Method for Translating Selenium Tissue Criterion Elements into Site-specific Water Column Criterion Elements for California, Version 2, December 2024

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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
$C_{water\ criterion\ element}$	translated site-specific water column criterion element
$C_{tissue\ criterion\ element}$	selenium fish tissue or bird egg criterion element
C_{water}	concentration of total dissolved selenium in water
CF	conversion factor
CFR	Code of Federal Regulations
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

A. Introduction

This document describes the performance-based approach (PBA) methodology for California's use in calculating site-specific water column criterion elements for the selenium aquatic life and aquatic-dependent wildlife criterion as promulgated in the Environmental Protection Agency's (EPA's) final rule, "Water Quality Standards; Establishment of a Numeric Criterion for Selenium for the State of California."[1] The PBA is a methodology that the State may use to translate the bird and fish tissue criterion elements into water column criterion elements on a site-specific basis. If the State follows the methodology as prescribed in the PBA, it is not required to adopt and submit the outcomes that result from using the PBA to the EPA for Clean Water Act (CWA) section 303(c) review in accordance with the procedures at 40 Code of the Federal Regulations (CFR) part 131. If the State chooses to use the PBA, the State will coordinate with the EPA at the beginning of the process. If the State calculates a site-specific water column criterion element using another methodology, that criterion element must be adopted by the State and submitted to the EPA for review under CWA section 303(c) in accordance with the procedures at 40 CFR part 131.

As provided in this document, if the State uses this PBA, it will follow 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 described by the EPA for the translation of tissue criterion elements into water column criterion elements in Appendix K of *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016* (USEPA 2021). Given that these are well established approaches for translating concentrations between tissue and water, the State of California will use these approaches for site-specific water column criterion element translations if it does not wish to submit translation outcomes to the EPA for review. The egg-ovary criterion element is the preferred fish tissue criterion element to be used in either approach to translate to a water column criterion element for protection of aquatic life, as the egg-ovary criterion element is most closely related to the toxicological effects of selenium observed in fish. The bird egg criterion element will be translated into a water column criterion element for the protection of aquatic-dependent wildlife, as birds are the most sensitive aquatic-dependent wildlife taxa and the bird egg is most closely related to the toxicological effects of selenium observed in birds. 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 tissue selenium concentrations.

The EPA derived the national recommended 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 using 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). The mechanistic model approach is described in detail in *Aquatic Life and Aquatic-Dependent Wildlife Selenium Water Quality Criteria for Freshwaters of California* (TSD) (USEPA 2024) and *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for*

[1] This final rule applies to certain waters of California in a manner consistent with the California Toxics Rule where the protection of aquatic life and aquatic-dependent wildlife are designated uses. The final rule does not apply to California waters where site-specific selenium criteria have been adopted, nor does it apply to California waters with selenium criteria promulgated in the National Toxics Rule.

Selenium – Freshwater 2016 (USEPA 2021). The EPA started with an equation that described bioaccumulation of selenium in animal tissues by assuming that net bioaccumulation is a balance between assimilation efficiency from diet, ingestion rate, rate of direct uptake in dissolved forms, loss rate, and growth rate. This equation was then simplified as described in section 3.2.1 of USEPA 2021. The growth rate factor was removed from the starting equation because high consumption rates of selenium-contaminated food may counteract the selenium dilution that occurs with the addition of body tissue during periods of fast growth. The factor accounting for direct aqueous uptake of selenium was also removed from the starting equation because aqueous exposure only accounts for a small proportion of selenium bioaccumulation by an organism (USEPA 2021). The resulting mechanistic model equation can be used to derive a selenium water column criterion element from fish egg-ovary or fish muscle criterion elements.

$$C_{water\ criterion\ element} = \frac{C_{fish\ egg-ovary\ or\ muscle\ criterion\ element}}{TTF^{composite} \times \mathrm{EF} \times \mathrm{CF}} \qquad \textbf{(Equation 1)}$$

Where:

$C_{water\ criterion\ element}$	=	translated site-specific water column criterion element (total dissolved µg/L),
$C_{fish\ egg\text{-}ovary\ or\ muscle\ criterion\ element}$	=	fish egg-ovary or muscle tissue criterion element (mg/kg dw),
$TTF^{composite}$	=	composite trophic transfer factor (*TTF*) is the product of the species-specific trophic transfer factor (*TTF*) values for selenium in each trophic level of the food web of the target fish or bird species (no units of measurement),
EF	=	enrichment factor is the proportional bioconcentration of total 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 concentration of selenium in the whole-body of the fish (no units of measurement, not needed if starting with the whole-body fish tissue criterion element or bird egg criterion element).

