

**Update to Kentucky Water Quality Standards for Protection
of Aquatic Life: Acute Selenium Criterion and
Tissue-Based Selenium Chronic Criteria**

Randall G. Payne, Environmental Scientist

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

Frankfort, Kentucky

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Sandy Gruzesky, Director
Division of Water

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TABLE OF CONTENTS

<u>Chapter</u>	<u>Page</u>
ABSTRACT	1
CHAPTER 1.0 INTRODUCTION	1
1.1 GEOCHEMISTRY AND TOXICITY OF SELENIUM IN THE ENVIRONMENT .	2
1.2 HISTORY OF SELENIUM AQUATIC LIFE CRITERIA DEVELOPMENT: 1976 to 2004	3
CHAPTER 2.0 UPDATING WATER QUALITY STANDARDS FOR SELENIUM IN KENTUCKY	5
2.1 ACUTE SELENIUM	6
2.2 CHRONIC SELENIUM	8
2.2.1 Updating the USEPA’s 2004 Draft Selenium Fish Tissue-Based Chronic Criterion with a Focus on Taxa Found in Kentucky.....	9
2.2.2 Calculating Tissue Thresholds.....	18
2.2.2.1 Bluegill (<i>Lepomis macrochirus</i>) and Other Centrarchidae Data	20
2.2.2.2 Trout and Other Salmonidae Data	22
2.2.2.3 Minnow (Cyprinidae) Data	23
2.2.2.4 Sucker (Catostomidae) Data	24
2.2.2.5 Data of Other Species	24
2.2.2.6 Data Summary	25
2.2.2.7 Chronic Criteria Calculations	25
2.3 IMPLEMENTATION OF THE CRITERIA	27
2.3.1 Acute Selenium Criterion	27
2.3.2 Chronic Selenium Criteria	28

2.3.3 Threshold Screening Value.....	29
LITERATURE CITED	30
APPENDIX A	36
APPENDIX B	40

LIST OF TABLES

Table 1. Freshwater selenium data from chronic toxicity tests. Reference (study) considered in USEPA 2004 draft chronic criterion =*	10
Table 2. Selenium toxicity data available for Kentucky fish species used to calculate GMCVs CV = Chronic Value, GMCV = Genus Mean Chronic Value, WB = whole body	21
Table 3. Calculation of the selenium final chronic values for fish whole body using the updated chronic data (N = 10 genera, R = sensitivity rank in database, P = rank / N+1	26
Table 4. Calculation of the selenium final chronic values for egg/ovary using the updated chronic data (N= 10 genera, R = sensitivity rank in database, P = rank / N+1)	26
Table A-1. Data used to derive the updated “All Species” (data for bluegill, fathead minnow, and cutthroat trout) translation equation. Bluegill and cutthroat trout data are as reported in Appendix 1 of GEI et al. (2008) and fathead minnow data are from GEI (2008).....	36

FIGURE

Figure A-1. Modified “all species” regression using log-transformed egg/ovary and whole body tissue selenium concentrations measured in bluegill, fathead minnow and cutthroat trout.....	38
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Aquatic Life Selenium Criteria for Kentucky

Abstract: The Kentucky Energy and Environment Cabinet developed chronic selenium criteria based on either whole body or egg/ovary concentrations. The current national recommended water quality chronic criterion for selenium (Se) of 5.0 µg/L will be used as a threshold, which if exceeded will trigger the collection of fish whole body or egg/ovary tissue to ascertain attainment with the appropriate tissue-based criterion. Since publication of the national recommended criterion in 1987 there have been ongoing toxicity studies. Current findings show the mode of chronic toxicity effects on fishes is based primarily on dietary uptake while direct exposure to aqueous concentrations is negligible. The whole body or egg/ovary criteria are based on fish tissue concentration of total selenium and presented herein. Either criterion will provide confirmation of potential toxicity effects in waters where exceeded.

The current USEPA national recommended acute total selenium criterion is based on an equation that accounts for the percent fractions of selenite and selenate in a water body. The current criterion updates the previous national recommended criterion (20 µg Se/L) that was based only on total selenium (USEPA 1987). Research conducted since the current recommended criterion was developed recognizes the differential toxicity of the two predominate selenium species that constitute total selenium in the water column, selenite and selenate, and shows that the presence of sulfate in the water column modifies or attenuates the potential acute toxicity effects of selenate. Therefore, Kentucky has partially incorporated this modifier calculation into the acute criterion equation.

1.0 Introduction

Selenium is an essential element, but it is toxic at elevated concentrations. Acute toxicity occurs at high concentrations in the water column with toxic response similar to those of other inorganic elements like metals. The toxic effects of selenium (Se) in the aquatic environment are well recognized through decades of research. The chronic toxicity of selenium is not primarily a response to water column concentrations, but a result of body accumulation of selenium from dietary intake (USEPA 1998). It is this mode of action for chronic toxicity that is reflected in the approach the Kentucky Energy and Environment Cabinet (Cabinet) has taken regarding the proposed modification to Kentucky's chronic criterion. The complexity of selenium bioavailability and toxicity results from various selenium species occurring in the food chain, the varied concentration and speciation of selenium in aqueous systems, hydrological characteristics (lentic or lotic environment), and the ecological nature of aquatic systems (Barceloux 1999).

Given the differential toxicity of species of selenium that occur in the environment under varying hydrological and redox (reduction-oxidation) conditions the development of proper criteria is complex. It is this complexity of the fate and role of selenium in biological systems that created difficulty in determining aquatic life criteria and implementation (Simmons and Wallschlager

2005). However, this complexity has driven environmental studies to address many of the outstanding questions related to the mode of toxicity, particularly the dietary role that is of primary consideration regarding chronic toxicity in the aquatic environment. Given these recent findings with regard to understanding modes of toxicity and data that support those findings, the Cabinet is proposing to update its acute selenium criterion with the current USEPA national recommended criterion and a sulfate modifier equation for selenate (USEPA 2004). We propose to change the current chronic water criterion of 5.0 µg/L whereby it becomes a threshold; which when exceeded will trigger fish tissue sampling to assure the tissue-based criterion concentration, either whole body or egg/ovary, is met and the aquatic life use is protected. The modifications (dietary-based) to the chronic toxicity criterion and updates to the acute criteria reflect the recent findings of toxicology research.

1.1 Geochemistry and Toxicity of Selenium in the Environment

The fate and transport of selenium is intimately related to the speciation of selenium, which is controlled by the pH and redox conditions of the environment. In natural environments, selenium can exist in four different oxidation states, Selenide (-II), elemental Selenium, Se (0), Selenite, SeO_3^{2-} (IV), and Selenate SeO_4^{2-} (VI) (McNeal and Balistrieri 1989; Elrashidi, et al. 1987). It generally is accepted that the order of toxicity of selenium species is as follows: Se-met (selenomethionine) (seleno-amino acids) > selenite > selenate (Simmons, et al. 2005). Presently, Se-met is believed to be the species of selenium that is most bioavailable to primary consumers (those organisms that consume producers (e.g. algae) of energy and nutrients) in the food chain (Bowie et al. 1996).

Thermodynamic data indicates that selenide should exist under reducing conditions as hydrogen selenide (H_2Se), and as metal selenides. The latter tend to be associated with metal sulfide minerals and, along with Se-sulfides, are very insoluble (Elrashidi et al. 1987). Similar to selenide, elemental selenium is only stable in reducing environments and is insoluble. Elemental selenium can be oxidized to selenite and trace amount of selenate by certain microorganisms.

Selenite has a strong affinity for sorption, particularly by iron (Fe) oxides such as goethite, amorphous Fe hydroxide, and aluminum (Al) sesquioxides (Merrill, et al. 1986; Balistrieri and Chao 1987). Adsorption of selenite⁻ depends on its concentration, pH, nature of particles, and the concentration of other competing anions (such as phosphate) (e.g. Balistrieri and Chao 1987; Ryden et al. 1987). In contrast to selenite, selenate is stable in well-oxidized environments, and not as strongly adsorbed as selenite by solid particles. The conversion of selenate to the less mobile form Se (selenite or elemental Se) is a slow process.

For the pH and redox conditions of most aquatic environments, selenite and selenate are the dominant species of selenium. Microbial processes can change the speciation of selenium through oxidation or reduction, or through the formation of organic selenium compounds.

Competition between sulfate and selenate has been observed in many animal species affecting the toxic potential to aquatic species; as sulfate concentration increases the acute selenate toxicity decreases. A similar modification occurs between certain metals and hardness; as hardness increases metal bioavailability decreases for aquatic species. The USEPA proposed draft selenium criteria (2004) presenting acute toxicity results conducted at varying sulfate concentrations to the freshwater species *Daphnia magna*, *Hyalella azteca*, *Gammarus pseudolimnaeus*, Chinook salmon and fathead minnow. The USEPA withdrew the 2004 draft criteria based on comments associated with the chronic criterion.

1.2 History of Selenium Aquatic Life Criteria Development: 1976 to 2004

The first nationally recommended criteria for selenium was published in 1976 and was stated for protection of aquatic life that concentration of selenium should not exceed 0.01 of the 96-hour LC₅₀ (lethal concentration resulting in mortality of 50 percent of a test population) determined by bioassays. Indicators used were *Escherichia coli*, *Microregma* sp. *Daphnia* sp. and *Scenedesmus* sp. The criterion was based on a 96-hour threshold effect at 2.5 mg/L (USEPA 1976). An update to the 1976 aquatic life criterion was published by USEPA (1980). For freshwater species a criterion was published for selenite at 35 µg/L as a 24-hour average and a not to exceed concentration of 260 µg/L. Data from bioassay results indicated the concentration of selenate was not-to-exceed 760 µg/L (acute); no data were available for chronic toxicity.

Partial updates occurred in 1987, 1995 and 1996. The 1987 update recommended a chronic criterion not to exceed 5.0 µg/L more than once in three years on average and an acute criterion of 20 µg/L not to be exceeded on a one-hour average more than once every three years. A partial update to the acute criterion occurred in 1995 with the recognition of differential acute toxicities of selenite and selenate, and accounted for the percentage of the species of selenium present. A formula was adopted as the national recommended criterion to account for this difference. That formula and current recommended national water quality criterion for selenium is:

$$CMC = \left[\frac{1}{\left(\frac{f1}{CMC1} \right) + \left(\frac{f2}{CMC2} \right)} \right]$$

where f1 and f2 are the fractions of selenite and selenate, respectively, in the water column and CMC1 (criterion maximum concentration) and CMC2 are 185.9 (selenite) and 12.82 (selenate) µg/L, respectively. These relative concentrations are based on calculations from the 1987 criteria recommendations (USEPA 2012). The current national recommended chronic criterion is 5.0 µg/L.

In 2002 and 2004 the USEPA published draft selenium criteria that accounts for the differential toxicity of selenite and selenate, but also incorporates data from assays that support water column sulfate as a modifier of selenate toxicity in recognition of the dietary pathway for chronic toxicity of selenium (Canton 1999; Brix et al. 2001a,b; USEPA 2002 and 2004). Selenium speciation of water column samples is important when determining the biological and geochemical cycling of a substance in the environment, along with exposure routes and potential for relative toxicity to aquatic organisms. USEPA's draft acute criterion accounted for the modifying effect of sulfate in the water column on acute toxicity of selenate due to the competition for the two substances in aquatic animals (Brix, et al. 2001a; Ogle and Knight 1989; Riedel and Sanders 1996). This relationship is akin to the resultant effect hardness has on the toxicity of certain metals (e.g. copper, lead and cadmium); as hardness increases toxicity of these metals decrease. This relationship reflects the sulfate-selenate acute toxicity effect wherein there is an inverse relationship as related to acute toxicity. Sulfate competes with selenate in the uptake into aquatic organisms (Ogle and Knight 1989; Riedel and Sanders 1996; Bailey et al. 1995; Hansen et al. 1993). Since the uptake into organisms of selenate is reduced as sulfate concentration increases, this affects the toxicity of selenate to the organisms (Brix et al. 2001a). Thus, sulfate is used for correction to the toxicity of selenate. When developing the sulfate correction equation, the USEPA took into account the variability of selenate toxicity to different life stages and test conditions of the studies used to determine the sulfate slope that all contribute to the uncertainty of the sulfate correction. The regression analysis (a statistical tool for the investigation of relationships between variables) showed significant, positive slope for five of six species that had precisely determined acute values. An F-test (statistical test) indicated that the null hypothesis could not be rejected. "Analysis of covariance thus confirmed it is correct to assume there is no significant variation in slopes among species and that the overall slope is a reasonable estimate of the relationship between sulfate concentration and selenate toxicity" (USEPA 2004). Note, an analysis of covariance is a measure of how much two variables change together and the strength of the relationship between them.

