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## TECHNICAL MEMORANDUM

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**To:** Sarah Armstrong and Guy Gilron (CCME Guideline Subcommittee)

**From:** David DeForest

**Subject:** Data Gaps Analysis for Translating a Fish Tissue-based Selenium Criterion to a Water-based Screening Criterion

**Date:** August 31, 2012

**cc:** Bill Adams and Ron Jones (NAMC-SWG Co-chairs)

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### 1 INTRODUCTION

Selenium (Se) toxicity to fish is primarily manifested via exposure of adult females to dietary organic Se and subsequent maternal transfer to the eggs, which, at sufficiently high concentrations, can result in larval deformities, edema, and mortality (Janz et al. 2010). Because the bioaccumulation potential of Se from the water column into the aquatic food web is highly dependent on site-specific biogeochemistry and food web characteristics, there is a wide range of water column Se concentrations across different site types that could result in a given Se concentration in fish tissue. Accordingly, there is a general consensus that fish tissue Se concentrations, especially in fish eggs, are most appropriate for evaluating whether Se concentrations in an aquatic system are posing risks to fish. Recently, DeForest et al. (2012) developed a proposed guideline for Se concentrations in fish eggs following the Canadian Council of Ministers of the Environment (CCME) protocol for developing guideline values. Although a fish tissue-based Se guideline is much more broadly applicable among sites than a water-based Se guideline, there is typically still a need to translate the fish tissue-based Se guideline to a site-specific water-based Se guideline because water Se concentrations can be more readily monitored and Se sources can be mitigated to achieve target Se concentrations in water. The objective of this evaluation is to review approaches for translating a fish tissue Se

concentration to a water Se concentration and to highlight key data gaps associated with the translations; if filled, the added information could reduce uncertainty in the available approaches.

## 2 SELENIUM BIOACCUMULATION MODELS

The issue of relating Se concentrations in fish tissue or aquatic bird eggs to water Se concentrations has been addressed in several publications over the last 20 years. Most of the Se bioaccumulation models are partitioning models that relate water Se concentrations to fish or bird tissue Se concentrations via multiple food chain steps (Skorupa and Ohlendorf 1991; Ohlendorf and Santolo 1994; Presser and Luoma 2010) or a single step (Adams et al. 1998; Brix et al. 2005; Toll et al. 2005). The multi-step models account for partitioning of Se from water to one or more food chain components and then into fish tissue or bird eggs, while the one-step models directly relate water Se concentrations to co-located fish tissue or bird egg Se concentrations. Regardless of the approach used, the key variable is the Se enrichment factor (EF), which relates Se concentrations at the base of the food web (e.g., detritus, algae) to water Se. The EF can be an explicit component of a multi-step model and is an implicit component of a single step model. The Se EF varies depending on site-specific physical-chemical parameters, which influence Se speciation and bioavailability, and site-specific biology, as Se bioaccumulation potential varies among different particulates at the base of the food chain. In general, Se bioaccumulation potential is higher in biologically productive systems that favor the reduction of selenate to selenite or organo-Se compounds. The one-step and multi-step Se partitioning models, and their relationship to each other, are shown mathematically as follows:

One-step partitioning approach

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$$C_{\text{fish}} = C_{\text{water}} \times \text{BAF}_{\text{fish}} \quad \text{Eqn. 1}$$

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$$C_{\text{fish}} = C_{\text{water}} \times K_d \times \text{TTF}_{\text{invert}} \times \text{TTF}_{\text{fish}} \quad \text{Eqn. 2}$$

↑

Multi-step partitioning approach

In general, Se EFs tend to be greater in lentic systems than in lotic systems, although the EF distributions for lentic and lotic sites substantially overlap and the EFs may still vary by greater than one order of magnitude within lentic and lotic systems. In terms of selecting an EF value that would result in a water Se guideline that is broadly protective of most, if not all sites,

selection of conservative EFs can result in largely over-protective water Se guidelines where Se is naturally elevated or where Se bioaccumulation potential is low. The benefit of the fish tissue-based Se guideline is to accommodate this site-to-site variability in Se bioaccumulation potential, but adoption of an unnecessarily low water Se-based guideline would result in an allocation of resources to conduct fish tissue sampling programs that are not necessary. Accordingly, it would be useful if the water-based Se guideline could be developed such that it accounts for site-specific factors that may modify the Se EF. The following summarizes Se partitioning through aquatic food webs and highlights key uncertainties and data gaps.

### 3 DATA GAPS ANALYSIS

For the purposes of this analysis, five primary sources of uncertainty and variability associated with the partitioning of Se from the water column to fish eggs were identified. These are identified in Figure 1, which provides a generalized schematic diagram of Se partitioning in an aquatic food web. The following data gaps analysis is organized by the five primary sources of uncertainty and variability, as outlined in Figure 1 (i.e., Sections 3.1 to 3.5).