A conversion factor is not needed when translating from either the bird egg or fish tissue whole-body criterion elements into a site-specific water column criterion element (because the conversion factor is only used to convert a fish muscle or fish egg-ovary value into a proportional fish whole-body value) so **Equation 2** can be used for translations from the bird egg or fish tissue whole-body criterion elements:

$$C_{water\ criterion\ element} = \frac{C_{bird\ egg\ or\ fish\ whole-body\ criterion\ element}}{TTF^{composite} \times \mathrm{EF}} \qquad \textbf{(Equation 2)}$$

Where:

$C_{water\ criterion\ element}$	=	translated site-specific water column criterion element (total dissolved µg/L),
$C_{bird\ egg\ or\ fish\ whole\text{-}body\ criterion\ element}$	=	bird egg or fish whole-body tissue criterion element (mg/kg dw),
$TTF^{composite}$	=	composite trophic transfer factor (*TTF*) is the product of the species-specific trophic transfer factor (*TTF*) values for selenium in each trophic level of the food web of the target fish or bird species (no units of measurement),
EF	=	enrichment factor is the proportional bioconcentration of total dissolved selenium at the base of the aquatic food web (L/g).

The State will use these equations to derive site-specific water column criterion elements when using the mechanistic model approach. When the State derives a site-specific water column criterion element for the protection of aquatic life, it will translate from the fish whole-body, muscle, or egg-ovary tissue criterion element to determine an appropriate water column criterion element using either Equation 1 (muscle or egg-ovary) or Equation 2 (whole-body). When the State is using the mechanistic model approach to determine a water column criterion element that is protective of the aquatic-dependent wildlife use, it will translate from the bird egg criterion element using Equation 2.

Alternatively, the State may use the BAF approach described in Appendix K of USEPA 2021 to translate tissue criterion elements into site-specific water column criterion elements. If so, the state will use **Equation 3** and **Equation 4** below to translate a fish tissue or bird egg criterion element to a water column criterion element. Equation 3 will be used to calculate a bioaccumulation factor by dividing the concentration of selenium in either fish or bird tissue by the concentration of selenium in the water column.

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

Where:

BAF	=	bioaccumulation factor for selenium 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 field-collected water (total dissolved µg/L)

The site-specific BAF will then be used in Equation 4 with the appropriate criterion element (fish egg-ovary, fish whole-body, fish muscle, or bird egg) that matches the tissue type collected to derive the BAF, to calculate a site-specific water column criterion element ($C_{water\ criterion\ element}$):

$$C_{water\ criterion\ element} = \frac{C_{tissue\ criterion\ element}}{BAF} \qquad \textbf{(Equation 4)}$$

Where:

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

B. Procedures

1.0 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 and rationale for the determination 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. A number of factors could be considered when defining a site, including hydrodynamics, water chemistry, and physical habitat. Natural breaks in these elements, such as the confluence of one river with another, can be used to determine boundaries of a site. The presence of a community with a unique taxonomic composition may also justify a designation as a distinct site (i.e., assemblage that is distinct from another site or presence of a rare, unique, or ecologically significant species such as a threatened or endangered species). If a selenium discharge from a point source or non-point source is part of a site, the site boundaries will 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 PBA described in this document is appropriate for single water bodies or water body segments. In contrast, if the State decides to set site-specific water column criterion elements for larger areas, the State must derive and adopt site-specific water column criterion elements for the EPA's review in accordance with the CWA and the EPA's implementing regulations at 40 CFR part 131 (not using the PBA).

2.0 Determination of Community Present at Site

After the State has defined the boundaries of the site, the State will determine what aquatic and aquatic-dependent species are present at the site, and whether the site includes the habitat of any threatened or endangered species. The State will first evaluate scientific publications and the State's monitoring data to determine what species, including threatened and endangered species, are present at the site and whether the site may include the habitat of any threatened or endangered species. If monitoring data or scientific publications are not available, then the State will contact local resource agencies to see if they have information regarding the fish and bird species present or reasonably expected to be 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 species present at the site. Fish sampling will be conducted both in the spring and 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. Bird monitoring will be conducted during the breeding season (typically April to August).

The site-specific standards setting process should be reflective of the existing and potential future beneficial uses at the site and the characteristics of the aquatic life and aquatic-dependent wildlife present or reasonably expected to be present at the site, including threatened and endangered species. If the State finds that fish are not present at the site and are not expected to be present, then the State cannot use the PBA to determine a site-specific water column criterion element for the aquatic life designated use. Instead, the State must either use the water column criterion elements of the statewide criterion for the site or derive and adopt a site-specific water column criterion element for the EPA's review in accordance with the CWA and the EPA's implementing regulations at 40 CFR part 131.

3.0 Target Species Selection

The State will target the fish species with the greatest bioaccumulation potential from the genera *Acipenser, Lepomis, Salmo,* and *Oncorhynchus* for sampling or for modeling to develop the site-specific water column criterion element. If no fish species from these genera are present at the site, then the species with the greatest bioaccumulation potential will be targeted for sampling or modeling. If all fish species at the site have similar bioaccumulation potential, then the State will target the species that is most sensitive to selenium. The State will target the bird species with the greatest bioaccumulation potential.

If the selected target species at the site is a threatened or endangered species, the State will use the mechanistic model approach to derive the site-specific water column criterion element. If sufficient information is not available and not able to be collected for the mechanistic model approach for the target threatened and endangered species, then the State will sample a closely related (e.g., order or closer) surrogate species with a similar dietary composition and bioaccumulative potential for use in the BAF model.