The USEPA recognized data from acceptable bioassays related to the effects of selenate in freshwater for 12 invertebrate species and 11 fishes. These species satisfied the requirement of eight families per *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses* (Stephan et al. 1985), hereafter referred to as the Guidelines.

The Cabinet adopted Kentucky's current aquatic life standards for selenium in 1990. The standards were a result of criteria published as national recommended criteria in 1987 (USEPA 1987) which are not based on laboratory-developed criteria. The national recommended chronic criterion was based on field observations from Belews Lake, North Carolina and the acute standard was determined by applying an acute-to-chronic ratio in reverse from the chronic value (Canton 1999). Given there are now available data from laboratory-derived bioassays that reflect

sound science, including data gleaned from species that reside in Kentucky, the Cabinet concluded it appropriate to propose acute aquatic life criteria for selenium based on current data that is protective of Kentucky's aquatic resources and that the cabinet can use to make sound decisions.

2.0 Updating Water Quality Standards for Selenium in Kentucky

States are given the option in Section 303(c) of the federal Clean Water Act to adopt national recommended standards or to develop site-specific (statewide in this instance) water quality standards that are protective of the local aquatic resources and the resident biota that inhabit or depend on those aquatic resources. The commonwealth's current criteria of 20 µg/L and 5.0 µg/L were published by USEPA in 1987 and are now over 25 years old. Given there have been considerable published data regarding selenium toxicity, and much of it meeting the Guidelines required to develop chronic or acute criteria, it is appropriate and in the best interest of all stakeholders for Kentucky to develop criteria based on the latest science.

There exists a dataset from the 2004 draft document published by USEPA. These data derive from bioassays that are scientifically defensible for use in updating acute and chronic selenium criteria for Kentucky standards. Resulting updates of acute criteria for selenite and selenate are summarized below. With regard to chronic toxicity, in addition to those toxicology studies in the 2004 draft, more published chronic tissue-based data are available since publication of the 2004 draft document. Chronic value updates from the 2004 draft and all subsequently published studies were compiled and are presented in Section 2.2.1 below.

A brief overview of the factors involved in the Guidelines is presented to help understand the derivation of the proposed criteria for selenium. The salient factors for considerations are:

- (1) Acute toxicity test data are gathered from all suitably developed studies. Data need to be available for species representing eight families from a diverse assemblage of taxa;
- (2) The FAV (Final Acute Value) is derived by extrapolation or interpolation to a hypothetical genus more sensitive than 95 percent of a diverse assemblage. The FAV represents the LC₅₀ or EC₅₀ (concentration causing observed toxicity effects on 50 percent of a test population) and is divided by two to obtain an acute criterion protective of nearly all individuals in such a genus;
- (3) Chronic toxicity test data (those test exposing taxa to longer-term survival, growth and reproductive success) require at least three taxa. The common approach to determine a chronic criterion is accomplished through an appropriate acute-chronic ratio (the ratio of acutely toxic concentrations to the chronically toxic concentrations) and applying that ratio to the FAV determined from factor 2 above; and

- (4) When necessary, the acute and/or chronic criterion may be lowered to protect critically important species (e.g. endangered species).

The primary chronic toxicity pathway for selenium is one of bioaccumulation through diet, a different mode of action than many toxicants. It is because of this pathway that Step 3 above from the Guidelines is not the appropriate approach to determine chronic criterion for a substance like selenium. The Guidelines incorporate language allowing for “appropriate modifications” of the procedures if necessary to obtain criteria that are based on sound science. The procedures followed are presented in Sections 2.2.1 and 2.2.2 below.

2.1 Acute Selenium

Currently Kentucky water quality standards include an acute value for total selenium of 20 µg/L. As discussed, selenium is found most commonly in the aquatic environment as either selenite or selenate and data from toxicity studies show there are differential toxicities for these two species to aquatic organisms. The 1995 acute criterion the USEPA (1996) published for protection of aquatic life was a formula that accounted for the relative proportions of selenite and selenate in a water body (USEPA 2012). The use of USEPA-approved methods for determination of selenium species is required by the Cabinet. This was a step forward in reaching an acute criterion based on scientific information, and substantially improved criterion compared to the previously discussed 20 µg/L total selenium criterion. Investigation into the draft acute criterion proposed by USEPA (2004) that was based on this formula found this formula would provide marked progression in setting criteria based on the best current understanding of the science of acute toxicity of selenium. The 2004 document went a step farther by taking into account the modifying effect of sulfate to selenate (Brix et al. 2001a,b); this modification factor is similar to accounting for the ameliorating effect of water hardness on the toxicity of many metals and different modes of toxicity between acute and chronic (dietary and bioaccumulation) exposures (Canton 1999). Given the new and additional studies that went into development of this proposed acute criterion the Cabinet considers adoption of this formula, as updated with more recent acute selenite and selenate data, to be a scientifically sound update to acute water quality standards for selenium.

Selenite Toxicity

The USEPA recognized 14 species of freshwater invertebrates and 20 species of fishes that had acceptable data on the acute effects of selenite. This satisfied the condition of eight families called for in the Guidelines. Invertebrates demonstrated toxic effects at both ends of the range by having species that are the most sensitive and least sensitive to selenite. The SMAV (Species Mean Acute Value) of the invertebrates ranged from 440 µg/L for the crustacean, *Ceriodaphnia dubia*, to 203,000 µg/L for the leech, *Nepheleopsis obscura*. The selenite SMAV for fishes ranged from 1,783 µg/L for the striped bass, *Morone saxatilis*, to 35,000 µg/L for the common

carp, *Cyprinus carpio*. The freshwater SMAV were then calculated as geometric means of the available acute values for selenite and GMAVs (Genus Mean Acute Values) were next calculated as the geometric means of the SMAVs. The most sensitive species with available mean acute values was *Hyalella* at 440 times more sensitive than the most tolerant, the leech *Nephelopsis*. The FAV of the four most sensitive taxa at the 5th percentile of those genera was calculated as 514.9 µg/L following methodology in the Guidelines. The resultant freshwater CMC for selenite is calculated to be 258 µg/L, or one-half the FAV.

Selenate Toxicity

As noted previously, selenate toxicity to aquatic life is dependent on the concentration of dissolved sulfate in freshwater (this relationship has not been found with regard to selenite). Studies reviewed by the USEPA for development of acute criteria for selenium indicate this relationship between selenate and sulfate (Brix et al 2001a,b). Freshwater taxa used in studies showing this relationship between selenate and sulfate included *Ceriodaphnia dubia*, *Daphnia magna*, *Hyalella azteca*, *Gammarus pseudolimnaeus*, Chinook salmon and fathead minnows. Data from these studies indicated selenate is more toxic in low sulfate water compared to higher sulfate water. Given this relationship, the concentration of sulfate in the water column is used as a correction to the toxicity of selenate.

In the criterion derivation by USEPA, 12 invertebrate species and 11 fish species were deemed of proper design and rigor for use of the acute effects data. These 23 species meet the eight family provision of the Guidelines. As with selenite, invertebrates had species that represented both the most and least sensitive taxa to the toxic effects of selenate adjusted for sulfate. The SMAVs ranged from 593 µg/L for the crustacean, *Daphnia pulicaria* to 15,154,616 µg/L for the leech *Nephilopsis obscura*. The SMAVs for fishes ranged from 10,305 µg/L for the razorback sucker, *Xyrauchen texanus*, to 226,320 µg/L for the channel catfish *Ictalurus punctatus*. Next, the GMAVs were calculated from the SMAVs. The most sensitive genus was the cladoceran *Ceriodaphnia*, with a sulfate adjusted GMAV of 842 µg/L. The amphipod *Hyalella* was the second most sensitive genus with a sulfate adjusted GMAV of 1,397 µg/L. The fourth most sensitive genus was the amphipod, *Gammarus*, with a sulfate adjusted GMAV of 2,522 µg/L. The GMAV for the catfish, *Ictalurus*, was 226,320 µg/L adjusted for sulfate.

Of the four most sensitive genera of invertebrates, the range of sensitivity spanned a factor of 3.0. At a sulfate concentration of 100 mg/L, the freshwater FAV, representative of the most sensitive 5th percentile genus, was calculated to be 834.4 µg/L for selenate. The resultant CMC for selenate is:

$$e^{(0.5812[\ln(\text{sulfate})] + 3.357)}$$

Set at a sulfate concentration of 100 mg/L, the yield is 417.2 µg/L, or one-half the FAV.

The current national recommended criterion to protect aquatic life from acute selenium is the formula:

$$CMC = \left[\frac{1}{\left(\frac{f1}{CMC1} \right) + \left(\frac{f2}{CMC2} \right)} \right]$$

where f1 and f2 are the fractions of total selenium considered selenite and selenate, respectively, and CMC1 and CMC2 are 185.9 µg/L and 12.82 µg/L, respectively. However, using the recent (USEPA 2004) test data and the sulfate modifier:

$$CMC2 = e^{(0.5812[\ln(\text{sulfate})] + 3.357)},$$

the resultant formula values are 258 µg/L selenite (CMC1) and CMC2 is $e^{(0.5812[\ln(\text{sulfate})] + 3.357)}$ (at 100 mg/L sulfate, the value is 417 µg/L [selenate]); f1 and f2 are the fractions of total selenium considered selenite and selenate, respectively, in a water body.

2.2 Chronic Selenium

A tissue-based criterion with chronic endpoints is an excellent protective value since tissue-based criteria are based on integration of exposure to the toxicant temporally and spatially (e.g. chemical reaction rates, organisms in the food chain and exchange rates between sediment, water and organism) (USEPA 1998). In the 2004 draft criterion for chronic protection, USEPA chose to base the value on whole-body tissue residue rather than organ-specific tissues such as ovary, liver, kidney or muscle. This was done in part with consideration of practical issues like obtaining organ-specific tissues and the recognition that many water bodies may have a limited community of primarily small-bodied fishes which would exacerbate the difficulty of collecting specific body tissues. While it is recognized the ovaries may be an excellent target tissue the direct transfer of selenium to the eggs and developing embryo and larva (the developmental stages are one of the most sensitive in the lifecycle for chronic effects), collecting egg/ovary tissue has inherent difficulties. Egg/ovary tissue is only available seasonally and is often difficult to extract from fishes in sufficient quantities for analysis, particularly in small fish; additionally, the maturity in ovaries may affect selenium concentrations.

The USEPA 2004 draft selenium criteria developed for protection of aquatic life in fresh water was a move forward toward setting a protective chronic criterion because it was tissue based. In working toward development of a tissue based chronic criterion for Kentucky it was necessary to evaluate USEPA's 2004 draft criterion. Given the Cabinet's development of proposed chronic criteria is Kentucky-specific, the Cabinet examined data from the USEPA draft for fish families that are resident in Kentucky, or may be expected to occur in Kentucky (e.g. economically and recreationally important species) (Thomas 2011). The most species-rich families in Kentucky

include Percidae, Centrarchidae, Cyprinidae, Catostomidae and Ictaluridae (Thomas 2011). The 2004 draft criteria document included chronic endpoints for three of these families: Centrarchidae, Cyprinidae and Catostomidae. Since the 2004 document was released additional studies have been published, that include additional data for taxa that occur in Kentucky.

To increase the number of studies available in which chronic effects could be compared, the Cabinet has considered studies that were based on egg/ovary tissue in addition to the whole-body studies. Following is a discussion of updates to the chronic selenium criterion that are a result of the 2004 draft USEPA document and new data published since then that are relevant to Kentucky-specific aquatic resources. In the course of our research the Cabinet generated a table of fish data the Cabinet used. Calculations were made for chronic values such as the no-effect concentration (NOEC), low-effect concentrations (LOEC) and the EC₁₀ (point estimate of effect concentration at the 10 percent level). Additionally, toxicity data summarized by DeForest and Adams (2011) and DeForest et al. (2012) were considered to ensure the data were complete.