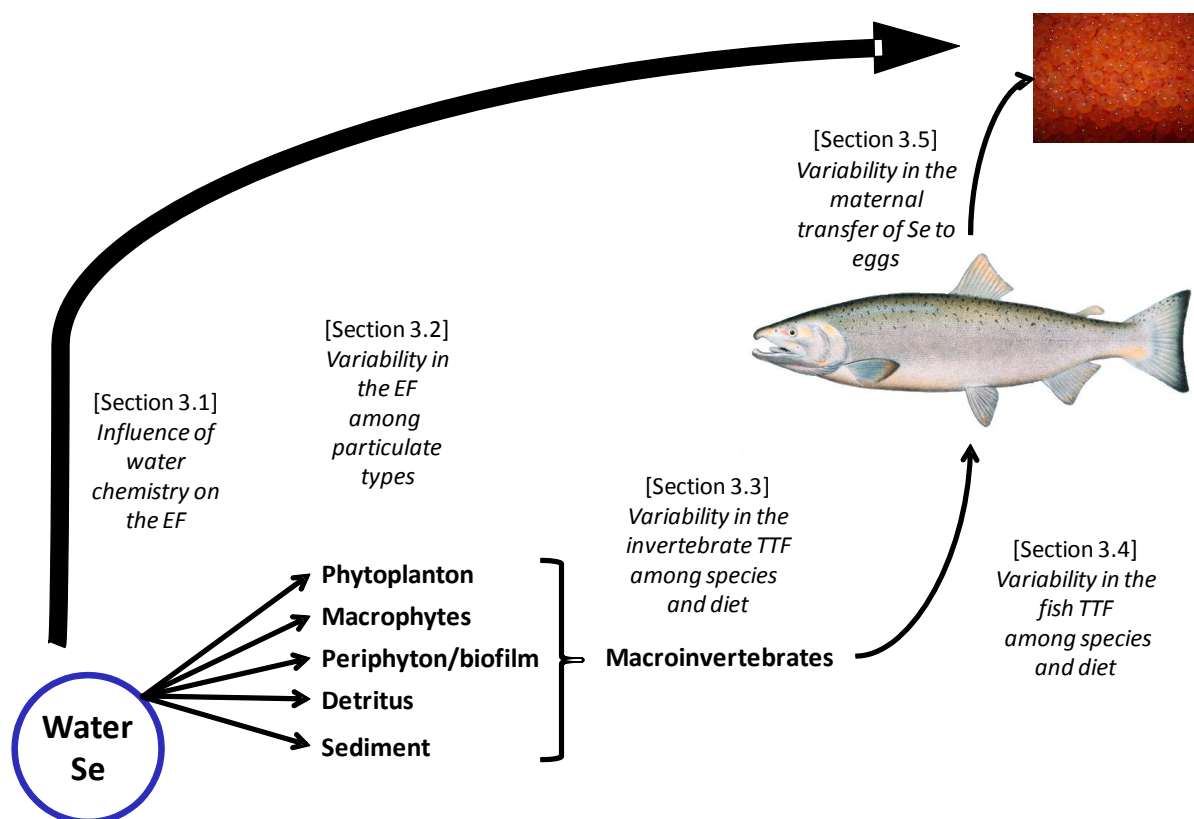


Figure 1. Schematic diagram of selenium bioaccumulation from water to fish eggs.

### **3.1 Influence of Water Chemistry on the Enrichment Factor**

There are at least three primary components of water chemistry that influence the magnitude of the EF for Se: (1) Se speciation; (2) modifying factors that influence Se uptake; and (3) the magnitude of the Se concentration in the water.

#### **3.1.1 Selenium Speciation**

In the water column, Se predominantly occurs as selenate, selenite, or as reduced organic Se compounds. In general, selenate and selenite tend to be predominant, with the relative proportion of selenate tending to be greater in well oxygenated waters, such as most streams, and the relative proportion of selenite tending to be greater in waters with reducing conditions, such as ponds and wetlands. Smaller proportions of organic Se may also occur in the water column. Selenium speciation in the water column has an important influence on the bioaccumulation potential of Se, as demonstrated by Besser et al. (1993), who exposed the alga *Chlamydomonas reinhardtii* to selenate, selenite, or selenomethionine. The EFs for each Se form clearly showed the following pattern of Se bioaccumulation potential: selenate < selenite < selenomethionine (Figure 2). Several other studies also have shown greater bioaccumulation potential of selenite compared to selenate. Riedel et al. (1996) found that the short-term uptake rate of selenite in a natural plankton community was 4-5 times faster than for selenate. Similarly, Riedel and Cole (1999) exposed periphyton to 10 µg Se/L as either selenate or selenite and observed that the selenite uptake rate was substantially greater (0.0112 L/g-hr for selenite versus 0.0045 L/g-hr for selenate). Kiffney and Knight (1990) and Malchow et al. (1995) similarly showed that the bioaccumulation potential of selenite was greater than selenate in a cyanobacterium and a green alga, respectively.

To-date, the relative bioaccumulation potential of selenate, selenite, and organic Se compounds has not been extensively studied for a wide variety of algae species or for other “particulates”, such as detritus, periphyton, biofilms, and macrophytes. A broader understanding of Se bioaccumulation potential among Se species, coupled with site-specific Se speciation data, may help to narrow the range of potentially relevant Se EFs for a given site.

