*Final Guide*

# Approach for Conducting Site-specific Assessments of Selenium Bioaccumulation in Aquatic Systems

Prepared for

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**FINAL GUIDE**

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## Executive Summary

The goal of this document is to provide a standard guide for field and laboratory assessments of selenium bioaccumulation that can be applied in different environmental settings relative to developing and interpreting a tissue-based selenium value. It describes a recommended tiered approach (or framework) for conducting assessments and includes discussion of decision trees, conceptual models and data quality objectives toward defining what should be done, and describes recommended sampling and monitoring for conducting the assessment. It also presents several case studies that illustrate the approaches used for site-specific assessments under a variety of conditions, including both large and small "sites" as well as consideration of various assessment endpoints.

This guide focuses on aquatic-dependent birds as well as aquatic organisms (primarily fish). It integrates some of the components of selenium assessment for aquatic systems that are presented in more detail in other guides that focus on selenium analyses (instrumental approaches and sample considerations) and on tissue endpoint assessment (appropriate tissue types, appropriate endpoints/life stages/sampling protocols/etc. for measuring effects, and association of tissue thresholds with population/community effects in the field).

Extensive data have been developed for both fish and bird species, and there is an emerging trend that there is a clear egg-selenium threshold at which effects begin to be observed. Maternal bioaccumulation and subsequent transfer of selenium to eggs may be the most important factor in understanding selenium toxicity. Given that there is a clear egg-selenium threshold at which effects begin to be observed, a unimodal model, such as logistic regression, may result in exaggerated confidence intervals, particularly in the tails.

Relationships between tissue selenium concentrations in fish and invertebrates and aqueous concentrations vary depending on site-specific conditions. In a flow-through treatment wetland for refinery effluent (Richmond Refinery Water Enhancement Wetland, California), selenium concentrations in invertebrates were positively related to variability in waterborne selenium concentrations with a 2-week time lag. In contrast, emerging data for selenium in fish tissues and aqueous selenium concentrations from stream systems in southeast Idaho are showing strong relationships between co-located water and tissue samples. Developing the association between tissue and aqueous selenium will be important for all sites where selenium is thought to be a potential concern.

While most State standards are based on a waterborne concentration, the state-of-the-science indicates that effects are more strongly related to tissue residues than water concentrations. Current U.S. Environmental Protection Agency (USEPA) guidance recommends that the

chronic criterion for selenium be evaluated by whole-body fish tissue concentrations. More recent information concerning the revision of the draft criterion suggests USEPA is leaning toward the use of a two-part criterion (first tier, a mature ovary measurement, second tier whole body). The mature ovaries are the target organ and measurements show less variability than whole body.

Because data for fish ovaries have not been developed from many sites, conversion factors will need to be developed that allow translation from whole-body tissue residues to ovary tissue residue for a species. Where work is contemplated or currently underway, it is advisable to develop the conversion factors for the site. For these reasons, it will become ever more important to develop the relationship between waterborne concentrations and fish tissue concentrations in order to translate between a tissue-based effects criterion and water. USEPA is currently working on implementation procedures to facilitate this process, but the currently held wisdom is that these relationships will be best developed on a site-specific basis.

This guide provides a general summary of relevant information concerning tissue thresholds/guidelines; more detailed information is presented in the Tissue Threshold Workgroup effort conducted concurrently with this guide. It also provides a general summary of appropriate analytical methods for doing reliable analyses of selenium in environmental media (water, sediment, etc.) and tissues, which are an essential component of conducting site-specific assessments of selenium bioaccumulation. More detailed information concerning analytical methods is presented in the Selenium Analysis Workgroup effort conducted concurrently with this guide.

Overall, we conclude that the approach for site-specific assessment must be flexible, depending on what is appropriate for the situation. It may be assumed that current suggested draft selenium criteria are conservative and that site-specific studies with a risk assessment approach will be needed to set realistically protective site-specific values. Risk management/remediation decisions should be based on integration of biology and chemistry data.

The guide recommends using a tiered approach because that provides the most resource-effective framework for conducting assessment of selenium in aquatic systems. The following are key components of the tiered approach:

- Comparison of tissue concentrations to tissue residue guidelines or adopted water quality criteria/guidelines;
- Reproductive toxicity testing; and
- Assessment of fish populations in the area of interest.

This approach is consistent with that used for conducting ecological risk assessments, and the ecological risk assessment paradigm is useful toward the goal of assessing exposure and effects of selenium. Using this approach, along with decision trees, conceptual models and data quality objectives (described in a later section), helps ensure that all the important work is done, but only that work that contributes to the decision-making process.

# Introduction

The goal of this document is to provide a standard guide for field and laboratory assessments of selenium bioaccumulation that can be applied in different environmental settings relative to developing and interpreting a tissue-based selenium value. It describes a recommended tiered approach (or framework) for conducting assessments and includes discussion of decision trees, conceptual models and data quality objectives toward defining what should be done, and describes recommended sampling and monitoring for conducting the assessment. It also presents several case studies that illustrate the approaches used for site-specific assessments under a variety of conditions, including both large and small "sites" as well as consideration of various assessment endpoints (see Attachment).

The focus of this guide includes aquatic-dependent birds as well as aquatic organisms (primarily fish). It integrates some of the components of selenium assessment for aquatic systems that are presented in more detail in other guides that focus on selenium analyses (instrumental approaches and sample considerations) and on tissue endpoint assessment (appropriate tissue types, appropriate endpoints/life stages/sampling protocols/etc. for measuring effects, and association of tissue thresholds with population/community effects in the field).

# Tiered Approach/Framework

A tiered approach provides the most resource-effective framework for conducting assessment of selenium in aquatic systems. This approach is consistent with that used for conducting ecological risk assessments (USEPA, 1997, 1998), and the ecological risk assessment paradigm is useful toward the goal of assessing exposure and effects of selenium. Using this approach, along with conceptual models and data quality objectives (described in a later section), helps ensure that all the important work is done, but only that work that contributes to the decision-making process. In essence, the approach allows the investigator to assess tissue concentrations and bioaccumulation under site-specific conditions and to stop when information is adequate for decision-making. It is important, however, to recognize that the approach must be able to "prove the negative" (that is, must be convincing), and that USEPA (or other regulatory/resource agencies) may differ in their application of tissue-based criteria, so evaluation on the basis of multiple criteria/lines of evidence may be needed to meet requirements for the assessment.

This section describes the overall framework, including comparison of tissue concentrations to tissue residue guidelines or adopted water quality guidelines/criteria, reproductive toxicity testing study design, assessment of fish populations in the area of interest, and the use of decision trees as pathways for conducting the assessment.

## Overall Framework

Approaches for site-specific assessment of selenium have recently been described by McDonald and Chapman (2007), Lemly (2007), and Lemly and Skorupa (2007). Of these, the weight-of-evidence approach described by McDonald and Chapman (2007) is most directly applicable to the approach we recommend and describe in more detail in the following sections. Below, we briefly describe the approaches from each of these authors.

McDonald and Chapman (2007) described two commonly used approaches for evaluation (comparison of tissue selenium concentrations to tissue residue guidelines and reproductive toxicity testing using field-collected fish) and some of the shortcomings of using each of those approaches on their own. Alone, neither of those lines of evidence provides sufficient information as to whether wild fish populations are affected. The authors discussed the limitations of each of those methods and provided recommendations for improving the relevance of each line of evidence. They also proposed and discussed the use of a third line of evidence, field measurement of fish population dynamics. They proposed integrating the use of all three lines of evidence into a framework, consistent with an ecological risk assessment methodology, for the design, application, and interpretation of selenium using a weight-of evidence approach.

Lemly (2007) described a procedure for evaluating site-specific data to determine whether permits should be issued in accordance with the National Environmental Policy Act (NEPA) for mining on U.S. Forest Service lands. The procedure includes five major components designed to gather information on operational parameters of the proposed mine as well as key aspects of the physical, chemical, and biological environment surrounding it (geological assessment, mine operation assessment, hydrological assessment, biological assessment, and hazard assessment). Although the procedure was developed for the Forest Service, the author considered it also to be useful for other federal land management agencies that conduct NEPA assessments.

Lemly's (2007) procedure recommends an approach for hazard assessment. Four levels of potential hazard could be identified, ranging from "minimal or no hazard" to "high hazard" for aquatic life from selenium exposure. Minimal or no hazard finding would result in the permit being issued and environmental monitoring being conducted. However, if the assessment indicates there would be "low," "moderate," or "high" hazard, a more detailed assessment would be required. Application of approaches described in this guide would be appropriate under those circumstances.

While Lemly's procedure provides a standardized assessment process, the hazard assessment portion of the procedure is limited to the use of specific thresholds to define hazards. May et al. (2006) used the Lemly hazard protocol to assess potential selenium issues for the Solomon River basin in North-central Kansas. They found selenium concentrations in water, fish, and prey items that exceeded the hazard thresholds; however, they also found young fish (with elevated levels of selenium) even though selenium concentrations were several times higher than Lemly's reproductive threshold of 4 µg/g. Similar findings for the Republican River basin were also reported. These authors speculate that Lemly's thresholds may be too conservative or that fish may have immigrated into the area from lower exposure areas, but whatever the reason a true assessment of potential impacts was not fulfilled.

Narloch et al. (2004) used an earlier version of the hazard protocol to evaluate selenium in the Blackfoot River basin in southeastern Idaho. These authors suggested that the proposed hazard assessment protocol for selenium may be applicable as a screening tool, but should not be used for the determination of safe watershed concentrations of selenium. McDonald and Chapman (2007) generally concurred with these types of findings, and thus, used many components of the Lemly hazard approach to develop a more flexible approach using several lines of evidence.

Lemly and Skorupa (2007) recognized that USEPA's approach of developing a water quality criterion for selenium on the basis of its concentration in whole-body fish offers advantages, and they discussed some of the technical issues associated with implementation of such a criterion. In addition, they outlined a strategy that incorporates both water column and tissue-based approaches for implementation. They suggested that a national generic "safety-net" water criterion of 2 µg/L could be combined with a tissue-based criterion for site-specific implementation. For most waters, monitoring of waterborne selenium concentrations would be adequate to meet compliance requirements without the expense and logistical difficulty associated with sampling and interpreting selenium concentrations in biological materials. Dischargers would do fish sampling intermittently (not as part of the routine monitoring) relative to the tissue-based criterion. Only when the fish tissue criterion is exceeded would full site-specific analysis, including development of intermedia translation factors, be necessary.

Currently, most States regulate chronic selenium in water at 5 µg/L. While a "safety-net" approach is an interesting concept, we see no reason at this time to circumvent the current regulatory criterion for water. As the revised tissue residue criterion becomes available, it will become increasingly important for industry and regulatory agencies to understand the relationships of aqueous selenium and fish tissue residues for their area streams/water bodies, what levels pose a threat, and what levels may become "trigger" values for increased monitoring frequency or alternative management strategies.

Overall, we conclude that the approach for site-specific assessment must be flexible, depending on what is appropriate for the situation. Risk management/remediation decisions should be based on integration of biology and chemistry data, with emphasis on the biological data. Use of specific thresholds of effects is limited, however, so this framework relies more on developing the lines of evidence for a site to define whether selenium concentrations are a concern using a weight-of-evidence approach as described by McDonald and Chapman (2007). While aqueous selenium data may provide the starting point for assessment, bioaccumulated (i.e., tissue) selenium will provide the best information to discern potential for effects at a particular site.

## Comparison of Tissue Concentrations to Tissue Residue Guidelines or Adopted Water Quality Criteria/Guidelines

A first step in the tiered framework is comparison of waterborne and tissue concentrations of selenium to water quality criteria/guidelines and tissue residue guidelines. Water quality criteria/guidelines have been established by USEPA, Environment Canada, and the Ministry of the Environment in British Columbia. Additionally, Lemly and Skorupa (2007) have recently recommended a generic "safety-net" criterion that would be applicable for most water bodies. The USEPA, Canadian, and Lemly and Skorupa (2007) guidelines, as well as relevant dietary and tissue toxicity thresholds, are discussed in this section.

**Water Quality Criteria/Guidelines for Selenium.** In the United States, the promulgated chronic National Recommended Water Quality Criteria for selenium are 5 µg/L (total recoverable Se) in freshwater and 71 µg/L in saltwater (USEPA, 2006a). In addition, USEPA (2004) recommended a whole-body tissue value of 7.91 µg/g dry weight (dw) as a chronic exposure criterion for selenium in both freshwater and saltwater fishes. Further, USEPA indicated that if whole-body fish tissue concentrations exceed 5.85 µg/g dw during the summer or fall, fish tissue should be monitored during the winter to determine if selenium concentrations exceed 7.91 µg/g dw. The USEPA (2004) draft criteria document is currently being revised to include additional study data generated since the draft criteria document was released, including a reassessment of the Lemly's (1993b) Winter Stress Syndrome study that was, in effect, the basis for the 2004 draft chronic criterion.

USEPA (2008) conducted a study designed to replicate Lemly's (1993b) test, and to further explore how temperature affects the toxicity of selenium. Two groups of juvenile bluegill were exposed to various (nominal) concentrations of selenium in water (background, 1.25, 2.5, 5.0, 10, 20, or 40 µg/L) and in diet (*Lumbriculus variegatus* with background, 1.25. 2.5, 5.0, 10, 20, or 40 µg/g dw); one group was exposed to 20ºC decreasing to 4-5ºC, and the second 20ºC decreasing to 9ºC. A third exposure group received a diet containing 5 µg/g dw (as seleno-L-methionine incorporated in TetraMin) in water containing selenium at 5 µg/L while the temperature was decreased from 20ºC to 4-5ºC (similar to Lemly's exposure conditions, except that photoperiod was not reduced). Projection of the selenium concentration associated with the onset of mortality (>10%) in bluegill receiving selenium from the *Lumbriculus variegatus* diet were in the range of 11-14 µg/g dw, with an $EC_{20}$ value of about 10 µg/g and an $EC_{10}$ of about 9.6 µg/g when temperature was lowered to 4-5ºC and $EC_{20}$ of 14 µg/g and $EC_{10}$ of 13 µg/g when temperature was lowered to 9ºC. Average selenium concentration in fish receiving selenium as seleno-L-methionine (in TetraMin) incorporated about 2.5 times more selenium in their tissues than those that had been fed the worms; at the end of the experiment the average concentration in tissues of those fish was 9.4-10 µg/g while the average in fish receiving selenium in worms for a similar time was 4.0 µg/g.

Canadian water quality criteria for selenium are provided by Environment Canada (Canadian Environmental Quality Guidelines [CEQG]) and British Columbia (Water Quality Guidelines), both of which are more stringent than the USEPA criteria. Environment Canada developed CEQGs for the protection of aquatic life. A guideline of 1 µg/L is reported for freshwater systems, but a guideline is not provided for marine environments (CEQG, 2003). In contrast, British Columbia developed a Water Quality Guideline of 2 µg/L for both freshwater and marine aquatic life (Nagpal and Howell 2001). The 2 µg/L guideline for the water column will protect aquatic life from direct toxic effects as well as from accumulating undesirable levels of selenium via the food chain. The guideline to protect from direct toxicity of selenium was based on the lowest observed effect level (LOEL) of 10 µg/L and a safety factor (SF) of 5, which is lower than 10 recommended in the Canadian Council of Ministers of the Environment protocol. This choice for the lower SF was in recognition of the facts that (a) selenium is an essential element for animal health, and (b) food (and not water) is the major source of selenium in the food chain. Another basis for this guideline is a fish tissue threshold of 1 µg/g wet weight (ww). Assuming an average moisture content for bony fish of 75 percent (USEPA, 1993), the dry-weight equivalent of this threshold is 4 µg/g.

As indicated above, the Lemly and Skorupa (2007) "safety net" is lower than USEPA's current chronic criterion, which has been adopted by most States as a chronic standard. Those authors recommended the 2 µg/L screening value on the basis of reviews of selenium literature since the promulgation of the 5 µg/L criterion by USEPA in 1987 and on issues related to the protection of species covered by the Endangered Species Act (briefly summarized by Lemly and Skorupa [2007]).

What is clear from a review of current literature is that no overwhelming evidence exists that toxic effects are present in lotic systems with selenium concentrations at or less than the current USEPA waterborne criterion of 5 µg/L, or with fish having selenium at the suggested tissue level of 4 µg/g (Simmons and Wallschläger 2005). As a conservative screening approach, one may choose to compare waterborne selenium concentrations to a water quality guideline of 2 µg/L. Using the State-approved water quality standard would also be a conservative approach because it is not adjusted for site-specific factors.