3.1 Food Web Modeling

As a species is primarily exposed to selenium through its diet, quantifying the dietary composition of each potential target 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 species in the genera *Acipenser*, *Lepomis, Salmo,* and *Oncorhynchus* (or all fish species if no species are present from *Acipenser*, *Lepomis, Salmo,* and *Oncorhynchus)* present at the site and all bird species present during the breeding season at the site by reviewing the relevant state and scientific literature. In addition, if anadromous salmonids are present, the diet of the juvenile anadromous salmonids, rather than adults, will be evaluated.

Dietary compositions of many fish and bird species are defined in the CWA section 304(a) recommended selenium criterion document (USEPA 2021) and the TSD (USEPA 2024) for the California selenium criterion final 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 may 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 was 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 species in the genera *Acipenser*, *Lepomis, Salmo,* and *Oncorhynchus* (or all fish species if these genera are not present at the site) and bird species present at the site during the breeding season are identified and quantified, the State will calculate the composite trophic transfer factor ($TTF^{composite}$) for each fish and bird species. For sites that include threatened or endangered species, rather than using an average diet for each fish or bird species, the State may use a dietary estimate within the species' range that reflects a greater proportion of consumption of prey items with high selenium bioaccumulation potential. 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 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 $TTF^{composite}$ utilizing **Equation 5** and, if needed, **Equation 6**.

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

Where:

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

When more than one species is 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):

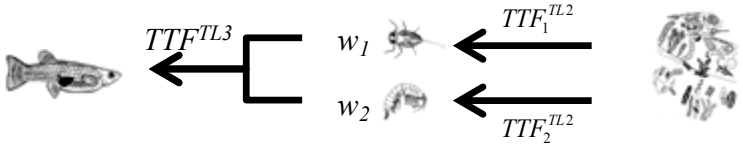
$$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$$

B) Four trophic levels (simple):

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

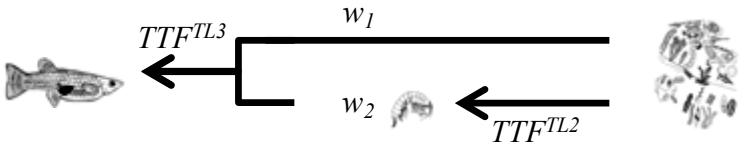
C) Three trophic levels (mix within trophic levels):

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



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):

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

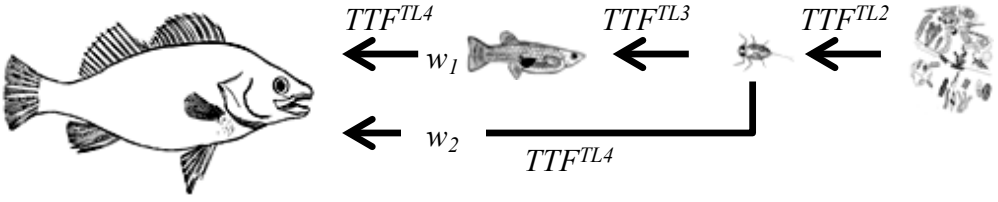
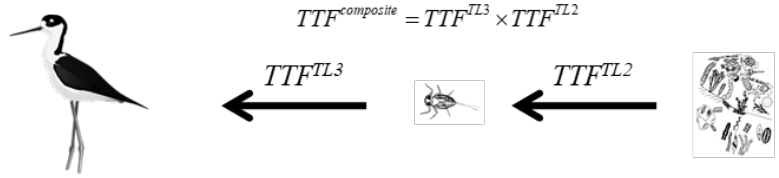


Figure 1. Example mathematical expressions of $TTF^{composite}$ representing different food-web scenarios for fish species. $TTF^{composite}$ quantitatively represents the trophic transfer of selenium through all dietary pathways of a targeted fish species. The mathematical expression of the food web 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.

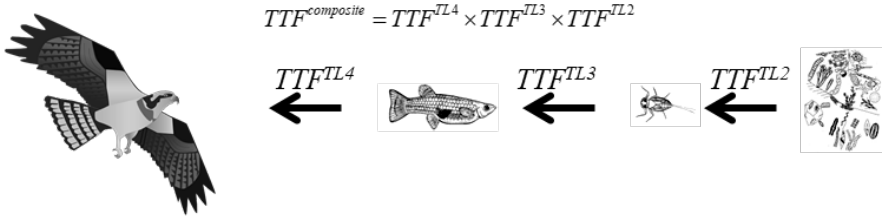
A) Three trophic levels (simple):

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



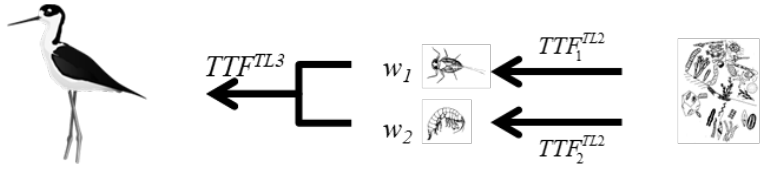
B) Four trophic levels (simple):

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



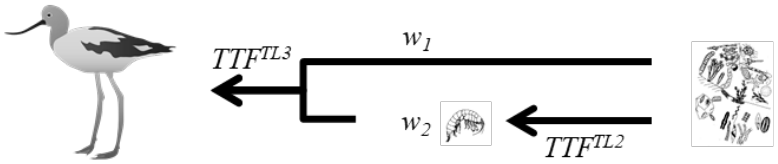
C) Three trophic levels (mix within trophic levels):