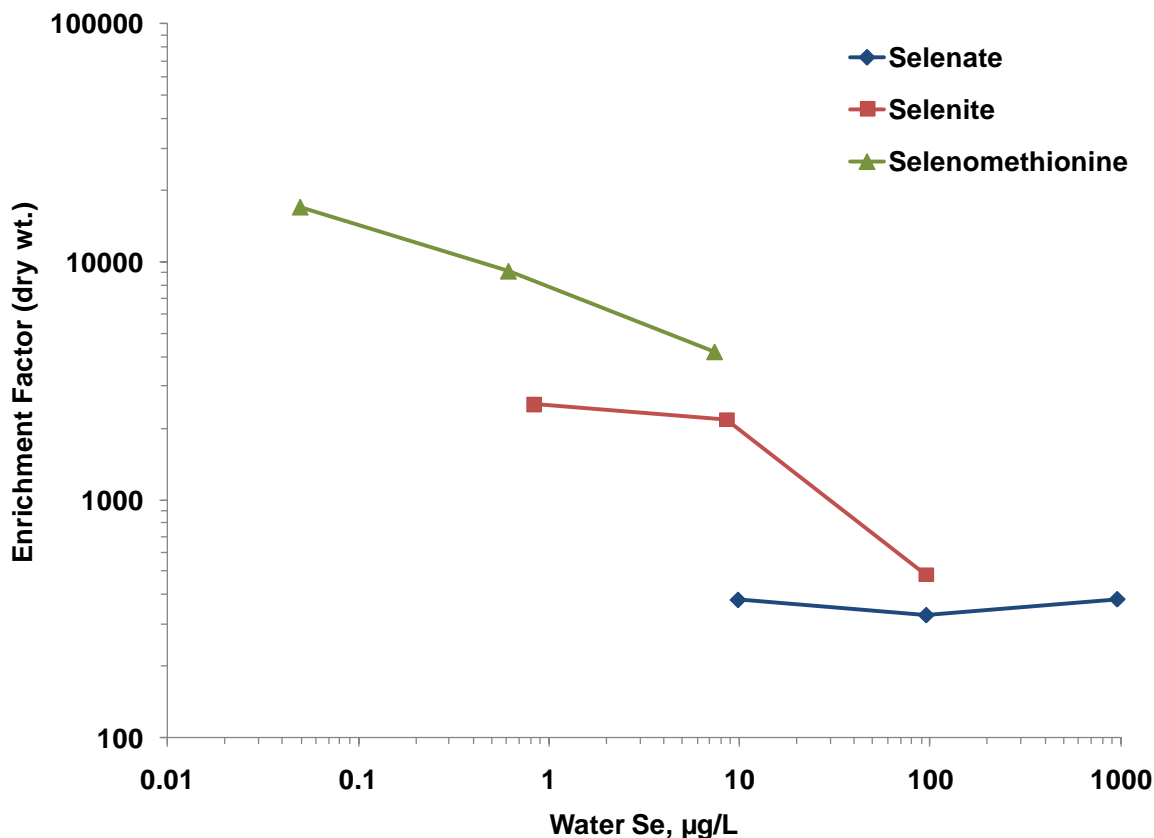


Figure 2. Selenium enrichment factors (EFs) for the freshwater alga *Chlamydomonas reinhardtii* exposed to selenate, selenite, or selenomethionine in the laboratory. Date from Besser et al. (1993).

### 3.1.2 Influence of Modifying Factors

There are potentially several modifying factors that may influence the bioavailability and bioaccumulation potential of Se, which in turn would influence the EF. The most well known modifying factor is the influence of sulphate ( $\text{SO}_4$ ) on selenate uptake. This was most clearly shown by Williams et al. (1994), who observed that Se bioaccumulation in green algae exposed to a water Se concentration of 11.3 µg/L was approximately four-fold greater when the water contained 3.3 mg  $\text{SO}_4$ /L compared to 33 mg  $\text{SO}_4$ /L (Figure 3). The influence of  $\text{SO}_4$  was even more pronounced when algae were exposed to a higher, but less environmentally relevant, Se concentration of 107 µg/L (Figure 3). Riedel and Sanders (1996) likewise observed a reduction in the uptake rate of selenate with increasing sulphate concentrations. For example, in the green alga *Chlamydomonas reinhardtii* exposed to 10 µg selenate/L, the Se uptake rate decreased by approximately 55% as the sulphate concentration increased from approximately 4.8 to 96 mg/L.

As opposed to selenate, Riedel and Sanders (1996) did not observe any influence of sulphate on the uptake rate of selenite, although they did observe that increasing phosphate concentrations substantially reduced the selenite uptake rate. Yu and Wang (2004a) similarly observed that increasing phosphate concentrations reduced selenite uptake by the alga *Scenedesmus obliquus*. Yu and Wang (2004b) exposed *C. reinhardtii* to a selenite concentration of 2 µg/L and a corresponding phosphate concentration of 3, 30, or 300 µg/L or a corresponding nitrate concentration of 70, 560, or 2800 µg/L. These phosphate and nitrate concentrations were intended to mimic different nutrient conditions in natural oligotrophic, mesotrophic, and hypertrophic lakes. Selenite uptake significantly decreased as the phosphate concentration increased from 3 to 300 µg/L, while nitrate had no clear influence on selenite uptake. Sulphate and selenate appear to compete for uptake because both are group VI oxyanions of the form  $XO_4$  (Brix et al. 2001).

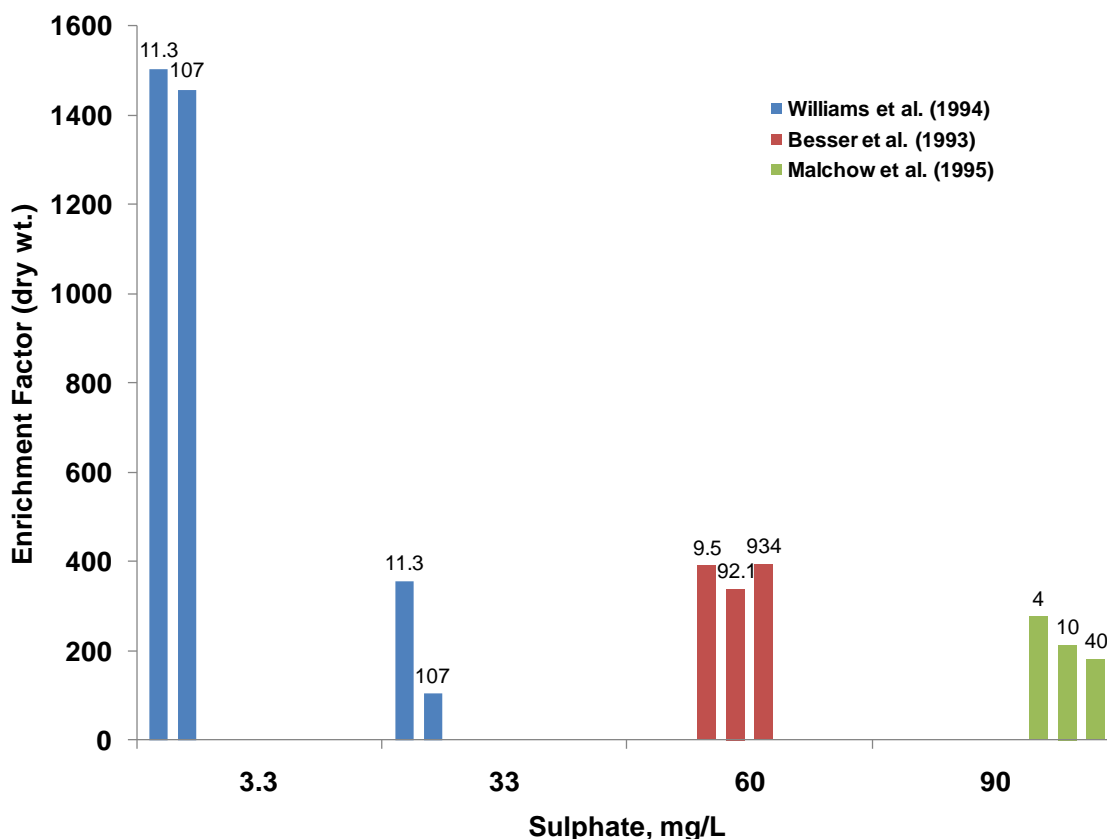


Figure 3. The influence of sulphate ( $SO_4$ ) on enrichment factors (EFs) for selenate ( $SeO_4$ ) in the algae *Selenastrum capricornutum* (Williams et al. 1994; Malchow et al. 1995) and *Chlamydomonas reinhardtii* (Besser et al. 1993). Values above bars are the selenate exposure concentrations in µg/L.