**Dietary and Tissue Thresholds/Guidelines.** This and the following subsections provide a general summary of relevant information; more detailed information concerning thresholds/guidelines is presented in the Tissue Threshold Workgroup effort conducted concurrently with this guide (Canton et al., 2008).

Selenium is an essential trace element for animals, and is a component of glutathione peroxidase (GSH-PX), which aids in the protection of tissues against peroxidation by destroying hydrogen peroxide or organic hydroperoxides (Ohlendorf, 2003). The presence of selenium at increased dietary levels results in the replacement of sulfur in some metabolic pathways disrupting certain biological processes. Studies describing the effects of selenium on fish and wildlife have been summarized in several publications such as Eisler (2000), Ohlendorf (2003), and Presser and Luoma (2006). Toxic endpoints for these studies included egg hatchability, growth, mortality of young, and teratogenesis, and effects to these endpoints have been associated with concentrations of selenium in both diet and tissue (for example, liver, muscle, whole-body, and egg tissue for fish and egg tissue for birds). Dietary and tissue thresholds presented in Tables 1 through 4 are primarily represented by those provided in Presser and Luoma (2006), with the addition of more recent information as available.

***Fish Diet.*** Eggs and larvae of fish and amphibians may be the most sensitive life-stages to direct exposure to waterborne selenium. Excess selenium in the diet of fish leads to substitution of selenium for sulfur during protein synthesis (Lemly, 1998a). This substitution disrupts normal chemical bonds resulting in improperly formed or dysfunctional proteins and enzymes affecting sub-cellular, cellular, organ, and system functions. Effects include teratogenicity in developing embryos, reduced survival of fry, and reduced health and survival of adult fish (Sorensen, 1986). Typical deformities include scoliosis, missing or deformed fins, missing or deformed gills and gill covers, abnormally shaped head, missing or deformed eyes, and deformed mouth (Lemly, 1998a). Parental transfer of selenium to eggs and larvae of fish can be lethal or teratogenic (Ohlendorf, 2003; McDonald and Chapman, 2007).

In general, fish studies indicate that when selenium concentrations are elevated, sensitive fish species disappear due to direct mortality or reproductive failure while a few tolerant species persist (Garrett and Inman, 1984; Vencil, 1986; Sorensen, 1988; NRC, 1989, Hamilton, 2004). Dietary exposure of fish to concentrations of selenium greater than 3 µg/g dw results in accumulation in developing eggs, with dietary concentrations of 5 to 20 µg/g dw exceeding the threshold for teratogenic effects in the embryo (Table 1). In two well-documented cases, most fish species were absent when their invertebrate prey reached selenium concentrations of 20 to 80 μg/g dw (Belews Lake) or 100 μg/g dw (Kesterson Reservoir).

*Bird Diet.* Multiple studies have investigated the effects of dietary exposure of selenium to birds (Table 2). Avian embryos are highly sensitive to the toxic effects of selenium (Poley and Moxon, 1938; Thapar et al., 1969; Arnold et al., 1973; NAS-NRC, 1976; El-Begearmi et al., 1977; Ort and Latshaw, 1978; Ohlendorf, 1989, 2003). Hatchability of fertile eggs is considered the most sensitive endpoint. Dabbling ducks, such as mallards (*Anas platyrhynchos*) and cinnamon teal (*A. cyanoptera*), are among the most sensitive species (USDI, 1998).

Several researchers have observed that selenium concentrations in bird eggs are similar to or greater than (1 to 4 times) the dietary concentration (Heinz et al., 1989; Ohlendorf, 1989). Based on this information, dietary concentrations greater than 3 μg/g dw could yield egg concentrations associated with embryo mortality. Additionally, dietary levels from 6 to 9 μg/g dw are known to reduce the hatchability of chicken eggs (Ohlendorf, 1989), but reproductive impairment can result from diets of only 3 to 8 μg/g dw (Wilber, 1980; Martin, 1988; Heinz, 1996; USDI, 1998).

Ohlendorf (2003) used the results of six studies with mallards to determine the selenium concentration in diet that was associated with reduced egg hatchability. A dietary concentration of 4.87 μg/g dw was associated with a 10 percent effect (reduction in hatchability) or $EC_{10}$. The 95 percent confidence intervals (CIs) for this $EC_{10}$ are 3.56 to 5.74 μg/g dw. It should be noted, however, that the mallard studies used a "dry diet" that had about 10 percent moisture. Ohlendorf (2003) used the reported dietary selenium concentrations without adjustment for that moisture content, but an upward adjustment of the values (by 11 percent) would be appropriate to account for the moisture content of the duck diet. Adams et al. (2003) used the same mallard studies and hockey-stick regression to model relationships between dietary selenium concentrations and adverse effects in order to derive toxicity thresholds, such as $EC_{10}$ values. Based on this analysis, an $EC_{10}$ of 4.4 μg/g dw was predicted, with 95 percent CIs of 3.8 to 4.8 μg/g dw. The use of hockey-stick regression results in a slightly lower threshold and a narrower CI range than using logistic regression. Based on prior use of hockey-stick regression to define a threshold when an underlying background level of response is unrelated to the dose, this model is thought to be relevant to naturally occurring elements that are essential to birds and a wide variety of other organisms. It appears to be particularly useful for elements such as selenium, which has a narrow range between levels that are essential and those that are toxic to birds so that variance around the inflection point (threshold) in the model is small.

*Fish Tissue.* Tissue concentrations that have been associated with adverse effects in fish are listed in Table 3. It should be noted that for fish and other animals, selenium is an essential nutrient, and concentrations lower than those associated with effects can be considered within the normal range needed to meet physiological needs. Data in Table 3 show that effects may occur at whole-body selenium concentrations as low as 4 to 6 μg/g dw, with consistent evidence of teratogenesis and reproductive failure at whole-body concentrations greater than 15 μg/g dw. It has also been shown that the occurrence of deformities increases rapidly when selenium concentrations in fish eggs are greater than 10 μg/g dw. As previously discussed, a fish tissue criterion of 7.91 μg/g dw was developed by USEPA

(2004). There has been some controversy as to whether this value is protective of both warm-water and cold-water anadromous fish, with some suggesting that cold-water fish are more sensitive. However, Chapman (2007) provides a summary of effects thresholds in fish eggs that indicates cold-water fishes are likely more tolerant to selenium than warm-water fish. Therefore, the USEPA criterion is expected to provide conservative protection for cold-water species.

*Bird Tissue.* Because the embryo is the avian life stage that is most sensitive to selenium, monitoring of eggs is a good approach for determining effects on avian species utilizing aquatic habitats. Table 4 presents egg tissue thresholds for adverse effects to avian species. Based on these studies, embryo or duckling mortality may occur at selenium concentrations in eggs a few times greater than background (which is 3 µg/g dw). The range of 12 to 16 µg/g dw, as reported by Adams et al. (2003), reflects different statistical approaches for analyzing the data. Egg hatchability and egg viability were found to be a more sensitive endpoint with thresholds ranging from 5.1 to 10 µg/g dw. Similar to the dietary values calculated by Ohlendorf (2003) for reproductive toxicity for mallards, the $EC_{10}$ in eggs (using a logistic regression model) was reported as 12.5 µg/g dw, with 95 percent CIs of 6.4 to 16.5 µg/g dw. The analysis of these data by Adams et al. (2003), using hockey-stick regression, resulted in a threshold of 9.8 µg/g dw, with 95 percent CIs of 9.7 to 13.6 µg/g dw. Given that there is a clear egg-selenium threshold at which effects begin to be observed, a unimodal model, such as logistic regression, may result in exaggerated confidence intervals, particularly in the tails.

**Thresholds for Invertebrates and Amphibians.** Development of selenium thresholds has largely focused on fish and birds. Invertebrates, when considered, are generally evaluated only as a dietary source to these higher trophic-level species. deBruyn and Chapman (2007) compiled waterborne, dietary, and tissue toxicity data for aquatic invertebrates to determine whether thresholds developed for fish and birds would also be protective of invertebrates. Significant reductions (50 percent decrease) in population size were observed at selenium concentrations as low as 10 µg/L in water and 1.5 µg/g dw in diet. Additionally, sublethal effects (for example, growth or reproduction) were observed at tissue concentrations as low as 1 µg/g dw. These data indicate that waterborne selenium thresholds based on fish may be protective of invertebrates, but dietary and tissue thresholds that are protective of fish and birds may not be sufficient to protect invertebrate communities.

Although the results reported in deBruyn and Chapman (2007) may be valid, the conclusion that is made should be considered preliminary and subject to verification from other studies (both lab and field). It should be noted that according to USEPA's draft revised selenium criteria document (USEPA, 2004), invertebrates are more sensitive than fish to the acute effects of both selenate and selenite, and the effect levels are considerably higher than 10 µg/L. These effect levels are based on lab studies, which typically result in lower effect levels than field exposures. Based on personal experience (R. Reash, American Electric Power, pers. comm.), invertebrates residing in selenium-enriched fly ash receiving streams are much more tolerant than fish.

As with invertebrates, little focus has been given to amphibians. Schuytema and Nebeker (1996) reported an effect level of 489 µg/L for embryonic amphibians. However, a recent study investigating the maternal transfer of selenium to amphibian eggs (Hopkins et al.,

2006) suggests that the larval life-stage may be more sensitive. Hopkins et al. (2006) reported a 19 percent decrease in offspring viability (as represented by hatching success, developmental abnormalities, and swimming abnormalities) in waters with an average selenium concentration of 3.93 µg/L. Selenium concentrations in amphibian eggs reared in this water ranged up to 100 µg/g dw. Although a statistical relationship between the concentration of selenium transferred to eggs and offspring viability was not observed, the study demonstrated that selenium is transferred to amphibian eggs at a high rate. It is important to note, however, that ash ponds such as the one used in this study typically have a mixture of metals and other constituents, so any effects observed (other than bioaccumulation of selenium) could be due in part to other chemicals.

**Discussion of Water Quality, Dietary, and Tissue Thresholds.** One consideration in determining effects thresholds is related to the hormetic effects of selenium. Hormesis is the phenomenon in which a low dose of a potential toxin results in a beneficial effect, but a high dose is detrimental (Calabrese, 2008). Consideration of the hormetic effects of selenium may result in lowering of thresholds (for hormetic substances and endpoints one has to distinguish between valid control responses and hormetic deficiency responses before a valid baseline to compare toxic responses against can be identified). However, it is considered unlikely that USEPA will make any adjustment to the selenium water quality criteria despite the empirical evidence that hormesis does occur.

In birds, although the hormetic bias in the laboratory study data used for the Ohlendorf (2003) and Adams et al. (2003) regressions was not reported, if such consideration were to result in changes, those changes would be in the direction of a downward shifting of the threshold confidence limits. Both Ohlendorf (2003) and Adams et al. (2003) used the results of six laboratory studies with mallards and determined that the threshold for reproductive effects ($EC_{10}$ for egg hatchability [Ohlendorf] or duckling mortality [Adams et al.]) was about 12 µg/g dw. Analyses by Beckon et al. (2008) that adjusted for hormetic effects in the mallard data from one of those laboratory studies yielded a revised $EC_{10}$ for egg hatchability of 7.7 µg/g dw. In the Elk Valley of British Columbia, Harding (2008) reported a hormetic effect of selenium on reproductive success of red-winged blackbirds (*Agelaius phoeniceus*), with an effects threshold of about 22 µg/g dw.

Finally, it should be noted that the use of water quality and/or tissue residue guidelines, on their own, usually does not provide sufficient evidence that risk management or remediation actions are warranted (McDonald and Chapman, 2007). Specifically, a tiered approach utilizing multiple lines of evidence should be used to develop weight-of-evidence conclusions regarding selenium impairment in a water body. Additional lines of evidence are discussed below. While a comprehensive weight-of-evidence approach is ideal for site-specific regulatory applications, it should be noted that most regulatory agencies will apply numeric criteria in a straightforward manner. Thus, the burden of accumulating data and information for a tiered weight-of-evidence approach will, in most cases, fall upon the regulated entity.

One other noteworthy observation from studies in aquatic systems is that tissue selenium concentrations in fish and invertebrates may be more highly correlated with waterborne concentrations a week or two before the biological samples were taken than with concurrently-collected water samples. For example, at the Richmond Refinery Water Enhancement Wetland (a flow-through treatment wetland for refinery effluent [see

Ohlendorf and Gala, 2000]) selenium concentrations in invertebrates were positively related to variability in waterborne selenium concentrations with a 2-week time lag (Chevron, 2002). This "lag" apparently is due to the bioaccumulation process, whereby the waterborne exposure level is not immediately reflected in tissue concentrations.

### Reproductive Toxicity Testing

McDonald and Chapman (2007) note that selenium accumulation in adult fish can result in increased frequency and severity of deformity in their offspring. This maternal transfer effect has been documented in fish exposed under field conditions (Gillespie and Baumann, 1986; Skorupa, 1998c) as well as laboratory-based experiments (Woock et al., 1987; Schultz and Hermanutz, 1990; Coyle et al., 1993). The role of paternal transfer of selenium to measured levels of selenium in eggs and fry (and observed toxic effects) is generally considered to be unimportant. Crossing studies indicate that males collected from selenium-contaminated water bodies do not confer high selenium levels or effects in fry after artificial fertilization (Gillespie and Baumann, 1986). Moreover, levels of selenium in testes of males collected from selenium-contaminated systems are typically lower compared to females collected from the same locality (Baumann and Gillespie, 1986; Lohner et al., 2001; Reash et al., 2006).

Some of the more current research, including Kennedy et al. (2000), Holm et al. (2003, 2005) de Rosemond et al. (2005), Muscatello et al. (2006), and Rudolph et al. (2008), has been conducted using wild fish captured in areas of elevated selenium where exposure to the parent fish has been integrated from aqueous and dietary pathways. Eggs from female adult fish have been raised in a laboratory setting to examine survival, growth, and potential for deformities. These studies typically included fish eggs collected from nearby reference or background settings as well for comparison. Hardy et al. (In Prep.) went one step further and collected wild fish, spawned the fish in the laboratory, and exposed the hatched fry to different dietary treatments. Fish were raised to sexual maturity at the different dietary exposures and spawned, with the subsequent fry being evaluated for similar endpoints described above.

Collectively, these studies have been used and are considered to provide key evidence that long-term maternal exposure via the diet and aqueous pathways may be the most important factor in understanding selenium toxicity. The level of maternal selenium tissue residue appears to be directly correlated to effects in young developing fry and to depend on the site-specific conditions that exist, such as the form of selenium, dietary uptake mechanisms, and the fish species being exposed. The reproduction endpoint is considered to be a sensitive endpoint that integrates long-term exposure, which may have adverse effects on young depending on the level of selenium exposure in the diet and aqueous environment.

The basic model for most of these studies has involved collection of wild parent fish, both males and females, in reproductive condition, spawning these fish, fertilizing the eggs, and rearing the eggs in a controlled laboratory environment. Recent studies in southeastern Idaho utilized an approach similar to those studies identified above to assess potential selenium toxicity and effects on reproductive success for brown trout (*Salmo trutta*). The approach described below is adaptable to most any setting where selenium in the aquatic environment may exceed levels considered to affect fish reproduction.

Priority one in conducting such studies is recognizing the potential limitations of field studies aimed at addressing effects due to selenium via reproductive testing using wild parents. Multiple stressors are present in a natural setting, so being able to discern contaminant effects from those related to natural conditions is an important consideration. Knowledge of the area being investigated, including selenium concentrations for multiple seasons or years, resident species characteristics, and habitats, will increase the chances for success of reproductive studies using wild fish eggs. Many factors should be considered before undertaking such an effort, but special attention should be given to the following:

- Concentrations of selenium in aqueous and dietary media;
- Selecting species to be tested;
- Mobility of the species to be tested and their exposure history;
- Sources of control or reference eggs;
- Timing of the spawn for the species of interest;
- Locations and habitats where species are likely to spawn relative to the selenium concentrations to be evaluated; and
- Collection of sufficient numbers of males and females to acquire eggs and milt for testing.

Because relatively few reproductive studies using wild fish have been conducted, there is no clear-cut “how to” guide for conducting these studies. Investigators should have a clear understanding of selenium concentrations in the aquatic environment before initiating reproductive toxicity testing. Site monitoring is critical to collecting the initial data that are needed to garner this understanding.