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



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):

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

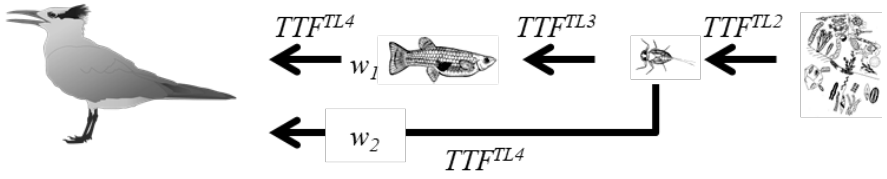


Figure 2. Example mathematical expressions of $TTF^{composite}$ representing different food-web scenarios for bird species. $TTF^{composite}$ quantitatively represents the trophic transfer of selenium through all dietary pathways of a targeted bird species. The mathematical expression of the food web 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.

3.2 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 that equation. To select proper *TTF* values, the State will first evaluate the list of *TTFs* below, from the CWA section 304(a) recommended selenium criterion document (USEPA 2021) and TSD for the California selenium criterion final rule (USEPA 2024), 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 the CWA section 304(a) recommended selenium criterion document (USEPA 2021) and Appendix B of the TSD for the California selenium criterion rule (USEPA 2024). The State may use the *TTF* values from these lists exclusively, or in conjunction with *TTF* values obtained from the other sources specified 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**)**:

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

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 *TTF* for a surrogate species will come either from Table 1, 2, or 3 above or from the peer-reviewed scientific literature. 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 median *TTF* from the matching table entries or published scientific literature will 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 procedures 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 in the field. The published studies that should be used will have paired organism and diet selenium measurements that have been collected at the same aquatic site in the field. Preferably these pairs will be collected concurrently. However, individual aquatic sites may have selenium loads and/or bioaccumulation characteristics that require different relative collection time criteria to accurately characterize selenium relationships. Therefore, data from published studies can be used if the time between collections is less than one year. The State will define the *TTF* value for any trophic level as:

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

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.

If the species consumes multiple dietary items, then the C_{food}^{TLn} will be determined using **Equation 9**.

$$C_{food}^{TLn} = \left(C_{prey\,1} \times w_1\right) + \left(C_{prey\,2} \times w_2\right) + \cdots + \left(C_{prey\,i} \times w_i\right) \qquad \textbf{(Equation 9)}$$

Where:

C_{food}^{TLn} = The selenium concentration (mg/kg dw) of the consumer organism's food,
$C_{prey\,i}$ = The selenium concentration (mg/kg dw) in the tissues of the i^{th} prey species
w_i = the proportion of the i^{th} species consumed

Species-specific *TTF* values will be derived from such measurements by using a combined median and regression approach. The State will use the median of the ratios calculated using **Equation 8** as the species-specific *TTF* value, but only if a positive direct 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 State will adopt the site-specific water column criterion element and submit it for the EPA's review in

accordance with the CWA and the EPA's implementing regulations at 40 CFR part 131 rather than deriving it through the PBA.

3.3 Selection of Target Species

Once the State has quantified the dietary composition and determined the appropriate species-specific *TTF*s, the State will calculate the composite *TTFs* for all fish species in the genera *Acipenser*, *Lepomis, Salmo,* and *Oncorhynchus* present at the site (or all the fish species if the genera *Acipenser*, *Lepomis, Salmo,* and *Oncorhynchus* are not present at the site) and bird species present at a site in the breeding season, using **Equation 5** and, if needed, **Equation 6.** The State will then compare the composite *TTF*s for all fish species in the genera *Acipenser*, *Lepomis, Salmo,* and *Oncorhynchus* (or all fish species if no species in these genera are present) and all bird species present at the site in the breeding season and select the fish and bird species with the greatest composite *TTF* (greatest bioaccumulation potential) to sample or model for the site-specific water column criterion element.

If all fish species have similar composite *TTF*'s, then the species with the lowest EC_{10} will be selected to sample or model for the site-specific water column criterion element. If the EC_{10} of a particular species is unavailable, sensitivity can be estimated from the EC_{10} of a closely related taxon.

The species with the highest composite *TTF* value will have the greatest bioaccumulation potential if selenium exposure is relatively equal throughout the site. 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.

If the State decides to use the BAF translation approach and 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 (i.e., areas with lentic properties, longest residence time). If the State is using the BAF approach and there is uncertainty in which species will be the highest bioaccumulator, 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.

4.0 Selection of Translation Approach

As stated previously, if the State chooses to use the PBA, the State will coordinate with the EPA at the beginning of the PBA process. Once the State has defined a site and selected a target species, the State will select which approach it will use to translate the tissue criterion element to a water column criterion element at the site. To make this decision, the State will evaluate what information it has available about the site and which approach is easier to accomplish logistically. The State will not use an approach for which it cannot acquire the proper data.