2.2.1 Updating the USEPA's 2004 Draft Selenium Fish Tissue-Based Chronic Criterion with a Focus on Taxa Found in Kentucky

It is the Cabinet's understanding that the forthcoming chronic criteria recommendation from USEPA will likely be a fish tissue-based value; technical updates from USEPA on the criteria development process point toward criteria based on egg/ovary tissue with a water column translator. This approach is akin to the 2004 draft chronic criterion that was based on whole body fish tissue. To use as many data points as possible, the USEPA translated the available data from whole body to egg/ovary and vice versa. Therefore, in Kentucky's analyses that follow, data were converted from whole body to egg/ovary and vice versa using the translation equations from: (1) USEPA (2004) for bluegill, (2) GEI (2008) for fathead minnow, and (3) GEI et al. (2008) for bluegill, cutthroat trout and both species combined to derive an *all species* equation.

However, since only bluegill and cutthroat trout data were used to derive the *all species* equation in GEI et al (2008), the *all species* equation was updated to include fathead minnow data from GEI (2008) along with the data for bluegill and cutthroat trout. Combined, these equations allow for derivation of a chronic selenium criterion based on either egg/ovary or whole body fish tissue data from all properly conducted studies and fish species. This not only increased the data available, but should be a benefit in the implementation of a tissue-based criterion given the challenges of collecting field-obtained fish egg/ovary tissue.

Table 1. Freshwater selenium data from chronic toxicity tests. Reference (study) considered in USEPA 2004 draft chronic criterion =*.

Species	Reference	Notes	Test Type	Toxicological Endpoint	Chronic Value mg/kg dw ^a	Useable/ Relevant
Egg/Ovary Data						
<i>Pimephales promelas</i> Fathead minnow	Schultz and Hermanutz 1990*	USEPA 2004 used 85% moisture for ovaries for this study	Dietary and waterborne (mesocosm-Monticello)	LOAEX for larval edema and lordosis	Ovary LOAEC: < 39.27	Y
Fathead minnow	Ogle and Knight 1989*		(lab)	NOEC for reproduction	OVARY NOEC > 10.92	N
Fathead minnow	GEI 2008	Translated from WB using GEI 2008 FHM equation	Dietary and waterborne (file Denver, CO)	EC ₁₀ larval skeletal and edema abnormality CV for larval deformities	Egg/Ovary EC ₁₀ : 45 Egg/ Ovary CV: 53.8	Y
Fathead minnow	Bennett et al. 1986*	Translated from WB using GEI 2008 FHM equation	Dietary	LOEC for growth	Ovary LOEC: < 57.75	N
Fathead minnow	Bertram and Brooks 1986*	Translated from WB using GEI 2008 FHM equation	Dietary	LOEC for growth	Ovary NOEC: > 3.94	N
Fathead minnow	Dobbs et al 1996*	Translated from WB using GEI 2008 FHM equation	Dietary	LOEC for growth	Ovary LOEC: <63.6 - <101.27	N
<i>Oncorhynchus mykiss</i> Rainbow trout	Holm et al. 2003*; Holm et al. 2005; EC ₁₀ and EC ₂₀ values calculated by DeForest and Adams (2011)	Values from DeForest and Adams	Dietary and waterborne (field Luscar River, Alberta)	EC ₁₀ for skeletal deformities EC ₂₀ for skeletal deformities	Egg NOEC : 17 Egg LOEC : 25 Egg EC ₁₀ : 23 Egg EC ₂₀ : 27	Y
<i>Oncorhynchus clarki bouvieri</i> Yellowstone cutthroat trout	Hardy et al. 2010		(lab)	NOEC for larval deformities, mortality	Egg NOEC : > 16.04	N
Yellowstone cutthroat trout	Formation Environmental 2011		(field)	MATC for alevin mortality	Egg MATC : 25	N
<i>Oncorhynchus clarki</i> Cutthroat trout	Hardy 2005		Dietary (lab)	NOAEC for embryo/larval deformities	Egg NOAEC > 16 – 18.0 ± 1.41	N
<i>Oncorhynchus clarki lewisi</i> Westslope cutthroat trout	Kennedy et al. 2000*		Dietary and waterborne (field – Fording River, BC)	NOAEC for embryo / larval deformities and mortality	Egg NOAEC : > 21.0 ± 18.3	N
Westslope cutthroat trout	Nautilus Environmental 2011		(field)	EC ₁₀ for alevin mortality	Egg EC ₁₀ : 24.8	N
Westslope cutthroat trout	Rudolph et al. 2008; EC ₁₀ and EC ₂₀ values calculated by DeForest and Adams (2011)	NOEC and LOEC for larval deformities; EC ₁₀ and EC ₂₀ for alevin mortality	Dietary and waterborne (field – Clode Pond, BC); No Se-related deformities; next highest [Se] tested (46.6 µg/g dw) did not produce viable fry	NOEC for larval edema LOEC for larval edema EC ₁₀ for alevin mortality EC ₂₀ for alevin mortality	Egg NOEC: 20.6 Egg LOEC: 46.8 Egg EC ₁₀ : 17 Egg EC ₂₀ : 23	N

Species	Reference	Notes	Test Type	Toxicological Endpoint	Chronic Value mg/kg dw ^a	Useable/ Relevant
<i>Salvelinus fontinalis</i> Brook trout	Holm 2002 ⁶ ; Holm et al. 2003 [*] ; Holm et al. 2005	CV for craniofacial deformities, calc assuming 75.84% moisture from Holm2002. Egg NOAEC given in Holm 2003 (6 mg/kg egg ww) but only for rainbow trout	Dietary and waterborne (field Luscar River, Alberta)	Combined 2000/2001 studies: NOEC for craniofacial deformities NOEC = > 20; EC ₀₆ = 20	Egg NOEC: > 20	Y
<i>Salvelinus malma</i> Dolly Varden	Golder 2009		Dietary and waterborne (field Kemess Mine NW BC)	EC ₁₀ for total deformities EC ₂₀ for total deformities	Egg EC ₁₀ : 54 Egg EC ₂₀ : 60	N
<i>Salmo trutta</i> Brown trout	NewFields 2009		Dietary and waterborne (field)	EC ₁₀ for larval survival EC ₂₀ for larval survival EC ₁₀ for larval deformities EC ₂₀ for larval deformities	Egg EC ₁₀ : 20.8 Egg EC ₂₀ : 23.1 Egg EC ₁₀ : 22 Egg EC ₂₀ : 23.4	Y
<i>Xyrauchen texanus</i> Razorback sucker	Hamilton et al. (2005a, 2005b) [*]	Larval deformities	(field)	NOEC for larval deformities LOEC for larval deformities MATC for larval deformities	Egg LOEC: 37.8 Egg NOEC: 46.5 Egg MATC: 41.9	N
<i>Catostomus commersonii</i> White sucker	De Rosemond et al. 2005	Larval deformities	(field)	EC ₁₃ for larval deformities	Egg EC ₁₃ : 25.6	Y
<i>Lepomis macrochirus</i> Bluegill	USEPA 2004; from Lemly 1993 ⁷	Translated from WB using bluegill equation in GEI et al. 2008	Lab	LOEC for mortality at 4°C	Ovary LOEC: 17.01 Ovary LOEC: 12.59	Y
Bluegill	Bryson et al. 1984 [*]		dietary and waterborne (field Hyco Reservoir, NC)	LOAEC for larval mortality	Ovary LOAEC: < 49	N
Bluegill	Bryson et al. 1985a [*]	Represents mean of 4 females from Hyco reservoir	Dietary and waterborne (field Hyco Reservoir, NC)	Chronic value for swim-up larvae	Ovary CV: <30 ± 3.4	N
Bluegill	Bryson et al. 1985b [*]		(field)	NOEC for hatchability, swim up LOEC for hatchability, swim up	Ovary NOEC: >14.8 Ovary LOEC: > 9.2	N
Bluegill	Gillespie and Baumann 1986 [*]		Dietary and waterborne (field – Hyco Reservoir, NC)	Chronic value for larval survival	OVARY CV: < 38.6	N
Bluegill	Doroshov et al. 1992; EC ₁₀ and EC ₂₀ values calculated by DeForest and Adams (2011)	Geometric means of ovary and egg EC _{10S} = 18.33	Dietary (lab)	NOEC for larval edema LOEC for larval edema EC ₁₀ for larval edema EC ₂₀ for larval edema	Ovary NOCE: 3.94 Ovary LOEC: 21.10 Ovary EC ₁₀ : 16 Ovary EC ₂₀ : 20	Y

Species	Reference	Notes	Test Type	Toxicological Endpoint	Chronic Value mg/kg dw ^a	Useable/ Relevant
					Egg NOEC: 8.55 Egg LOEC: 25.81 Egg EC ₁₀ : 21 Egg EC ₂₀ : 23	
Bluegill	Coyle et al. 1993 [*] ; EC ₁₀ and EC ₂₀ values calculated by DeForest and Adams (2011)	Wb, ovary and egg NOECs; LOECs; EC ₁₀ s and EC ₂₀ s for larval mortality; geometric mean of ovary and egg EC ₁₀ s = 23	Dietary and waterborne (lab)	NOEC for larval survival LOEC for larval survival EC ₁₀ for larval survival EC ₂₀ for larval survival	WB NOEC: 7 WB LOEC: 16 WB EC ₁₀ : 8 WB EC ₂₀ : 8.5 Ovary NOEC: 20 Ovary LOEC: 35 Ovary EC ₁₀ : 24 Ovary EC ₂₀ : 27 Egg NOEC: 22.5 Egg LOEC: 41.3 Egg EC ₁₀ : 22 Egg EC ₂₀ : 26	Y
Bluegill	Hermanutz et al. 1992 [*] ; Hermanutz et al. 1996 [*] ; EC ₁₀ and EC ₂₀ values calculated by DeForest and Adams (2001)		Dietary and waterborne (mesocosm – Monticello)	NOEC for larval edema LOEC for larval edema EC ₁₀ for larval edema EC ₂₀ for larval edema	WB NOEC: 4.4 WB LOEC: 21.8 WB EC ₁₀ : 7.7 WB EC ₂₀ : 9.7 Ovary NOEC: 17.3 Ovary LOEC: 69 Ovary EC ₁₀ : 30 Ovary EC ₂₀ : 36	Y
		Back-calc ovaries from USEPA 2004 criterion value given for parent tissue (17.35) using bluegill equation in GEI et al. (2008)	Dietary (mesocosm – Monticello)	NOAEC for larval survival, edema, lordosis and hemorrhaging	Ovary NOAEC: >36.8	N
Bluegill	WVDEP 2010	No measure of toxicity; egg [Se] only from 2009 (max sample 13.8% deform, avg 5.38%). Some species had egg [Se], but only larval deformity data for bluegill. Eggs for deformity studies not from same females that had eggs excised so egg [Se] are not truly indicative of reproductive impairment.	Dietary and waterborne (field, Upper Mud River, WV)	NOAEC for larval deformities	Egg NOAEC: < 9.8	N
Bluegill	McIntyre et al. 2008	Translated from WB using bluegill equation in GEI et al. 2008	Dietary	EC ₁₀ for mortality at 4°C EC ₂₀ for mortality at 4°C EC ₁₀ for mortality at 9°C EC ₂₀ for mortality at 9°C	Ovary EC ₁₀ : 18.3 Ovary EC ₂₀ : 19.8 Ovary EC ₁₀ : 28.6 Ovary EC ₂₀ : 30.8	Y