### **3.1.3 *Influence of Selenium Exposure Concentrations***

The magnitude of the Se concentration in water also influences the magnitude of the EF, as EFs tend to be inversely related to the Se concentration in water (i.e., higher EFs are often observed at lower water Se concentrations, in both laboratory and field exposures). In the laboratory, the inverse relationship between the EF and exposure concentration is usually, but not always observed. The data from Williams et al. (1994) and Malchow et al. (1995) demonstrate an inverse relationship (Figure 3), as do the data from Besser et al. (1993) for selenite and selenomethionine, but not selenate (Figure 2). Conley et al. (2009) observed increasing Se concentrations in a complex periphyton community when exposed to increasing selenite concentrations for nine days. In this experiment, periphyton were exposed to an initial water Se concentration that was not renewed over the exposure period. Because the waterborne Se was rapidly depleted in the low Se treatments, there was less Se available for uptake by the periphyton. Using a similar exposure method, Conley et al. (2011) reported that the Se EF was lowest in the highest selenite concentrations tested (19.2 to 23.1  $\mu\text{g/L}$ ), but the relative magnitudes of the EFs for the low Se (1.1 to 3.4  $\mu\text{g/L}$ ) and moderate Se (5.9 to 8.9  $\mu\text{g/L}$ ) were variable.

The inverse relationships among the EFs and water Se concentrations often observed emphasizes that constant EFs cannot be assumed in Se partitioning models, even in waters that otherwise have the same water chemistry and conditions.

### **3.2 *Particulate-specific Variability in Selenium Enrichment Factors***

In addition to the various factors that can influence Se EFs for a given particulate (e.g., Se speciation, modifying factors, water Se concentration), the Se EF may also vary substantially among particulates collected from the same site. Many field data sets demonstrate this variability in Se EFs among particulate types. A good example is Saiki et al. (1993), who measured Se concentrations in water and three different particulates (sediment, detritus, and filamentous algae) collected from the San Joaquin River, Salt Slough, and Mud Slough (California) in spring and fall. Selenium concentrations in detritus were approximately 10- to 165-fold greater than in sediment (Figure 4). This demonstrates the importance of not only understanding how Se concentrations vary among particulate types at a site, but also understanding the site-specific food web. In this study the authors noted that the food web was detritus-based. If this was not known or considered, and if it was assumed that the Se EF for sediment was a representative surrogate, then obviously the Se enrichment potential based on

measurement of Se in sediment alone would have resulted in underestimating the Se bioaccumulation potential in this study area.

The data from Saiki et al. (1993) also demonstrate the potential seasonal variability in Se EFs. This variability is most important where water Se concentrations vary seasonally, which is a common occurrence in areas where Se tends to be mobilized by seasonal weather or irrigation events. In Saiki et al. (1993), seasonal EFs varied by a factor of approximately 3 to 10 for the three water bodies. Time-varying Se concentrations and the kinetics of partitioning into the aquatic food web are important issues for relating Se concentrations in fish tissue back to water. Understanding the site-specific food web, as well as seasonal variability in the EFs, is therefore critical for identifying representative EFs for a site.

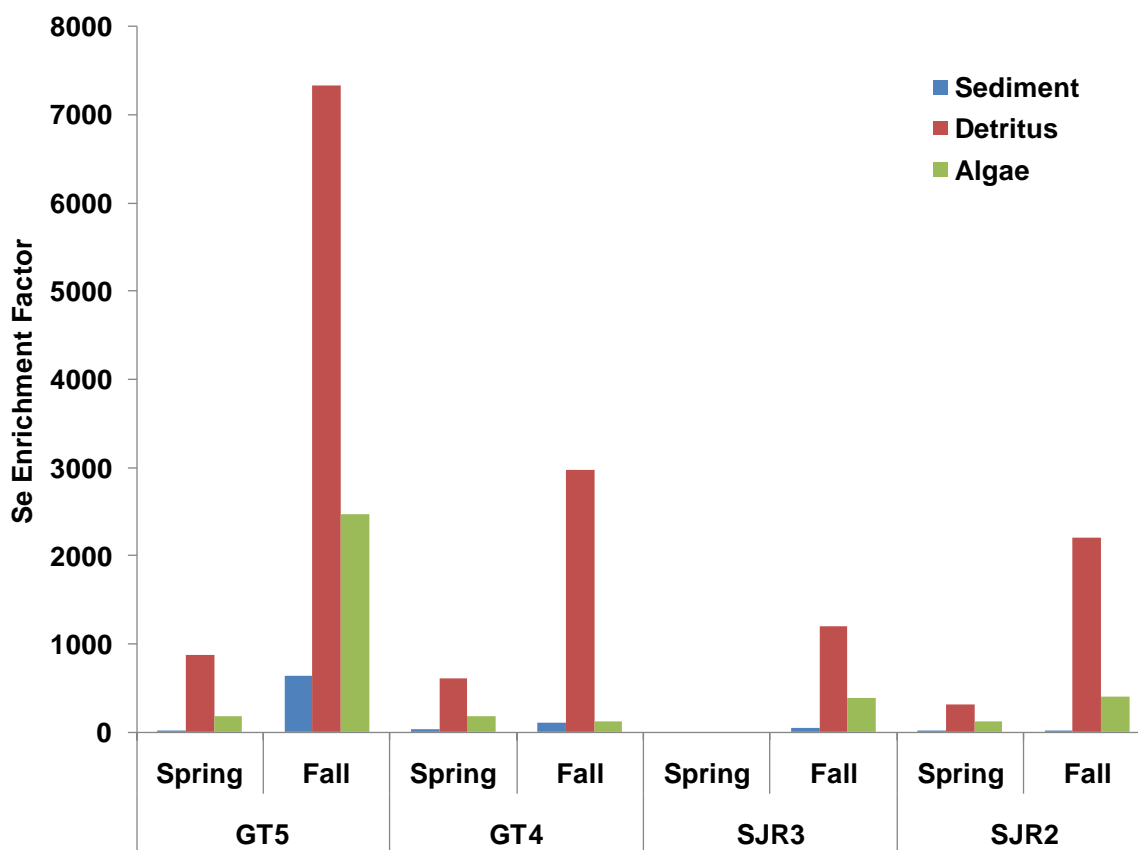


Figure 4. Comparison of Se enrichment factors (EFs) for different particulate types. GT5 = Mud Slough; GT4 = Salt Slough; SJR = San Joaquin River. Data from Saiki et al. (1993).