Selecting the species to be tested is one of the more important considerations to be made before undertaking this type of study. Factors that should be considered during the species selection process include, among others, ability to maintain/raise the eggs in a laboratory setting, species distribution across the area of interest, difficulty of collecting parent fish and or eggs, mobility of the species, species sensitivity, trophic guild of the species, and the ability to obtain either reference or control parents for spawning. Personnel collecting, spawning, and raising eggs must have experience with the species selected. If the species selected has not been used or the eggs raised in a laboratory setting before, success of reproductive toxicity testing using wild fish may be low. Investigators must also consider the difficulty in obtaining the wild species of interest in adequate numbers. One may collect hundreds of fish, but not all will be ripe; thus, selective culling will be necessary to obtain enough ripe fish from the larger number of fish that will ultimately be collected.

Species mobility and range are other important considerations given that some species of fish have relatively narrow and small home ranges while others are transient and may move across relatively large exposure areas. Fish with narrow home ranges would be ideal, since exposure history would likely be consistent with their surrounding environment where they are collected. But what if that species is not overly sensitive to selenium—is it still an appropriate test species? Also, because selenium is primarily accumulated via the dietary pathway, feeding guilds of the fish species should be considered. Is an insectivore more or less appropriate than a higher-level predator? Is an omnivorous fish a better candidate? Thus far, most studies have focused on fish species with management implications (that is, game species) that have a history of sensitivity to other metals/metalloids, and have known culture histories in the laboratory.

Knowledge of the spawning conditions (for example, temperature, flow, and so forth), habitat, timing, and actual locations where site species of interest spawn is a critical first component in undertaking reproductive testing of wild fish. Reconnaissance-level surveys should be conducted in prior seasons to identify these conditions, such that when the field team is on site, specific areas can be targeted to increase the potential for success. For instance, for species that create nests, previous identification of where these nests are located will aid in identifying potential new nesting areas in subsequent seasons when spawning fish are to be collected. For example, if trout species are being investigated, locating areas with suitable spawning gravels or where redds have previously been excavated will provide key evidence for where to collect parental fish during future surveys. Conversely, collection of eggs from broadcast-spawning fish requires knowledge of timing of the spawn, local water temperatures and/or flows that may serve as the environmental cues for spawning.

Rather than targeting only a high and a low or background/reference testing scenario, it may be preferable to identify potential spawning areas at a range of locations exhibiting a range of selenium exposure concentrations. By collecting a number of fish across a range of concentrations, a regression analysis can be used that relates test endpoints to parental body burdens. Thus, adverse effects levels can be estimated or elucidated using a regression approach based on multiple points in the relationship, not just the upper and lower ends. Determining the sample size needed for such an approach requires knowledge of the range of tissue concentrations present for a site.

Since the focus of the study is assessing the potential effects of selenium on the reproductive success for the species in question, parent fish must be collected from exposure areas as well as non-exposure areas to provide for an adequate comparison. Sufficient habitat must be available in the exposure area, not only for spawning but for residence as well. In a stream setting, locations downgradient of a known source may provide varying levels of exposure and habitat suitable for study purposes. In a lake or pond setting, gradients of higher to lower contamination can often be located. Determining the actual spatial and temporal exposure of a field population, while certainly desirable in terms of relating residue levels to toxicity endpoints, is often problematic. Large water body settings (lakes, large rivers, and estuaries) are especially difficult when estimating actual exposure. Creative techniques may be required to validate the magnitude of selenium exposure from a source, or evaluate demographic isolation between "exposed" and "reference" populations.

Fish from a natural location unaffected by elevated selenium exposure should be obtained as "controls" (that is, reference fish) for reproductive toxicity testing. Hatchery-raised fish can be used as controls if reference fish are not available, but there are some important considerations one should account for when using hatchery fish, including the following: hatchery fish experience few stressors similar to the natural environment (such as limited food availability); hatchery spawners will tend to be larger than wild-captured fish of the same age; and egg production of hatchery fish will likely be higher than wild-captured fish. All these factors may affect the results of the data analysis.

Methods for field collection will vary depending upon the habitats to be sampled. Collected fish should be checked for ripeness and retained if ripe and released if unripe. Eggs and milt should be expressed in the field and the eggs fertilized immediately, if possible. Higher success rates of egg fertilization are achieved when eggs are immediately fertilized. Following fertilization, eggs must be immediately transported to the laboratory because they become increasingly sensitive to disturbance 48 hours post-fertilization; this sensitivity lasts until the eyed stage.

Egg rearing should be conducted according to ASTM (2005) Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes (E1241-05). Temperature modifications may be necessary based on site-specific conditions. Testing/observation should continue until hatch or beyond, depending upon the species. Some of the potential effects caused by long-term selenium exposure are related to craniofacial deformities, which may affect a young fish’s ability to feed following the yolk-sac stage. Therefore, it may be appropriate to evaluate a period of feeding past the yolk-sac stage to assess whether young fish are successful in transitioning from endogenous to exogenous feeding.

Test endpoints may include many of the following:

- Fecundity;
- Percent fertilization;
- Survival at the swim-up stage;
- Incidence of deformities or other physical abnormalities;
- Growth (based on weight and length) prior to exogenous feeding;
- Growth (based on weight and length) following exogenous feeding; and
- Selenium tissue residues.

One of the key endpoints listed above is presence of deformities. Maternal exposure to selenium that is passed on to fry during the development stages can produce deformities in fry that may eventually lead to mortality, although they may initially survive. Length, weight, and any deformities (craniofacial, finfold, skeletal and yolk sac malformations, among others) should be recorded for each fish at the swim-up stage. A graduated severity index (GSI) for ranking deformities should be used because this approach measures both the magnitude and the frequency of the deformity. Holm et al. (2003, 2005) and Kennedy et al. (2000) describe the GSI for measuring and ranking deformities. Briefly, larval fish are rated as “0” for normal, “1” for slight defect of size or structure, “2” for moderate defect or multiple defects, and “3” for severe defect or multiple moderate defects. Although edema can be considered a teratogenic effect, it is reversible and thus is not considered a true teratogenic effect.

McDonald and Chapman (2007) reviewed the importance of edema and its inclusion or exclusion as a teratogenic effect. They argue that all deformities are not equal in terms of their effect on the ability of an individual fish to feed, escape predation, and reproduce. Equating a potentially reversible measurement with permanent teratogenic deformities is questionable. Holm et al. (2003) found that edema contributed nearly 50 percent to the GSI scores in larval rainbow trout; conclusions regarding the frequency and severity of deformities are clearly influenced by the edema measurement. Consistent with recommendations from McDonald and Chapman (2007), methods (described below) include observation of edema to be evaluated separately.

Several investigators are currently developing methods with more specific definitions for each of the deformity assessment categories to provide for repeatability and consistency. Golder Associates is developing a standard operating procedure for Teck Coal, while Dr. David Janz independently developed a similar procedure for the Tissue Threshold Workgroup effort conducted concurrently with this guide (Canton et al., 2008). Likewise, Dr. Kevin Bestgen at the Colorado State University Larval Fish Laboratory is similarly developing an independent procedure to assess deformities (not published). Collectively, as these assessment procedures become available, they will likely promote a more unified assessment strategy that future deformities assessments can utilize to increase comparability of results.

McDonald et al. (2008) presented an assessment of defensible selenium tissue residue guidelines based on larval fish deformity data. They found that the uncertainty in residue response relationships was higher than expected and that quality assurance and control in studies where larval deformities were evaluated and ranked were lacking. To reduce these uncertainties, they proposed QA/QC guidelines that include the following:

- Create and consistently apply an *a priori* description of each deformity type and magnitude;
- Non-sequential assessment is preferred, and blind labeling is required;
- Expand uncertainty assessments in reports and provide raw data to allow future recalculation as science advances; and
- Implement formal QC checks that include the following:
  - 10 percent internal re-check (or 100 fish) to confirm observational drift is not an issue;
  - Minimum 10 percent external recheck;
  - Suggested data quality objectives include:
    - No actions if overall precision is > 90 percent
    - Expand QA check if precision is between 75 and 90 percent; consider implications on TRG derivation
    - Consider re-doing assessment if precision is <75 percent.

As this work is the first assessment of these types of data that we are aware of, it seems practical to take precautionary steps that ensure deformity data used to derive tissue residue guidelines are of the highest quality.

USEPA (2004) opted to use a regression analysis to define the dose-response relationship to derive a draft chronic value for fish tissue. The $EC_{20}$ was used and defined as a reduction of 20 percent in the response observed for controls. Rationale for use of the $EC_{20}$ as the chronic value, rather than, for example, an $EC_{10}$, was that it represents a low level of effect that is generally significantly different from the control (USEPA, 1999). Smaller reductions in growth, survival, or other endpoints only rarely can be detected statistically. Effect concentrations associated with such small reductions have wide uncertainty bands, making them unreliable for criteria derivation (USEPA, 2004). Site-specific laboratory studies will likewise develop $EC_{20}$s for test endpoints. In its revision of the 2004 draft selenium criterion, USEPA is contemplating the use of $EC_{10}$s for long-term exposure criteria for tissues (C. Delos, USEPA, pers. comm.). Merits of the different effects level endpoints will be evaluated in terms of the effects levels found in laboratory tests and how those levels relate to the site-specific conditions.

### Assessment of Fish Populations in the Area of Interest

Reproductive toxicity testing of wild-captured fish from a site provides information for effects to individuals, not populations or communities. Ideally, it stands to reason that if the progeny of an individual are severely affected and do not survive, this would ultimately have an effect on the population for that species. In other words, effects on individuals are significant such that population-level parameters will be expressed above some threshold considered within normal temporal variability. McDonald and Chapman (2007) suggest that a population-level survey, if properly conducted with adequate statistical power, can be used to clarify, refine, and "ground truth" the findings derived from analytical or reproductive toxicity lines of evidence. In order for population-level studies to be effective in meeting this goal, these authors recognize that certain factors may influence how results are interpreted, including the following:

- The time scale over which changes in populations are evaluated;
- Emigration/immigration – influence of fish barriers; and
- Habitat carrying capacity of the area under investigation.

Each of these factors, independently or combined, can influence a population survey such that discerning any type of trend may not be possible. Detecting changes in fish populations may take many years to define baseline population-level changes despite the influence of a contaminant, and then several more years to define population-level changes due to a contaminant. For example, understanding the baseline characteristics of a trout population may necessitate repeated population measurements over the course of that species' life cycle. If one were to consider cutthroat trout, for example, their life cycle ranges from five to seven years.

Fish populations are dynamic and influenced by many factors that can change annually as well as seasonally. However, the goal here is not so much to define that a population is changing through time, or even predict potential future changes; rather, it is to corroborate the evidence provided by the reproductive toxicity testing. As another line of evidence, population-level surveys could provide a level of confirmation or consistency with observations of reproductive effects (that is, locations where fish may exhibit lower or higher reproductive success). For example, if reproductive testing indicates that for some selenium level found at a location there is a decreased measure of reproductive success, information gained from population-level surveys could be used to ground truth such findings, through observations such as population statistics (standing crop biomass or number per unit area, condition factors, and/or length frequency statistics for the species of interest). In addition, species population age structure for a given area can provide unique insights. Understanding if there is a reasonable/comparable distribution of age classes at an exposure area versus a non-exposure area, or if age classes are missing may reveal evidence confirming observations from laboratory reproductive studies. As noted previously, population studies are recommended as a complementary line of evidence, not an absolute requirement.

Use of a three-pass removal or mark and recapture style approaches over several seasons will provide information on population stability and variability. Without such initial assessments, deriving a sample estimate with adequate statistical power may be difficult for a site because site-specific population variability may be unknown. Again, similar to studies for reproductive toxicity testing, there are limitations or considerations investigators should be familiar with, including species mobility, seasonal changes in population dynamics, sampling similar habitats and flow regimes, and comparability to reference and background sites. Background sites should not be confused with reference sites, as most states view these as having different properties. The study design should consider the use of one or both of these types of comparative sites, how they are defined within the state where the water body is being evaluated, and what questions are being addressed. Comparison of a potentially affected site to a reference site may address different questions than comparisons to an unaffected or affected background site.

Selecting sites based on similarity of habitats as well as proximity to a selenium contaminant source is a key factor to consider early on in the study design. Changes in fish communities and populations will be affected by habitat quality and quantity, predator density, food availability and, potentially, selenium exposure. Understanding the fish community and habitat quality and quantity will be important variables in understanding changes in fish populations' dynamics. Use of habitat-based models that predict fish standing crop, usable area for a life stage, or habitat suitability will be useful tools to consider.

Lemly and Skorupa (2007) provide the following guiding principles to consider when selecting what species to monitor:

- Consider the chemical sensitivity of species as well as the candidate species' life history aspects that contribute to their exposure and vulnerability. The type of diet (for example, detritivore, omnivore, insectivore, piscivore, planktivore) may greatly influence the intake of selenium and thus result in different tissue concentrations among the species available for sampling (Lemly, 1985; Barwick and Maher, 2003). In some studies, selenium concentrations were found to increase up through the food chain, but the increases are not always predictable. It is therefore important that both species sensitivity and life history be considered since some species may bioaccumulate higher levels of selenium due to life history (i.e., foraging behavior) but be less sensitive to accumulated selenium.

- Species with long life cycles and low reproductive rates are often more vulnerable to increases in mortality than species with short life cycles and high reproductive rates (Matthews, 1998; Meyers et al., 1999).

- For the initial monitoring effort it would be prudent to sample multiple trophic levels and different life stages (juvenile and adult) in order to ensure that the range of tissue selenium concentrations present in the aquatic system is identified. This range-finding would be useful for selecting species and life stages for sampling in subsequent monitoring efforts.

## Representative Decision Trees

An accurate characterization of selenium bioaccumulation requires a focused study approach driven by choices made at key decision points (for example, see Figure 1 of McDonald and Chapman [2007] and Figure 3 of Lemly [2007]). In fact, it may be assumed that the current draft selenium criteria are conservative and that site-specific studies with a risk assessment approach will be needed to set realistically protective site-specific values (for example, see Chapman, 2000, 2007). It is assumed that prior investigations have identified soil, sediment, or waterborne selenium concentrations of toxicological concern or in exceedance of guidance screening values. (Soil data may be appropriate if they represent an area that will be inundated in the future.) The following major questions may influence design choices for bioaccumulation investigations and determining tissue concentrations:

- To what degree are the draft water quality criteria chronic tissue value or waterborne acute concentration (that is, USEPA [2004]) exceeded? (These are used only as general indicators of magnitude of potential effect, rather than strict differentiators of risk. This could be used as a screening value as offset with continued, low-frequency monitoring [Lemly and Skorupa, 2007].)
- What is the primary risk driving the project – human health or ecological risk?
- Are fish, aquatic-dependent birds, or amphibians present and important members of the ecosystem?
- What are the important and/or most sensitive receptor species for characterizing risk? Are those receptors available for sampling? If not, are adequate surrogate species available? Are their dietary items available to sample?
- In general, are field-collected samples of the appropriate biota readily available and easily sampled?
- Can the spatial area of greatest exposure to selenium be identified, delineated, and readily sampled for both abiotic (sediment, water) as well as biotic (whole-body or tissue) samples?
- Is the system highly seasonal, with only ephemeral stream flows?
- Is the system easily characterized as part of a conceptual model of sources, transport and fate, exposure, and risk from selenium (see next section)?

A decision tree approach can be applied to these questions and to help list the choices available when conditions do not match literature-based documentations of risk (Figure 1). For example, the draft national recommended aquatic life criteria for selenium are values established for chronic exposure of fish (as measured by whole-body fish tissue). However, cases exist where fish are not present but exposure to aquatic birds through their aquatic invertebrate diet is of most concern (see, for example, the case study for Great Salt Lake). In the latter case, management decisions would require the adoption of water quality standards as determined by exposure and risk to wildlife, rather than fish.

