaluating the mean-to-variance relationship in stream fish selenium concentrations (Figure 2). Numbers of individual whole-body samples are given for Central Stoneroller (CS), Creek Chub (CC), and Green Sunfish (GS) among sampling events. Site locations are mapped in Figure 1. Data were provided by USGS and West Virginia Department of Environmental Protection (WVDEP).

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