### 3.3 Variability in Invertebrate Selenium Trophic Transfer Factors

Whereas Se EFs may range over two to three orders of magnitude, or more, Se TTFs tend to vary between approximately 0.5 and 2.5, although lower and higher TTFs may be observed in



some cases. Invertebrate Se TTFs were compiled from laboratory trophic transfer studies and from field studies with co-located measurements of Se in invertebrates and their diets (Figures 5 and 6). Invertebrate Se TTFs were compiled from a range of invertebrate taxa, including amphipods, cladocerans, crayfish, and insects (caddisflies, chironomids, crane flies, damselflies, mayflies, stoneflies, water boatmen). The median invertebrate Se TTF was 1.2, with 10<sup>th</sup> and 90<sup>th</sup> percentile TTFs of 0.5 and 2.4, respectively (Figure 5). There are not any clear patterns in how TTFs vary among taxa, as insect taxa, for example, are distributed fairly evenly throughout the dataset. One factor not reflected in Figure 5 is the influence of the dietary exposure concentration on the TTF, which may be inversely related (just like the inverse relationship often observed among EFs and water Se concentrations). The TTFs that can be derived from laboratory tests are usually inversely related to the dietary exposure concentration, although this slope is not always statistically significant ( $p < 0.05$ ). An interesting result from the Conley et al. (2011) study is the influence of the dietary dose on the TTF, who observed that TTFs in the mayfly were approximately 60% less in mayflies that were provided twice as much periphyton as a food source. The authors attributed this to reduced Se concentrations in mayflies due to growth dilution in mayflies provided more food.

Even though the magnitude and variability in Se TTFs for invertebrates is much less than that observed for Se EFs, the invertebrate TTF still is an important factor when relating a fish tissue-based Se guideline back to a water Se concentration. In a multi-step model, a factor of two difference in the TTF results in a factor of two difference in the water Se concentration (e.g., a difference between a guideline of 5  $\mu\text{g}/\text{L}$  versus 10  $\mu\text{g}/\text{L}$ , which can be important in managing Se).

### **3.4 Variability in Fish Selenium Trophic Transfer Factors**

Overall, Se TTFs for whole body fish tissue tend to be lower for fish than for invertebrates, and less variable among species. Whole body fish Se TTFs are almost always less than 2.0, with the median and 90<sup>th</sup> percentile TTFs from laboratory studies being 0.7 and 1.5, respectively (Figure 7). The two highest whole body fish TTFs of 4.4 and 5.4 are from low fish provided low Se diets containing 0.4 and 0.23  $\mu\text{g Se}/\text{g dry wt.}$ , respectively. The high TTFs reflect the essential nature of Se, with fish actively regulating their internal Se concentrations. The whole body Se TTF for fish represents a relatively minor uncertainty. Presser and Luoma (2010), for example, recommended assuming a whole body fish TTF of 1.1 across species. A

greater source of variability is the Se TTF in fish eggs or ovaries, as discussed in the following section.

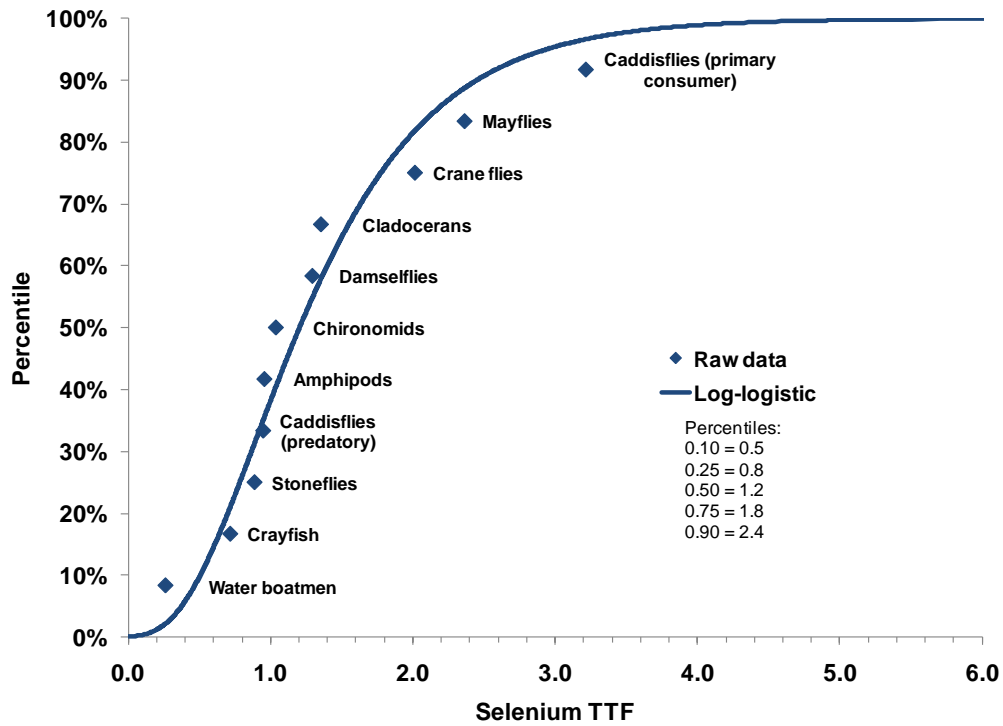


Figure 5. Cumulative distribution of Se trophic transfer factors (TTFs) for invertebrates. Data from Besser et al. (1989; 1993), Birkner (1978), Casey (2005), Conley et al. (2009; 2011), Guan and Wang (2004), Malchow et al. (1995), Saiki et al. (1993), and Thomas et al. (1999).

