Draft Translation of Selenium Tissue Criterion Elements to Site-Specific Water Column Criterion Elements for California Version 1, August 8, 2018

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List of Acronyms and Abbreviations

AE	assimilation efficiency
BAF	bioaccumulation factor
°C	degrees Celsius
$C_{particulate}$	concentration of selenium in particulate material
C_{target}	translated site-specific water column criterion element
$C_{tissue\ criterion\ element}$	selenium fish tissue or bird egg criterion element
C_{water}	concentration of dissolved selenium in water
CF	conversion factor
d	day
dw	dry weight
EF	enrichment factor
FAO	Food and Agriculture Organization
g	gram
IR	ingestion rate
k_e	elimination rate constant
kg	kilogram
L	liter
m	meter
mg	milligram
ml	milliliter
Se	selenium
TTF	trophic transfer factor
$TTF^{composite}$	composite trophic transfer factor
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
ww	wet weight
µg	microgram
µm	micrometer

1.0 Introduction

This document describes the performance-based approach methods that the state will utilize for calculating a site-specific water column criterion element for the selenium aquatic life and aquatic-dependent wildlife criterion. The state will use one of two approaches to translate a fish tissue criterion element or bird egg criterion element into a water column criterion element, either the mechanistic model approach or the empirical bioaccumulation factor (BAF) approach. These two approaches are recommended by the EPA for the translation of tissue criterion elements into water column criterion elements in Appendix K of *Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016* (USEPA 2016). While some other methods are available for translations, no other methods have been tested or utilized in the regulatory context for selenium. The BAF approach is the typical empirical procedure used to establish the site-specific relationship between water concentrations and tissue concentrations of bioaccumulative parameters. For selenium specifically, the mechanistic model has been widely used in the peer-reviewed, published literature to translate between tissue and water concentrations. Given that these are well established procedures for translating concentrations between tissue and water, the state of California will utilize these approaches for their site-specific water column criterion element translations. The egg-ovary criterion element is the preferred fish tissue criterion element to be used in either approach to translate to a water column element as the egg-ovary criterion element is most closely related to the toxicological effects of selenium observed in fish. A sampling plan for the collection of data to be used for either the mechanistic model or empirical BAF approach will consider the temporal, spatial, and biogeochemical factors affecting water column, food web, and fish tissue selenium concentrations.

The EPA derived the national recommended Clean Water Act (CWA) section 304(a) selenium water-column criterion elements by modeling selenium bioaccumulation in aquatic systems. The EPA worked with the United States Geological Survey (USGS) to derive a translation equation utilizing a mechanistic model of bioaccumulation previously published in peer-reviewed scientific literature (Luoma et. al., 1992; Wang et. al, 1996; Luoma and Fisher, 1997; Schlekat et al. 2002; Wang 2002; Luoma and Rainbow 2005; Presser and Luoma 2006; Presser and Luoma 2010; Presser 2013). For the 2016 national recommended CWA section 304(a) selenium criterion, the EPA translated the selenium egg-ovary criterion element into two sets of site-specific water column concentration values (lentic and lotic) and used the distributions of those water column values to derive the respective water column criterion elements (USEPA 2016). The mechanistic model approach is described in detail in the technical support document accompanying this proposed rule, *Aquatic Life and Aquatic-Dependent Wildlife Selenium Water Quality Criteria for Freshwaters of California* (TSD), and *Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016* (USEPA 2016).

The mechanistic model utilizes the following equation to translate a tissue criterion element to a water column criterion element.

$$C_{target} = \frac{C_{tissue\ criterion\ element}}{TTF^{composite} \times EF \times CF} \qquad \textbf{(Equation 1)}$$

Where:

C_{target} = translated site-specific water column criterion element (µg/L),
$C_{tissue\ criterion\ element}$ = fish tissue or bird egg criterion element (µg/g),
$TTF^{composite}$ = product of the species-specific trophic transfer factor (TTF) values in each trophic level of the food web of the target fish or bird species related to the tissue criterion element ($C_{tissue\ criterion\ element}$) (no units of measurement),
EF = enrichment factor is the steady-state proportional bioconcentration of dissolved selenium at the base of the aquatic food web (L/g),
CF = conversion factor is the species-specific proportion of selenium in fish eggs, fish ovaries or fish muscle relative to the average concentration of selenium in the whole-body of the fish (no units of measurement).

When the state is using the mechanistic model approach to translate a site-specific water column criterion element for the protection of aquatic life, they will translate from the fish whole-body, muscle, or egg-ovary tissue criterion element to determine an appropriate water column criterion element. When the state is using the mechanistic model approach to translate a water column criterion element that is protective of the aquatic dependent wildlife use, they will translate from the bird egg criterion element. A conversion factor is not needed when translating a water column criterion element from either the bird egg or fish tissue whole-body criterion elements so the state will use the following equation for those translations:

$$C_{target} = \frac{C_{tissue\ criterion\ element}}{TTF^{composite} \times EF}$$ (**Equation 2**)

The BAF approach uses the following equations to translate a fish tissue or bird egg criterion element to a water column criterion element.

$$BAF = \frac{C_{tissue}}{C_{water}}$$ **(Equation 3)**

Where:

BAF = bioaccumulation factor derived from site-specific field-collected samples of tissue and water (L/g)
C_{tissue} = concentration of selenium in field collected fish tissue or bird egg (µg/g dw)
C_{water} = ambient concentration of selenium in water (µg/L)

The site-specific BAF can then be applied to the criterion element (fish egg-ovary, fish whole-body, fish muscle, or bird egg) that matches the tissue type collected to derive the BAF, to solve for a site-specific water column criterion element (C_{target}):

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

(Equation 3)

Where:

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

2.0 Site Definition

The state will provide a clear definition of the site for which the site-specific water column criterion element applies, including a description of the site boundaries. The site will be defined on the basis of expected changes in selenium's biological availability and/or toxicity due to physical and chemical variability of the site water and variability in the aquatic community. Communities with a unique taxonomic composition may justify a designation as a distinct site. If a selenium discharge from a point source or non-point source is part of a site, the site boundaries should reflect the magnitude and geographic extent of contamination based on the influence of the discharge. As selenium bioaccumulation is largely dependent on site specific conditions (e.g., *EF* and food web structure), the performance-based approach described in this document is appropriate for single water bodies or water body segments. The state will derive and adopt site-specific water column criterion elements through the typical water quality standards adoption process (not using the performance-based approach) if they decide to set site-specific water column criterion elements for larger areas. The state will maintain a publicly available list of sites with descriptions of their geographic extents on their website. The state will also provide a list of all site-specific criterion elements to relevant CWA implementing programs.

3.0 Determination of Aquatic Life Community Present at Site

After the state has defined the boundaries of the site, the state will determine what aquatic and aquatic-dependent communities are present at the site. The state will first evaluate the state's monitoring data and scientific publications to determine what species are present at the site. If monitoring data or scientific publications are not available, then the state will consult with local resource agencies to see if they have information regarding the fish and bird communities present. If no information is available, the state will perform appropriate fish (e.g. seining, electrofishing, and gillnets) and bird (e.g., point count surveys and nest monitoring) monitoring to determine the aquatic and aquatic-dependent communities present at the site. Aquatic community sampling should be conducted both in the spring and in the fall season. If sampling is not possible in the spring due to unsafe spring run-off flows, sampling will be conducted in late spring or early summer, once it is safe to conduct monitoring activities. Aquatic-dependent community (bird) monitoring will be conducted during the breeding season (typically April to August). If the state finds that fish species are not present at the site, then the state will not use the performance-based approach to determine the site-specific water column criterion element for the aquatic life designated use and instead will derive and adopt the site-specific water column element through the typical water quality standards adoption process.