Species	Reference	Notes	Test Type	Toxicological Endpoint	Chronic Value mg/kg dw ^a	Useable/ Relevant
<i>Micropterus salmoides</i> Largemouth bass	CP&L 1997	Maternal transfer	(lab)	EC ₁₀ : for larval mortality EC ₂₀ : for larval mortality	Ovary EC ₁₀ : 22 Ovary EC ₂₀ : 24	Y
<i>Esox lucius</i> Northern pike	Muscattello et al. 2006		Dietary and waterborne (field Saskatoon, Sask.)	NOEC larval deformities LOEC larval deformities EC ₁₀ larval deformities EC ₂₀ larval deformities	Egg NOEC: 3.8 Egg LOEC: 31.3 Egg EC ₁₀ : 20.4 Egg EC ₂₀ : 33.6	Y
<i>Gambusia affinis</i> Western mosquitofish	Saiki et al. 2004	Translated from WB using updated "all species" equation derived by GEI, see Appendix A	Field MT	NOEC for fry mortality and deformities	Egg:> 37.2	Y
<i>Acipenser transmontanus</i> White sturgeon	Tashjian et al. 2006	Translated from WB using updated "all species" equation derived by GEI, see Appendix A	Dietary	NOECs, LOECs, EC ₁₀ s and EC ₂₀ s for growth	Egg EC ₁₀ : 30.6 Egg EC ₂₀ : 52.7	
Whole-Body Data						
Fathead minnow	Schultz and Hermanutz 1990	USEPA 2004 used 85% moisture for ovaries for this study; translated from ovary using GEI 2008 FHM equation	Dietary and waterborne (mesocosm – Monticello)	LOAEC for larval edema and lordosis	WB LOAEC: < 28.99	Y
Fathead minnow	Ogle and Knight 1989		Lab MT	NOEC for reproduction	WB NOEC: .75 Ovary NOEC: >10.92	N
Fathead minnow	GEI 2008		Dietary and waterborne (field Denver, CO)	EC ₁₀ larval skeletal and edema abnormality CV larval deformities	WB EC ₁₀ : 33 WB CV: 40	Y
Fathead minnow	Bennett et al. 1986		Dietary	LOEC for growth	WB LOEC: < 43.0	N
Fathead minnow	Bertram and Brooks 1986		Dietary	NOEC for growth	WB NOEC: > 2.2	N
Fathead minnow	Dobbs et al. 1996		Dietary	LOEC for growth	WB LOEC: < 47.5 - < 76.0	N
Rainbow trout	Hodson et al. 1980	Exposed to [selenite] 5.5 – 53 µg/L; only measured tissue in 53 µg/L treatment; negligible effects in 53 µg/L treatment; water-only exposure in inorganic Se not environmentally relevant; not used by DeForest and Adams	Aqueous exposure		WB:>1.8	N
Rainbow trout	Hunn et al. 1987	Not used by DeForest and Adams because exposed to mixture of elevated elements and water-only exposure to inorganic Se not environmentally relevant	Aqueous exposure	NOEC LOEC	WB: 2.6 WB: 4.3	N
Rainbow trout	Holm et al. 2003; Holm et al. 2005; EC ₁₀ and EC ₂₀ values calculated by DeForest and	Translated from egg using trout equation in GEI et al. 2008	Dietary and waterborne (field Luscar River,	EC ₁₀ for skeletal deformities EC ₂₀ for skeletal	WB NOEC: 9.18 WB LOEC: 12.26 WB EC ₁₀ : 11.52	Y

Species	Reference	Notes	Test Type	Toxicological Endpoint	Chronic Value mg/kg dw ^a	Useable/ Relevant
	Adams (2011)		Alberta)	deformities	WBEC ₂₀ :12.99	
Cutthroat trout	Hardy 2005*	Translated from egg using trout equation in GEI et al. 2008	Dietary (lab)	NOAEC for embryo/larval deformities	WB NOAEC > 8.77 – 9.58	N
Brook trout	Holm 2002; Holm et al. 2003; Holm et al. 2005	CV for craniofacial deformities, calc assuming 75.84 moisture from Holm 2002. Egg NOAEC given in Holm 2003 (6mg/kg egg ww) but only for rainbow trout. Translated from egg using trout equation in GEI et al. 2008	Dietary and waterborne (field Luscar River, Alberta)	NOEC for craniofacial deformities; NOEV > 20; EC ₀₆ = 20	WB NOEC: > 10.34	Y
Brown trout	NewFields 2009	Translated from egg using trout equation in GEI et al. 2008	Dietary and waterborne (field)	EC ₁₀ for larval survival EC ₂₀ for larval survival EC ₁₀ for larval deformities EC ₂₀ for larval deformities	WB EC ₁₀ : 10.68 WB EC ₂₀ : 11.55 WB EC ₁₀ : 11.14 WB EC ₂₀ : 11.67	Y
White sucker	De Rosemond et al. 2005	Translated from egg using updated “all species” equation derived by GEI, see Appendix A	(field)	EC ₁₃ for larval deformities	WB EC ₁₃ : 13.05	Y
Bluegill	USEPA 2004; from Lemly 1993	Draft criterion; 40% overwinter mortality in juveniles (winter stress)	Lab	LOEC for mortality at 4°C	WB:7.91 WB: 5.85	Y
Bluegill	Cleveland et al. 1993*	Water-only exposure to inorganic Se is not environmentally relevant; not used by DeForest and Adams	Aqueous exposure	NOEC for mortality LOEC for mortality	WB NOEC: 3.8 WB LOEC: 5.0	N
Bluegill	McIntyre et al. 2008	Mortality (winter stress syndrome); EC ₁₀ and EC ₂₀ at 4°C and 9°C	Dietary	EC ₁₀ for mortality at 4°C EC ₂₀ for mortality at 4°C EC ₁₀ for mortality at 9°C EC ₂₀ for mortality at 9°C	WB EC ₁₀ :9.56 WB EC ₂₀ :10.16 WB EC ₁₀ : 13.29 WB EC ₂₀ : 14.02	Y
Bluegill	Coyle et al. 1993* EC ₁₀ and EC ₂₀ values calculated by DeForest and Adams (2011)	Wb, ovary, and egg NOECs, LOECs, EC ₁₀ s, and EC ₂₀ s for larval mortality	Dietary and waterborne (lab)	NOEC for larval survival LOEC for larval survival EC ₁₀ for larval survival EC ₂₀ for larval survival	WB NOEC: 7 WB LOEC: 16 WB EC ₁₀ : 8 WB EC ₂₀ : 8.5 Ovary NOEC: 20 Ovary LOEC: 35 Ovary EC ₁₀ : 24 Ovary EC ₂₀ : 27 Egg NOEC: 22.5 Egg LOEC: 41.3 Egg EC ₁₀ :22 Egg EC ₂₀ :26	Y
Bluegill	Hermanutz et al. 1992; Hermanutz et al. 1996; EC ₁₀ and EC ₂₀ values calculated by DeForest and Adams (2011)		Dietary and waterborne (mesocosm – Monticello)	NOEC for larval edema LOEC for larval edema EC ₁₀ for larval edema EC ₂₀ for larval edema	WB NOEC: 4.4 WB LOEC: 21.8 WB EC ₁₀ : 7.7 WB EC ₂₀ : 9.7	Y

Species	Reference	Notes	Test Type	Toxicological Endpoint	Chronic Value mg/kg dw ^a	Useable/ Relevant
					Ovary NOEC: 17.3 Ovary LOEC: 69 Ovary EC ₁₀ : 30 Ovary EC ₂₀ : 36	
Bluegill	Bryson et al. 1984*	Translated from ovary using bluegill equation in GEI et al. 2008	Dietary and waterborne (field – Hyco Reservoir, NC)	LOAEC for larval mortality	WB CV: <19.67	N
Bluegill	Bryson et al. 1985a*	Represents mean of 4 females from Hyco reservoir. Translated from ovary using bluegill equation in GEI et al. 2008	Dietary and waterborne (field – Hyco Reservoir, NC)	Chronic value for swim-up larvae	WBCV: < 13.75	N
Bluegill	Bryson et al. 1985b*	Translated from ovary using bluegill equation in GEI et al. 2008	(field)	NOEC for hatchability, swim-up LOEC for hatchability, swim-up	WB NOEC: > 8.21 WB LOEC: > 5.80	N
Bluegill	Gillespie and Baumann 1986*	Translated from ovary using bluegill equation in GEI et al. 2008	Dietary and waterborne (field – Hyco Reservoir, NC)	Chronic value for larval survival	WB CV: < 16.53	N
Bluegill	Doroshov et al. 1992; EC ₁₀ and EC ₂₀ values calculated by DeForest and Adams (2011)	Translated from ovary or egg using bluegill equations in GEI et al. 2008 (geometric mean of translated ovary and egg values was calc.)	Dietary (lab)	NOEC for larval edema LOEC for larval edema EC ₁₀ for larval edema EC ₂₀ for larval edema	WB NOEC: 3.25 WB LOEC: 9.85 WB EC ₁₀ : 8.12 WB EC ₂₀ : 9.17	Y
Largemouth bass	CP&L 1997	Maternal transfer, Translated from ovary using bluegill equation in GEI et al. 2008	(lab)	EC ₁₀ for larval mortality EC ₂₀ for larval mortality	WB EC ₁₀ : 10.96 WB EC ₂₀ : 11.68	Y
Northern pike	Muscattello et al. 2006	Translated from egg using updated “all species” equation derived by GEI, see Appendix A	Dietary and waterborne (field Saskatoon, Sask.)	EC ₁₀ larval deformities EC ₂₀ larval deformities	WB EC ₁₀ : 10.92 WB EC ₂₀ : 16.16	Y
<i>Gambusia holbrooki</i> Eastern mosquitofish	Staub et al. 2004		Field MT	NOEC for brood size/offspring viability	WB: > 11.85	N
Western mosquitofish	Saiki et al. 2004		Field MT	NOEC for fry mortality and deformities	WB: 17.5	Y
White sturgeon	Tashjian et al. 2006		Dietary	NOWECs, LOECs, EC ₁₀ s and EC ₂₀ s for growth	WB NOEC: 14.7 WB LOEC: 22.5 WB EC ₁₀ : 15 WB EC ₂₀ : 23	Y
Other Data (E.g., Synthesis Studies)						
Various Species	Lemly 1996*	Reproductive failure	Synthesis	Reproductive failure	Egg: 10	N
Cold FW fish	Chapman 2007	Range in “effects thresholds” for coldwater species	Synthesis		Egg:> 16-40	N
Bluegill and fathead minnow	DeForest and Adams (2011)	EC ₁₀ for larval mortality and edema	Synthesis		Ovary EC ₁₀ : 17	N
Various species	Lemly 1996*	Reproductive failure	Synthesis		Ovary: 10	N

Species	Reference	Notes	Test Type	Toxicological Endpoint	Chronic Value mg/kg dw ^a	Useable/ Relevant
Bluegill and fathead minnow	DeForest and Adams (2011)		Synthesis	EC ₁₀ for larval mortality and edema	WB EC ₁₀ :8.1	N
Various species	Hamilton 2002 [*] ; Lemly 1996 [*]		Synthesis	Juvenile mortality and reproductive failure	WB: 4	N
Chinook salmon	Hamilton et al. 1990 Hamilton 2002, 2003		Lab and synthesis	Swim-up larval growth and reproductive failure	WB: 4 – 6.5	N
Rainbow trout, Brook trout	Holm et al. 2003	Rapid rise in edema and deformities in fry (parental exposure); Egg (52% moisture); Muscle translation	Field (eggs/milt) Lab (fish rearing)	Larval edema/deformities	Egg: 12.5 Muscle: 4.3	N
Rainbow trout	Holm et al. 2005 from Chapman 2007	Threshold between 8-10 µg/g ww; converted to dw using 75% moisture	Field		Egg: 32-40	N
Rainbow trout	Vidal et al. 2005	NOEC and LOEC could not be identified because dose-response data anomalous	Dietary	NOEC and LOED for larval deformities	WB NA	N
Brook trout	Holm et al. 2005 from Chapman 2007	No increase in larval deformities at 6.6 and 7.8 µg/g ww; converted to dw using 75% moisture	Field	Larval deformities	Egg: >26.4 – 31.2	N
Bluegill	Cleveland et al. 1993 [*]	NOEC and LOEC could not be identified because dose-response data anomalous	Dietary	NOEC and LOEC for mortality	WB NA	N
Various species	USDI 1998 from various studies	Background; no risk to aquatic life	Synthesis		WB: <4	N
Various species	Engberg et al. 1998	Range of concern	Synthesis		WB: 4-12	N
Various species	Lemly 2002 [*]	Max allowable [Se]; values are recommendations by Lemly based on synthesis and interpretation of literature cited	Synthesis	Protective of reproduction	WB: 4 Muscle: 8 Liver: 12 Egg: 10	N
Centrarchids, Fatheads, minnows, Salmonids, Percichthyids	Lemly 1998; cited Hoffman et al. 1988, Lemly 1985a, 1993b, c, 1997b,c Ohlendorf 1989, Ohlendorf et al. 1986a, b, 1988, Skorupa and Ohlendorf 1991, Skorupa et al. 1996	Diagnostic residues for reproductive impairment	Synthesis	Larval/fry deformity or mortality	WB: 5-7 Muscle: 6-8 Liver: 15 -20 Egg: 5- 10 Larvae/Fry: 8-12	N
Perch and Bluegill	USDI 1998; 4-6 has Marginal Risk in Presser et al. 2004	Reproductive impairment in sensitive species	Synthesis	EC ₁₀ for reproductive impairment	WB: 4-6 Gonad/Egg: 7-13	N
Various species	DeForest et al. 1999	Recommended toxicity guidelines	Synthesis	EC ₁₀ for toxicity	WB: 6 (coldwater) WB: 9 (warmwater) Ovary: 17	N
Centrarchids	Lemly 1993	Rapid rise in deformities (terata)	Synthesis	Deformities	Egg:10 Eggs: 6-17	N
Bluegill	Lemly 1993 from Chapman 2007	Equivalent to wb 4 µg/g dw	Synthesis		Egg:10	N
Various species	USEPA 2004 from Chapman	21 studies of 8 fish species (warm	Synthesis		Egg: 17	N