The first step in any such assessment should include determining the adequacy of existing data for the characterization of bioaccumulation. The decision tree illustrated in Figure 1 is based on the concept that existing data are inadequate to characterize the bioaccumulation of selenium at the site but that ambient media concentrations suggest the possibility of risk from selenium (that is, beginning at Step 2 of the weight-of-evidence framework presented in Figure 1 of McDonald and Chapman, [2007]). This decision tree provides the rationale for study design and future field sampling plans. Major decision choices include the characterization of whole-body fish tissue concentrations for assessment of exposure and risk to fish (directly), versus the characterization of whole-body fish tissue concentrations as a measure of exposure through diet to piscivorous wildlife, or the characterization of plants and invertebrates (with or without fish) as a measure of exposure and risk to omnivorous or invertivorous wildlife receptor species. In addition, bird eggs may be sampled, if available, as a direct measure of bioaccumulation in key receptor species. Where fish are not available as a measure of direct exposure, bird eggs should be sampled along with their plant and invertebrate dietary items and media of environmental exposure (sediment, water).

The choice of tissue type is important, as well:

- Fish may be analyzed as individuals or composites of several individuals as whole-body samples (preferred for ecological risk assessment). Alternatively, fish tissues may be analyzed for specific tissue concentrations, particularly from larger fish if smaller ones are not readily sampled. Example tissues include eggs, muscle, or livers. Fish muscle tissue analysis provides human health screening information. Eggs provide a measure of accumulation most directly related to reproductive toxicity (Lemly, 1993a). However, sampling of eggs is seasonally limited, depending on the biology of the species present.
- Invertebrates are usually analyzed as whole-body composites of several to many individuals. However, bivalves and snails are usually removed from the shell for analysis of soft tissues that would contain biologically available selenium.
- Aquatic plants should be analyzed as composites from several individuals. However, larger plants are best analyzed as selected tissues that may be likely forage items, such as seeds, leaves, or roots (rather than larger stems or whole plants).
- Algae (filamentous, periphytic, or phytoplankton) are typically analyzed as composite samples.

In almost all cases, the optimal time for selenium bioaccumulation sampling coincides with the reproductive period of the key receptors. Spring-summer (bird nesting time) is appropriate for collecting bird eggs and many fish species as well as the invertebrate and plant dietary items for those species. Some fish are fall spawners, so if exposure and effects to those species are of concern, then sampling of fish and associated environmental media (water, invertebrates, etc.) should be conducted in late summer or fall.

# Conceptual Models

Conceptual models are important as a way of describing sources, transport, and fate of selenium as related to biological exposure and bioaccumulation. Freshwater and estuarine embayment ecosystem examples are shown in Figures 2 and 3, respectively. In a comprehensive comparison between selenium ecotoxicity in lentic and lotic environments, no general or consistent differences were discovered, but there are important differences in selenium cycling (briefly discussed below; see also Simmons and Wallschläger, 2005). Those authors recognized the scarcity of data available for lotic systems as compared to lentic systems and suggested that ecology, hydrology, and biochemistry of these systems are different and cause differences in bioaccumulation. They provided a useful figure illustrating simplified biogeochemical cycles of selenium in lentic versus lotic environments.

In general, complete background characterizations of waterborne and sediment selenium concentrations on a seasonal basis are needed to accurately design the site conceptual models for bioaccumulation sampling. Although it is not necessary to collect data on all physical and chemical processes in the selenium cycle to be able to characterize bioaccumulation, it is important to recognize major transport and fate pathways so that important data collection is not missed. When adapted for site-specific conditions, conceptual models of selenium transport, fate, and exposure may be significantly different from the examples shown here. However, the important considerations are to capture major pathways, fate, and reservoirs for selenium in the ecosystem (for example, see Maier and Knight, 1994). Note that the Figure 2 example of freshwater pathways in the Newport Bay, California watershed includes wetland (lentic) as well as flowing water (lotic) environments. Differences between those environments that may be important in designing the sampling program are discussed below. A more generic example showing the complexity of selenium transformation pathways, as well as example choices for bioaccumulation sampling, is shown in Figure 4.

One of the main reasons for incorporating a conceptual model to structure individual investigations is the narrow range between essential physiological requirements and toxicity of selenium and the complexity and dynamic nature of its environmental chemistry (Lemly, 1993a; USEPA, 2004) as well as the variation in exposure and risk among different receptors (Peterson and Nebeker, 1992; deBruyn and Chapman, 2007). These factors taken together dictate the need for a weight-of-evidence approach and site-specific studies in evaluating selenium effects (Maier and Knight, 1994; Chapman, 2000, 2007; McDonald and Chapman, 2007). Such factors as water and sediment chemistry and toxicity testing may need to be considered in relation to tissue selenium concentrations in evaluating exposure and risk. Such conceptual models, including transfer terms from water and sediment to biota and between species, form the basis for detailed biogeochemical and food web modeling of selenium (for example, Bowie et al., 1996; Presser and Luoma, 2006).

## Lentic

Lentic habitats (ponds or lakes) have specific characteristics due to their lack of flowing water that influence the degree and extent of selenium exposure. Ponds and lakes tend to have a developed plankton community including small, free-swimming organisms and may have a degraded or reduced benthic community due to deep water oxygen depletion. Work in lotic habitats often requires knowledge of thermal or chemical vertical stratification of the water column as a means of understanding the seasonality of the system, uptake and loss processes, and routes of exposure for selenium (for example, see Figure 2). The sediment-trapping nature of lentic environments supports enhanced sediment microbial activity with subsequent conversion of selenium to more bioavailable, volatile, or sequestered forms. The lack of flowing water often provides biotically-rich shallow-water habitats for feeding and nesting of waterfowl and shorebirds, and the spatial extent of foraging areas may often be defined by the pond or lake, particularly in the case of nesting birds. For fish, as well, the chemical characterizations of small ponds or lakes (in particular) can be viewed as average conditions of exposure and risk for the entire habitat. Typical freshwater organisms for bioaccumulation sampling (and receptor consumption) from pond sediment or vegetation, not as often available from flowing water, include midge larvae (chironomids), *Daphnia* or other micro-crustacean zooplankters, amphipods, various odonates (damselfly and dragonfly nymphs), and various water column hemipterans (waterboatmen [corixids], backswimmers [notonectids]). Most of the selenium toxicity research used in standard-setting and populations studied for development of the tissue-based standards originated from lentic populations (USEPA, 2004; Simmons and Wallschläger, 2005).

Estuarine and marine habitats (embayments) are of special concern for selenium contamination because of enhanced runoff of selenium from terrestrial systems (for example, San Francisco and Newport bays, California). These habitats assume lentic characteristics but incorporate stream loading as an important part of their conceptual model (Figure 3). Important receptors may follow different marine food chains that influence their exposure and risk from selenium; for example, benthic bivalves to sturgeon and waterfowl in San Francisco Bay (Presser and Luoma, 2006) versus plankton to top-water fish to terns or skimmers in Newport Bay (for example, see Byron et al., 2007).

The seasonal characterization of pond, lake, or bay water column chemistry and physical/chemical stratigraphy, as well as nearshore sediment chemistry, is needed to create an accurate conceptual model for the lentic environment that would help structure a bioaccumulation sampling program.

## Lotic

Lotic habitats (flowing water) are highly variable in their routes of exposure of selenium to key receptors and care must be taken to adequately create a conceptual model of exposure and risk. Figure 2 is an example that includes flowing as well as wetland freshwater habitats. Variations in lotic habitats include shallow, high-elevation streams with rocky substrates and high-velocity, clear-water flows to low-elevation, deep, slow-moving rivers with muddy substrates, turbid water, and more-developed midwater and plankton communities. This variability in lotic environments dictates variation in degree of uptake, loss, transformation, or movement of selenium through the system as well as variability in the availability of food items for exposure to aquatic-dependent birds or mammals.

In general, creeks and other streams may be assumed to provide less productivity and availability of dietary items than downstream lakes or ponds. However, as observed in many river/lake/bay systems, the lotic environments have higher waterborne concentrations of selenium than downstream receiving waters (for example, Salton Sea, Newport Bay, San Francisco Bay, Great Salt Lake). As a consequence, the limited freshwater biota of the lotic sections may experience higher levels of exposure to selenium as compared to biota in lentic areas. Alternatively, the flowing–water selenium may tend to favor oxidized inorganic forms, such as selenate, that may be less bioavailable than more chemically-reduced and bioaccumulative species such as selenite or organo-selenium compounds (as might be produced in lentic environments).

# Data Quality Objectives

The USEPA Data Quality Objective (DQO) process (USEPA, 2006b) provides a useful tool toward assessing what decisions must be made, what information is available toward making those decisions, what additional information is needed, and how that information will be used in making decisions for the site. Using the DQO process along with developing an overall conceptual model (described above) helps show how the physical/chemical and ecological components of the environment are related, as well as providing context to the work that is being done or planned. Figure 5 briefly describes the components of each DQO step.

Using the DQO process along with developing an overall conceptual model can also be useful in getting agreement about definition of the problem that requires assessment, how important tissue vs. waterborne selenium concentrations are to agencies/other stakeholders, what decisions require the collection of data, how data that are to be collected will be used, and how those data will be collected. The process should be initiated at the beginning of the site-specific assessment and include the agencies or other interested stakeholders so they can “buy into” the process as it is being conducted.

Example DQOs from studies conducted for development of a site-specific standard for selenium for Great Salt Lake are presented in Table 5. (The DQOs were developed following a previous version of the DQO guidance document, but they are generally similar to what would result following the current guidance.) These were the overall DQOs that provided the structure for the studies to be conducted to define sources and loads of selenium; transport/partitioning/loss of selenium in the water column, sediment, volatilization; food-chain bioaccumulation; and uptake and effects in birds. DQOs for the individual studies were developed and are available at http://www.deq.utah.gov/Issues/GSL_WQSC/selenium.htm.

# Sampling and Monitoring

## Sample Collection Methods

### Surface Water and Sediment

Sample collection methods for contaminants in surface water and sediments are reasonably standardized, and there are no unusual handling requirements for the measurement of selenium in these environmental media. If possible, collection of biotic (tissue samples and biological monitoring data such as benthic community or fish community data) and abiotic (sediment and surface water) samples should be co-located from a number of locations across the gradient of selenium concentrations at a site, from background to exposed to reference, such that investigators can garner a clear picture of concentrations of selenium in the abiotic and biotic media to which receptors are exposed. Gathering these types of site-specific data over several seasons will allow for relationships to be developed among water, sediment, and biotic components of the aquatic environment.

Surface water samples for selenium should be accompanied by a suite of in-situ measurements such as dissolved oxygen, temperature, conductivity, pH, and oxidation reduction potential. Additional water quality parameters should also include sulfate, hardness, alkalinity, and organic carbon.

For abiotic samples, composite samples within the area or site to be sampled should provide the level of data quality necessary for characterization and monitoring to assess future trends. In a flowing water setting, a depth- or width-integrated sample for a site may be desired if incomplete mixing of a discharge is thought to be an issue. Similarly, a depth-integrated sample may be needed if a deep-water discharge occurs in a reservoir. In other words, the type of sample should be consistent with the needs of the characterization.

Composite sediment samples are preferred in order to provide a more spatially integrated sample for a site. Collecting multiple sediment grab samples within the vicinity of a site will provide for greater spatial representativeness due to the small area typically sampled from a single sediment grab or core sample.

### Fish, Benthic Invertebrates, Birds Eggs

Sample size and type for biotic samples is similarly dependent on the type of sample and media being evaluated. Common methods for fish collection include electrofishing, seining, gill nets, or traps. Fish collection methods will be determined by the habitats to be sampled. Electrofishing either from wading or boat methods is an effective, non-destructive tool as is seining. Gill nets may be an alternative in larger open waters. If smaller minnow species are to be evaluated, a combination of electrofishing and seining may be effective.

Whole-body selenium apparently does not vary with age (as determined by size) or sex of the fish (McIntyre et al., 2006). For more mobile species, monitoring of younger age class fish, which may tend to be resident to the site of interest, may be preferred.

To evaluate effects in fish, samples are preferentially analyzed as individual fish unless there is insufficient biomass for analytical testing. In contrast, it is usually most appropriate to analyze composite samples to evaluate dietary exposure of piscivorous birds and mammals. Composite samples, if needed, should be composites of similar fish size and the same species. It is often preferable, however, to conduct analyses for selenium residues on individual fish tissue samples. By submitting fish individually, the variability of selenium residue concentrations in tissues can be defined. This is extremely important in developing relationships of tissue concentrations to water concentrations. The intended use of the fish tissue data will determine whether the samples should be submitted for whole body analysis or fillet analysis. For game or recreational species, within the legal catch size limits, it is practical to excise a plug of skin-on fillet for separate analysis from the remaining whole body analysis. Submittal of a fillet plug and the remainder of the fish as whole body will satisfy future data needs for either human health or ecological risk assessments.

Current USEPA draft guidance recommends that the chronic criteria for selenium be based on whole-body fish tissue concentrations (USEPA, 2004). More recent information concerning the revision of the draft criterion suggests USEPA is leaning toward the use of a two-part criterion (first tier, a mature ovary measurement; second tier, whole body). The mature ovaries are the target organ and measurements show less variability than whole body. Because data from many sites have not been developed for fish ovaries, conversion factors will need to be developed that allow translation from whole-body tissue residues to ovary tissue residue for a particular species. Where work is contemplated or currently underway, it is advisable to develop the conversion factors for the site.

McIntyre et al. (2006) recommended monitoring either bluegill or rainbow trout. If neither is available, they recommended monitoring another species in the genus *Lepomis* or *Oncorhynchus*. If the genus is not available, select a species within the same family (Centrarchidae or Salmonidae). If centrarchids, salmonids, and sensitive endemic species are all absent from the site, a minimum of two species should be monitored. Carmichael and Chapman (undated) used sculpin as an indicator species to monitor long-term changes at different locations. Due to high site fidelity and widespread use at other sites in Canada, sculpin were considered to be an appropriate monitoring species to monitor trends.

McIntyre et al. (2006) recommended use of two invertebrate species for monitoring if no fish are present, due to the potentially high variability among species. Data from Butler et al. (1994) and deBruyn and Chapman (2007) confirm not only variability in residues among species but also sensitivity. However, it is important to note that if invertebrates are used to monitor selenium residues, species that will provide sufficient biomass necessary for conducting analyses should be sampled.

For site characterization/assessment purposes, it is preferable to collect a random sample of bird eggs by taking one egg per clutch (nest) after the clutch is complete. For typical sites, it is our experience that eight eggs per species usually provide a reasonable sample size for screening purposes. If the site is small relative to the size of the foraging range of the study species, variability will be higher and a larger sample size will be needed. For assessment of reproductive success, it is desirable to mark the nests after collecting a sample egg and determine reproductive success on as many nests as feasible. Species to be sampled depend on the particular site, but eggs of any aquatic-dependent species would be appropriate (for example, waterfowl, shorebirds, and so forth). If possible, the eggs should be collected after the midpoint of the incubation period, in order to allow for evaluation of the presence of developmental abnormalities. However, when there is high probability of nest loss due to predation, an egg can be collected as soon as a nest is found. More details of sampling and assessment of avian reproductive success are provided by Ohlendorf (1989, 2003) and Ohlendorf et al. (1989).

## Analytical Methods

Reliable analyses of selenium in environmental media (water, sediment, etc.) and tissues are an essential component of conducting site-specific assessments of selenium accumulation. This section provides a general summary of relevant information; more detailed information concerning analytical methods is presented in the Selenium Analysis Workgroup effort conducted concurrently with this guide (Ralston et al., 2008).

The choice of analytical methods, with careful consideration for matrix interference and digestion/extraction problems, is important to a well-designed selenium bioaccumulation sampling program. In addition, the sampling and analysis of biota tissue concentrations may benefit from associated, additional data collection of water or sediment. For example, finer sediment grain size and higher total organic carbon (TOC) content are likely to be positively associated with the sediment-biota accumulation potential for selenium and should be measured. Similarly, pH, dissolved oxygen, and total dissolved solids or salinity are likely to be important determinants of waterborne uptake of selenium to biota and also should be measured.

As described in the Decision Tree flowchart (Figure 1) fish may be analyzed as whole-body tissue concentrations for most ecological risk applications but can also be sampled for muscle tissue (fillets) or as fillets in combination with "remainder" tissues that can be added as total selenium content with the fillet, post-analysis, to recreate the whole-body fish concentration. In the latter case, the fish can be used to characterize both human health as well as ecological risk (from larger fish).

Once the choices of plant, invertebrate, fish, or bird egg tissues are decided, further decisions are required for:

- Minimum sample size (with replicates/repeats) for the analytical method to be applied;
- Sample preparation, preservation, and storage;
- Laboratory methods for digestion, extraction, and chemical analysis; and
- Nature and extent of laboratory QA/QC including certified reference materials (CRMs) to be submitted along with samples.