4.0 Target Species Selection

The state will target fish and bird species (or closely related (e.g., order or closer) surrogate species with similar dietary compositions) with the greatest bioaccumulation potential for sampling or for modeling to develop the site-specific water column criterion. If the species with the greatest bioaccumulation potential at the site is threatened or endangered, then the state will either use the mechanistic model approach to derive the site-specific water column criterion element or the state will sample a closely related surrogate species with a similar dietary composition.

4.1 Food Web Modeling

As a species is primarily exposed to selenium through its diet, quantifying the dietary composition of each species will help determine the bioaccumulation potential of each fish and bird species present at that site. The state will begin by defining the diets of all fish and bird species (only bird species that are present during the breeding season will be evaluated) present at the site by reviewing the relevant state and scientific literature. Dietary compositions of many fish and bird species are defined in the USEPA 2016 CWA section 304(a) recommended selenium criterion document and the TSD for the California selenium criterion proposed rule. The state may also use publicly available databases such as NatureServe (http://www.natureserve.org) and FishBase (http://www.fishbase.org) to estimate the dietary composition of the fish species present at the site. FishBase is a relational database developed at the World Fish Center in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and many other partners. The state will use publicly available databases such as North America Birds Online (https://birdsna.org) to quantify the dietary composition of bird species present at the site. The North America Birds Online database is developed by the Cornell Lab of Ornithology in collaboration with the American Ornithological Society and is available through member subscription. The Handbook of Freshwater Fishery Biology, volumes 1, 2, and 3 (Carlander 1969-1997) and the Wildlife Exposure Factors Handbook, volumes I and II (USEPA 1993) may also be consulted for diet information.

Once an average diet for each fish and bird species present at the site is identified and quantified, the state will estimate the composite trophic transfer factor ($TTF^{composite}$) for each fish and bird species. Bioaccumulation of selenium from one trophic level to the next is quantified by a trophic transfer factor (*TTF).* A *TTF* is a single value that represents the steady-state proportional concentration of selenium in the tissue of an organism relative to the concentration of selenium in the food it consumes. The parameter $TTF^{composite}$ quantitatively represents all dietary pathways of selenium exposure for a particular fish or bird species within an aquatic system. The parameter is derived from species-specific *TTF* values representing the food web characteristics of the aquatic system and the proportion of each species consumed. The state will calculate a $TTF^{composite}$ for each fish and bird species present at the site utilizing **Equation 5**.

$$\mathbf{TTF^{composite} = TTF^{TL2} \times TTF^{TL3} \times \ldots \times TTF^{TLn}} \qquad \textbf{(Equation 5)}$$

Where:

$TTF^{composite}$ = the product of all *TTF* values at all trophic levels.
TTF^{TLn} = the *TTF* value of the highest trophic level.

Where more than one species are consumed at the same trophic level, the state will calculate the *TTF* for that trophic level as the weighted average of the *TTFs* of all species consumed using **Equation 6**. Examples of how a $TTF^{composite}$ will be calculated by the state are presented in Figures 1 and 2.

$$\overline{TTF}^{TLx} = \sum_i \left(TTF_i^{TLx} \times w_i \right) \qquad \textbf{(Equation 6)}$$

Where:

TTF_i^{TLx} = the trophic transfer factor of the i^{th} species at a particular trophic level
w_i = the proportion of the i^{th} species consumed.

A) Three trophic levels (simple):

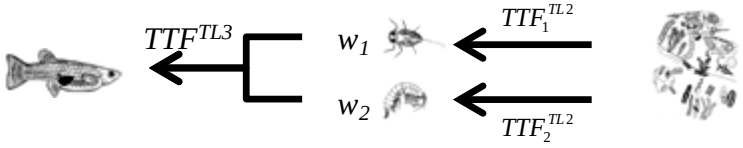
$$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$$

B) Four trophic levels (simple):

$$TTF^{composite} = TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2}$$

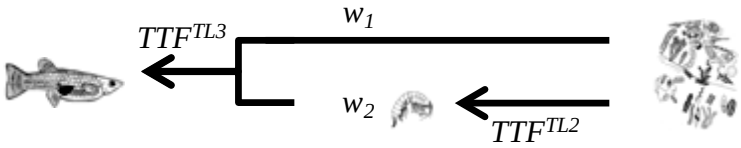
C) Three trophic levels (mix within trophic levels):

$$TTF^{composite} = TTF^{TL3} \times \left[\left(TTF_1^{TL2} \times w_1\right) + \left(TTF_2^{TL2} \times w_2\right)\right]$$



D) Three trophic levels (mix across trophic levels):

$$TTF^{composite} = \left(TTF^{TL3} \times w_1\right) + \left(TTF^{TL3} \times TTF^{TL2} \times w_2\right)$$



E) Four trophic levels (mix across trophic levels):

$$TTF^{composite} = \left[\left(TTF^{TL4} \times TTF^{TL3} \times w_1\right) + \left(TTF^{TL4} \times w_2\right)\right] \times TTF^{TL2}$$

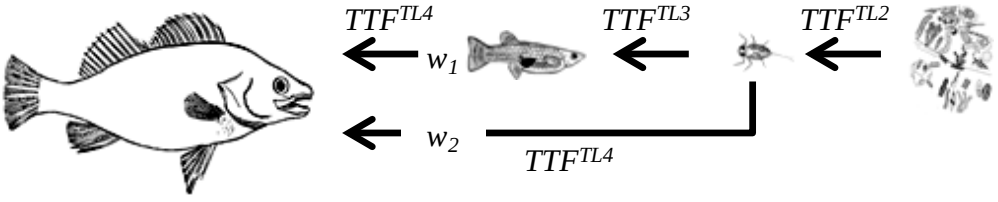
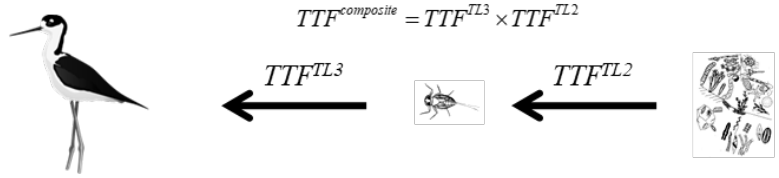


Figure 1. Example mathematical expressions of $TTF^{composite}$ representing different food-web scenarios for fish species. $TTF^{composite}$ quantitatively represents the trophic transfer of selenium through all dietary pathways of a targeted fish species. The mathematical expression of the food web model is used to calculate a value for $TTF^{composite}$ using appropriate species-specific *TTF* values and the proportions of each species consumed at each trophic level.

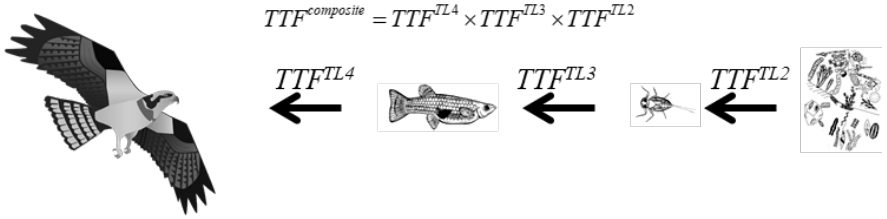
A) Three trophic levels (simple):

$$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$$



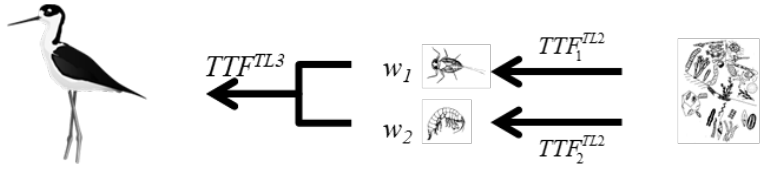
B) Four trophic levels (simple):

$$TTF^{composite} = TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2}$$



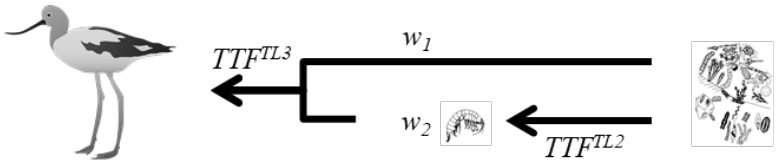
C) Three trophic levels (mix within trophic levels):

$$TTF^{composite} = TTF^{TL3} \times \left[\left(TTF_1^{TL2} \times w_1\right) + \left(TTF_2^{TL2} \times w_2\right)\right]$$