Species	Reference	Notes	Test Type	Toxicological Endpoint	Chronic Value mg/kg dw ^a	Useable/ Relevant
	2007	and cold water); used USEPA (2004) to convert 7.9 µg/g dw wb to egg				
Various Species	Cumbie and Van Horn 1978; Lemly 1985, 1997, 1998a, 2002	16 species extirpated 10-70% rates of teratogenesis	Field	Species extirpation; teratogenesis	WB: 40-125 Muscle: 25-200 Egg:20-170	N

¹Studies USEPA considered when determining the 2004 draft chronic criterion update

Equations used to translate between whole-body and egg/ovary:

$$FHM_{[Se]dwWB} = (0.75826 \times FHM_{[Se]dw\ ovary}) - 0.78645 \quad (\text{GEI 2008})$$

$$BG_{\log[Se]dw\ WB} = (0.73 \times BG_{\log[Se]dw\ ovary}) + 0.06 \quad (\text{GEI et al. 2008})$$

$$BG_{\log[Se]dw\ WB} = (0.90 \times BG_{\log[Se]dw\ egg}) - 0.31 \quad (\text{GEI et al. 2008})$$

$$TROUT_{\log[Se]dw\ WB} = (0.75 \times TROUT_{\log[Se]dw\ egg}) + 0.04 \quad (\text{GEI et al. 2008})$$

$$ALL\ SPECIES_{\log[Se]dw\ WB} = (0.7851 \times ALL\ SPECIES_{\log[Se]dw\ egg}) + 0.01 \quad (\text{modified herein from GEI et al. 2008; see [Appendix A](#)})$$

$$BG_{[Se]dwWB} = (0.46337 \times BG_{[Se]dw\ ovary}) + 0.01728 \quad (\text{USEPA 2004})$$

Considerable information has become available since the 1987 chronic criterion of 5.0 µg/L was issued, including information on the route of exposure. Studies show diet is the principle route of exposure that causes chronic toxicity effects to fish, the group of organisms considered most sensitive to chronic selenium exposure (Coyle et al 1993; Hamilton et al. 1990; Hermanutz et al. 1996). Tests studying chronic toxicity effects only through water exposure have had questionably low selenium tissue residue (USEPA 2004). Given these results, currently only studies that expose test organisms to selenium in the diet or selenium in the diet and water column were considered valid in the derivation of a chronic value.

2.2.2 Calculating Tissue Values for Criteria

To develop a selenium chronic criterion based on tissue residue the USEPA (2004) considered and evaluated the available aquatic life tissue-based studies. Generally, chronic values have been defined as the geometric mean of the greatest concentration of a toxin that results in no observable adverse effect (highest no observed adverse effect concentration, NOAEC) and the lowest concentration of the toxic substance that causes an adverse effect (lowest observed adverse effect concentration, LOAEC). The significance of observed effects is determined by statistical tests comparing response of organisms to natural concentrations of the toxin (control) to responses of organisms exposed to elevated concentrations. Subsequent to this evaluation USEPA proposed a criterion of 7.91 mg/Kg whole-body (wb) dry weight (dw) based on a single study using bluegill (*Lepomis macrochirus*) by Lemly (1993). A proposed criterion based on a single study and single species is not consistent with the Guidelines for developing criteria, which holds that criteria must be based on the EC₁₀ or EC₂₀ from multiple studies on numerous species. The USEPA used the EC₂₀ as the chronic value as it represents a departure of 20 percent from the control response observed (USEPA, 2009, 2004, 1999). To increase the margin of safety, the Cabinet's approach to the development of the Kentucky criterion was based on the EC₁₀ for selenium. This approach is also consistent with other recent approaches (e.g. DeForest and Adams 2011).

In the draft criterion document (USEPA 2004) USEPA presented the following for selecting datasets from studies for inclusion in the analysis:

- (1) The experiment had a control treatment, making it possible to define response levels at natural concentrations of selenium;
- (2) The experiment must have had at least four treatment concentrations of selenium;
- (3) The greatest tested concentration of selenium resulted in >50 percent observed effects compared to the control treatment; and
- (4) At least one tested selenium concentration resulted in <20 percent observed effects relative to the control treatment to ensure that the EC₂₀ was bracketed by tested

concentrations of selenium. In updating the selenium chronic criteria for Kentucky the Cabinet included all recent data fitting the USEPA Guidance criteria in calculations to develop a final value that represents Kentucky species. The calculations are based on the most inclusive set of data, including data developed since the 2004 USEPA draft criterion document.

Included in the derivation of chronic criteria several prior studies were reviewed that summarized selenium-effects values from a variety of coldwater and warmwater fishes, including some non-species-specific values from synthesis papers DeForest and Adams 2011, DeForest et al 2012). In DeForest and Adams (2011) their analysis included some recalculation of endpoints and new calculations of previously unreported endpoints, such as EC₁₀ and EC₂₀ values, which the original researcher may not have calculated. Through this evaluation of summaries, additional data were added to the Cabinet's analysis from studies not cited or not available at the time of publication of these studies.

Many of the species available in selenium toxicity studies do not include Kentucky-representative species (either actual or surrogate species) (e.g. Chinook salmon, Yellowstone cutthroat trout and eastern mosquitofish, Table 1). Those associated data are not relevant to species or aquatic habitat (water quality) characteristics occurring in Kentucky and thus, are not appropriate to use in the derivation of specific criteria for Kentucky water resources. Other parameters established for data evaluation for the Kentucky-specific criteria were:

- Exclusion of tests using only aqueous selenium exposure given the irrelevance of those data for derivation of chronic criteria based on the known dietary pathway for exposure to chronic levels of selenium toxicity (GEI et al. 2008, DeForest and Adams 2011).
- The EC₁₀ values were used to err on the side of conservative values and for consistency with recent approaches (DeForest and Adams 2011).
- When both egg and ovary data were available for a study, the geometric mean of the two values was used to calculate the chronic value for egg/ovary tissue.

The studies described below contain data generated from selenium toxicity bioassays that included fish species appropriate for review and consideration in the development of Kentucky-specific chronic criteria. Those studies reviewed and salient associated information are presented in Table 1, above; a number of those studies were considered irrelevant due to the species evaluated and/or the design of those studies were not based on criteria established previously that were required to produce data relevant or protective to selenium chronic toxicity. Studies that reported relevant and usable selenium toxicity data for species that occur in Kentucky or represent a closely related species (e.g. from the same genus) are reported in Table 2 below.

2.2.2.1 Bluegill (*Lepomis macrochirus*) and Other Centrarchidae Data

In the 2004 USEPA draft document the application of the values based on Lemly (1993) was made as follows:

“Given the uncertainty of juvenile fish concentrating selenium over the winter, an FCV [final chronic value] of 7.91 ug Se/g dw is recommended. However, if the concentration of selenium in whole body fish tissues approaches 5.85 $\mu\text{g Se/g dw}$ during summer or fall months, it is recommended fish be sampled during winter to determine if they exceed the FCV of 7.91 $\mu\text{g Se/g dw}$.”

This was the only study at the time to evaluate possible winter stress (water temperature at 4°C) or seasonal variation on the effects of selenium toxicity. The USEPA conducted a similar study (McIntyre et al. 2008) using water temperatures of 4°C and 9°C and reported EC_{10s} of 9.56 and 13.3 $\mu\text{g/g wb dw}$, respectively. There were additional studies that evaluated selenium exposure in outdoor microcosms that commenced in late summer and continued through winter and spawning in the spring (Hermanutz et al. 1996, Hamilton et al. 2002). These studies included a winter conditions component in natural environments, which is closer to representing real-life conditions than modeling winter stress conditions in the laboratory. Each study exposed test organisms to multiple water and dietary selenium concentrations; however, neither study reported excessive additional mortality of selenium-exposed test organisms during winter months. Therefore, these studies do not support sole application of the Lemly (1993) “winter stress” study to Kentucky waters.

Given these recent study results it is not thought the winter stress component of the USEPA’s 2004 draft chronic criterion is applicable to all species or locations. Therefore, simply including the Lemly (1993) values into an overall SMCV calculation with other appropriate data on bluegills is in keeping with the Guidelines. Many values were derived from other sources that represent offspring mortality endpoints, often considered more sensitive endpoints than juvenile or adult mortality for many species (Gillespie and Baumann 1986; Schultz and Hermanutz 1990; Coyle et al. 1993; Holm et al. 2003).

Table 1 presents chronic values that are available for bluegill. The USEPA did not include chronic values from studies that included eggs and larvae obtained from bluegill adults previously exposed to selenium for multiple generations (Bryson et al. 1984 and 1985a,b; Gillespie and Baumann 1986). Hence, those values were excluded in chronic value calculation for all Centrarchidae.

Table 2. Selenium toxicity data available for Kentucky fish species used to calculate GMCVs.
 CV = Chronic Value, GMCV = Genus Mean Chronic Value, WB = whole body.

Scientific Name	Common Name	Endpoint	Reference	Whole-body			Egg/Ovary		
				CV μg/g	GMCV μg/g	WB Rank	CV μg/g	GMCV μg/g	Egg/Ovary Rank
<i>Lepomis macrochirus</i>	Bluegill	Juvenile mortality LOEC	Lemly 1993	7.91	8.9	1	17.01	22	3/4/5
		Larval edema EC ₁₀	Hermanutz et al. 1992, 1996	7.7			30		
		Larval survival EC ₁₀	Coyle et al. 1993	8			23		
		Larval edema EC ₁₀	Doroshov et al. 1992	8.12			18.3		
		Juvenile mortality 4°C EC ₁₀	McIntyre et al. 2008	9.56			18.3		
		Juvenile mortality 9°C EC ₁₀	McIntyre et al. 2008	13.29			28.6		
<i>Salvelinus fontinalis</i>	Brook trout	Craniofacial deformities NOEC	Holm 2000; Holm et al. 2003; Holm et al. 2005	>10.34	10.3	2	>20	20	1
<i>Esox lucius</i>	Northern pike	Larval deformities EC ₁₀	Muscattello et al. 2006	10.92	10.92	3	20.4	20.4	2
<i>Micropterus salmoides</i>	Largemouth bass	Larval mortality EC ₁₀	CP&L	10.96	11	4	22	22	3/4/5
<i>Salmo trutta</i>	Brown trout	Larval deformities EC ₁₀	NewFields 2009	11.14	11.1	5	22	22	3/4/5
<i>Oncorhynchus mykiss</i>	Rainbow trout	Skeletal deformities EC ₁₀	Holm 2000; Holm et al. 2003; Holm et al. 2005	11.52	11.5	6	23	23	6
<i>Catostomus commersonii</i>	White sucker	Larval deformities EC ₁₃	de Rosemond et al. 2005	13.05	13.05	7	25.6	25.6	7
<i>Acipenser transmontanus</i>	White sturgeon	Larval growth EC ₁₀	Tashjian et al. 2006	15	15	8	30.6	30.6	8
<i>Gambusia affinis</i>	Western mosquitofish	Larval deformities/mortality NOEC	Saiki et al. 2004	17.5	17.5	9	>32.7	37.2	9
<i>Pimephales promelas</i>	Fathead minnow	Larval deformities EC ₁₀	GEI 2008	33	31	10	45	42	10
		Larval edema/lordosis LOEC	Schultz and Hermanutz 1990	< 28.99			<39.27		

In addition to excluding data points from potentially acclimated test organisms, the USEPA did not include chronic values from Lemly (1993) of $>6.0 \mu\text{g/g}$, Cleveland et al. (1993) and Hermanutz et al. (1996) in the *Lepomis* SMCV calculation, without giving a detailed explanation of why they were excluded. The exclusion of the Lemly data point was understandable since the other reported tissue value from the same study at which a significant effect was observed was in the database and used in the SMCV calculations. The exclusion of Cleveland et al. (1993) data was prudent given the exposure of the fishes was to aqueous concentrations of selenium and did not include the important dietary exposure relevant to a bioaccumulative toxicant. The reasoning behind exclusion of the Hermanutz et al. (1996) data was not so apparent given their values were well within the range reported for this species. One of the toxicological endpoints was larval edema (abnormal fluid accumulation); often a selected manifestation used by the USEPA over other data used from fish species for calculations of the SMCV (e.g. fathead minnows). It was for this reason and to maintain consistency that the data point was included in the calculation of a revised SMCV for bluegills. The Lemly (1993) and McIntyre et al. (2008) usable data were translated to whole body concentrations using the bluegill ovary-to-whole body translation equation found in GEI et al. (2008); this equation updated the Equation II used in USEPA (2004).