The narrow range between physiological requirements and toxic levels of selenium requires accurate and precise measurements of environmental concentrations in all media. Sediment and tissue may be analyzed by the same laboratory because these are solid media requiring digestion, homogenization, and extraction steps that are often similar and not done well by many laboratories. However, sample preparation for tissue is different from sediment; many laboratories that offer soil analysis are also equipped to analyze wet sediment but have no experience with tissue homogenization (e.g., grinding whole-body fish) and digestion. The laboratory's ability to measure selenium contents of CRMs (ideally at a rate of 1 per 20 samples, but at least 2-3 per batch) with acceptable accuracy and precision should be evaluated before initiating contracts for sample analysis. Similarly, not all tissue laboratories can process sediment, particularly for important associated analyses such as TOC and grain size.

Analytical methods yielding good results by media are briefly described below.

### Water

Water is most often analyzed as total recoverable and dissolved fraction (filtered to < 0.45 or < 0.22 μm) selenium but can also be reported as total selenium or as selenium species in the dissolved fraction. Total selenium as well as individual selenium species in water samples can be determined at sub μg/L levels by hydride generation atomic fluorescence spectrometry (HG-AFS) methods (Ipolyi et al., 2001). Total selenium is measured directly, but for speciation the molecular forms present in the samples are first separated by high-performance liquid chromatography (HPLC) before UV irradiation for generation of the hydride from that is measured by atomic fluorescence.

Selenite can be determined directly by hydride generation; total inorganic selenium (that is, selenate plus selenite) is then determined after chemical reduction of selenate followed by hydride generation. Selenate is then determined by difference. Samples can be oxidized to convert all organoselenides to selenate and then reduced to determine total selenium by hydride generation. Organoselenide concentration is then determined by difference.

A recent improvement to the method of Cutter (1978) was developed that uses manganese as a sacrificial oxidant to halt the oxidation of organoselenide at selenite (Zhang et al., 1999). This makes it possible to quantify organoselenide as the difference between organoselenide plus selenite and selenite-only measurements (that is, a difference between two relatively smaller values). This modification was developed by the University of California, Riverside, and has been applied in recent watershed assessments to quantify selenium speciation (Meixner et al., 2004), but is not commonly used.

Technological advances in separation of selenium species by HPLC and detection by inductively coupled plasma-mass spectrometry (ICP-MS) may now rival the capabilities of hydride, and potentially can offer more detailed information on individual organoselenide species. Anion exchange chromatography (AEC) can directly quantify selenite and selenate, resulting in selenate detection limits that are lower than achievable by a difference method. More recent HPLC developments, when coupled to ICP-MS detection, may be able to produce comparable or better selenium speciation data than hydride generation, and are also attractive because of the capacity to automate such systems (Wallschläger and Roehl, 2001).

The development of collision/reaction cell (CRC) technology is a significant advance in ICP-MS analysis that can lower detection limits and eliminate interferences (Mazan et al., 2002). Because ICP-MS depends on mass/charge (m/z) ratios, polyatomic ions formed in the argon plasma that have nearly the same m/z as selenium can give a false positive signal. A CRC-equipped ICP-MS introduces helium, hydrogen, ammonia or other gases into a cell containing a multiple (typically quadrupole, hexapole or octapole) through which the ion beam passes before entering the mass analyzer. Interfering polyatomic species are eliminated either through collisional dissociation, charge transfer, or kinetic energy discrimination. By reducing spectral interferences, not only is the risk of false positives diminished, but complex matrices such as seawater need not be diluted as much, resulting in lower detection limits. Detection limits are also reduced because isotopes with higher natural abundance (such as $^{80}$Se) may be used because high background count rates from argon dimmers are reduced.

In summary, total selenium in water is readily characterized by hydride generation atomic absorption spectroscopy (HG-AAS) as per Cutter (1978) or HG-AFS as described by Ipolyi et al. (2001). Water can be analyzed for speciation of major selenium species as shown above, using the method developed by Zhang et al. (1999) that incorporates a persulfate digestion, reduction to Se (IV) and Se (VI) and analysis by HG-AAS or using AEC separation and ICP-CRC-MS, HG-AFS, or HG-AAS analysis. Alternatively, excellent results for water can be obtained by inductively-coupled plasma CRC mass spectrometry (ICP-CRC-MS) with total and filtered fractions analyzed by ion chromatography inductively coupled plasma mass spectrometry (IC-ICP-MS) (Wallschläger & Roehl, 2001). It is important to note, however, that samples for Clean Water Act/Safe Drinking Water Act/National Pollutant Discharge Elimination System compliance typically cannot be analyzed using CRC technology.

The volatilization of selenium compounds from water (or plants) may be important in some systems and the analysis and characterization of this component may require the direct trapping of volatile species and analysis of speciation in water (for example, see Lin and Terry, 2003).

### Sediment

Sediment analyses for total selenium are accomplished following strong acid digestion (nitric and perchloric) of the sample followed by HG-AAS (Gao et al., 2000), HG-AFS, or ICP-MS. Various fractions, such as soluble, ligand exchangeable, carbonate, organic matter, or elemental selenium may be analyzed from sediment, as well. Details on methodology are provided by Gao et al. (2000, 2003). Detailed methodology of sequential soil extractions using HG-AAS (as could be applied to sediment) are also provided by Wright et al. (2003). Total Se in sediment can also be analyzed by ICP-CRC-MS following digestion using nitric and hydrofluoric acids. Samples of pulverized rock, soil, or sediment can also be directly analyzed for total selenium contents using instrumental neutron activation analysis (INAA) as can soluble selenium forms in solutions obtained from sequential extractions.

### Tissue

Tissue selenium analysis can be accomplished by graphite furnace atomic absorption spectrometry (GF-AAS; e.g., Krynitsky, 1987), HG-AFS (e.g., Kaneko and Ralston, 2007; Ralston et al., 2007), HG-AAS, ICP-MS, and instrumental neutron activation analysis (INAA; Peterson et al., 2008 in press). For GF-AAS, HG-AAS, ICP-MS, and HG-AFS, tissue sample materials need to be digested with acid treatment before analysis, but samples for INAA are run without the need for organic destruction or pretreatment (see individual articles for differences in sample preparation methods). Total selenium in tissue can also be analyzed using ICP-CRC-MS with appropriate measures taken to correct for physical matrix effects (see Ralston et al., 2008).

It is also possible to speciate selenium in tissue as a means of evaluating transformations and effects of dietary tissues on predators. X-ray absorption spectroscopy (XAS) may be used to characterize various inorganic and organic selenium compounds in tissue (Andrahennadi et al., 2007). Because this method requires time on a properly equipped beamline at a synchrotron radiation facility, it is not a method that is applicable for routine analyses. Various other methods for selenium speciation in biological matrices are available and rely on hyphenated techniques using liquid chromatography, gas chromatography, and either ICP-MS or electrospray ionization-tandem mass spectrometry (ESI-MS-MS) (see Ralston et al., 2008).

## Endpoints and Detection/Reporting Limits

As discussed above, endpoints should focus on the health, reproduction, and survival of the aquatic receptors of concern. Of these, reproduction usually has been found to be the most sensitive endpoint. Collection of biological samples would include tissues that best represent the desired endpoint in addition to those dietary items that are the best indication of exposure to the receptor. For example, concentrations of selenium in fish and bird eggs would provide a better indication of potential reproductive impairment than measurement of selenium in whole-body animals or in selected organs. Similarly, selection of fish and invertebrate species that are likely components of the receptor diet provides a better indication of exposure than a random sampling of fish and invertebrates in the area or than analysis of water or sediment.

It has recently been recommended by USEPA (M. Greenberg, USEPA, pers. comm.) that target analytical reporting limits should be 10 percent of the selected thresholds (water, diet, or tissue). This gives confidence that useful results will be obtained and that detection limits are sufficiently low to provide meaningful comparisons to guidelines or thresholds. Additionally, labs should demonstrate their proficiency according to rigorous QC standards (see Ralston et al., 2008).

Concentration of selenium in all tissue samples should be reported on a dry-weight basis, along with the moisture content of the tissue. This is important because moisture content of plant and animal tissues can vary widely, especially when collecting and handling samples under field conditions, and normalization to dry-weight basis reduces the variability in selenium concentrations.

## Relationship between Selenium Tissue Burdens and Point-source Loadings

Establishing a quantifiable link between point-source loadings and the bioaccumulation of selenium in resident biota can be challenging. However, recent USEPA guidance recommends the water quality chronic criteria for selenium be determined by whole-body fish tissue concentrations (USEPA, 2004). In general, USEPA has suggested that tissue-based standards may be used in a regulatory setting as tissue, as tissue and water, or as water alone (USEPA, 2006c). Regulating waterborne selenium loads requires translating the fish tissue levels in receiving waters to waterborne loading or concentrations from individual discharges. This is a two-part process to (1) establish the quantitative link between ambient water concentrations and fish or birds, and (2) quantify the relationship between the water concentrations contributing to receptor exposure and the specific upstream water concentrations and loads of concern for the regulation of point sources.

Waterborne selenium concentrations may remain stable and additive from multiple sources in some fast-flowing lotic environments. In that example, selenium can be traced through the system using a simple mass balance approach, with resulting calculations of concentration and load for the point source discharge. However, even in those conditions, selenium tissue concentrations in fish are not easily predicted from ambient waterborne concentrations. The primary route of uptake and exposure of selenium to fish is through diet, and the varied and seasonal components of fish diet may be highly variable in terms of uptake of ambient selenium. Establishing ratios or regression relationships between a series of co-located water and tissue samples may provide a simple way of translating between the two media but with significant error and variability in prediction.

When both fish tissue and aqueous selenium concentrations are available for a site, developing a simple X-Y plot of water concentrations (on X axis) vs. fish tissue concentrations (Y axis) provides a simple graphical approach to explore potential relationships. Derivation of the regression equation for the data distribution provides an estimate of the relative strength of the relationship; however, the strength of the relationship will be governed in large part by the quality and quantity of data used in the analysis. When site-specific data are available, this method is preferred, but due to the various factors influencing selenium bioaccumulation, and potentially limited availability of fish resident to the exposure area, there are no guarantees that a useful relationship will be observed. If not, more data may be needed or a more sophisticated model as described below may be required to translate fish tissue concentrations to aqueous concentrations.

In contrast, more complex biodynamic exposure and uptake models incorporate loss and growth rates as well as environmental flux to yield accurate predictions of the level of bioaccumulation in relation to exposure (Peterson and Nebeker, 1992; Luoma and Rainbow, 2005). Each system should be evaluated for whether simple or more complex mechanistic models are needed to predict bioaccumulation, but selenium characterizations are best served by site-specific approaches (for example, see Chapman, 2000).

Despite the ease of relating concentrations from co-located tissue and water samples, most aquatic environments are not so simply modeled. In ponds, lakes, or marine or estuarine embayments selenium is often rapidly sequestered by phytoplankton and other biota and translocated to the sediment. Under those circumstances, the water column concentrations do not match a mass balance estimate based on loads, and a simple mass balance approach will not work. Instead, a more mechanistic model must be constructed that incorporates all selenium gain and loss processes such as:

- Summed multiple sources influent to the receiving water body;
- Atmospheric deposition direct to the water body surface;
- Losses to permanent sediment burial;
- Losses to volatilization;
- Continued internal loading from sediments to the water column (even with no new inputs); and
- Relative concentrations of selenium as selenium species and particulate versus dissolved fractions.

This list shows the factors needed to estimate water column concentrations in the receiving water body, as both seasonal average concentrations and rate processes. Discharge limits are then applied to individual influent sources that are quantitatively linked, through the model, to receiving water concentrations and ultimately to fish tissue bioaccumulation (for example, see Presser and Luoma, 2006).

Only when these factors are accounted for in a detailed mechanistic model can selenium tissue concentrations be related to ambient waterborne concentrations and be quantitatively related to upstream individual discharge concentrations and loads. An example of the multiple factors that determine the quantitative links between fish tissue and waterborne load concentrations is depicted in Figure 4.

## Community-level Fish and Invertebrate Evaluations

Fish and/or invertebrate community sampling may precede reproductive toxicity testing in order to gain an understanding of the species present across selenium gradients and habitats. Structure and function of these communities provides yet another line of evidence in understanding selenium impacts on the aquatic system. Similar to population surveys for key fish species, changes in fish and/or benthic invertebrate community dynamics may be an indicator of contaminant stress. Such measures are already used by USEPA and most states as part of their bioassessment programs to monitor water quality and aquatic ecosystem integrity. Due to the specificity of State biomonitoring programs for waters within their areas of responsibilities, it is recommended that the methods and approaches used to assess selenium impacts for the site of interest consider the State biomonitoring program guidance for area waters. Many States have developed reference site comparison indices to evaluate benthic and fish community impacts for specific regional areas within their borders. These State-specific metrics incorporate habitat quality along with fish and benthic community evaluation metrics, which may be refined for a specific site to aid in teasing out potential impacts due to selenium. Selecting sites based on similarity of habitats as well as proximity to a selenium contaminant source are key factors to consider early on in the study design. Changes in fish communities and populations will be affected by habitat quality and quantity, predator density, food availability and, potentially, selenium exposure. Understanding the fish community and habitat quality and quantity will be important variables in understanding changes in fish population dynamics. Use of habitat-based models that predict fish standing crop, usable area for a life stage, or habitat suitability will be useful tools to consider.

Some key questions that may be addressed by this line of evidence include:

- Is the structure and function of macroinvertebrate or fish communities impaired by selenium contamination?
- Are some of the expected species or species groups missing?
- Can sensitivity to selenium be inferred based on these observations?

To our knowledge, indices or metrics to assess fish or invertebrate community structure and function relative to selenium impacts have not been developed. However, large-scale studies are currently being conducted, such as in the Elk River Valley of British Columbia, that may provide new insights.

Long-term studies of benthic macroinvertebrate response to selenium exposure are limited. Swift (2002) conducted long-term (>1 year) experimental dosing studies of stream mesocosms and found no significant effect on benthic community abundance, diversity, or richness in the high (30 µg/L nominal) and moderate (10 µg/L nominal) experimental units, but *Tubifex* and isopod numbers were reduced. deBruyn and Chapman (2007) examined the literature to assess selenium sensitivity of macroinvertebrates and found that some invertebrates may be sensitive at body burdens similar to those protective of fish.

## Laboratories

Requirements for low-level analysis of selenium in water, sediment, and tissue and the advanced capabilities (sometimes needed) to analyze water for selenium speciation or concentrations in suspended particulate fractions are not often met by a single laboratory. As noted elsewhere (above and in Ralston et al., 2008), the laboratory to be used on a particular project should demonstrate that it can achieve accurate and reproducible results for the particular types of samples to be analyzed. In addition, it should be noted that reporting limits may vary significantly within a laboratory depending on matrix interferences that vary from site to site and among media. This is of particular concern when analyzing samples of sediment or water from marine or hyper-saline environments.

Suggested laboratories for analysis of total or dissolved fractions of selenium in water are laboratories that can usually achieve reporting limits of less than 1 µg/L (preferably 0.1 µg/L when the waterborne criterion is 1 µg/L).

Laboratories for sediment or tissue analysis of selenium must be able to achieve adequately low reporting limits (less than 1 µg/g dw) and pass routine QA/QC tests including the analysis of reference material.

### Recommended Laboratories

Based on recent experience, the following laboratories (presented in alphabetical order) have performed satisfactorily in analyzing samples of various media. It is not the intent of this guide to give blind endorsement of these laboratories, however, because some good laboratories may go bad, and other laboratories become good; this depends strongly on the personnel doing the work.