D) Three trophic levels (mix across trophic levels):

$$TTF^{composite} = \left(TTF^{TL3} \times w_1\right) + \left(TTF^{TL3} \times TTF^{TL2} \times w_2\right)$$



E) Four trophic levels (mix across trophic levels):

$$TTF^{composite} = \left[\left(TTF^{TL4} \times TTF^{TL3} \times w_1\right) + \left(TTF^{TL4} \times w_2\right)\right] \times TTF^{TL2}$$

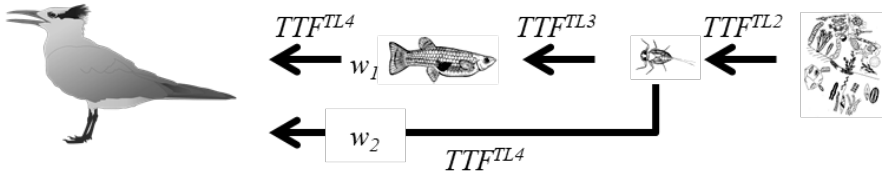


Figure 2. Example mathematical expressions of $TTF^{composite}$ representing different food-web scenarios for bird species. $TTF^{composite}$ quantitatively represents the trophic transfer of selenium through all dietary pathways of a targeted bird species. The mathematical expression of the food web model is used to calculate a value for $TTF^{composite}$ using appropriate species-specific *TTF* values and the proportions of each species consumed at each trophic level.

4.2 Selection of TTF Values

Once the state has determined the proper equation to calculate the $TTF^{composite}$ for a species, the state will select proper *TTFs* to populate the equation. To select proper *TTF* values, the state will first evaluate the list of *TTFs* below, from USEPA 2016 CWA section 304(a) recommended selenium criterion document and TSD for the California selenium criterion proposed rule, to see if there is a known *TTF* for the species included in the $TTF^{composite}$ equation. Examples of $TTF^{composite}$ calculations can be found in Appendix B section 3 of USEPA 2016 CWA section 304(a) recommended selenium criterion document and Appendix B of the TSD for the California selenium criterion proposed rule. The state may use the *TTF* values from these lists exclusively, or in conjunction with *TTF* values obtained from other sources (see below).

Table 1. EPA-derived Trophic Transfer Factor (TTF) values for freshwater aquatic invertebrates.

AE = Assimilation efficiency (%), IR = Ingestion rate (g/g-d), k_e = Elimination rate constant (/d).

Common name	Scientific name	AE	IR	k_e	TTF
Crustaceans					
amphipod	*Hyalella azteca*	-	-	-	1.22
copepod	copepods	0.520	0.420	0.155	1.41
crayfish	*Astacidae*	-	-	-	1.46
water flea	*Daphnia magna*	0.406	0.210	0.116	0.74
Insects					
dragonfly	*Anisoptera*	-	-	-	1.97
damselfly	*Coenagrionidae*	-	-	-	2.88
mayfly	*Centroptilum triangulifer*	-	-	-	2.38
midge	*Chironimidae*	-	-	-	1.90
water boatman	*Corixidae*	-	-	-	1.48
Mollusks					
asian clam[a]	*Corbicula fluminea*	0.550	0.050	0.006	4.58
zebra mussel	*Dreissena polymorpha*	0.260	0.400	0.026	4.00
Annelids					
blackworm	*Lumbriculus variegatus*	0.165	0.067	0.009	1.29
Other					
zooplankton	zooplankton	-	-	-	1.89

[a] Not to be confused with *Potamocorbula amurensis*

Table 2. EPA-derived Trophic Transfer Factor (*TTF*) values for freshwater fish.
AE = Assimilation efficiency (%), IR = Ingestion rate (g/g-d), k_e = Elimination rate constant (/d).

Common name	Scientific name	AE	IR	k_e	TTF
Cypriniformes					
blacknose dace	*Rhinichthys atratulus*	-	-	-	0.71
bluehead sucker	*Catostomus discobolus*	-	-	-	1.04
longnose sucker	*Catostomus catostomus*	-	-	-	0.90
white sucker	*Catostomus commersonii*	-	-	-	1.11
flannelmouth sucker	*Catostomus latipinnis*	-	-	-	0.98
common carp	*Cyprinus carpio*	-	-	-	1.20
creek chub	*Semotilus atromaculatus*	-	-	-	1.06
fathead minnow	*Pimephales promelas*	-	-	-	1.57
red shiner	*Cyprinella lutrensis*	-	-	-	1.31
redside shiner	*Richardsonius balteatus*	-	-	-	1.08
sand shiner	*Notropis stramineus*	-	-	-	1.56
Cyprinodontiformes					
western mosquitofish	*Gambusia affinis*	-	-	-	1.21
northern plains killifish	*Fundulus kansae*	-	-	-	1.27
Esociformes					
northern pike	*Esox lucius*	-	-	-	1.78
Gasterosteiformes					
brook stickleback	*Culaea inconstans*	-	-	-	1.79
Perciformes					
black crappie	*Pomoxis nigromaculatus*	-	-	-	2.67
bluegill	*Lepomis macrochirus*	-	-	-	1.03
green sunfish	*Lepomis cyanellus*	-	-	-	1.12
largemouth bass	*Micropterus salmoides*	-	-	-	1.39
smallmouth bass	*Micropterus dolomieu*	-	-	-	0.86
striped bass	*Morone saxatilis*	0.375	0.335	0.085	1.48
walleye	*Sander vitreus*	-	-	-	1.60
yellow perch	*Perca flavescens*	-	-	-	1.42
Salmoniformes					
brook trout	*Salvelinus fontinalis*	-	-	-	0.88
brown trout	*Salmo trutta*	-	-	-	1.38
mountain whitefish	*Prosopium williamsoni*	-	-	-	1.38
cutthroat trout	*Oncorhynchus clarkii*	-	-	-	1.12
rainbow trout	*Oncorhynchus mykiss*	-	-	-	1.07
Scorpaeniformes					
mottled sculpin	*Cottus bairdi*	-	-	-	1.38
sculpin	*Cottus sp.*	-	-	-	1.29
Siluriformes					
black bullhead	*Ameiurus melas*	-	-	-	0.85
channel catfish	*Ictalurus punctatus*	-	-	-	0.68

Table 3. EPA-Derived Trophic Transfer Factor (*TTF*) Values for Aquatic-Dependent Birds.

Common name	Scientific name	*TTF*
Non-Migratory		
American coot	*Fulica americana*	1.89
red winged blackbird	*Agelaius phoeniceus*	0.86
Migratory		
American avocet	*Recurvirostra americana*	1.44
cinnamon teal	*Anas cyanoptera*	1.79
eared grebe	*Podiceps nigricollis*	2.00
gadwall	*Anas strepera*	1.78
pied billed grebe	*Podilymbus podiceps*	0.78
yellow headed blackbird	*Xanthocephalus xanthocephalus*	1.04

If the state cannot obtain required *TTF* values from Tables 1, 2, or 3, the state will derive species-specific *TTF* values from existing data. The state will do this by determining the species-specific physiological coefficients representing food ingestion rate (*IR)*, selenium efflux rate (k_e), and selenium assimilation efficiency (*AE*) from the scientific literature to calculate a *TTF* value using **Equation 7** (Reinfelder et al. 1998**)** given as:

$$TTF = \frac{AE \times IR}{k_e} \qquad \textbf{(Equation 7)}$$

Where:

TTF	=	species-specific trophic transfer factor
AE	=	species-specific assimilation efficiency (%)
IR	=	species-specific ingestion rate (g/g-d)
k_e	=	species-specific efflux rate constant (/d)

If *TTF* values are not available from the above tables or cannot be calculated because the physiological coefficients are unavailable, the state will extrapolate a new *TTF* value from a surrogate species with an empirically derived *TTF* value. The surrogate species considered should have a similar dietary composition and if possible be taxonomically related (within the same order). If the lowest matching taxon of the species of interest is common to more than one of the available *TTF* values, the average *TTF* from the matching table entries could be used. The use of taxonomic hierarchies in this way utilizes evolutionary relationships to infer biological similarities among organisms (Suter 1993).