Three other studies, Doroshov et al. (1992) and Coyle et al. (1993) and McIntyre et al. (2008) were determined to be usable in addition to the two studies noted above. The recent studies from the WVDEP (2010) were not usable due to lack of matched adult and egg/ovary tissue concentrations and larval response.

Data are available from another common centrarchid species that inhabits a wide-range of aquatic habitats, the largemouth bass, *Micropterus salmoides* (Carolina Power & Light 1997). The data point was published as an ovary concentration and was translated to whole body selenium concentration using the bluegill ovary to whole-body translation equation (GEI et al. 2008) as there is not another translation equation for this species.

2.2.2.2 Trout and Other Salmonidae Data

Given data available are for trout species that occur in Kentucky, those species of trout that do not occur in the commonwealth were not used in the Kentucky update for selenium chronic criteria development. Those studies are presented in Table 1 as a review of the literature available and initially considered for all species of this family.

There are no trout endemic to Kentucky waters (Burr and Warren 1986); however the Kentucky Department of Fish and Wildlife Resources have done an exceptional job of managing a trout fishery in Kentucky's coldwater and tailwater habitats. Several species may be found in these habitats, which include rainbow, brown and brook trout. There are now a number of reproducing

populations of these trout species. For those three species that do occur in Kentucky, additional data exist and were used in the SMCV calculations.

Chronic toxicity data exist for rainbow trout in studies by Holm et al. 2003; Holm et al. 2005; Hodson et al. 1980; Hunn et al. 1987; Vidal et al. 2005. Brook trout data are found in Holm 2002; Holm et al. 2003 and brown trout data in NewFields 2009. The data points for brook and brown trout were determined usable; however, only one of the rainbow trout data points (the EC₁₀ value derived from data presented in Holm 2000; Holm et al. 2003; and Holm et al. 2005) is usable for criteria calculation (Table 1). The two data for rainbow trout not usable were aqueous-only derived values which is not relevant to bioaccumulatives such as selenium (DeForest and Adams 2011).

The usable data for these trout species were published as egg selenium concentrations. These values were translated to whole body concentrations using the trout egg-to-whole-body translation equation in GEI et al. (2008).

2.2.2.3 Minnow (Cyprinidae) Data

There are many data points available for the fathead minnow, but most of these data were determined unusable for the reasons presented in the discussion presented in this Section. The Bertram and Brooks (1986) study on fathead minnows was not used for criteria development. They reported a whole body no observed effect concentration (NOEC) for growth of >2.2 µg/g. However, the authors were only assessing the uptake of selenium and depuration and not selenium toxicity. The growth of fathead minnows was monitored to determine if the model assumption that organisms do not grow was met. The determination that the data produced in this study were not usable for the purpose of developing chronic criterion was based on two reasons: (1) the test concentrations of selenium were very low compared to concentrations used to evaluate toxicity effects, and (2) the study was to evaluate the selenium uptake and depuration, not to measure selenium toxicity effects on growth.

The USEPA draft selenium criteria document (2004) reviewed four studies for chronic effects on cyprinids. These studies evaluated toxic effects in adult and larval fathead minnows. Chronic effect estimates were obtained from three laboratory studies (Bennett et al. 1986; Dobbs et al. 1996; Ogle and Knight 1989) and one field and mesocosm study (Schultz and Hermanutz 1990). All laboratory studies involved selenium concentrations in the water and added to the food. Analyzing larval fish for growth effects involved modeling with respect to whole body selenium concentrations. Reduced larval growth was the effect chosen in the respective laboratory study; chronic values were <43, <76, and >7.5 µg/g wb dw, respectively. Those three laboratory studies proved to be unreliable for USEPA criteria development due to extreme range in chronic values, dietary exposure uncertainties and endpoint issues.

The mesocosm study conducted by Schultz and Hermanutz (1990) considered both waterborne and dietary exposures. Adult fathead minnows were initially exposed to selenite that was added to artificial streams. Embryo samples were collected from spawning platforms and reared in the laboratory in natural water containing 10 µg/L selenium. Edema and lordosis (curvature of the spine) were observed in about 25 percent of the larvae. The mean selenium residue in the ovaries of the females from the treated stream was 39.27 µg/g. Whole body samples of maternal minnows were not analyzed. To estimate whole body selenium tissue concentrations, the ovary-to-whole body tissue model derived using fathead minnow data (GEI 2008) was used. The whole body chronic value for this study was estimated to be <28.99 µg/g dw (note that USEPA [2004] reported this value as <18.21 based on translation from their whole body regression based primarily on bluegill data. It is believed the fathead regression is more appropriate. Although an unidentified value derived from an ovary-to-whole body regression, this was the only fathead minnow chronic study that was deemed acceptable by USEPA for criteria development. The study by GEI (2008) provided an additional fathead minnow chronic value for both whole body and egg/ovary (Table 2).

2.2.2.4 Sucker (Catostomidae) Data

Data for the white sucker (native to Kentucky) are available from a study conducted by de Rosemond et al. (2005). This data point was published as egg selenium concentration and was translated to whole body concentration using the modified “all species” egg-to-whole body translation equation (Table 2) (modified from GEI et al. 2008, as described above).

Data for the razorback sucker (Hamilton et al 2005a,b) were not used given this species does not occur in Kentucky. Earlier studies conducted by Beyers and Sodergren (2001a) found no reduction in survival and growth of larval razorback suckers after 28 day exposure. The chronic value for this study was based on selenium concentration found in waterborne and spiked food and was measured at >12.9 µg Se/g dw. A second study, Beyers and Sodergren (2001b) exposed larval razorback suckers to control water and three varying site waters containing differing concentrations of selenium. Fish were fed rotifers cultured in test water and control water. There were no reductions in survival or larval growth of those fishes exposed to both site water and site diet compared to fishes exposed to control water and diet. The USEPA (2004) did not use those data in the draft chronic criterion.

2.2.2.5 Data of Other Species

Data points for three other fish species that occur or represent species that could occur in the state are available: (1) northern pike (*Esox lucius*, introduced to Kentucky waters) (Muscatello et al. 2006), (2) western mosquitofish (*Gambusia affinis*, a native species) (Saiki et al. 2004), and (3) the white sturgeon (*Acipenser transmontanus*, used as a surrogate for native sturgeon species in Kentucky) (Tashjian et al. 2006).

Data for the northern pike were originally published as egg selenium concentrations. This value was translated to whole body concentration using the modified “all species” egg-to-whole body translation equation. The white sturgeon and western mosquitofish data were originally published as whole body selenium concentrations; these values were translated to egg/ovary concentrations using the same equation.

2.2.2.6 Data Summary

Genus mean chronic values (GMCVs) were derived for 10 species (Table 2). Based on this analysis of relevant data for Kentucky-specific chronic selenium criteria, the bluegill remains the most sensitive taxa of species in Kentucky when whole body data are considered (Table 2), whereas the brook trout is the most sensitive taxa based on egg/ovary data.

In making comparisons among the taxa considering sensitivity rank between whole body and egg/ovary chronic values, the Salmonidae or Centrarchidae each had a taxon that ranked one or two, depending on the tissue residue considered. In addition to these two families representing the most sensitive taxa of Kentucky fishes, species in these families are arguably representative of some of the most recreationally, and by extension economically, valuable fishes in the commonwealth. The white sucker is distributed statewide and is found in wadeable streams, excluding the lowland streams of the coastal plain in west Kentucky; note, the western mosquitofish is found in the streams of that province. Importantly from a distributional and aquatic community composition perspective, the fathead minnow is ubiquitous in a large portion of Kentucky’s headwater streams.

2.2.2.7 Chronic Criteria Calculations

The calculated FCV was made for both whole body and egg/ovary tissues using the GMCVs (Table 2) for the four most sensitive genera in the revised chronic data (Tables 3 and 4). The calculations followed the USEPA methods for criteria determination in the Guidelines. The FCV for whole body fish tissue is 8.6 $\mu\text{g/g dw}$ and the egg/ovary tissue FCV is 19.3 $\mu\text{g/g dw}$.

Table 3. Calculation of the selenium final chronic values for fish whole body using the updated chronic data (N = 10 genera, R = sensitivity rank in database, P = rank / N+1).

Rank	Genus	GMCV	ln GMCV	(ln GMCV) ²	P = R/(N+1)	√P
1	Lepomis	8.92	2.1883	4.7886	0.0909	0.3015
2	Salvelinus	10.34	2.3360	5.4570	0.1818	0.4264
3	Esox	10.92	2.3906	5.7149	0.2727	0.5222
4	Micropterus	10.96	2.3943	5.7324	0.3636	0.6030
SUM			9.3092	21.6929	0.9090	1.8531

Calculations Table 3:
Chronic Whole body Criterion

$$S^2 = \frac{\sum(\ln \text{GMCV})^2 - (\sum \ln \text{GMCV})^2/4}{\sum P - (\sum \sqrt{P})^2/4} = \frac{21.6929 - (9.3092)^2/4}{0.9090 - (1.8531)^2/4} = 0.5465 \quad S = 0.7393$$

$$L = [\sum \ln \text{GMCV} - S(\sum \sqrt{P})]/4 = [9.3092 - 0.7393(1.8531)]/4 = 1.9848$$

$$A = S(\sqrt{0.05}) + L = (0.7393)(0.2236) + 1.9848 = 2.1501$$

$$\text{Final Chronic Value} = \text{FCV} = e^A = 8.5858$$

Table 4. Calculation of the selenium final chronic values for egg/ovary using the updated chronic data (N= 10 genera, R = sensitivity rank in database, P = rank / N+1).

Rank	Genus	GMCV	Ln GMCV	(ln GMCV) ²	P = R/(N+1)	√P
4	Micropterus	22	3.0910	9.5543	0.3636	0.6030
3	Lepomis	22	3.0910	9.5543	0.2727	0.5222
2	Esox	20.4	3.0155	9.0932	0.1818	0.4264
1	Salvelinus	20	2.9957	8.9744	0.0909	0.3015
Sum			12.1934	37.1769	0.9191	1.8531

Calculations Table 4:
Chronic Egg/Ovary Criterion

$$S^2 = \frac{\sum(\ln\text{GMCV})^2 - (\sum\ln\text{GMCV})^2/4}{\sum P - (\sum\sqrt{P})^2/4} = \frac{37.1769 - (12.1934)^2/4}{0.9090 - (1.85317)^2/4} = 0.1406 \quad S = 0.3750$$

$$L = [\sum\ln\text{GMCV} - S(\sum\sqrt{P})]/4 = [12.1934 - 0.3750(1.85317)]/4 = 2.8746$$

$$A = S(\sqrt{0.05}) + L = (0.3850)(0.2236) + 2.8746 = 2.9585$$

$$\text{Final Chronic Value} = \text{FCV} = e^A = 19.2681$$

2.3 Implementation of the Criteria

2.3.1 Acute Selenium Criterion

The Cabinet proposes to update the 20 µg/L acute standard in its regulations with the current National Recommended Water Quality Criterion per USEPA (2012) for acute aquatic life protection for total selenium by including a sulfate modifier equation:

$$\text{CMC} = 1/[f1/\text{CMC1}] + (f2/\text{CMC2}),$$

where f1 and f2 are the fractions of total selenium that consists of selenite and selenate, respectively; CMC1 is the selenite criterion of 258 µg/L and CMC2 is the selenate criterion per the equation $\text{CMC2} = e^{(0.5812[\ln(\text{sulfate})] + 3.357)}$ and is incorporated. This is consistent with the 2004 (USEPA) draft selenium document update that was proposed with regard to the current national recommended criterion equation. Assuming speciation of total selenium is not done, attainment evaluations would use the more conservative selenite value of 258 µg/L (conservatively assumes that all of the total selenium is in the more toxic selenite form).

In addition to the above, the Cabinet chose to further implement a more conservative approach than the 2004 (USEPA) draft selenium document update by capping the sulfate modifier for selenate at 44 mg/L which is the sulfate level at which the CMC would equate to 258 µg/L. Consequently, 258 µg/L would apply as the site acute criterion (wherever sulfate is ≥44 mg/L at a site). If sulfate is <44 mg/L at a site then the acute criterion will move lower than 258 µg/L based on the equation variables applicable to the site location.