**Applied Speciation and Consulting, LLC: Water, water speciation, sediment, tissue**
Russell Gerads, (206) 219-3779, russ@appliedspeciation.com
953 Industry Drive
Tukwila, WA 98188
http://www.appliedspeciation.com

**Battelle, Marine Sciences Laboratory: Water**
Brenda LaSorsa, (360) 681-4565, brenda.lasorsa@pnl.gov
Marine Sciences Laboratory
Pacific Northwest National Laboratory
1529 West Sequim Bay Road
Sequim, WA 98382

**Brooks Rand: Water, sediment, tissue**
Elizabeth Madonick, (206) 632-6206, elizabeth@brooksrand.com
3958 6th Ave. NW
Seattle, WA 98107
www.brooksrand.com

**CH2M HILL, Applied Sciences Laboratory: Water, sediment.**
Kathy McKinley, (541) 768-3144, kmckinle@ch2m.com
2300 North West Walnut Boulevard
Corvallis, OR 97330

**Frontier: Water**
(206) 622-6960
414 Pontius Avenue North
Seattle WA 98109
http://www.frontiergeosciences.com/

**Laboratory and Environmental Testing (LET), Inc.: Tissue, sediment**
Ed Hinderberger, (573) 874-2481, edhindermo@aol.com
3501 Berrywood Drive
Columbia, MO 65201

**Pace Analytical: Tissue**
Tod Noltemeyer, (608) 232-3300, Tod.Noltemeyer@pacelabs.com
6409 Odana Road, Suite B
Madison, WI 53719

**Trent University: Water, soil, sediment, tissue, water speciation, soil speciation, sediment speciation, tissue speciation (limited), development of new specialized analysis methods**
Dirk Wallschläger, (705) 748-1011 x 7378, DWallsch@TrentU.ca
1600 West Bank Dr
Peterborough, ON K9J 7B8, Canada

**U.C. Davis, California Animal Health and Food Safety Laboratory: Tissue**
Birgit Puschner, (530) 752-6322, bpuschner@ucdavis.edu
West Health Sciences Dr.
Davis, CA 95616

**U. of Kentucky Department of Plant and Soil Sciences Soil: water, tissue**
Jason Unrine, (859) 257-1657, jason.unrine@uky.edu
N212-N Agricultural Science Center North
Lexington, KY 40546-0091

**U. of Missouri Research Reactor: Soil, water tissue**
J. David Robertson, (573) 882-5346, RobertsonJo@missouri.edu
University of Missouri Research Reactor
Research Park Drive
Columbia, MO 65211

**U.N.D. Energy & Environmental Research Center: Soil, water, tissue**
Nick Ralston, (701) 777-5066, nralston@undeerc.org
15 North 23rd Street
Grand Forks, ND 58202

# Conclusions and Recommendations

The goal of this document is to provide a standard guide for field and laboratory assessments of selenium bioaccumulation that can be applied in different environmental settings relative to developing and interpreting a tissue-based selenium value. It describes a tiered approach (or framework) for conducting assessments and includes discussion of decision trees, conceptual models and data quality objectives toward defining what should be done, and describes recommended sampling and monitoring for conducting the assessment.

This guide focuses on aquatic-dependent birds as well as aquatic organisms (primarily fish). It integrates some of the components of selenium assessment for aquatic systems that are presented in more detail in other guides that focus on selenium analyses (instrumental approaches and sample considerations) and on tissue endpoint assessment (appropriate tissue types, appropriate endpoints/life stages/sampling protocols/etc. for measuring effects, and association of tissue thresholds with population/community effects in the field).

Extensive data have been developed for both fish and bird species, and there is an emerging trend that there is a clear egg-selenium threshold at which effects begin to be observed. Maternal bioaccumulation and subsequent transfer of selenium to eggs may be the most important factor in understanding selenium toxicity. Threshold levels for reproductive effects have been estimated for various fish and bird species, though they vary considerably among species and test conditions. For fish, there also appear to be differences between cold water species and warm water species.

Relationships between tissue selenium concentrations in fish and invertebrates and aqueous concentrations vary depending on site-specific conditions. In a flow-through treatment wetland for refinery effluent (Richmond Refinery Water Enhancement Wetland, California), selenium concentrations in invertebrates were positively related to variability in waterborne selenium concentrations with a 2-week time lag. In contrast, emerging data for selenium in fish tissues and aqueous selenium concentrations from stream systems in southeast Idaho are showing strong relationships between co-located water and tissue samples. Developing the association between tissue and aqueous selenium will be important for all sites where selenium is thought to be a potential concern.

While most State standards are based on a waterborne concentration, the state-of-the-science indicates that effects are more strongly related to tissue residues than waterborne concentrations. Current USEPA draft guidance recommends that the chronic criteria for selenium be based on whole-body fish tissue concentrations (USEPA, 2004). More recent information concerning the revision of the draft criterion suggests USEPA is leaning toward the use of a two-part criterion (first tier, a mature ovary measurement; second tier, whole body). The mature ovaries are the target organ and measurements show less variability than whole body.

Because data for fish ovaries have not been developed from many sites, conversion factors will need to be developed that allow translation from whole-body tissue residues to ovary tissue residue for a species. Where work is contemplated or currently underway, it is advisable to develop the conversion factors for the site. For these reasons, it will become ever more important to develop the relationship between waterborne concentrations and fish tissue concentrations in order to translate between a tissue-based effects criterion and water. USEPA is currently working on implementation procedures to facilitate this process, but the currently held wisdom is that these relationships will be best developed on a site-specific basis.

Reliable analyses of selenium in environmental media (water, sediment, etc.) and tissues are an essential component of conducting site-specific assessments of selenium accumulation. This guide provides a general summary of relevant information; more detailed information concerning analytical methods is presented in the Selenium Analysis Workgroup effort being conducted concurrently with this guide (Ralston et al., 2008).

Overall, we recommend that the approach for site-specific assessment must be flexible, depending on what is appropriate for the situation. Risk management/remediation decisions should be based on integration of biology and chemistry data. While a comprehensive weight-of-evidence approach is ideal for site-specific regulatory applications, it should be noted that most regulatory agencies will apply numeric criteria in a straightforward manner. Thus, the burden of accumulating data and information for a tiered weight-of-evidence approach will, in most cases, fall upon the regulated entity.

The guide recommends using a tiered approach because that provides the most resource-effective framework for conducting assessment of selenium in aquatic systems. The following are key components of the tiered approach:

- Comparison of tissue concentrations to tissue residue guidelines or adopted water quality criteria/guidelines;
- Reproductive toxicity testing; and
- Assessment of fish populations in the area of interest.

This approach is consistent with that used for conducting ecological risk assessments, and the ecological risk assessment paradigm is useful toward the goal of assessing exposure and effects of selenium. Using this approach, along with decision trees, conceptual models and data quality objectives, helps ensure that all the important work is done, but only that work that contributes to the decision-making process.

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# Tables

TABLE 1
Thresholds for Selenium Effects (Health, Reproductive, Teratogenesis, or Survival) in Fish Based on Concentrations of Selenium in Diet

| Dietary Concentration (µg/g, dw) | Approach | Effects | Reference(s) |
|---|---|---|---|
| 2–4 | Synthesis | Threshold ranges for reproductive failure | Engberg et al., 1998 |
| 3 | Synthesis | Maximum allowable concentrations (protective of reproduction) | Lemly, 2002 |
| 2–4 | Synthesis | Diagnostic residues; ecosystem contamination sufficient to cause reproductive impairment | Lemly, 1998b |
| 3–7 | Synthesis | Range of concern: toxicological and reproductive effects a certainty if upper limit exceeded: impaired development and survival in larval fish | Engberg et al., 1998; USBR et al., 2004, Hamilton et al., 1996; Hamilton, 2004; Presser et al., 2004 |
| 3–8 | Synthesis | Reproductive impairment threshold (LOAEL) via lethal larval exposure (salmon, bluegill, razorback sucker) | Skorupa, 1998a |
| 2–5.9 | Belews Lake, North Carolina | Teratogenesis in fry of four recovering fish species (common carp [*Cyprinus carpio*], bluegill [*Lepomis macrochirus*], largemouth bass [*Micropterus salmoides*], mosquitofish [*Gambusia affinis*]) | Lemly, 1997b, 2002 |
| 4.6 | Lab | Mortality in razorback sucker (*Xyrauchen texanus*) larvae | Hamilton, 2002, 2004; Hamilton et al., 2005b |
| 3.2–5.3 | Lab | Reduced growth in Chinook salmon (*Oncorhynchus tshawytscha*) swim-up larvae | Hamilton et al., 1990 |
| 5.1 | Lab | 40% overwinter mortality (winter stress) in juvenile bluegill | Lemly, 1993b |
| 5–20 | Synthesis | Sufficient to load eggs beyond teratogenic threshold | Lemly, 1997a, 1998a, 2002 |
| 30–35 | Synthesis | Complete reproductive failure (100% effect level) in bluegill; parental exposure | Coyle et al., 1993; Woock et al., 1987 as cited in Skorupa, 1998a |
| 15–57 | Belews Lake, North Carolina (1973-1984) | Massive poisoning of fish community (16 of 20 species disappeared; two species rendered sterile, but persisted as aging adults; one occasionally re-colonized as adults; and one unaffected; deformities in survivors; some recovery after selenium removal) | Cumbie and Van Horn, 1978; Lemly, 1985, 1997b, 1998a |
| 155–290 | Kesterson Reservoir (Pond 2), California | Massive poisoning of fish and birds, including deformities in birds | Saiki and Lowe, 1987; Ohlendorf, 1989; Presser and Ohlendorf, 1987 |

Notes:

Source: Presser and Luoma, 2006

µg/g = microgram per gram
dw = dry weight
LOAEL = lowest observable adverse effect level

TABLE 2
Thresholds for Selenium Effects (Health, Reproductive, Teratogenesis, or Survival) in Birds Based on Concentrations of Selenium in Diet

| Dietary Concentration (µg/g, dw) | Approach or Site | Effects | Species | Reference(s) |
|---|---|---|---|---|
| 4.87 (CI 3.56–5.74) | Synthesis of lab data | Hatchability in mallards (10% effect level / 95% confidence boundaries) | Mallard (*Anas platyrhynchos*) | Ohlendorf, 2003 |
| 4.4 (CI 3.8–4.8) | Synthesis of lab data | $EC_{10}$ for duckling mortality | Mallard | Bill Adams analyses presented in Ohlendorf, 2007 |
| 3.85–7.7 (diet based on 10% moisture) | Lab | Reduced hatching success in mallards (33% at 7.7 µg/g); reduced growth and weight in hatchlings | Mallard | Stanley et al., 1996 |
| 7.7 (diet based on 10% moisture) | Lab | Reduction in number of surviving mallard ducklings produced per female | Mallard | Stanley et al., 1996 |
| 8.8/4.4/6.2 (diet based on 10% moisture) | Lab | 8.8 - LOAEL, 4.4 - NOAEL, 6.2 - Geometric Mean Reduction (17%) in survival of mallard ducklings; mean decrease (43%) in number of 6-day-old ducklings | Mallard | Heinz et al., 1989 |
| 6.0 | Lab | Adverse effect on body condition of male American kestrels | American kestrels (*Falco sparverius*) | Yamamoto and Santolo, 2000 |
| 7.7–8.8 (diet based on 10% moisture) | Lab | Dietary threshold of teratogenic effects in mallards; above upper threshold, rate of deformity rises sharply | Mallard | Stanley et al., 1996 |
| 7.7–8.8 (diet based on 10% moisture) | Lab | Dietary threshold of mallard duckling mortality (parental exposure) | Mallard | Stanley et al., 1996 |

Notes:

Source: Modified from Presser and Luoma (2006); data compiled by the Great Salt Lake Science Panel (see Ohlendorf 2007)

µg/g = microgram per gram
CI = 95% confidence interval
dw = dry weight
$EC_{10}$ = 10% effect concentration
LOAEL = lowest observed adverse effect level
NOAEL = no observed adverse effect level

TABLE 3
Thresholds for Selenium Effects (Health, Reproductive, Teratogenesis, or Survival) in Fish Based on Selenium Concentrations in Tissue of Fish

| Tissue Concentration (μg/g, dw) | Location | Effect/Threshold | Reference(s) |
|---|---|---|---|
| 4–12 (whole-body) | Synthesis | Range of concern; toxicological and reproductive effects a certainty if upper limit exceeded/whole-body | Engberg et al., 1998 |
| 4 (whole-body)<br>8 (muscle)<br>12 (liver)<br>10 (egg) | Synthesis | Maximum allowable concentration (protective of reproduction) | Lemly, 2002 |
| 5–7 (whole body)<br>6–8 (muscle)<br>15–20 (liver)<br>5–10 (egg)<br>8–12 (larvae and fry) | Synthesis | Diagnostic residues for reproductive impairment (deformity or mortality of larvae/fry); applies to centrarchids, fathead minnows, salmonids, percichthyids | Lemly, 1998b |
| 4–6 (whole-body)<br>7–13 (gonad/egg) | Synthesis | Reproductive impairment (10% effect level) in sensitive species (perch, bluegill, salmon) | Skorupa, 1998a; Presser et al., 2004 |
| 4–6.5 (whole-body) | Lab and synthesis | Growth and survival (swim-up Chinook salmon larvae) | Hamilton et al., 1990; Hamilton 2002, 2003 |
| 3.6–8.7 (whole-body) | Field | Survival (razorback sucker larvae) | Hamilton et al., 1996, 2005a, b; Hamilton, 2002, 2004 |
| 5.85 (whole-body) | Lab | 40% overwinter mortality in juvenile bluegill (winter stress) | Lemly, 1993b |
| 6 cold-water (whole-body)<br>9 warm-water (whole-body)<br>17 (ovary) | Synthesis | Recommended toxicity guidelines (10% effect level) | DeForest et al., 1999 |

TABLE 3
Thresholds for Selenium Effects (Health, Reproductive, Teratogenesis, or Survival) in Fish Based on Selenium Concentrations in Tissue of Fish

| Tissue Concentration (µg/g, dw) | Location | Effect/Threshold | Reference(s) |
|---|---|---|---|
| 10 (egg)<br>6–17 (egg) | Synthesis | Rapid rise in deformities (terata) for centrarchids | Lemly, 1993b |
| 12.5 (egg based on 52% moisture) | Field (eggs and milt) | Rapid rise in edema and deformities in rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) fry (parental exposure) | Holm et al., 2003 |
| 4.3 (muscle translation) | Lab (fish rearing) | | |
| 18–22 (egg based on 52% moisture) | Field(eggs and milt) | Range of 15% effect level (edema, skeletal or craniofacial deformities) in rainbow trout swim-up fry | Holm et al., 2005 |
| 6.4–7.6 (muscle translation) | Lab (fish rearing) | | |
| 40–125 (whole-body)<br>25–200 (muscle)<br>20–170 (egg) | Field | 16 species extirpated; 10–70% rates of teratogenesis | Cumbie and Van Horn, 1978; Lemly 1985, 1997b, 1998a, 2002 |
| 10 (egg) | Synthesis | Centrarchids (bluegills); Equivalent to a whole body value of 4 µg/g dw | Lemly, 1993b as reported in Chapman, 2007 |
| 17 (egg) | Synthesis | Threshold determined using 21 studies representing 8 fish species (warm-water and cold-water); using the procedure in USEPA (2004) to convert the 7.9 µg/g dw whole-body draft criterion value to an egg value | USEPA, 2004 as reported in Chapman, 2007 |
| >16–18 (egg) | Lab | Cutthroat trout (*Oncorhynchus clarki*); mean values, no effects | Hardy, 2005 as reported in Chapman, 2007 |
| >20.6 (egg) | Field/Lab | Cutthroat trout; no selenium-related deformities; next highest concentration tested, 46.6 µg/g dw, did not produce viable fry | Rudolph et al., 2008; Chapman, 2007 |
| 21.2 (egg) | Field | Cutthroat trout; mean value; no effects found at egg selenium concentrations as high as 81.3 µg/g dw | Kennedy et al., 2000 as reported in Chapman, 2007 |

TABLE 3
Thresholds for Selenium Effects (Health, Reproductive, Teratogenesis, or Survival) in Fish Based on Selenium Concentrations in Tissue of Fish

| Tissue Concentration (µg/g, dw) | Location | Effect/Threshold | Reference(s) |
|---|---|---|---|
| 25.6 (egg) | Field/Lab | White sucker; mean value; corresponds to a mean frequency of deformity of 12.8% | de Rosemond et al., 2005 as reported in Chapman, 2007 |
| >26.4–31.2 (egg) | Field | Brook trout; no increase in larval deformities at 6.6 and 7.8 µg/g ww; converted to dw based on 75% moisture | Holm et al. 2005, as reported in Chapman, 2007 |
| 32–40 (egg) | Field | Rainbow trout; threshold between 8-10 µg/g ww; converted to dw based on 75% moisture | Holm et al. 2005, as reported in Chapman, 2007 |
| 33.6 (egg) | Lab | Northern pike: $EC_{20}$ for larval deformities relative to reference | Muscatello et al. 2006, as reported in Chapman, 2007 |
| 4–6 Marginal effects<br>>6 Substantive effects | Synthesis | Whole-body thresholds | Presser et al., 2004 |
| 7.91 (whole-body)<br>5.85 (whole-body) | Synthesis | Draft criterion, winter stress conditions; a concentration of 5.85 µg/g dw measured in the summer or fall would trigger repeated monitoring in the winter | USEPA, 2004 |