If the state cannot derive a *TTF* using one of the approaches described above, the state will derive species-specific *TTF* values by assessing the relationship between the selenium concentration in the tissue of organisms and the selenium concentration in the food they consume using paired measurements from published field studies. Species-specific *TTF* values should not be derived using paired measurements from controlled laboratory experiments as these measurements will likely not accurately represent selenium bioaccumulation in organisms at the

site. The published studies that should be utilized will have paired selenium measurements that have been collected in the field concurrently at the same aquatic site. However, individual aquatic sites may have selenium loads and/or bioaccumulation characteristics that require different relative collection time criteria to accurately characterize selenium relationships. Data from published studies will not be used if the time between collections exceeds more than one year. Species-specific *TTF* values will be derived from such measurements by using a combination median and regression approach. The state will define the *TTF* value for any trophic level as:

$$\boldsymbol{TTF^{TLn} = \frac{C_{tissue}^{TLn}}{C_{food}^{TLn}}} \quad \textbf{(Equation 8)}$$

Where:

TTF^{TLn} = The trophic transfer factor of a given trophic level,
C_{tissue}^{TLn} = The selenium concentration (mg/kg dw) in the tissues of the consumer organism,
C_{food}^{TLn} = The selenium concentration (mg/kg dw) in the consumer organism's food.

The state will use the median of the ratios calculated using **Equation 8** as the species-specific *TTF* value, but only if an empirical relationship between the paired measurements is confirmed by linear regression analysis. Using the median of the individual ratios provides an estimate of central tendency for that relationship that is less sensitive to potential bias from measurements taken from aquatic systems with very high or very low selenium concentrations. The state will consider the relationship acceptable if a linear regression of tissue selenium concentrations and food selenium concentrations resulted in both a statistically significant fit of the slope (p-value < 0.05) and a positive slope (i.e., selenium concentrations in the consumer increases with increasing selenium in food). A significant positive linear regression confirms that the relationship between selenium in organisms and the food they ingest is adequately represented by the available data. Outlier analysis may be performed to make sure that all data included are appropriate for use in analyses. In addition, the data may be transformed to better reflect the underlying distribution of the data.

If *TTF*s need to be calculated by performing additional studies, then the site-specific water column element will be determined through the typical water quality standards adoption process rather than through the performance-based approach.

4.3 Selection of Target Species

Once the state has quantified the dietary composition and determined the appropriate species-specific *TTF*s for all fish and bird species present at a site, the state will calculate the composite *TTF*s for the species using **Equation 5.** The state will then compare the composite *TTF*s for all fish species and all bird species present at the site and select the fish and bird species with the greatest bioaccumulation potential to sample or model for the site-specific water column translation. The species with the highest composite *TTF* value will have the greatest bioaccumulation potential if selenium exposure is relatively equal throughout the site. For the

BAF approach, if exposure is not equal throughout the site, the state will sample the species with the highest $TTF^{composite}$ located in the area of highest bioaccumulation potential. If there is uncertainty in which species will be the highest bioaccumulator using the BAF approach, the state will sample multiple fish or bird species to determine which has the highest selenium concentrations and use the species with the highest selenium concentration to calculate the BAF. If the highest bioaccumulator at a site is an anadromous salmonid, then the smolt stage of the fish species will be modeled or sampled as a whole-body sample. Smolts will be sampled because adult fish will not be exposed to selenium from the site through their diet due to their migratory behavior.

5.0 Selection of Translation Approach

Once a state has defined their site and selected a target species, the state will select which approach they will use to translate the tissue criterion element to a water column criterion element at each site. To make this decision, the state will evaluate what information they have available about the site and which approach is easier to accomplish logistically.

6.0 Sources of Potential Uncertainty in Translated Water Column Values

Some species of fish and birds exhibit spatial and temporal variabilities in feeding biology and have unique breeding behaviors. These variabilities introduce uncertainty that will be considered by the state when translating the fish and bird tissue criterion elements to a water column element. For instance, the life history characteristics of migratory fish and bird target species may increase the uncertainty in the translated water column element compared to target species that are year-round residents. Additionally, water column elements translated from the bird tissue element may be more uncertain than water column elements translated from the fish tissue criterion elements due to the wider foraging range of birds compared to fish. Therefore, all of these sources of potential uncertainty will be taken into consideration by the state when translating the fish and bird tissue elements into a water column element, particularly in target species selection and sampling plan development.

7.0 Mechanistic Modeling Approach

7.1 Fish Tissue Type Selection

When the state is translating to a site-specific water column criterion element from a fish tissue criterion element using the mechanistic model, the state will translate from any of the fish tissue criterion elements. As the egg-ovary criterion element is most closely related to the toxicological effects of selenium observed in fish, the egg-ovary criterion element is the preferred fish tissue criterion element to be used to translate to a water column element. The state will select which tissue criterion element they will translate from based on what data they have available for the translation (e.g., CFs).

7.2 Selection of Conversion Factor Value

Once the state has determined the proper fish tissue criterion element to translate to the water column criterion element for a site, the state will select proper *CF* values to populate

Equation 1. To select a proper *CF* value, the state will use known species-specific *CF* values in Table 4 or Table 5 (reproduced from the USEPA 2016 CWA section 304(a) recommended selenium criterion document). If a species-specific *CF* value is not available in the Table 4 or Table 5 from USEPA 2016, a *CF* value from a closely related surrogate species (within the same order) will be used. If the lowest matching taxon of the target species is common to more than one of the available *CF* values, the average *CF* values from the matching table entries will be used.

The EPA derived species-specific *CF* values (Table 4) by using empirical measurements of selenium concentrations in different tissues of the same fish. To derive whole body to egg-ovary *CF* values, the EPA defined matched pairs of selenium measurements from the whole body and from the eggs or ovaries measured from the same individual fish or from matched composite samples. Egg-ovary concentration was defined as a measurement from either the eggs or the ovaries. If multiple measurements from both eggs and ovaries of the same individual or matched composite sample were available, the average value was used. *CF* values were calculated using matched tissue measurements from all available sites and studies for a given species. The EPA had sufficient egg-ovary and whole-body selenium measurements to directly derive egg-ovary to whole body *CF* values for 13 species of fish. However, matched pairs of selenium measurements in eggs and/or ovaries and muscle (M) tissue, and matched pairs of selenium measurements in muscle and whole body were also available. To derive *CF* values for additional fish species, the EPA used either the additional data or a taxonomic classification approach to estimate *CF*. The EPA derived 13 *CF* values directly from matched pairs of egg-ovary and whole-body selenium measurements and an additional seven *CF* values by multiplying EO/M and M/WB conversion factors. For more details on *CF* values for fish see Section 3.2.2.2 and Appendix B in USEPA 2016 CWA section 304(a) recommended selenium criterion document. For the process of translating the bird egg criterion element or fish whole-body criterion element to a water column concentration, *CF* values were not necessary.

Table 4. EPA-Derived Egg-Ovary to Whole Body Conversion Factor (*CF*) Values (USEPA 2016).