5.0 Mechanistic Modeling Approach

5.1 Tissue Type Selection

The State can translate from any of the fish tissue criterion elements or the bird egg criterion element into a site-specific water column criterion element. As the fish 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 fish tissue criterion element it will translate from based on what data are available for the translation (e.g., *CF*s).

5.2 Selection of $TTF^{composite}$ Value

The $TTF^{composite}$ for the target species determined in Section 4.0 will be used in the model for the water column translation. The *TTF* calculated using methods in Sections 4.1 Food Web Modeling and 4.2 Selection of *TTF* Values and the information from the CWA section 304(a) recommended selenium criterion document (USEPA 2021), the TSD for the California selenium criterion rule (USEPA 2024), and the scientific literature will be used for the mechanistic model translation.

5.3 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 the proper *CF* value to populate **Equation 1**, if the State has selected the egg-ovary or muscle criterion element. To select a proper *CF* value, the State will use known species-specific *CF* values in Table 4 or Table 5 (reproduced from USEPA 2021 CWA section 304(a) recommended selenium criterion document). If a species-specific *CF* value is not available in Table 4 or Table 5 from USEPA 2021, 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 median *CF* values from the matching table entries will be used. If the target species is a threatened or endangered species, or the water body includes habitat for threatened or endangered species, the State could use the highest *CF* from the lowest matching taxon rather than the median of those *CF* values. If a *CF* value is not available for a closely related surrogate species, then the State will conduct the translation from the whole-body criterion element.

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 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 paired egg-ovary/muscle and muscle/whole body data or a taxonomic classification approach to estimate the *CF*. The EPA derived an additional seven *CF* values by multiplying egg-ovary/muscle and muscle/whole body conversion factors. For more details on *CF* values for fish see Section 3.2.2.2 and Appendix B in USEPA 2021 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 are not necessary.

Table 4. EPA-Derived Egg-Ovary to Whole Body Conversion Factor (*CF*) Values (USEPA 2021).

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			
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 (USEPA 2021) for a presentation of the data for both species.

Table 5. EPA-Derived Muscle to Whole Body Conversion Factor (*CF*) Values (USEPA 2021).

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

5.4 Data Collection for the Calculation of the *EF*

The parameter *EF* represents the proportional concentration of selenium in particulate material relative to the concentration of total dissolved selenium in water and is calculated as the ratio of the concentration of selenium in particulate material and the concentration of total dissolved selenium in water. The EPA defines particulate material as the mixture of living and non-living entities at the base of the aquatic food web, including phytoplankton, periphyton, detritus, inorganic suspended material, biofilm, sediment and/or attached vascular plants (Presser and Luoma 2010).

The *EF* varies more widely across aquatic systems than any other parameter in the mechanistic model equation 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 (USEPA 2021). Because the *EF* can vary greatly between water bodies, this parameter has the greatest potential to introduce uncertainty to 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 using the mechanistic model 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*. Therefore, the State will either collect site-specific field data or use appropriate site-specific data (see below) to calculate the *EF*.

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

$$EF = \frac{C_{particulate}}{C_{water}} \qquad \textbf{(Equation 10)}$$

Where:

$C_{particulate}$ = Concentration of selenium in particulate material (µg/g dw)
C_{water} = Concentration of total dissolved selenium in water (µg/L)
EF = Enrichment Factor (L/g)

The State will determine site-specific *EFs* by a) deriving an *EF* value from field measurements at the site, or b) deriving an *EF* value from appropriately collected existing site data, if the conditions at the site have not changed greatly since the *EF* data was collected (e.g., no significant new inputs of selenium such as new mine expansion or petroleum refining facility). If conditions have greatly changed since the *EF* data was collected, the State will not use the existing data and will collect new field data to derive the *EF*. If the State uses existing data, it will follow the same temporal bounds determined in the data requirements of the USEPA 2021 selenium criterion (see Section 3.2.2.3 of the USEPA 2021) and will assure that at least eight paired data points are available for the site. For large sites, the State may use more than eight paired data points for the calculation, if eight will not sufficiently represent the variability at the site and additional paired data points are available. The EPA used sites with selenium measurements in particulate and water collected within 1 year of each other as inputs to the EPA's model 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 2021). However, site-specific *EF* values using particulate and water samples that are as spatially and temporally coincident as possible are considered the most robust. Therefore, to calculate the *EF* the State will use spatially and temporally coincident samples or will collect particulate and water samples at the same time and location; if coincident samples are not available or cannot be collected, the State will use existing data from that location if the data were collected within one year of each other. The State will use the most closely related spatially and temporally coincident water and particulate data to determine *EF* values, to ensure the data represents the same conditions for both the water and particulate samples.

Where the State decides to collect new data, at each site the State will decide which particulate material(s) is most appropriate to sample for the site and sample that material(s) using the methods listed below. If enough samples can be collected from algae and detritus (organic media), then sediment will not be sampled or used. The State may sample multiple media for particulate material and combine the *EFs*, if appropriate for the site. Consistent with the EPA's 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 2021). The *EF* for the site will not be determined using data from sediment samples alone.

5.4.1 Particulate Sampling

Using the methods below, the State will ensure that enough particulate material is collected to perform selenium analyses. The State will discuss with the analytical laboratory that will be performing the selenium analysis what amount of particulate matter is needed to conduct the selenium analysis. All samples will be labeled with site, date, material collected, and initials of the sampler.