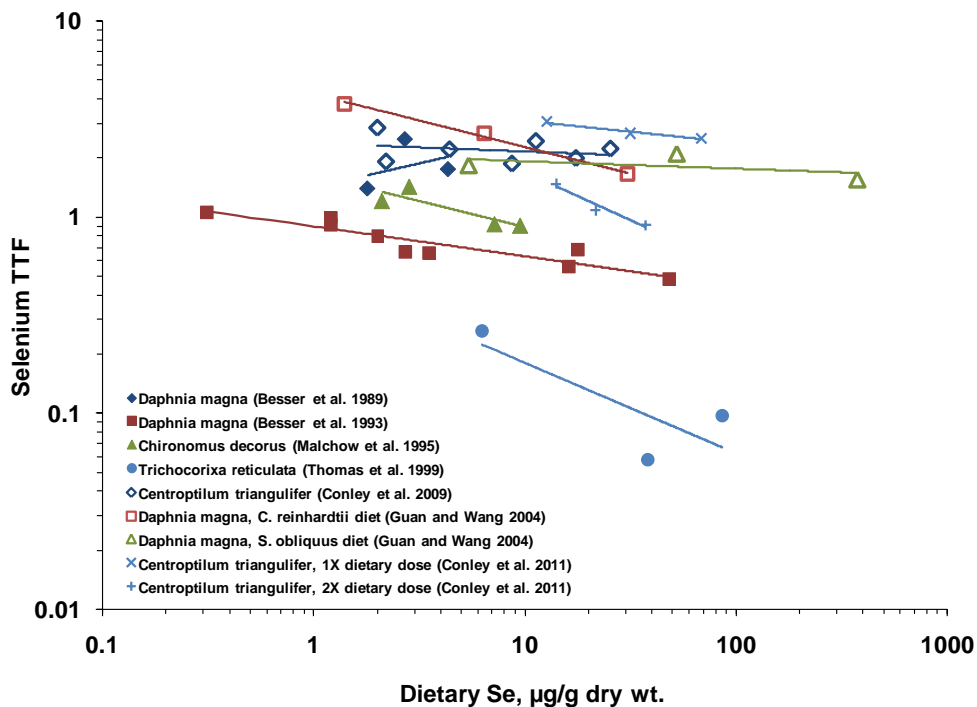


Figure 6. Relationship among Se trophic transfer factors (TTFs) for invertebrates versus the dietary Se concentration. Inverse relationships among TTFs and dietary concentrations are observed for most species, but slope is significant only for the *D. magna* data from Besser et al. (1993).

### 3.5 Fish Species-specific Maternal Transfer of Selenium to the Eggs

Because the Se concentration in fish eggs or ovaries is considered the most reliable indicator of potential Se toxicity to fish, there is a need to understand TTFs based on these tissues, or how relationships between whole body Se and egg or ovary Se concentrations vary among species. However, many fewer egg or ovary Se data are available for developing egg or ovary-based Se TTFs than whole body-based Se TTFs. From laboratory studies, ratios between egg or ovary Se and whole body Se can be determined for bluegill (Coyle et al. 1993; Hermanutz et al. 1996), cutthroat trout (Hardy et al. 2010), and fathead minnow (Ogle and Knight 1989), with the means from each study ranging from approximately 1.3 to 2.4 (with an overall mean of 2.0) (Figure 8). This would indicate that, on average, the whole body Se concentration will be approximately one-half of the egg-based Se criterion. However this ratio does vary by species, so any assumptions among species are tenuous. Diet-to-egg and diet-to-ovary Se TTFs can also be derived from some of the same laboratory studies. Mean egg- or ovary-based Se TTFs range from 0.7 for the fathead minnow to 1.5 for bluegill, with an overall mean of 1.1 (Figure 9). Note

that a fathead minnow TTF of 15.2 is not included; this very high TTF is based on the control organisms and is driven by the very low dietary Se concentration of 0.4  $\mu\text{g/g}$  dry wt. As discussed above for whole body fish (and invertebrates), there is an overall inverse relationship between the egg or ovary Se TTFs and dietary Se concentrations (Figure 10).

Orr et al. (2012) measured and modeled Se concentrations in the ovaries of westslope cutthroat trout in lentic and lotic water bodies. In lentic water bodies, ovary Se concentrations increased linearly with increasing benthic invertebrate Se concentrations. Based on the linear regression relationship, the diet-to-ovary Se TTFs range from approximately 1.7 to 2.8 over the range of the benthic invertebrate Se concentrations measured, with an inverse relationship observed. The TTF was 2.0 at an egg Se concentration of 17  $\mu\text{g/g}$  dry wt. No relationship between ovary Se and dietary Se was observed in lotic systems (i.e., ovary Se concentrations did not increase with increasing dietary Se concentrations), meaning that the TTF is strongly inversely related to the dietary Se concentration (the mean TTF was 2.5).