Notes:

Source: Modified from Presser and Luoma (2006) and Chapman (2007)

µg/g = microgram per gram
dw = dry weight
$EC_{20}$ = 20% effect concentration
ww = wet weight

TABLE 4
Thresholds for Selenium Effects (Health, Reproductive, Teratogenesis, or Survival) in Birds Based on Selenium Concentrations in Bird Eggs

| **Egg Concentration (µg/g, dw)** | **Approach or Site** | **Effects** | **Species** | **Reference(s)** |
|---|---|---|---|---|
| 12.5<br>(CI 6.4–16.5) | Synthesis of lab data | Hatchability in mallards (10% effect level / 95% confidence boundaries) | Mallard (*Anas platyrhynchos*) | Ohlendorf, 2003 |
| 10 | Synthesis of lab data | NOAEL | Mallard | Adams et al., 2003 |
| 12 - 16 | Synthesis of lab data | $EC_{10}$ for duckling mortality | Mallard | Adams et al., 2003 |
| 9.0 | Synthesis of lab data | Impaired clutch viability (8.2% effects level) | Mallard | Lam et al., 2005 |
| 14 | Synthesis of field data | Reduced egg hatchability (11.8% effects level) | Black-necked stilt (*Himantopus mexicanus*) | Lam et al., 2005 |
| 8.2<br>(egg based on 73% moisture) | Field | 16% depression in egg viability (2.2 µg/g ww and 7.3 µg/g dw in paper) | Spotted sandpiper (*Actitis macularia*) | Harding et al., 2005 |
| 6.0 | Synthesis of field data | Threshold (3% effect level) of hatchability | Black-necked stilt | Skorupa, 1998b, 1999 |
| 5.1<br>(egg based on 78.4% moisture) | Field | 15% depression in egg viability (1.1 µg/g ww and 8.4 µg/g dw in paper) | American dipper (*Cinclus mexicanus*) | Harding et al., 2005 |
| 22 | Field | Threshold for reduced egg hatchability | Red-winged blackbird (*Agelaius phoeniceus*) | Harding, 2008 |

Notes:

Source: Modified from Presser and Luoma (2006); data compiled by the Great Salt Lake Science Panel (see Ohlendorf, 2007)

µg/g = microgram per gram
CI = 95% confidence interval
dw = dry weight
$EC_{10}$ = 10% effect concentration
NOAEL = no observed adverse effect level

TABLE 5
Data Quality Objectives for Developing a Site-specific Selenium Standard for Great Salt Lake

| Step | DQO Guidance of Purpose and Outputs of Step | Great Salt Lake Project |
|---|---|---|
| 1. Problem Statement | **Purpose:** Clearly define the problem that requires new environmental data so that the focus of the study will be clear and unambiguous.<br><br>**Outputs From This Step**<br>• A concise description of the problem.<br>• A list of the planning team members and identification of the decision maker.<br>• A summary of available resources and relevant deadlines for the study. | **Problem:** The open waters of the GSL are protected for their current beneficial uses (identified as "Aquatic Wildlife") through the application of a narrative criteria clause, rather than a numerical value. Due to the highly individual nature of the Great Salt Lake's water, the Utah Department of Environmental Quality (DEQ) has not yet identified a numeric water quality standard (i.e., selenium concentration) specific to the GSL. The goal of this project is to complete a group of interrelated studies that will contribute to the establishment of an interim standard for selenium by the third quarter of 2007.<br><br>Essential components of the GSL ecosystem, as related to the establishment of the site-specific standard, have been identified in a detailed conceptual model for selenium cycling in the GSL (Bill Johnson et al., CWECS, University of Utah). These DQOs (including those for each of the individual projects) and the accompanying scopes of work describe the approach for obtaining information that is needed for establishment of the site-specific standard. The main focus of the work is to determine loading of selenium to GSL, its distribution within the lake, and transfer factors from one medium to another (e.g., from diet to bird eggs).<br><br>**Planning team members:** Dr. Mike Conover, Clay Perschon, Dr. Wayne Wurtsbaugh, Brad Marden, Dr. David Naftz, and Dr. William Johnson (Principal Investigators); Dr. Harry Ohlendorf, Dr. Earl Byron, Gary Santolo, and Daniel Moore (Project Advisors); Jeff DenBleyker (Project Manager); with ultimate decision authority by Utah DEQ, considering input by the GSL Steering Committee and GSL Science Panel.<br><br>**Resources:** Current estimated budget for this work is about $1,342,000 (plus $124,000 in USGS cost-sharing). Technical expertise for conducting the field studies is available from the CWECS team members, who also will provide needed equipment. Analytical laboratory services are available from a limited number of commercial laboratories for completing the selenium and other analyses. Selenium-related expertise and project management support will be provided by CH2M HILL project advisors and the project manager.<br><br>**Deadlines:** Although deadlines vary among the individual projects, the initial focus is to provide sufficient information by late 2006 or early 2007 for Utah DEQ to establish an interim standard for selenium by the third quarter of 2007. It is expected that initial results will be available for selenium concentrations (and their significance) in bird eggs (California gulls, American avocets, black-necked stilts), common invertebrates (brine flies and brine shrimp), inflowing waters from various sources, ambient waters (as waterborne selenium concentration and also as dissolved gas) of Gilbert Bay (i.e., the "open waters" of GSL), and sediment (including bed sediment and material being deposited to the sediment). |

TABLE 5
Data Quality Objectives for Developing a Site-specific Selenium Standard for Great Salt Lake

| Step | DQO Guidance of Purpose and Outputs of Step | Great Salt Lake Project |
|---|---|---|
| 2. Decision Statements | **Purpose**: Define the decision(s) that will be resolved using data to address the problem.<br><br>**Approach**: Identify the key question that the study attempts to address and alternative actions that may be taken, depending on the answer to the key study question.<br><br>**Outputs From This Step**<br><br>• A statement of the decision that must be resolved using data in order to address or solve the problem.<br>• A list of possible actions or outcomes that would result from each resolution of the decision statement.<br><br>*Note from EPA guidance on DQO: If the principal study question is not obvious and specific alternative actions cannot be identified, then the study may fall in the category of exploratory research, in which case this particular step of the DQO Process may not be needed.* | **Decisions:** The overall question to be resolved can be stated as "What is the acceptable waterborne concentration of selenium that will be appropriately protective of beneficial uses of Great Salt Lake waters?" More specific questions that support this overall decision are presented in the individual project-specific DQOs. I general, they include the following:<br><br>• What are the transfer factors that describe relationships between selenium concentrations in bird diets and the concentrations found in bird eggs?<br>• What is the relative importance (based on selenium concentrations and their availability) of various food-chain exposure pathways for aquatic wildlife?<br>• Are significant ecological effects occurring in aquatic wildlife? If so, to which ones and at which locations? What are the associated selenium concentrations in tissues (including bird blood, liver, and eggs)?<br>• What are the sources of waterborne selenium entering GSL, and what is the relative significance of each of the various sources?<br>• What are the most important processes that affect the partitioning, cycling, and release of selenium in the GSL open waters?<br><br>**Possible outcomes:**<br><br>• Information is adequate to quantify relationships among trophic levels and to conclude that current selenium loadings to GSL have a measurable adverse effect on aquatic wildlife in the open-water GSL ecosystem. Steps should be taken to reduce present and future selenium loadings by establishing a more protective site-specific standard for selenium.<br>• Information is adequate to quantify relationships among trophic levels and to conclude that current selenium loadings to GSL have no measurable adverse effect on aquatic wildlife in the open-water GSL ecosystem. Future selenium loadings to GSL can be maintained at this level or increased concurrent with low-intensity water-quality and biological monitoring.<br>• Information is not adequate to quantify relationships among trophic levels or to determine whether current selenium loadings to GSL have a measurable adverse effect on aquatic wildlife in the open-water GSL ecosystem. Further studies are needed to make a defensible conclusion about the significance of effects. |

TABLE 5
Data Quality Objectives for Developing a Site-specific Selenium Standard for Great Salt Lake

<table>
<tr><th>Step</th><th>DQO Guidance of Purpose and Outputs of Step</th><th>Great Salt Lake Project</th></tr>
<tr><td>3. Inputs to the Decision</td><td>Purpose: The purpose of this step is to identify the informational inputs that will be required to resolve the decision, and to determine which inputs require environmental measurements.<br>Activities<br>• Identify the information that will be required to resolve the decision.<br>• Determine the sources for each item of information identified.<br>• Identify the information that is needed to establish the action level for the study.<br>• Confirm that appropriate field sampling techniques and analytical methods exist to provide the necessary data.<br>Outputs From This Step<br>• A list of informational inputs (including sources and potential action levels) needed to resolve the decision.<br>• The list of environmental variables or characteristics that will be measured.</td><td>Informational inputs:<br>• Trophic transfer factors from diet to birds and ecological significance of selenium to avian aquatic wildlife at representative locations; Project 1<br>• Selenium bioaccumulation in brine flies (including larvae, pupae, and adults) and brine shrimp (including cysts as well as whole-body tissue) and the seasonal and spatial availability of these food-chain organisms for aquatic wildlife; Project 2<br>• Selenium concentrations and flow volume from sources entering GSL; Project 3<br>• Selenium flux from the water column to the atmosphere and to the lake bottom as well as remobilization from sediment to the water column; Project 4<br>Variables/characteristics to be measured:<br>• Selenium in the following media (see project-specific DQOs for more details):<br>– Gull, avocet, and stilt blood, liver, and eggs<br>– Eared grebe tissues (blood and liver)<br>– Duck tissues (blood and liver)<br>– Periphyton and brine flies (larvae, pupae, and adults)<br>– Seston and brine shrimp (whole-body tissues and cysts)<br>– Inflow water<br>– Ambient waters of Gilbert Bay (as waterborne total, dissolved volatile, and vapor selenium concentrations)<br>– Particulate phase in water column<br>– Sediment (submerged sediment cores and exposed sediment)<br>• Other variables (see project-specific DQOs for more details):<br>– Incidence of embryo mortality and abnormalities in nesting birds<br>– Body condition of grebes and ducks<br>– Periphyton/detrital biomass<br>– Brine fly larval and pupal density<br>– Brine shrimp population characteristics (e.g., biomass, abundance, age structure)<br>– $^{13}C$ and $^{15}N$ to correlate with selenium concentrations in seston and brine shrimp<br>– Flow of water from various sources<br>– Sediment flux (via sediment traps)<br>– Mixing of Deep Brine Layer with Shallow Layer between and during storm and wind events (using turbidimeter and thermistor strings)</td></tr>
</table>

TABLE 5
Data Quality Objectives for Developing a Site-specific Selenium Standard for Great Salt Lake

| Step | DQO Guidance of Purpose and Outputs of Step | Great Salt Lake Project |
|---|---|---|
| 4. Study Boundaries | **Purpose:** Specify the spatial and temporal circumstances that are covered by the decision.<br>**Activities**<br>• Define the domain or geographic area within which all decisions must apply.<br>• Specify the characteristics that define the population of interest.<br>• When appropriate, divide the population into strata that have relatively homogeneous characteristics.<br>• Define the scale of decision making.<br>• Determine when to collect data.<br>• Determine the time frame to which the study data apply.<br>• Identify any practical constraints on data collection.<br>**Outputs From This Step**<br>• Characteristics that define the domain of the study.<br>• A detailed description of the spatial and temporal boundaries of the decision.<br>• A list of any practical constraints that may interfere with the study. | **Spatial:** The project area is defined as the open waters of the Great Salt Lake (also referred to as Gilbert Bay) located north and west of Farmington Bay, west of the Weber River input, and south of Promontory Point, Bear River Bay, and the North Arm (bounded by the railroad causeway).<br>**Temporal:** The maximum period of data collection will be from mid-April 2006 through March 2007 for Project 1 and through February 2008 for Project 3. However, it is expected that other projects will provide initial results by late 2006 and will be completed in 2007.<br>**Practical constraints on data collection:** Weather is the major constraint for all of the projects, because storms can limit our ability to conduct any of the sampling and measurement activities on the lake. Availability of boats and other field equipment, as well as equipment functionality, also may limit some activities. Methodology for quantitative sampling of brine fly larvae and pupae has not yet been tested on the lake. |

TABLE 5
Data Quality Objectives for Developing a Site-specific Selenium Standard for Great Salt Lake

| Step | DQO Guidance of Purpose and Outputs of Step | Great Salt Lake Project |
| --- | --- | --- |
| 5. Decision Rules | **Purpose**: The purpose of this step is to integrate the outputs from previous steps into a single statement that describes the logical basis for choosing among alternative actions.<br>**Activities**<br>• Specify the parameter that characterizes the population of interest.<br>• Specify the action level for the study.<br>• Combine the outputs of the previous DQO steps into an "if...then..." decision rule that defines the conditions that would cause the decision maker to choose among alternative actions.<br>**Outputs From This Step**<br>• An "if...then..." statement that defines the conditions that would cause the decision maker to choose among alternative courses of action. | • If information is adequate to quantify relationships among trophic levels and to conclude that current selenium loadings to GSL have a measurable adverse effect on aquatic wildlife in the open-water GSL ecosystem, then the Science Panel will assist the Utah DEQ and the Steering Committee in establishing a site-specific selenium standard to reduce selenium loading.<br>• If information is adequate to quantify relationships among trophic levels and to conclude that current selenium loadings to GSL have no measurable adverse effect on aquatic wildlife in the open-water GSL ecosystem, then the Science Panel will assist the Utah DEQ and the Steering Committee in establishing a site-specific selenium standard, presumably maintaining the current level or increasing it, concurrent with low-intensity water-quality and biological monitoring.<br>• If information is not adequate for the Science Panel to quantify relationships among trophic levels or to determine whether current selenium loadings to GSL have a measurable adverse effect on aquatic wildlife in the open-water GSL ecosystem, then further studies will be recommended to provide the needed information for the Science Panel. |

TABLE 5
Data Quality Objectives for Developing a Site-specific Selenium Standard for Great Salt Lake

| Step | DQO Guidance of Purpose and Outputs of Step | Great Salt Lake Project |
|---|---|---|
| 6. Tolerable Limits on Decision Rules | **Purpose**: Specify the decision maker's acceptable limits on decision errors, which are used to establish appropriate performance goals for limiting uncertainty in the data.<br><br>**Activities**<br>• Determine the possible range of the parameter of interest.<br>• Define both types of decision errors and identify the potential consequences of each.<br>• Specify a range of possible parameter values where the consequences of decision errors are relatively minor (gray region).<br>• Assign probability values to points above and below the action level that reflect the acceptable possibility for the occurrence of decision errors.<br>• Check the limits on decision errors to ensure that they accurately reflect the decision maker's concern about the relative consequences for each type of decision error.<br><br>**Outputs From This Step**<br>• The decision maker's acceptable decision error rates based on a consideration of the consequences of making an incorrect decision. | These outputs are more applicable to specific studies than to the overall DQOs, and are presented as the study-specific limits applicable to the precision, accuracy, representativeness, completeness, and comparability of the data, including an appropriate quality assurance/quality control plan, for Projects 1, 2, 3, and 4. |

TABLE 5
Data Quality Objectives for Developing a Site-specific Selenium Standard for Great Salt Lake

| Step | DQO Guidance of Purpose and Outputs of Step | Great Salt Lake Project |
|---|---|---|
| 7. Optimization of the Sampling Design | **Purpose**: Identify the most resource-effective sampling and analysis design for generating data that are expected to satisfy the DQOs.<br>**Activities**<br>• Review the DQO outputs and existing environmental data.<br>• Translate the information from the DQOs into a statistical hypothesis.<br>• Develop general sampling and analysis design alternatives.<br>• For each design alternative, formulate the mathematical expressions needed to solve the design problems.<br>• For each design alternative, select the optimal sample size that satisfies the DQOs.<br>• Select the most resource-effective design that satisfies all of the DQOs.<br>• Document the operational details and theoretical assumptions of the selected design in the Sampling and Analysis Plan.<br>**Outputs From This Step**<br>• The most resource-effective design for the study that is expected to achieve the DQOs, selected from a group of alternative designs generated during this step. | These outputs are more applicable to specific studies than to the overall DQOs, and are presented as the study-specific limits applicable to the precision, accuracy, representativeness, completeness, and comparability of the data, including an appropriate quality assurance/quality control plan, for Projects 1, 2, 3, and 4. |