5.4.1.1 Periphyton Sampling

When selected as a medium to sample, 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 where it is present. The proportion of periphyton collected from each substrate/habitat type will correspond to the relative abundance of each habitat type in the site.

To collect periphyton, the State 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 4.3.2 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 deionized water. 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 non-wadeable rivers and streams, 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.

5.4.1.2 Macroalgae Sampling

If the State is collecting macroalgae (filamentous algae), it 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.

5.4.1.3 Phytoplankton Sampling

Phytoplankton samples will be collected for large rivers and 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 0.5 m to 1 m depth range). Water samples will be prefiltered through 53 µm mesh and then phytoplankton will be collected on pre-weighed .65 µm polyvinylidene fluoride filters (Graves et al. 2021). Filters will then be folded into quarters with filtered biomass inside and placed in a plastic sampling bag. 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.

If large volumes of water need to be collected to get a sample with sufficient mass (as indicated by the lab processing the sample) for analysis or filtering of the water in the field is impractical, 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.

5.4.1.4 Sediment Sampling

The method in Section 4.3.4 of Moulton et al. 2002 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.

5.4.2 Water Sampling

The State will make the greatest effort to sample water concurrently with particulate samples or use water data that was collected concurrently with particulate data. However, if spatially and temporally coincident samples cannot be collected or are not available, the State will use water measurements for the calculation of an *EF* that were collected within one year of particulate material being collected. The State will use the most closely related spatially and temporally coincident water and particulate data to determine *EF* values, to ensure the data represents the same conditions for both the water and particulate samples.

Water samples will be collected using a peristaltic pump from mid-water column in wadeable streams. If water is being sampled from a lake or non-wadeable stream, 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 syringe 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 of less than 2. Samples will be transported on ice from the field to the lab and then stored at 4°C until processing.

5.4.3 Time of Year for Sampling

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

5.4.4 Location of Sampling and Number of Samples

The State will collect eight particulate samples within each site. Composite periphyton samples will be composed of periphyton material from all periphyton habitats found throughout the site. Other types of particulate material will be collected from randomly selected locations within the site where that particulate material is located. The State will also collect eight water samples.

If the water samples are being collected with periphyton samples, they will be collected from randomly selected locations throughout the site. Otherwise, water samples will be collected from the site where the particulate material is collected.

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. For large sites, the State may collect more samples, if eight will not sufficiently represent the variability at the site.

5.5 Chemical Analysis

The State will use an EPA or other published method for chemical analysis of total dissolved selenium in water samples. The State will measure selenium concentrations in particulate materials using methods described in Appendix L of USEPA 2021 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 particulate material concentrations as dry weight concentrations.

5.6 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 calculation. 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 5.3.

The *EF* value will be calculated using field collected data or appropriate existing site-specific data and **Equation 10.** 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 to represent the *EF* for that particulate material. The State will then calculate the geometric mean of the median ratios for each category of particulate material (e.g., algae, periphyton, etc.) as the site *EF* value. If enough measurements can be collected from other media, then sediment measurements will not be used to calculate the *EF*. If enough measurements cannot be collected from other media, the State will only use sediment measurements if the majority of the other measurements are from other organic particulate material (algae, periphyton, phytoplankton or detritus).

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

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

Current water concentration (total dissolved µg/L)	5.00
Current particulate concentration (mg/kg dw)	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 criterion element (mg/kg dw)	15.1

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

$$EF = \frac{4.25 \frac{\text{mg}}{\text{kg dw}}}{5.00\ \mu\text{g/L}}$$

$$= 0.85\ \text{L/ g}$$

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

$$= 1.03 \times 1.22$$

$$= 1.26$$

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

$$C_{water\ criterion\ element} = \frac{15.1 \frac{\text{mg}}{\text{kg}} \text{dw}}{1.26 \times 0.85 \frac{\text{L}}{\text{g}} \times 2.13}$$

$$= 6.62\ \mu\text{g /L total dissolved selenium}$$

Example 2
Fathead minnow (*Pimephales promelas*) that consume mostly copepods in a river:

Current water concentration (total dissolved µg/L)	5.00
Current particulate concentration (mg/kg dw)	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 criterion element (mg/kg dw)	15.1

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

$$EF = \frac{4.25 \frac{\text{mg}}{\text{kg}} \text{dw}}{5.00\ \mu\text{g/L}}$$

$$= 0.85\ \text{L/g}$$

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

$$= 1.57 \times 1.41$$

$$= 2.21$$

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

$$C_{water\ criterion\ element} = \frac{15.1 \frac{\text{mg}}{\text{kg}} \text{dw}}{2.21 \times 0.85\ \text{L/g} \times 1.40}$$

$$= 5.74\ \mu\text{g/L total dissolved selenium}$$

Example 3
Bluegill (*Lepomis macrochirus*) that consume mostly aquatic insects in a lake:

Current water concentration (total dissolved µg/L)	5.0
Current particulate concentration (mg/kg dw)	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 criterion element (mg/kg dw)	15.1