Common name	Scientific name	*CF*	Std. Dev.[a]
Acipenseriformes			
white sturgeon	*Acipenser transmontanus*	1.69	
Cypriniformes			
bluehead sucker	*Catostomus discobolus*	1.82	0.19
flannelmouth sucker	*Catostomus latipinnis*	1.41	0.20
white sucker	*Catostomus commersonii*	1.38	0.36
desert pupfish	*Cyprinodon macularius*	1.20	0.10
common carp	*Cyprinus carpio*	1.92	0.49
roundtail chub	*Gila robusta*	2.07	0.29
fathead minnow	*Pimephales promelas*	1.40	0.75
creek chub	*Semotilus atromaculatus*	1.99	1.00
razorback sucker	*Xyrauchen texanus*	3.11	
Esociformes			

Common name	Scientific name	*CF*	Std. Dev.[a]
northern pike	*Esox lucius*	2.39	
Perciformes			
bluegill	*Lepomis macrochirus*	2.13	0.68
green sunfish	*Lepomis cyanellus*	1.45	0.23
smallmouth bass	*Micropterus dolomieu*	1.42	0.19
Salmoniformes			
brook trout	*Salvelinus fontinalis*	1.38	
Dolly Varden	*Salvelinus malma*	1.61	
brown trout	*Salmo trutta*	1.45	1.81[b]
rainbow trout	*Oncorhynchus mykiss*	2.44	
cutthroat trout	*Oncorhynchus clarkii*	1.96	2.03[b]
mountain whitefish	*Prosopium williamsoni*	7.39	

[a] Standard deviation for *CF* values for those species that had egg-ovary and whole body selenium concentrations.
[b] The brown trout and cutthroat trout standard deviations for *CF* values of 1.81 and 2.03 are considerably higher than the other standard deviations in this table. The brown trout data were taken from two studies, Formation Environmental (2011) and Osmundson et al. (2007). *CF* values for three of the four fish samples from Osmundson et al. were four to six times greater than the median. Also, the Formation Environmental data consisted of samples collected from natural streams and samples collected from a fish hatchery. The *CF* values for the fish hatchery samples were four to seven times lower than the median value. Although collectively, the data set meets the criteria for including the brown trout *CF*, the *CF* values for Osmundson et al. and Formation Environmental hatchery samples may be anomalously high and low, respectively. Excluding these potentially anomalous data reduces the brown trout standard deviation to 0.47. The cutthroat trout *CF* values are from two sources (Formation Environmental 2012 and Hardy 2005). The reason for the higher variability in the cutthroat trout *CF* values is due to the relatively higher *CF* values in the hatchery fish from the Formation study. The standard deviation for cutthroat trout drops to 0.62 if the hatchery fish are excluded. See Appendix B of (U.S. EPA 2016a) for a presentation of the data for both species.

Table 5. EPA-Derived Muscle to Whole Body Conversion Factor (*CF*) Values (USEPA 2016).

Common name	Scientific name	Median ratio
Bluegill	*Lepomis macrochirus*	1.32
Bluehead sucker	*Catostomus discobolus*	1.23
Common carp	*Cyprinus carpio*	1.61
Flannelmouth sucker	*Catostomus latipinnis*	1.46
Green sunfish	*Lepomis cyanellus*	1.23
Roundtail chub	*Gila robusta*	1.05
Smallmouth bass	*Micropterus dolomieu*	1.23
White sucker	*Catostomus commersonii*	1.34

7.3 Sampling Plan

In instances where the state is using the mechanistic model approach, the state will use information from USEPA 2016 CWA section 304(a) recommended selenium criterion document, the TSD for the California selenium criterion proposed rule and the scientific literature to define the $TTF^{composite}$ (as discussed in Sections 4.1 Food Web Modeling and 4.2 Selection of TTF Values) for the model. To determine the enrichment factor (*EF*), the state will collect field data if no appropriate site-specific data are available. The *EF* is the ratio of the concentration of selenium in particulate material and the concentration of selenium dissolved in water. The base of the aquatic food web includes phytoplankton, periphyton, detritus, inorganic suspended material, biofilm, sediment and/or attached vascular plants (Presser and Luoma, 2010). The EPA refers to this mixture of living and non-living entities as particulate material. The parameter *EF* is a single value that represents the steady-state proportional concentration of selenium in particulate material relative to the concentration of selenium dissolved in water. This parameter varies more widely across aquatic systems than any other parameter and is influenced by the source and form of selenium, water residence time, the biogeochemical characteristics of the water body, and the type of particulate matter collected (USEPA, 2016). Because the *EF* can vary greatly between water bodies, this parameter has the greatest potential to introduce uncertainty in the translation from a tissue criterion element to a site-specific water column criterion element. The greatest reduction in uncertainty when translating a tissue criterion element to a water column criterion element is achieved when spatially and temporally coincident site-specific empirical observations of dissolved and particulate selenium of sufficient quality and quantity are used to accurately characterize the *EF*.

The EPA recommends that site-specific EFs be determined by a) deriving a site-specific *EF* value from field measurements at the site, or b) deriving an appropriate *EF* value from appropriately collected existing site data. The state will generally collect field data to determine the *EF*, however, the state may use existing data if it follows the same temporal bounds determined in the data requirements of the USEPA 2016 selenium criterion (see Section 3.2.2.3 of the USEPA 2016 selenium criterion). The EPA used sites with selenium measurements in particulate and water collected within 1 year of each other as inputs to the EPA model (Presser and Luoma 2010) to derive national lotic and lentic water column criterion elements. The EPA's analysis of particulate and water samples from a sample population of aquatic systems found that samples taken within one year of each other, based on data availability, were appropriate in deriving the national criterion (Figure 3.5 in USEPA 2016) (USEPA 2016). However, site-specific *EF* values using particulate and water samples that are as spatially and temporally coincident as possible are considered the most robust. If possible, a site-specific *EF* value will ideally involve collecting particulate and water samples at the same location and time to ensure their representativeness of site-specific conditions.

The *EF* is the ratio of the concentration of selenium in particulate material and the concentration of selenium dissolved in water given as:

$$EF = \frac{C_{particulate}}{C_{water}} \quad \textbf{(Equation 9)}$$

Where:

$C_{particulate}$ = Concentration of selenium in particulate material (µg/g)

C_{water} = Concentration of selenium dissolved in water (µg/L)

EF = Enrichment Factor (L/g)

At each site the state will decide which particulate material is most appropriate to sample for the site, and sample that material using the methods listed below. The state may sample multiple media for particulate material and combine the *EFs* if appropriate for the site. Consistent with the EPA's 2016 national recommended selenium 304(a) criterion the state will only use selenium particulate concentrations from sediment if the majority of the other measurements are from algae or detritus because sediment samples were found to have a significantly lower correlation to selenium in water than algae or detritus (USEPA 2016).

7.3.1 Particulate Sampling

7.3.1.1 Periphyton Sampling

When sampling particulate material from wadeable streams, the state will collect periphyton. Periphyton will be collected during periods of stable stream flow and will not be sampled for 3 weeks after a high, bottom-scouring stream flow. The state will collect a small amount of periphyton from all substrate types and habitat types within the site. The amount of periphyton collected from each substrate/habitat type should correspond to their relative abundance in the site. If the state is collecting periphyton then they will follow the standard methods described in *Revised Protocols for Sampling Algal, Invertebrate, and Fish Communities as Part of the National Water-Quality Assessment Program* developed by USGS (Moulton et al. 2002). The methods in the following sections of Moulton et al. 2002 will be used for sampling periphyton from rocks, wood, plants, and sand/silt, respectively: section 4.3.1 sampling methods for epilithic habitats, section 4.3.1 sampling method for epidendric habitats, section 4.3.3 sampling method for epiphytic habitats, and section 4.3.4 sampling method for epipsammic/epipelic habitats. For each of these methods, rinse water will be filtered native stream water. If the state collects periphyton from multiple habitats, then they will follow the methodology in section 4.4 qualitative multihabitat to combine periphyton samples. These methods will be followed except for the quantification of the area from which the periphyton was collected. The area does not need to be quantified for selenium analysis. No preservative solutions will be added to these periphyton samples. Rather samples will be stored on ice for transport from the field to the lab, where they will be frozen at -20°C until analysis. Samples will be held no longer than 6 months before analysis.