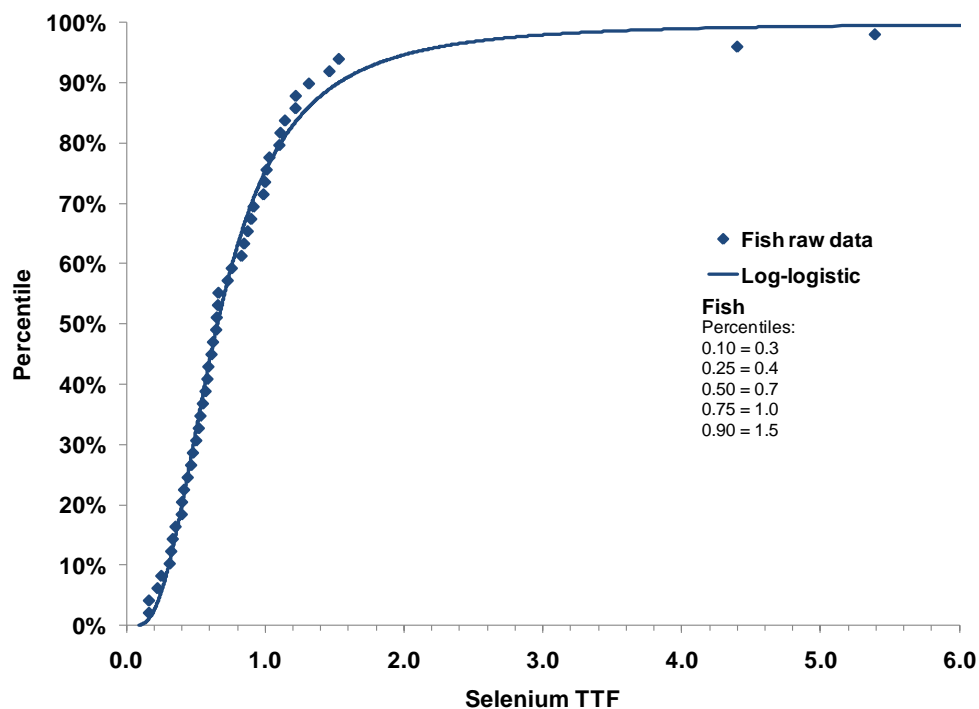


Figure 7. Cumulative distribution of laboratory-based Se trophic transfer factors (TTFs) for whole body fish. Data from Bennett et al. (1986), Bertram and Brooks (1986), Cleveland et al. (1993), Coyle et al. (1993), Hardy et al. (2010), Hamilton et al. (1990), Lemly (1993), Ogle and Knight (1989), and Vidal et al. (2005).

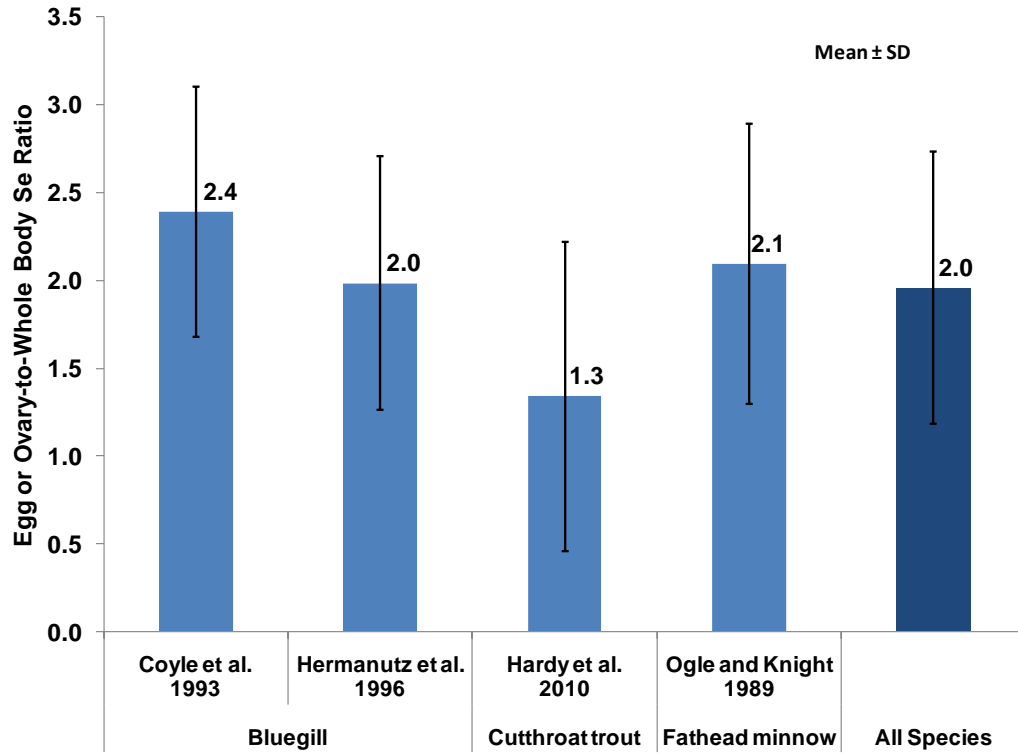


Figure 8. Mean ratios of selenium concentrations in fish eggs or ovaries to whole body selenium.

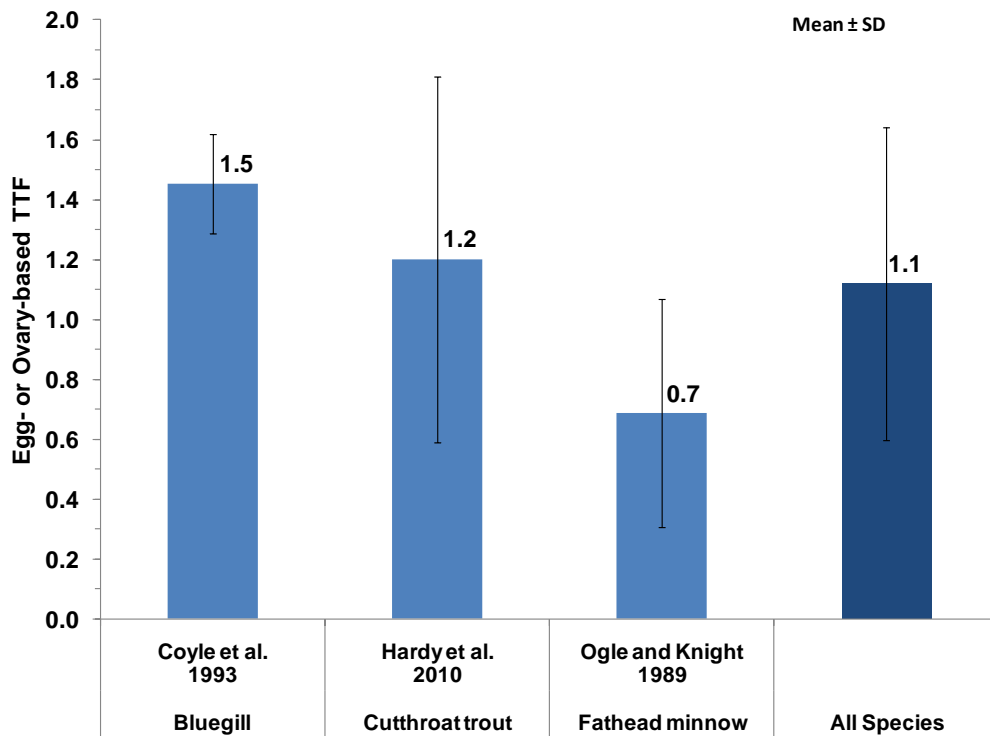


Figure 9. Mean egg- or ovary-based Se TTFs for fish.