This cap on the sulfate modifier also reflects sulfate levels typical of Kentucky waters. Sulfate data from the Kentucky Division of Water (DOW) 72-station ambient water quality network had a mean sulfate concentration of 95 mg/L for the period of 2007 through 2011. Of those 72 stations, 43 had mean sulfate concentrations less than 44 mg/L.

2.3.2 Chronic Selenium Criteria

The current national recommended water quality criterion for chronic selenium was issued in 1987 as 5.0 µg/L. This criterion was based on aqueous selenium concentration from an uncontaminated portion of the Belews Lake, but studies since then clearly demonstrate the primary route of exposure for chronic toxicity effects to fish is through diet. Studies have shown that through aqueous exposure alone, an extreme concentration of selenium in water greater than 300 µg/L has been necessary to result in a body burden sufficient to elicit a chronic toxicity response from fish (Cleveland et al. 1993; Gissel-Nielsen and Gissel-Nielsen 1978; cited in USEPA 2004). It is due to the current science that USEPA proposed the departure of tying a revised chronic criterion from an aqueous concentration to whole body tissue concentration in 2004, and continues to move toward a tissue-based criterion at this time. Since fish are believed to be the most sensitive aquatic group of organisms to selenium toxicity, and the chronic toxic effects are diet-born, consideration was given to organisms that fish prey on. That ultimately proved inappropriate for two reasons, (1) the concentration of selenium in the diet is an indirect measure of effects observed in the test species and this type of criterion does not consider feeding variables of the target species and (2) the selection of appropriate organisms to monitor for protection of the fish community is problematic given the variability of the range of prey species that are represented across the diverse fish community.

Based on the current science the Cabinet concludes taking a tiered approach for the chronic standard is advisable. Kentucky is utilizing a 5.0 µg/L water column value as threshold for screening purposes as a first step to determining potential selenium toxicity concerns in the waterbody (see 2.3.3 below). If the threshold (screening value) of 5.0 µg/L total selenium in the water column is not exceeded, then the water body is considered in attainment of the selenium criterion. If 5.0 µg/L total selenium in the water column is exceeded, this would trigger sampling of fish tissue. If the results of the selenium tissue concentrations do not exceed the criterion associated with the tissue type sampled (whole body or egg/ovary) then the designated use of aquatic life is protected for selenium.

Given the potential difficulties with implementing this tissue-based standard, the Cabinet proposes criteria for both whole body and egg/ovary tissue. The whole body or egg/ovary tissue concentration for total selenium will be based on the FCVs calculated in Section 2.2.2.7. This tiered approach will follow the steps outlined below:

- Step 1. Determine whether the water column concentration at the site exceeds 5.0 µg/L threshold.
 - If the water column concentration for total selenium is ≤ 5.0 µg/L the water body is meeting its aquatic life use.

- If the water column concentration for total selenium is $>5.0 \mu\text{g/L}$ proceed to Step 2.

Step 2. Determine whether the site is in attainment of the tissue criterion (whole body [$8.6 \mu\text{g/g}$ total selenium dw] or egg/ovary tissue [$19.3 \mu\text{g/g}$ total selenium dw]).

- If each species-composite fish tissue has a selenium concentration less than the appropriate tissue-based criterion, the water body is meeting the chronic standard for selenium.
- If a species-composite fish tissue has a selenium concentration that exceeds the tissue criterion the site is considered in non-attainment of the water quality standard.

2.3.3 Threshold Screening Value

Kentucky is utilizing a $5.0 \mu\text{g/L}$ water column value as a threshold for screening purposes as a first step to determining potential selenium toxicity concerns in the waterbody. This threshold serves as a trigger to require fish tissue analysis to determine whether the chronic criteria for selenium are being met. This threshold has been determined to be appropriate for screening waterbodies for potential selenium toxicity and falls within the range of threshold values in numerous other studies. The rationale for the use and appropriateness of the threshold value is presented in Appendix B: Validation and Utilization of the Selenium Chronic Threshold Value; *Utilization of Risk-Controlling Measures Provided in the Proposed Selenium Chronic Criteria for Aquatic Life* (Payne 2013b).

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Appendix A

**Data used in derivation of an updated “All Species”
egg/ovary-to-whole-body translation equation**

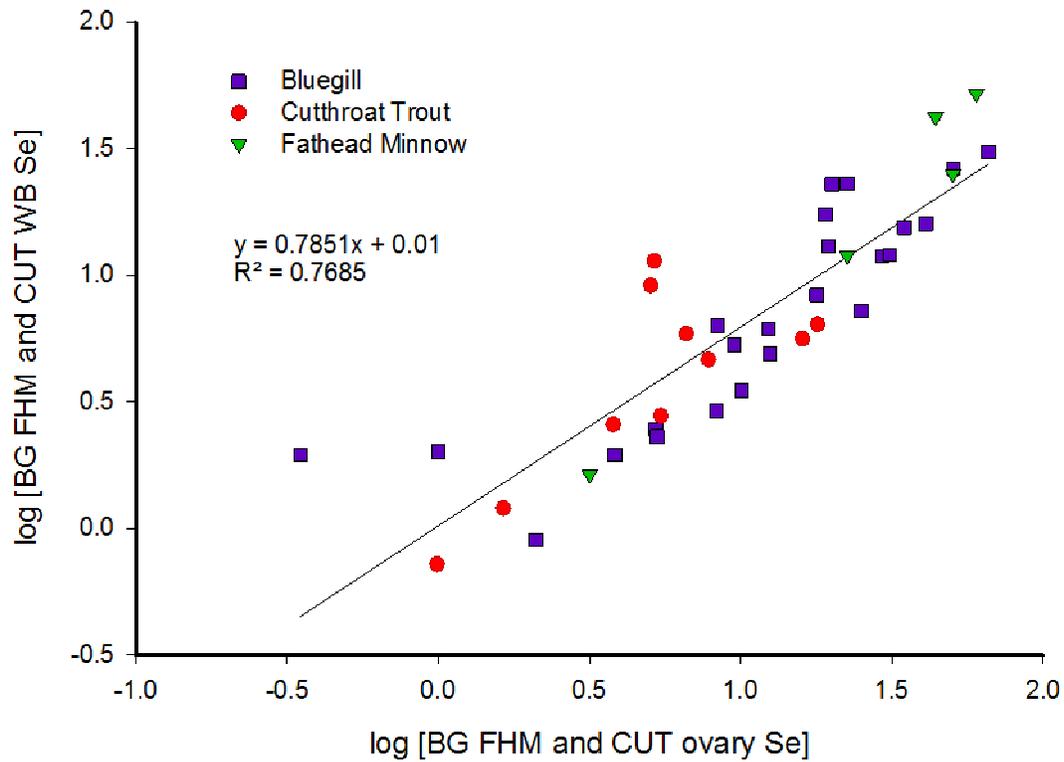
Table A-1. Data used to derive the updated “All Species” (data for bluegill, fathead minnow, and cutthroat trout) translation equation. Bluegill and cutthroat trout data are as reported in Appendix 1 of GEI et al. (2008) and fathead minnow data are from GEI (2008).

Study	Species	[Ovary Se]	[Whole body Se]	Log[Ovary Se]	Log[Whole body Se]
Coyle et al. 1993	Bluegill Sunfish	2.1	0.9	0.3222193	-0.045757
		2.1	0.9	0.3222193	-0.045757
		8.3	2.9	0.9190781	0.462398
		12.5	4.9	1.09691	0.6901961
		25	7.2	1.39794	0.8573325
		41	16	1.6127839	1.20412
Hermanutz et al. 1996	Bluegill sunfish	0.35	1.95	-0.455932	0.2900346
		20.05	22.85	1.3021144	1.3588862
		5.25	2.45	0.7201593	0.3891661
		3.85	1.95	0.5854607	0.2900346
		10.1	3.5	1.0043214	0.544068
		12.35	6.15	1.091667	0.7888751
		34.8	15.45	1.5415792	1.1889285
		50.5	26.45	1.7032914	1.4224257
		29.35	11.85	1.4676081	1.0737184
		66	30.6	1.8195439	1.4857214
		5.3	2.3	0.7242759	0.3617278
		8.4	6.3	0.9242793	0.7993405
		9.5	5.3	0.9777236	0.7242759
		31.15	12	1.4934581	1.0791812
		19.55	13	1.2911468	1.1139434
		17.85	8.35	1.2516382	0.9216865
19.1	17.35	1.2810334	1.2392995		

Study	Species	[Ovary Se]	[Whole body Se]	Log[Ovary Se]	Log[Whole body Se]
Hermanutz et al. 1992	Bluegill sunfish	1	2.0	0	0.30103
		22.5	23.0	1.3521825	1.3617278
Hardy 2005*	Cutthroat trout	0.99	0.72	-0.004365	-0.142668
		3.8	2.57	0.5797836	0.4099331
		5.45	2.78	0.7363965	0.4440448
		18.0	6.4	1.2552725	0.80618
		1.64	1.2	0.2148438	0.0791812
		7.82	4.64	0.8932068	0.666518
		6.61	5.87	0.8202015	0.7686381
		5.05	9.1	0.7032914	0.9590414
		5.18	11.37	0.7143298	1.0557605
		16.04	5.61	1.2052044	0.7489629
GEI 2008	Fathead Minnow	3.17	1.63	0.5010593	0.2121876
		22.52	11.96	1.3525684	1.0777312
		44.12	42.17	1.6446355	1.6250036
		50.33	25.15	1.7018269	1.400538
		60.26	52.22	1.7800291	1.7178369

*Data from this study were reported as ovary or egg, so were combined with the ovary data for the equation derivation

Figure A-1. Modified “all species” regression using log-transformed egg/ovary and whole body tissue selenium concentrations measured in bluegill, fathead minnow and cutthroat trout.



Appendix B

Validation and Utilization of the Selenium Chronic Threshold Value

Utilization of Risk-Controlling Measures Provided in the Proposed Selenium Chronic Criteria for Aquatic Life

Randall G. Payne, Environmental Scientist

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

Frankfort, Kentucky
March 22, 2013

TABLE OF CONTENTS

<u>Chapter</u>	<u>Page</u>
CHAPTER 1.0 INTRODUCTION	42
CHAPTER 2.0 THE THRESHOLD VALUE: PART OF A TWO-STEP STRATEGY FOR THE PROTECION OF AQUATIC LIFE.....	42
Chapter 3.0 REACHING A PROTECTIVE AQUATIC LIFE THRESHOLD	44
3.1 EVALUATION OF THE APPROPRIATENESS OF 5.0 µg/L TOTAL SELENIUM AS A THRESHOLD VALUE	45
CHAPTER 4.0 REVIEW AND CONCLUSION.....	46
LITERATURE CITED	48

1.0 Introduction

The Kentucky Energy and Environment Cabinet solicited and received comments on the proposed aquatic life selenium criteria. One issue that received particular attention was whether the threshold, or screening used to implement the chronic criteria was adequate to protect aquatic life, in particular fish. The threshold value proposed is a water column concentration of 5.0 µg/L total selenium. This threshold is the current nationally recommended chronic water quality criterion for protection of aquatic life ([U.S.EPA](#), accessed March 19, 2013) and falls within threshold values in other studies. Additional concerns regarding this threshold value and the tiered implementation strategy include that the approach will not protect sensitive fish species, fishless streams will be exempt from the criteria and in-turn will not protect other aquatic organisms in these streams.

The primary concern expressed regarding the proposed threshold value is that it is too high to prevent concentrations in fish tissue from reaching the proposed concentrations for chronic criteria. The Cabinet has proposed a whole body criterion of 8.6 µg/g dry wt total selenium concentration and 19.3 µg/g dry wt total selenium egg/ovary tissue. As discussed in the proposed aquatic life selenium criteria document for Kentucky (Payne 2013) the primary mode for chronic toxicity to aquatic organisms is through dietary exposure. Therefore, it is important that the threshold value be sufficiently low to provide a meaningful water column concentration that once reached will trigger fish tissue sample collection and analysis to verify the fish community is not adversely effected. The rationale for the soundness for this threshold value is presented in this document.