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

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

= 1.75 L/g

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

= 1.03 x 2.14

= 2.20

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

$$C_{water\ criterion\ element} = \frac{15.1\ \frac{\text{mg}}{\text{kg}}\text{dw}}{2.20 \times 1.75\ \text{L/g} \times 2.13}$$

= 1.84 µg/L total dissolved selenium

Example 4
Fathead minnow (*Pimephales promelas*) that consume approximately ⅔ copepods and ⅓ aquatic insects in a river:

Current water concentration (total dissolved µg/L)	5.0
Current particulate concentration (mg/kg dw)	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 $TTF^{TL2} = \sum_{i=1}^{n}\left(TTF_i \times w_i\right)$ $= (1.41 \times ⅔) + (2.14 \times ⅓)$ $= 1.65$	1.65
Egg-ovary to whole-body conversion factor for fathead minnow (*CF*)	1.40
Selenium egg-ovary criterion element (mg/kg dw)	15.1

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

$$EF = \frac{4.25\,\frac{\text{mg}}{\text{kg}}\,\text{dw}}{5.00\ \mu\text{g/L}}$$

= 0.85 L/g

$TTF^{composite} = TTF^{TL3} \times TTF^{TL2}$
$= 1.57 \times 1.65$
$= 2.59$

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

$$C_{water\ criterion\ element} = \frac{15.1\,\frac{\text{mg}}{\text{kg}}\,\text{dw}}{2.59 \times 0.85\ \text{L/g} \times 1.40}$$

= 4.90 µg/L total dissolved selenium

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

Current water concentration (total dissolved µg/L)	5.0
Current particulate concentration (mg/kg dw)	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 criterion element (mg/kg dw)	15.1

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

$$EF = \frac{4.25\,\frac{\text{mg}}{\text{kg}}\,\text{dw}}{5.00\ \mu\text{g/L}}$$

= 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

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

$$C_{water\ criterion\ element} = \frac{15.1\,\frac{\text{mg}}{\text{kg}}\,\text{dw}}{2.29 \times 0.85\ \text{L/g} \times 1.95}$$

= 3.98 µg/L total dissolved selenium

Example 6
Largemouth bass (*Micropterus salmoides*) that consume mostly Western mosquitofish (*Gambusia affinis*) that consume approximately ¾ insects and ¼ crustaceans in a large river:

Current water concentration (total dissolved µg/L)	5.0
Current particulate concentration (mg/kg dw)	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 criterion element (mg/kg dw)	15.1

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

$$EF = \frac{4.25\,\frac{\text{mg}}{\text{kg}}\,\text{dw}}{5.00\ \mu\text{g/L}}$$

= 0.85 L/g

$TTF^{composite} = TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2}$
= 1.39 × 1.21× 1.96
= 3.30

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

$$C_{water\ criterion\ element} = \frac{15.1\,\frac{\text{mg}}{\text{kg}}\,\text{dw}}{3.30 \times 0.85\ \text{L/g} \times 1.42}$$

= 3.79 µg/L total dissolved selenium

6.0 Bioaccumulation Factor Approach

6.1 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 in the genera *Acipenser, Lepomis, Salmo*, and *Oncorhynchus* (or all the fish species if the genera *Acipenser, Lepomis, Salmo,* and *Oncorhynchus* are not present at the site) for sampling that feed in areas with sediment and flow characteristics that will lead to the greatest selenium bioaccumulation potential. Therefore, 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.

6.2 Fish Tissue Type Selection

When the State is using 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 and practical, as egg concentrations have the strongest correlation to toxicity effects compared to all the tissue types. If egg samples are not available or impractical to collect, 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 and when the fish is large enough to easily sample eggs. The State will contact 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 contact local fish biologists to determine the spawning time period for the target fish species. They 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) to avoid collecting fish tissue that is depurated of selenium, since selenium is transferred to fish eggs during egg development. If a small asynchronous spawning species is being sampled where it is difficult to identify one specific spawning period and difficult to sample eggs, the State will collect whole-body samples of fish (with eggs, if present) and perform the BAF translation from the whole-body tissue criterion element.

6.3 Sampling Plan

6.3.1 Fish Tissue Sampling

The State will collect composite egg, whole-body or muscle samples. Those composite samples will be at least 20 g ww, unless impractical due to fish size or limited number of fish at a location. In those instances, the State will discuss with the analytical laboratory that will be performing the selenium analysis what mass of tissue is needed to conduct the selenium analysis and related QA/QC protocols and collect that mass. For the composites, the fish tissue 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 is no less the 75% of the total length of the largest individual. All samples will also be collected within a week of each other.

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. If a fish is showing signs of physical damage, mortality, or other sources of stress, the sign of stress will be documented, and no eggs will be collected from the fish. 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 pre-cleaned steel bowls 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, composited, and frozen (-20°C) for storage (if not analyzed immediately) and shipped for laboratory percent moisture and selenium analysis when appropriate (Janz and Muscatello 2008). All samples will be labeled with site, date, fish species sampled, material collected, and initials of the sampler. 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 and sacrificed using an overdose of tricaine methanesulphonate (MS-222). The length, weight, species, 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 will 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. All samples will be labeled with site, date, fish species sampled, material collected, and initials of the sampler. Once packaged, samples will be immediately placed on ice for transport back to the lab. In the lab, samples will be composited and frozen at -20°C (if not immediately analyzed) until selenium and percent moisture analysis. 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 and sacrificed using an overdose of MS-222. The length, weight, species, 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 will 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. All samples will be labeled with site, date, fish species sampled, material collected, and initials of the sampler. 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). Samples will then be composited and frozen at -20°C until selenium and percent moisture analysis. Samples will be held for no longer than 6 months.