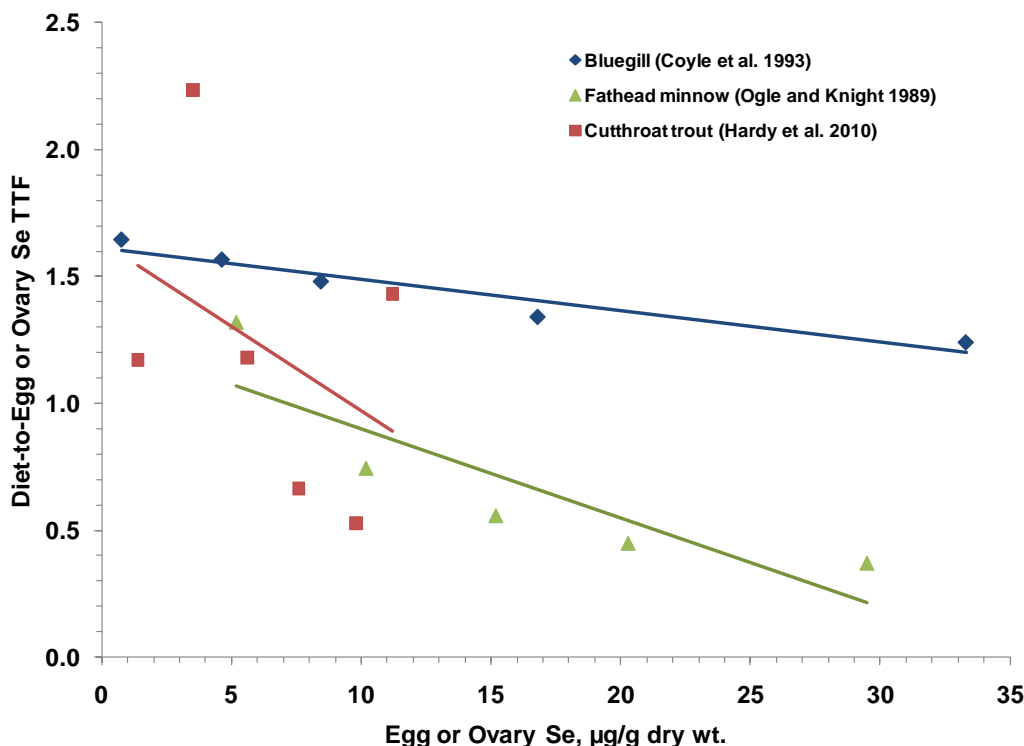


Figure 10. Relationship between egg- or ovary-based Se TTFs for fish and dietary selenium concentrations.

#### 4 SUMMARY AND CONCLUSIONS

In a simplified food chain there are four primary steps for the transfer of Se from the water column to fish eggs/ovaries: water → particulate → invertebrate → fish → fish eggs/ovaries. In nature, food webs obviously vary in their complexity, where the base of the food web will consist of multiple particulates and there may be multiple steps within each discrete food chain step (e.g., predatory insects feeding on primary consumer insects). Because it will not likely be possible to know or fully understand Se transfer within the food web in the foreseeable future, it will generally be necessary to develop TTFs, for example, at a broad scale (e.g., use of composite benthic invertebrate samples to describe the link between particulate Se and fish Se). As noted above, in addition to modeling Se bioaccumulation in fish using a multi-step model, Se bioaccumulation can be evaluated using a one-step model that describes the relationship between water Se and fish Se. Both models have their utilities, as multi-step models help to better understand the process of Se bioaccumulation in a food web, while a one-step model integrates some of the uncertainties in each step of the multi-step model (rather than

compounding them). The following highlights key uncertainties in the bioaccumulation and trophic transfer of Se from water to fish eggs and ovaries.

1. The influence of Se speciation, modifying factors, and exposure concentration on the EFs for a variety of relevant particulate types has not been well studied. Most studies have focused on unicellular algae – how do the EFs vary for particulates such as detritus, periphyton, biofilm, and macrophytes?
2. Can we develop more refined categories of generic water-based Se guidelines based on key water chemistry and food chain characteristics?
3. Linking time-varying water Se concentrations to Se concentrations in fish ovaries and eggs – what are the appropriate EFs and TTFs (multi-step model) or BAFs (one-step model)?
4. Selenium TTF data for eggs and whole body tissue in a broader range of fish species are needed.
5. Methods are needed to develop a site-specific Se guideline for water. It is assumed that fish eggs would initially be sampled at a site to determine compliance with an egg-based Se guideline (presumably after a water-based Se screening threshold is exceeded). If the egg-based Se guideline is exceeded, how do you develop a water-based Se guideline? Options exist, such as the Bayesian one-step model approach described in Brix et al. (2005) and the multi-step model approach described in Presser and Luoma (2010).

The studies being conducted by Nautilus Environmental, Natural Resources Canada (NRCan), and Golder Associates will help to address some of the above questions and data gaps highlighted above. Nautilus Environmental is testing the influence of sulphate on Se uptake (i.e., the EF) by a diatom, which will help us to better understand Se uptake in an additional particulate with limited data (i.e., diatoms) and how varying sulphate concentrations influences the uptake of varying Se concentrations. NRCan is using field-collected Se-enriched sediments to evaluate the uptake, trophic transfer, and toxicity of Se to the benthic insect *Chironomus dilutus*. This will provide useful information on Se fate and effects at the base of the food chain. Finally, Golder Associates will use existing data to develop a Se bioaccumulation model for use in the derivation of water quality guidelines for Se. This model will consider variation in Se bioaccumulation among sites (e.g., lotic vs. lentic), between species, and within species. The resulting model may be compared to other Se bioaccumulation modeling approaches, such as

(Toll et al. 2005) and (Presser and Luoma 2010). The ultimate goal is that the information from the above three studies will contribute to a framework for relating a tissue-based Se guideline to a appropriate site-specific water quality guidelines for Se.

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