When periphyton is being sampled from large rivers, the state will follow the protocols in sections 5.4, 5.4.1, and 5.4.2 of *Concepts and Approaches for the Bioassessment of Non-wadeable Stream and Rivers* (Flotemersch et al. 2006). Samples will not be preserved as described in these methods, but rather will be placed on ice for transport from the field to the lab, where they will be frozen at -20°C. Samples will be held no longer than 6 months before analysis.

7.3.1.2 Macroalgae Sampling

If the state is collecting macroalgae (filamentous algae), they will follow methods outlined in Moulton et al. (2002) in section 4.4.2 macroalgae. Macroalgae samples will not have any preservative solutions added to them, rather, they will be stored on ice for transport from the field to the lab. Samples will then be frozen at -20°C until analysis. Samples will be held no longer than 6 months before analysis.

7.3.1.3 Phytoplankton Sampling

Phytoplankton samples will be collected for either large rivers or for lentic water bodies. Whole water samples will be collected using either a subsurface grab or a depth/width-integrating sampler. In productive waters, 1 liter of water will likely be sufficient, but 5 or more liters of water may need to be collected from unproductive water bodies (Moulton et al. 2002). Water samples will be collected from the photic zone of the water body (likely in the .5 m to 1 m depth range). Water samples will be prefiltered through 125 µm filters and then phytoplankton will be collected on .45 µm flatstock, cellulose acetate filters. Filters will then be folded into quarters with filtered biomass inside and placed in a plastic sampling bag with a chain of custody tag. Samples will be placed on ice for transport from the field to the lab, where the sample will be frozen between -25°C and -30°C until analysis. Filters will be pre-weighed to allow for determination of dry weight of the phytoplankton so that the concentration of selenium in the sample can be determined. If large volumes of water need to be collected to get a sample with sufficient mass for analysis, unfiltered water samples will be transported on ice to the lab for processing. Large quantities of water will be processed using a high volume, continuous centrifuge to concentrate the phytoplankton in the water samples. That phytoplankton will then be freeze dried and sent for selenium analysis.

7.3.1.4 Sediment Sampling

The method for epipsammic/epipelic habitats will also be used if sediment is sampled. Sediment will only be sampled from depositional zones or habitats. Sediment will only be sampled in addition to another particulate material. No preservative solutions will be added to these samples. Rather samples will be stored on ice for transport from the field to the lab, where they will be frozen at -20°C until analysis. Samples will be held no longer than 6 months before analysis.

7.3.2 Water Sampling

The state will make the greatest effort to sample water concurrently with particulate samples, however, the state will use water measurements for the calculation of an *EF* that were collected within one year of particulate material being collected. Water samples will be collected using a peristaltic pump from mid-water column in wadeable streams. If water is being sampled from a deep-water body, then a surface, middle, and bottom water sample will be collected and composited. Water samples that are collected will be filtered through a 0.45 µm high capacity cartridge filter and collected in a high-density polyethylene bottle. If large particulates are present, the water will also be prefiltered through a 125 µm filter. 250 ml of water will be

collected. Water samples will be preserved with nitric acid to a pH less than 2. Samples will be transported on ice from the field to the lab and then stored at 4 °C until processing.

7.3.3 Time of Year for Sampling

Particulate samples will be collected during the algae growing season only (likely limited to spring and summer).

7.3.4 Location of Sampling

The state will collect composite samples from eight locations within each site (one water and one composite particulate sample at each location) and will ensure that enough water and particulate material is collected to perform chemical analyses. If a selenium discharge is present at the site, the state will make sure the sampling locations capture areas of potentially high exposure, based on the physical, chemical, and biological characteristics of the water body.

7.3.5 Number of Samples

The state will collect eight particulate samples and eight water samples for the site (one for each location sampled within the site). For large sites, the state may collect more samples, if eight will not sufficiently represent the variability at the site.

7.4 Chemical Analysis

The state will use an EPA-approved method for chemical analysis of dissolved selenium in water samples. The state will measure selenium concentrations in particulate materials using methods described in Appendix L of USEPA 2016 CWA section 304(a) recommended selenium criterion document. The state will also verify that the methods being used have method detection limits and quantitation limits sufficiently sensitive to quantify the selenium concentration within the sample. The state will report all particulate material concentrations as a dry weight concentration.

7.5 Data Analysis for the Mechanistic Modeling Approach

The state will calculate a site-specific water column criterion using the mechanistic model approach by applying appropriate input values to **Equation 1,** if translating from the fish egg-ovary criterion element or fish muscle criterion element, or **Equation 2,** if translating from the fish whole-body criterion element or bird egg criterion element. The state will use the $TTF^{composite}$ previously calculated during the target species analysis in this equation. The tissue criterion element will either be the bird egg or one of the fish tissue criterion elements. If the egg-ovary or fish muscle criterion element is being used, then the *CF* value included will be the one selected or derived as described in section 7.2. The *EF* value will be calculated using field collected data (or appropriate existing site-specific data) and **Equation 9.** To calculate a site-specific *EF* value, the state will first calculate the ratio of each individual particulate measurement and its associated water measurement (if more than one water measurement is available for any given particulate measurement, the state will use the median water

measurement). If more than one ratio for any given category of particulate material is available (e.g., more than one ratio of algae to water), the state will use the median of the ratios. The state will then calculate the geometric mean of the median ratios for each category of particulate material as the site EF value. The state will only use sediment measurements if there is at least one measurement from other organic particulate (algae, periphyton, phytoplankton or detritus).

Below are example calculations of site-specific water column criterion elements using the mechanistic model approach.

Example 1
Bluegill (*Lepomis macrochirus*) in a river that consume mostly amphipods:

Current water concentration (µg/L)	5.00
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor for bluegill (TTF^{TL3})	1.03
Trophic transfer factor for amphipods (TTF^{TL2})	1.22
Egg-ovary to whole-body conversion factor for bluegill (CF)	2.13
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$\boldsymbol{C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}}$$

$\mathbf{TTF^{composite} = TTF^{TL3} \times TTF^{TL2}}$

= 1.03 × 1.22

= 1.26

$$C_{water} = \frac{15.1}{1.26 \times 0.85 \times 2.13}$$

= 6.62 µg/

Example 2

Fathead minnow (*Pimephales promelas*) in a river that consume mostly copepods:

Current water concentration (µg/L)	5.00
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor for fathead minnow (TTF^{TL3})	1.57
Trophic transfer factor for copepods (TTF^{TL2})	1.41
Egg-ovary to whole-body conversion factor for fathead minnow (CF)	1.40
Selenium egg-ovary FCV (mg/kg)	15.1

$$\boldsymbol{EF = \frac{C_{particulate}}{C_{water}}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite}\ x\ EF\ x\ CF}$$

$\mathbf{TTF^{composite} = TTF^{TL3} \times TTF^{TL2}}$

= 1.57 × 1.41

= 2.21

$$C_{water} = \frac{15.1}{2.21 \times 0.85 \times 1.40}$$

= 5.74 µg/L

Example 3

Bluegill (*Lepomis macrochirus*) in a lake that consume mostly aquatic insects:

Current water concentration (µg/L)	5.0
Current particulate concentration (mg/kg)	8.75
Trophic transfer factor for bluegill (TTF^{TL3})	1.03
Trophic transfer factor for aquatic insects (median of Odonates, Water boatman, Midges, and Mayflies) (TTF^{TL2})	2.14
Egg-ovary to whole-body conversion factor for bluegill (CF)	2.13
Selenium egg-ovary FCV (mg/kg)	15.1

$$\boldsymbol{EF = \frac{C_{particulate}}{C_{water}}}$$

$$EF = \frac{8.75}{5.00}$$

= 1.75 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite}\ x\ EF\ x\ CF}$$

$TTF^{composite}$ $= TTF^{TL3} \times TTF^{TL2}$

= 1.03 x 2.14

= 2.20

$$C_{water} = \frac{15.1}{2.20 \times 1.75 \times 2.13}$$

= 1.84 µg/L

Example 4

Fathead minnow (*Pimephales promelas*) in a river that consume approximately ⅔ copepods and ⅓ aquatic insects:

Current water concentration (µg/L)	5.0
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor for fathead minnow (TTF^{TL3})	1.57
Trophic transfer factor for copepods and aquatic insects (TTF^{TL2}) Copepods = 1.41 Average of all aquatic insects = 2.14 $TTF^{TL2} = \sum_{i=1}^{n}(TTF_i \times w_i)$ $= (1.41 \times ⅔) + (2.14 \times ⅓)$ $= 1.65$	1.65
Egg-ovary to whole-body conversion factor for fathead minnow (CF)	1.40
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$

$= 1.57 \times 1.65$

$= 2.59$

$$C_{water} = \frac{15.1}{2.59 \times 0.85 \times 1.40}$$

= 4.90 µg/L

Example 5
Flathead chub (*Platygobio gracilis*) in a river with a diet of approximately 80% aquatic insects and 20% algae:

Current water concentration (µg/L)	5.0
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor of flathead chub: Lowest matching taxon is the family Cyprinidae. Therefore, the TTF value of Cyprinidae is used (TTF^{TL3})	1.20
Trophic transfer factor for insects (TTF^{TL2}) Average of all aquatic insects = 2.14	2.14
Egg-ovary to whole-body conversion factor for flathead chub (species-specific value not available, so median CF for family Cyprinidae is used). (CF)	1.95
Selenium egg-ovary FCV (mg/kg)	15.1

$$EF = \frac{C_{particulate}}{C_{water}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$$TTF^{composite} = [TTF^{TL3} \times TTF^{TL2} \times w_1] + [TTF^{TL3} \times w_2]$$

Where:

w_1 = Proportion of fathead chub diet from insects; and

w_2 = Proportion of fathead chub diet from algae

$$TTF^{composite} = [1.20 \times 2.14 \times 0.8] + [1.20 \times 0.2]$$

= 2.29

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

$$C_{water} = \frac{15.1}{2.29 \times 0.85 \times 1.95}$$

= 3.98 µg/L

Example 6

Largemouth bass (*Micropterus salmoides*) in a large river that consume mostly Western mosquitofish (*Gambusia affinis*) that consume approximately ¾ insects and ¼ crustaceans:

Current water concentration (µg/L)	5.0
Current particulate concentration (mg/kg)	4.25
Trophic transfer factor of largemouth bass (TTF^{TL4})	1.39
Trophic transfer factor of Western mosquitofish (TTF^{TL3})	1.21
Trophic transfer factor for insects and crustaceans (TTF^{TL2}) Median all Insects – 2.14 Median all Crustaceans – 1.41 $TTF^{TL2} = \sum_{i=1}^{n} \left(TTF_i^{TL2} w_i\right)$ = (2.14 x 0.75) + (1.41 x 0.25) = 1.96	1.96
Egg-ovary to whole-body conversion factor for largemouth bass (species-specific value not available, so median CF for genus Micropterus is used) (CF)	1.42
Selenium egg-ovary FCV (mg/kg)	15.1

$$\boldsymbol{EF} = \frac{\boldsymbol{C_{particulate}}}{\boldsymbol{C_{water}}}$$

$$EF = \frac{4.25}{5.00}$$

= 0.85 L/g

$TTF^{composite} = TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2}$

= 1.39 × 1.21× 1.96

= 3.30

$$C_{water} = \frac{C_{egg-ovary}}{TTF^{composite} \; x \; EF \; x \; CF}$$

$$C_{water} = \frac{15.1}{3.30 \times 0.85 \times 1.42}$$

= 3.79 µg/L

8.0 Bioaccumulation Factor Approach

8.1 Additional Target Species Considerations for BAF - Exposure at the Site

The state will consider differences in exposure at the site when selecting which fish and bird species will be sampled for the BAF approach. In order to fully assess which species has the greatest bioaccumulation potential, selenium exposure at the site, in addition to diet, will be considered when selecting a target species. The state will make the greatest effort to target species for sampling that feed in areas with sediment and flow characteristics that will lead to the greatest selenium bioaccumulation potential. If the site is a lotic site but has areas that have lentic properties, the state will target a species for sampling that utilizes these lentic locations for feeding, as selenium has the potential to bioaccumulate more in lentic areas.

8.2 Fish Tissue Type Selection

When the state is utilizing the BAF approach to derive a site-specific water column criterion element to protect the aquatic life designated use, the state will collect fish egg samples, if available, as egg concentrations have the strongest correlation to toxicity effects compared to all the tissue types. If egg samples are not available, then the state will collect whole-body or muscle samples.

Fish egg samples will be collected when the state can sample the fish at the appropriate time of the year. The state will consult with local fish biologists to determine the spawning time periods for their target fish species and will then collect egg samples from those target fish species in the pre-spawn time period, when the eggs are mature but the fish have not yet released their eggs.

If the state is not able to collect egg samples during this pre-spawn period either due to resource limitations or safety concerns due to high flows during spring snow melt, the state will instead collect whole-body or muscle samples of fish. If the state is collecting whole-body or muscle tissue samples, the state will consult with local fish biologists to determine the spawning time period for the target fish species, and will make sure that for whole-body or muscle samples, the fish are collected outside of that spawning period and also not collected directly post spawn (~ 1 month after spawning) as to avoid collecting fish tissue that is depurated of selenium, since selenium is transferred to fish eggs during egg development. If possible to identify in the field, the state will sample male fish rather than females for whole-body or muscle samples.

8.3 Sampling Plan

Although a site-specific, field-measured BAF is a direct measure of bioaccumulation, its predictive power depends on a number of important factors being properly addressed in the design of the field sampling effort. The preferred approach for using a BAF to implement the selenium fish and bird tissue criterion elements is to calculate a site-specific, field-measured BAF from data gathered at the site of interest, and to apply that BAF to that site. Uncertainty can be introduced when BAFs are derived from water and fish or bird tissue concentration measurements that do not closely represent the temporal and spatial variability based on a particular site’s characteristics, particularly for lotic systems which tend to be more dynamic.

8.3.1 Fish Tissue Sampling

The state will collect composite egg, whole-body or muscle samples. Those samples will be at least 20 g ww. For fish that are being composited, the fish tissue that is collected will be from fish that are all the same species. If whole-body or muscle tissue is being collected, the fish will all be similar in size such that the smallest individual in no less the 75% of the total length of the largest individual. All samples will also be collected within a week.

For egg samples, gravid females will be collected using appropriate fish collection techniques for the water body (e.g. seines, hoop nets, electrofishing, angling etc.). The state will make sure that they are not sampling any undersized juveniles. Once the fish are collected they will be carefully observed for signs of physical damage, mortality or other sources of stress. Since any handling of the fish will remove the protective body layer of slime, fish will be handled as little as possible using dip nets and soft material gloves. Adult fish for egg collection will be held in live wells until the eggs are sampled. Egg collection tools will all be cleaned and dried before use. Female fish will be randomly selected from the live well and the area around the urogenital opening will be dried with paper towels. The length and weight of the female fish will be measured and recorded. The eggs will then be expressed from the fish by applying gentle pressure to the lower half of the fish from behind the pectoral fins and along the fish towards the anus. This application of pressure will be repeated until all the eggs have been expressed. Eggs will be collected in steel bowls that have been pre-cleaned and stored in a cool place. Eggs will be examined to make sure that they are free of fecal matter, urine, and blood. Any eggs that have other substances attached will be discarded using a clean plastic pipette. Samples will be transferred to resealable plastic bags and placed on ice for transport back to the lab where eggs will be weighed to the nearest gram using a top-loading digital scale, frozen (-20°C) for storage, and shipped for laboratory analysis when appropriate (Janz and Muscatello 2008). Samples will be frozen at -20°C in plastic, borosilicate glass, quartz or PTFE bottles. Sample will be held for a maximum of 6 months.