6.3.2 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 eight bird egg samples for the target 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, date, species information, and initials of sampler 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 analysis (Evers 2009). Samples will be held for no longer than 6 months.

6.3.3 Water Sampling

The State will make the greatest effort to sample water concurrently with fish tissue and bird egg samples or use water data that were collected concurrently with tissue data. However, if temporally coincident samples are not available or cannot be collected, the State will use water measurements that were collected within one year of the tissue material collected. The State will use the most closely related temporally coincident water and tissue data to ensure the data represents the same conditions for both the water and tissue samples.

Water samples will be collected using a peristaltic pump from mid-water column in wadeable streams. If water is being sampled from a non-wadeable 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 syringe 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. All samples will be labeled with site, date, material collected, and initials of the sampler. Samples will be transported on ice from the field to the lab and then stored at 4°C until processing. Samples will be held for no longer than 6 months.

6.3.4 Time of Year for Sampling

The State will determine what time of year to collect fish samples based on which tissue type and fish species the State decides to sample. The State will contact local fish biologists to determine the spawning period for target species at the site. If the State is collecting fish egg samples, the State will collect them during the appropriate pre-spawn period for the target species, which will be when the eggs are mature. 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. Fish will also not be sampled during winter months. If the site has characteristics that will cause significant 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. The State will concurrently collect water samples. If concurrent samples cannot be collected or are otherwise not available, the State will use water samples collected within a year of fish and bird egg samples. The State will use the most closely related temporally coincident water and tissue data to ensure the data represents the same conditions for both the water and tissue samples.

6.3.5 Location of Sampling

Once the State has defined the site for the site-specific water column criterion element, the State will identify locations from within the site that correspond to the target fish and bird species feeding habitats, home ranges, and/or nesting areas. Locations within those areas will then be randomly selected for sampling fish tissue and bird eggs. Water samples paired with bird egg samples will be collected from random locations within the bird species aquatic feeding habitat. Water samples paired with fish tissue samples will be collected from the same location where the fish was sampled.

6.3.6 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 (one egg per nest after the clutch is complete) and eight water samples for the site (Hitt and Smith 2015, BCMOE 2014). If the State is not able to find sufficient fish to create eight composites of three fish, or that will result in negatively impacting the fish population at the site in question, then the State will either create eight composites of two fish or sample eight individual fish (if they are of sufficient size). For large sites, the State may collect more samples, if eight will not sufficiently represent the variability at the site.

6.4 Chemical Analysis

The State will use an EPA or other published method for chemical analysis of total dissolved selenium in water samples. The State will measure selenium concentrations in fish and bird tissue using methods described in Appendix L of USEPA 2021 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.

6.5 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 account for uncertainty and produce a defensible outcome. First, if the State collected data from more than one species of fish or bird, it will calculate the median BAF for each species using **Equation 3**. The State will select the species (one fish species and one bird species) with the highest BAFs for the calculation of the water column criterion element. Next, the State will use all paired water and fish samples or paired water and bird egg samples to calculate BAFs for the selected species using **Equation 3**. A BAF will be generated for each fish/water pair and bird/water pair. The State will then select the 80th percentile of the distribution of calculated BAFs to derive the water column criterion element,

using **Equation 4**, to ensure protection of sensitive and highly exposed species at the site. If the target species is a surrogate for a threatened or endangered species, or the water body includes the habitat of any threatened or endangered species, the State may select a higher percentile of the distribution of calculated BAFs to use in deriving the water column criterion element. The fish tissue criterion element used in **Equation 4** will be for 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 element for a water body impacted by selenium where bluegill samples were collected (USEPA 2021).

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

Solve for the BAF:

$$BAF = \frac{Site - specific\ egg\ Se\ concentration}{Ambient\ dissolved\ selenium\ water\ column\ concentration}$$

$$AF = \frac{22.0\ \frac{mg\ Se}{kg}}{4.0\ \frac{\mu g\ Se}{L}} = 5.5\ \frac{kg\ Se \cdot L}{kg}$$

Solve for site-specific water column criterion element:

$$Water\ column\ criterion\ element\ = \frac{Fish\ tissue\ criterion\ element}{BAF}$$

$$Water\ column\ criterion\ element\ = \frac{15.1\ \frac{mg\ Se}{kg}}{5.5\ \frac{kg\ Se\ \cdot L}{kg}} = 2.75\ \mu g\ Se/L$$

Water column criterion element = 2.75 µg/L total dissolved selenium.

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, including any impacts to threatened and endangered species in the downstream waters. 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 eggs at the downstream site.

Finally, the State may consider revising the site-specific water column criterion element if conditions at the site change (such as hydrodynamics) such that fish tissue or bird egg concentrations increase despite constant water concentrations.

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