2.0 The Threshold Value: Part of a Two-Step Strategy for the Protection of Aquatic Life

To provide assurance that the aquatic habitat is protected from potential chronic toxicity effects of selenium, a two-step monitoring approach is utilized. The most sensitive organisms in the aquatic environment are egg-laying vertebrates (Chapman et al. 2010); therefore, for fish, two levels of protection should be set with regard to chronic selenium toxicity:

- 1) an appropriate level of protection that will provide reasonable certainty there will be no deleterious effects, (e.g. water quality criteria) and
- 2) a lower level of protection that if exceeded, will trigger focused monitoring to determine whether there is reason to expect that there may be adverse effects in advance of the primary level of protection (e.g. screening value) (Chapman 2005).

Based on the current science, and for programmatic implementation, the Cabinet concludes taking a tiered approach to the implementation of the chronic standard is advisable. This proposed approach is itself a two-step strategy. The tiered approach is designed to provide an

additional margin of safety and confidence that there are no adverse effects to fish occurring due to chronic selenium toxicity. If the threshold value of 5.0 µg/L aqueous total selenium is not exceeded, then the water body is considered in attainment of the selenium chronic criteria. Additionally, since this is a screening threshold that if reached will trigger fish tissue collection and analyses, exceeding the threshold does not indicate adverse effects are occurring or likely to occur. Rather, exceeding the threshold only indicates that the selenium concentrations in the environment are reaching a point where the margin of safety is reduced to a point where an additional level of assurance is warranted. In this stepwise approach, if total selenium values in the water column exceed the threshold of 5.0 µg/L monitoring of fish tissue will occur. If the total selenium concentrations in fish tissue are below the applicable criteria, 8.6 µg/g dry wt whole body or 19.3 µg/g dry wt egg/ovary, no adverse effects have occurred. This margin of safety is inherent in the methodology employed by utilizing the most sensitive fish species to derive chronic criteria (Stephan et al. 1985).

Given the potential difficulties with implementing this tissue-based standard, the Cabinet proposes criteria for both whole body and egg/ovary tissue. The whole body or egg/ovary tissue concentration for total selenium are based on the calculated FCVs (final chronic values) and presented in Section 2.2.2.7 (Payne 2013). This tiered approach will follow the steps outlined below:

- Step 1. Determine whether the water quality at the site is attaining a concentration of 5.0 µg/L threshold.
 - If the water column concentration for total selenium is ≤ 5.0 µg/L the water body is meeting its aquatic life use.
 - If the water column concentration for total selenium is > 5.0 µg/L proceed to Step 2.
- Step 2. Determine whether the site is in attainment of the tissue criteria (whole body [8.6 µg/g dw] or egg/ovary tissue [19.3 µg/g dw]).
 - If each species-composite of fish tissue has a selenium concentration less than the appropriate tissue-based criterion, the water body is meeting the chronic standard for selenium.
 - If a species-composite fish tissue has a selenium concentration that exceeds the appropriate tissue criterion the site is considered in non-attainment of the water quality standard.

3.0 Reaching a Protective Aquatic Life Threshold Value

Based on the current science and supporting studies from the scientific community that recommend a tiered approach to assure selenium levels do not pose a chronic toxicity threat to the aquatic community, the Cabinet concluded that taking a tiered approach for the chronic standard is advisable. Since the current nationally recommended chronic criterion was established at 5.0 µg/L total selenium, that value has been used in studies and monitoring of spills as a screening value, and recently associated with the Kingston, Tennessee coal-ash spill (U.S. EPA 2009). The current body of literature regarding selenium chronic toxicity for both threshold and ambient water quality criteria (DeForest and Adams 2011; U.S. EPA 2002 and 2004) are based on total selenium concentrations. Given that dietary exposure is the primary route for chronic toxicity effects, a low water column concentration threshold will provide additional assurance that tissue monitoring is triggered prior to potential bioaccumulation levels that may result in chronic effects on fish populations and exceedence of the standard.

The primary sources of selenium are well known, phosphorites, marine sedimentary rocks, especially black shales and petroleum source rocks. Selenate is most commonly the dominant species (form) of selenium in agricultural runoff and/or drainage, and discharges from coal mining operations (Presser and Luoma 2010). When water column samples are analyzed from streams total selenium is commonly reported, but that value does not take into account the species of selenium present. Selenium exists in four different oxidation states in the environment. Those forms are selenide (-II), elemental selenium (0), selenite (IV), and selenate (VI) (McNeal and Balistrieri 1989; Elrashidi et al. 1987). It is generally accepted that the order of toxicity of selenium species is as follows: Se-met (selenomethionine) (seleno-amino acids) > selenite > selenate (Simmons and Wallschläger 2005). In the physical habitat, selenium is found in one of several inorganic species; whereas, organoselenium is present within the cellular material of organisms. Of the most bioavailable inorganic forms, selenite is the most bioavailable and therefore toxic (i.e. readily bioaccumulates); however, under oxidation conditions (found most frequently in lotic habitats) selenate is the common species encountered. Of the inorganic bioavailable forms of selenium, selenate is 10 times less bioavailable than selenite (Besser et al. 1993; Milne 1998). To illustrate this differential toxicity, Cleveland et al. (1993) exposed bluegill to water column selenate:selenite (6:1) mixture to a maximum of 1,100 µg/L for 60 days and reported whole body total selenium concentration of approximately 10 µg/g dry wt, below the Cabinet's proposed 8.6 µg/g dw whole body criterion (and the equivalent criterion of 19.3 µg/g dw egg/ovary concentration). In this same study, a selenite concentration of 640 µg/L was required to reach a whole body total selenium concentration of approximately 5 µg/g dry wt in bluegill; note fish tissue would be collected and analyzed upon the water column concentration reaching 5.0 µg/L threshold for *total* selenium. By using total selenium as the threshold value, all species of selenium are considered equal with regard to potential toxicity.

This conservative approach is inline with a statement by Presser and Luoma (2006) who note that generalizations about comparative (differential) toxicity effects should be used with caution.

With the biogeochemical processes and phase transformations presented above, there are clear technical-based reasons why the Cabinet's selection of total selenium as the threshold value will provide another margin of assurance in the protection of the aquatic habitat from the effects of chronic selenium toxicity. Not the least of which is with consideration of the predominant species (forms) of selenium, particularly in lotic habitats. For example, potential discharges from mining operations are generally to lotic habitats where, as discussed above, selenate is the most common species of selenium.

3.1 Evaluation of the Appropriateness of 5.0 µg/L Total Selenium as a Threshold Value

In response to the December 2008 failure of a dike that contained coal ash at the Kingston Fossil Plant site adjacent the Emory River near Kingston, Tennessee, the U.S. Senate Environment and Public Works Committee staff requested a briefing regarding the related environmental consequences. The U.S. EPA (2009) prepared a Science Panel Review paper for this briefing and response to committee staff. Two areas of particular focus addressed in this paper are:

- testing and monitoring to determine the fate and transport of selenium released from this incident (water and wildlife) considering both short- and long-term endpoints; and
- the evaluation of possible chronic selenium toxicity levels of concern for response action.

In order to respond to the committee staff and inform the public whether any environmental toxicity by selenium as a result of the spill was ongoing or likely to occur, the U.S. EPA proposed a risk-based tiered monitoring approach to provide answers to these questions and concerns.

In an effort to identify the sources and environmental fate of selenium, and to maximize monitoring efficiency and target the appropriate media for determination of potential toxicity effects or likelihood of future occurrence, a conceptual model was designed by the U.S. EPA (2009). Sediment and surface water were the two primary media of the aquatic habitat that could serve as a conveyance of selenium to the aquatic food web at chronic concentrations and exposure durations. As such, each medium was identified as a complete pathway for potential selenium toxicity exposure to fish. The contamination of sediments and water column by potentially high levels of selenium serve as reservoir for dietary uptake and assimilation first into the periphyton and bacteria, then into the primary consumers, and ultimately the entire aquatic food web; this biotic pathway into the food web was identified as the significant exposure route for selenium. The contamination of the water column with high levels of selenium via the coal-

ash spill was recognized as a complete pathway for exposure to the food web to chronic levels of toxicity of selenium, but was considered a minor pathway compared to the dietary pathway. The contamination of sediments and water column provide a reservoir for potential selenium deposition, re-suspension and transfers between trophic levels of the aquatic community. This cycle can lead to exposures of duration that can result in chronic toxicity effects.

Upon identifying the appropriate monitoring strategy based on the conceptual exposure pathway model, fish tissue samples were collected subsequent to the coal-ash spill between January 9, 2009 and February 12, 2009 immediately downstream of the spill site in the Emory River. The mean muscle tissue concentrations of total selenium in fishes sampled were 2.9 $\mu\text{g/g}$ dry wt TVA (Tennessee Valley Authority) data (January 9), and 2.6 $\mu\text{g/g}$ dry wt TDEC (Tennessee Department of Environment and Conservation) data (February 12) in largemouth bass. Channel catfish at this location had a mean total selenium residue in muscle tissue of 1.7 and 1.2 $\mu\text{g/g}$ dry wt whole body. A second monitoring point near the mouth of the Emory River (mile point 0.5) indicated muscle tissue concentrations of total selenium in largemouth bass were little changed for the same period, 2.9 and 2.8 $\mu\text{g/g}$ dry wt whole body, respectively. The U.S. EPA (2009) concluded that the selenium levels in the aquatic habitat did not reach a toxic level to aquatic life utilizing the water column screening values ranging from 1 to 5 $\mu\text{g/L}$ (U.S. EPA 2009).

The tissue concentrations reported above were associated with water column total selenium concentrations that ranged between 1.3 $\mu\text{g/L}$ to 3.6 $\mu\text{g/L}$ (of the TDEC analyzed water samples, 32 of 353 samples collected had concentrations in this range, the remaining were below detection). The TDEC laboratory established a Method Detection Limit (MDL) of 1.3 $\mu\text{g/L}$ and a Method Quantification Limit (MQL) of 5.0 $\mu\text{g/L}$. The TVA collected 919 water column samples from December 22, 2008 through July 1, 2009. At time of the U.S. EPA (2009) report the TVA had validated results for 285 samples and verified the rest. The TVA MDLs ranged from 0.1 $\mu\text{g/L}$ to 3 $\mu\text{g/L}$ and MQLs ranged from 1 $\mu\text{g/L}$ to 20 $\mu\text{g/L}$. Dissolved selenium was detected in 6 of 916 samples; concentrations ranged from 2.3 $\mu\text{g/L}$ to 5.12 $\mu\text{g/L}$. All of these results were from samples collected in early January, but for one that was collected on March 25, 2009. The MDL for samples where selenium was detected was reported as 0.3 $\mu\text{g/L}$ with an MQL of 2 $\mu\text{g/L}$ (U.S. EPA 2009). While the water quality and fish-tissue data sets are limited, these data indicate a threshold value of 5.0 $\mu\text{g/L}$ would have been protective of the fishes in the Emory River. Additionally, the practical or analytical limitations (i.e. MDL and MQL) of reporting selenium concentrations below 5 $\mu\text{g/L}$ would not have provided data at lower levels of rigor to assure protection.

4.0 Review and Conclusion

To reiterate a significant point concerning the geochemical processes in various aquatic habitats, in oxidizing environments, such as lotic water bodies, it is understood selenate is the primary form of selenium in the water column, and it is the least reactive of the three common forms

(organoselenium, selenite and selenate) in short residence time environments (e.g. streams and rivers) (Presser and Luoma 2010). This point is emphasized as it relates to a threshold value for Kentucky-specific water bodies. These chemical phase transformations related to ecological and hydrological characteristics in water body types influence the bioaccumulation of selenium (Brix et al. 2005; Orr et al. 2006). Therefore, in flowing waters there is short residence time, reduced recycling, low ratio of particulate matter (i.e. food) and/or dissolved selenium and lower concentrations of selenium entering the food web (Presser and Luoma 2010).

To conclude, the biogeochemical processes associated with selenium are complex within the aquatic environment. The processes and fate of selenium in the aquatic environment are a consequence of the physical, chemical and hydrological characteristics in the environmental setting. Because the sources of anthropogenic releases of selenium into the aquatic environment are predominately into lotic habitats, the predominant bioavailable form of selenium will be selenate, which is the least bioavailable form and therefore least toxic. The proposed threshold value of 5.0 µg/L total selenium (Payne 2013) has been used elsewhere as a screening value, and recently was considered within the acceptable range of threshold values by U.S. EPA (2009). With the biogeochemical processes, the presented data and the proposed two-step monitoring approach presented, the Cabinet's threshold adds an additional margin of safety to both implement the tissue-based criteria and assure the protection of Kentucky's aquatic habitats from potential adverse effects of selenium toxicity.

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