For whole-body samples, fish (male or female) will be collected using appropriate fish collection techniques for the water body. The length, weight, and sex of whole fish samples will be measured and recorded as each fish is collected. Fish will then be individually wrapped in extra heavy-duty aluminum foil. Spines on fish should be sheared to minimize punctures in the aluminum foil packaging (Stober, 1991). Each individual fish will be placed into a waterproof plastic bag and sealed. Once packaged, samples will be immediately placed on ice for transport back to the lab. Samples will then be frozen until analysis at -20°C. Samples will be held for no longer than 6 months.

For muscle samples, fish (male or female) will be collected using appropriate fish collection techniques for the water body. The length, weight, and sex of the fish will be measured and recorded as each fish is collected. Fish will then be individually wrapped in extra heavy-duty aluminum foil. Spines on fish should be sheared to minimize punctures in the aluminum foil packaging (Stober, 1991). Each individual fish will be placed into a waterproof plastic bag and sealed. Once packaged, samples will be immediately placed on ice for transport back to the lab. Once in the lab, fish will be filleted according to methods in section 7.2.2 Processing Fish Samples in *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1 Fish Sampling and Analysis* (USEPA 2000).

8.3.2 Bird Egg Sampling

The state will sample individual bird eggs from a site by collecting one egg per clutch (nest) after the clutch is complete. The state will collect a limited amount of bird egg samples (8 egg samples for each species sampled at the site; Ohlendorf et al. 2008) to reduce the impact of sampling while still attaining an estimate of variability across the site. If the site is small relative to the total foraging area of the target species being sampled, the variability will likely be greater and a larger sampling size may be required. Egg samples should be free of debris (e.g., feathers and nest material) and fecal matter. All egg samples will be labeled with site and species information and placed in resealable plastic bag. The egg samples will be placed on ice for transport back to the lab where the eggs will be measured for length and breadth (to the nearest 0.01 millimeter) and weighed for total egg weight (to the nearest 0.07 gram). All egg samples will be stored in a freezer at -20ºC until selenium determination (Evers 2009).

8.3.3 Water Sampling

The state will make the greatest effort to sample water concurrently with fish tissue and bird egg samples, with a maximum allowable time period of one year between water collection and tissue collection. Water samples will be collected using a peristaltic pump from mid-water column in wadeable streams. If water is being sampled from a deep-water body, then a surface, middle, and bottom water sample will be collected and composited. Water samples that are collected will be filtered through a 0.45 µm high capacity cartridge filter and collected in high density polyethylene bottle. If large particulates are present, the water will also be prefiltered through a 125 µm filter. 250 ml of water will be collected. Water samples will be preserved with nitric acid to a pH less than 2. Samples will be transported on ice from the field to the lab and then stored at 4 °C until processing.

8.3.4 Time of Year for Sampling

The state will determine what time of year they collect fish samples based on which tissue type and fish species they have decided to sample. If the state is collecting egg samples, then they will need to collect them during the appropriate pre-spawn period for the target species, which will be when the eggs are mature and have the highest concentrations of selenium. The state will consult with local fish biologists to determine the spawning period for their target species at the site. Whole-body samples or muscle samples will be collected outside of the spawning period and post-spawning period (at least a month after spawning). For most fish species, this will likely be late summer or early fall. The state will consult local fish biologists to determine the spawning period for target fish species and make sure that whole-body and muscle tissue collections are conducted outside that period and the period directly post-spawn. Fish will also not be sampled during winter months. If the site has characteristics that will cause temporal variability in selenium concentrations, the state will consider sampling in multiple seasons or multiple years. As the bird tissue criterion element is based on bird eggs, bird samples will only be collected during the breeding season of the target species. Water samples will be collected within a year of fish and bird egg samples, with the state targeting the concurrent collection of samples if possible.

8.3.5 Location of Sampling

Once a state has defined the site for the site-specific water column criterion element, the state will select locations from within the site to sample fish and birds. To determine where to sample fish and birds, the state will first start by identifying any point or non-point sources of selenium entering the water body. If there are sources of selenium entering the water body, the state will determine the selenium concentrations in water near the points of entry of selenium for the site to identify where the highest concentrations of selenium are located. The state will then evaluate the selenium concentrations at these high exposure locations to determine where the highest exposure is occurring. Fish and birds will be sampled from the area with the highest exposure to selenium. The state may also sample fish and birds from areas where they anticipate high selenium bioaccumulation based on sediment type and flow dynamics of the system. If this is a lotic site with some areas within the site with lentic properties, the state will sample tissue from those areas with lentic properties. If there are no point sources or non-point sources of selenium at the site, the state will select locations based on feeding habitats and home ranges and/or nesting areas of the target species.

8.3.6 Number of Samples

The state will collect eight composite fish samples, composed of three fish (or more if needed to have adequate tissue mass for chemical analysis) or eight bird egg samples and eight water samples for the site (Hitt and Smith 2015, BCMOE 2015). For large sites, the state may collect more samples, if eight will not sufficiently represent the variability at the site.

8.4 Chemical Analysis

The state will use an EPA-approved method for chemical analysis of dissolved selenium in water samples. The state will measure selenium concentrations in fish and bird tissue using methods described in Appendix L of USEPA 2016 CWA section 304(a) recommended selenium criterion document or other published methods. The state will also verify that the methods being used have method detection limits and quantitation limits sufficiently sensitive to quantify the selenium concentration within the sample. The state will report all tissue concentrations as a dry weight concentration.

8.5 Data Analysis for the Empirical BAF Approach

Several considerations in the analysis of the available data to derive a BAF-based site-specific criterion must be addressed in order to address uncertainty and produce a defensible outcome. The state will use all paired water and fish samples or paired water and bird egg samples to calculate BAFs using **Equation 3**. The state will then select the 90^{th} percentile of the distribution of calculated BAFs to derive the SSC, using **Equation 4**, to insure protection of sensitive and highly exposed species at the site. The fish tissue criterion element used in **Equation 4**, will be the same tissue type that was collected to calculate the BAF.

Below is an example of the derivation of a site-specific water column criterion for a water body impacted by selenium using bluegill as an example (USEPA 2016).

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

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

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

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

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

Target water concentration = 2.75 µg/L.

Another factor that the state will consider is the impact of selenium inputs to downstream waters where conditions for selenium bioaccumulation are more favorable (e.g., a selenium input to a lotic system (e.g., river) that flows into a lentic receiving water (e.g., lake)). In such a circumstance, the state will ensure that the site-specific water column criterion element for the upstream site accounts for potential impacts on the downstream site. The state may collect fish tissue samples or bird egg samples from the downstream site to ground-truth the conditions at the receiving water and help to determine if the selenium input from the upstream site is having an impact to selenium concentrations in the fish tissue or bird egg at the downstream site.

Finally, the presence of other conditions such as rising fish tissue or bird egg concentrations (based on historical tissue data for the site) due to existing inputs of selenium at a particular site could constitute a basis for concluding that the SSC derived for that site should be adjusted to account for the potential of further increases in fish tissue such that the applicable tissue criterion threshold will be exceeded in the future, depending on the particular characteristics of the site.

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