# Figures

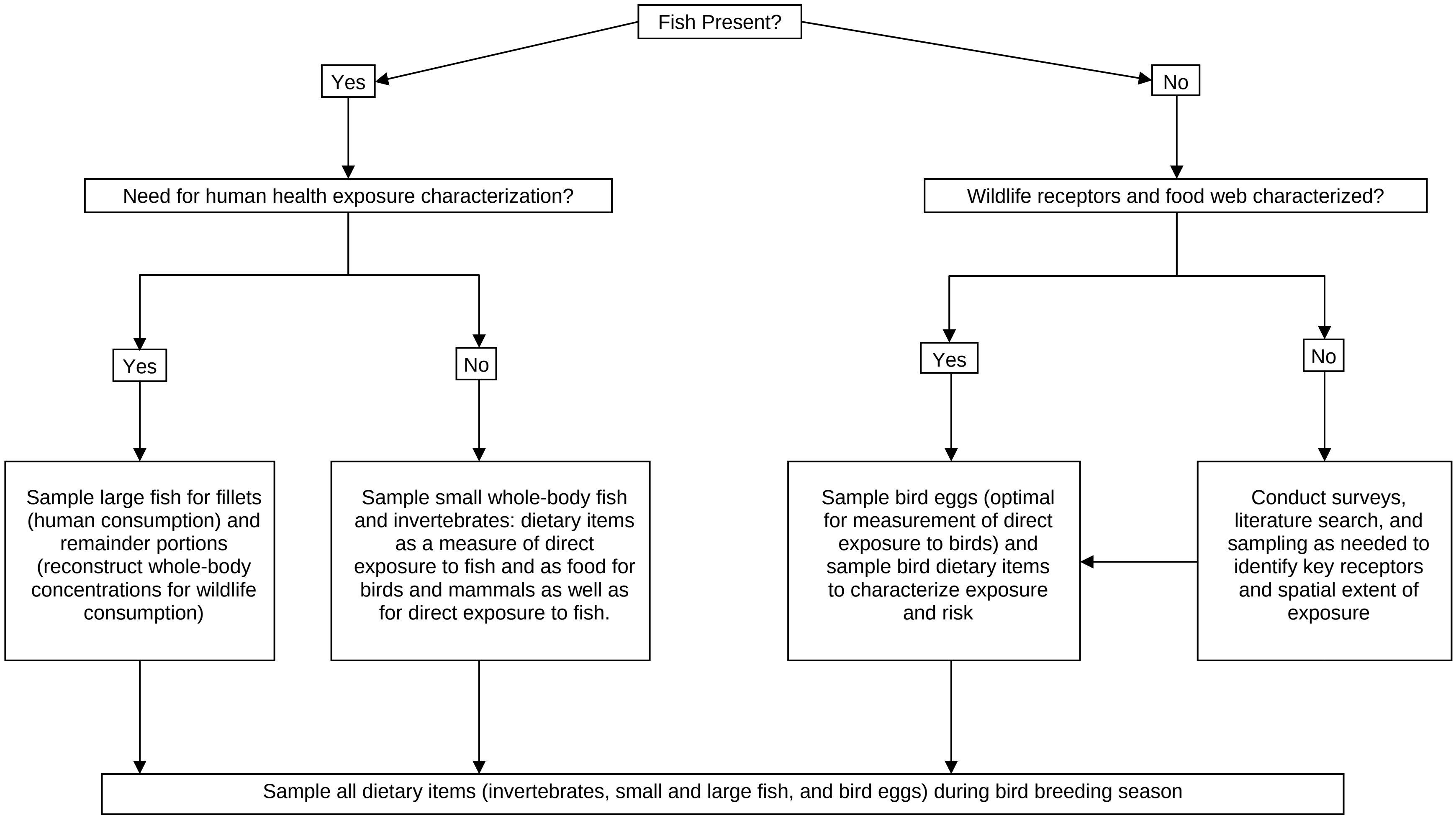
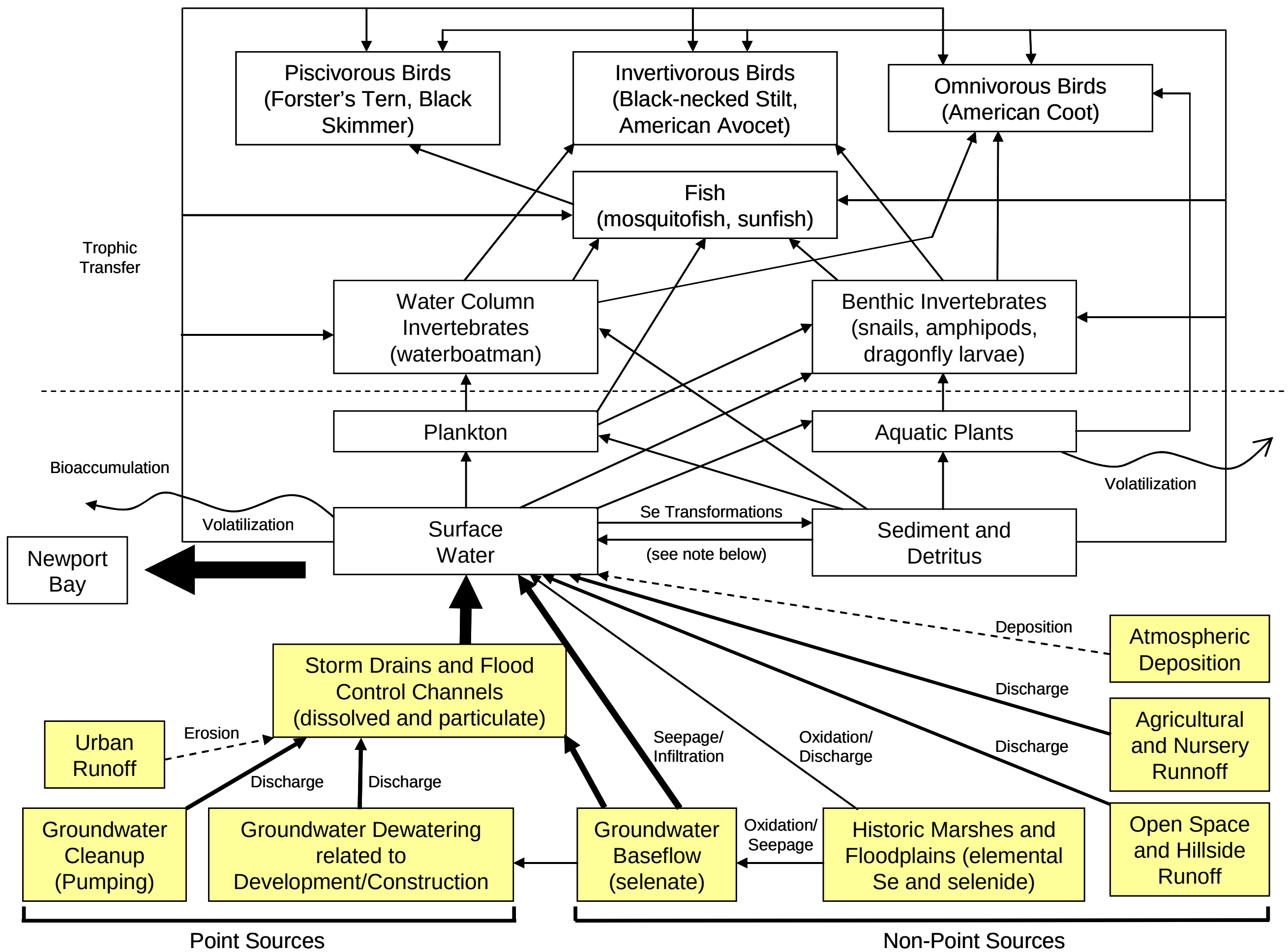
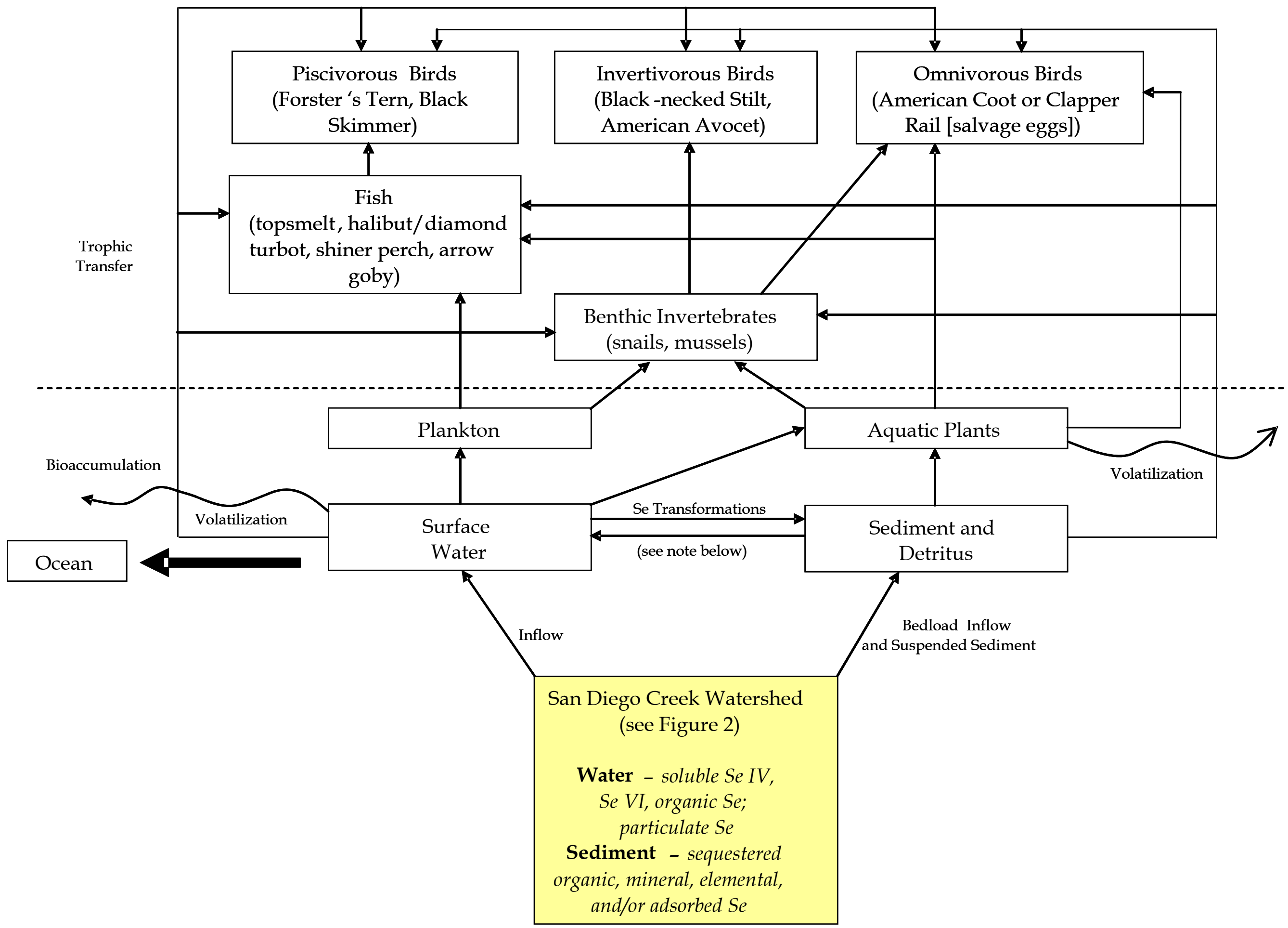



FIGURE 1
Decision Tree for Site-specific Selenium Bioaccumulation Assessment: Aquatic Habitats



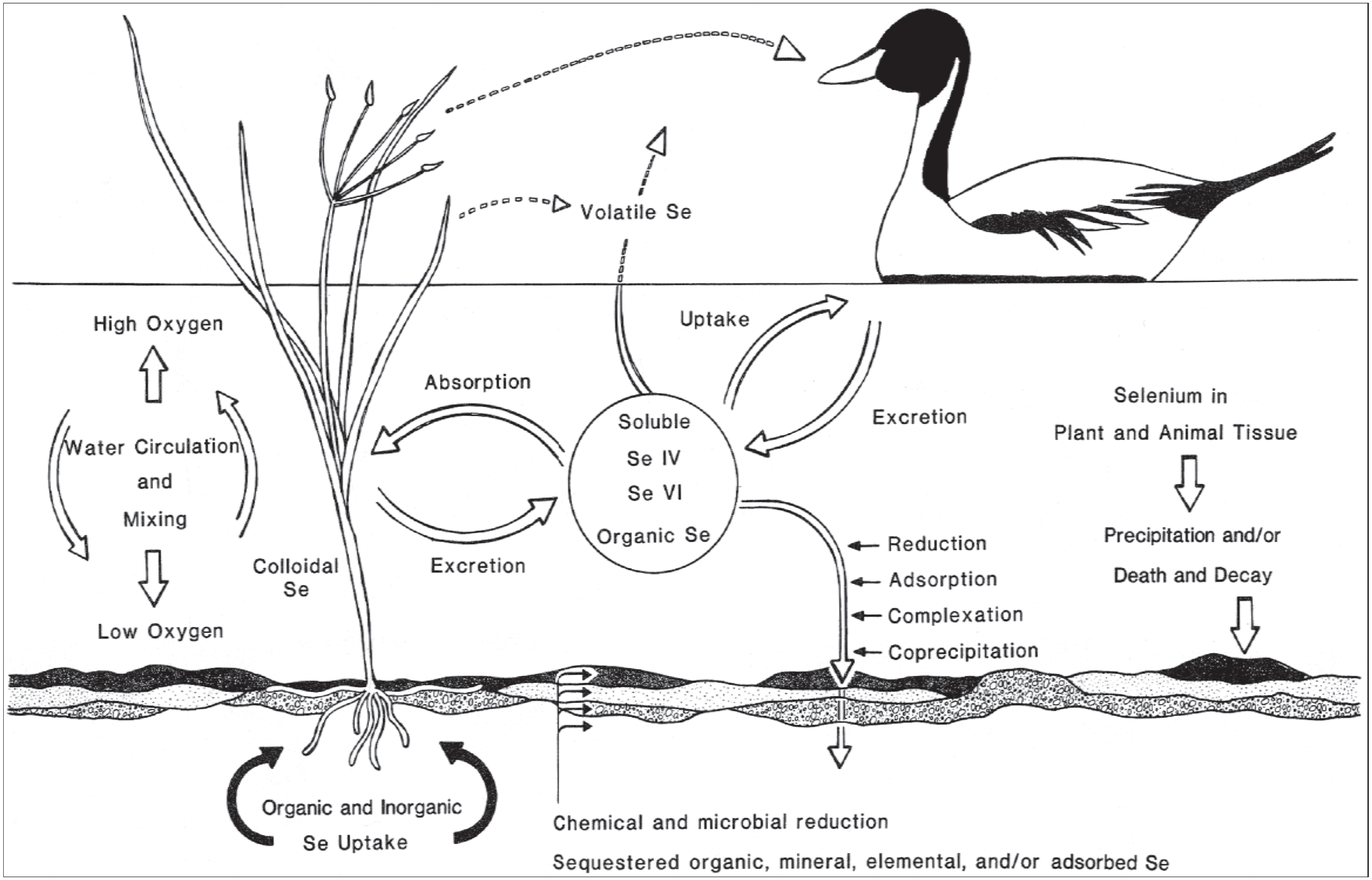

Notes:
Shaded Boxes = sources to the watershed
Weight of line from source indicates significance of contribution of selenium to the watershed (for example, dotted line indicates insignificant contribution, whereas a heavy line indicates significant contribution).
The Conceptual Model Report (Orange County NSMP, 2006) provides details on selenium transformations between sediment and surface water (for example, bacterial processes), as well as details on loss due to volatilization.

FIGURE 2
Conceptual Model, Exposure Pathways, and Food-Web Relationships for Freshwater Creek and Wetland Habitat within the San Diego Creek Watershed



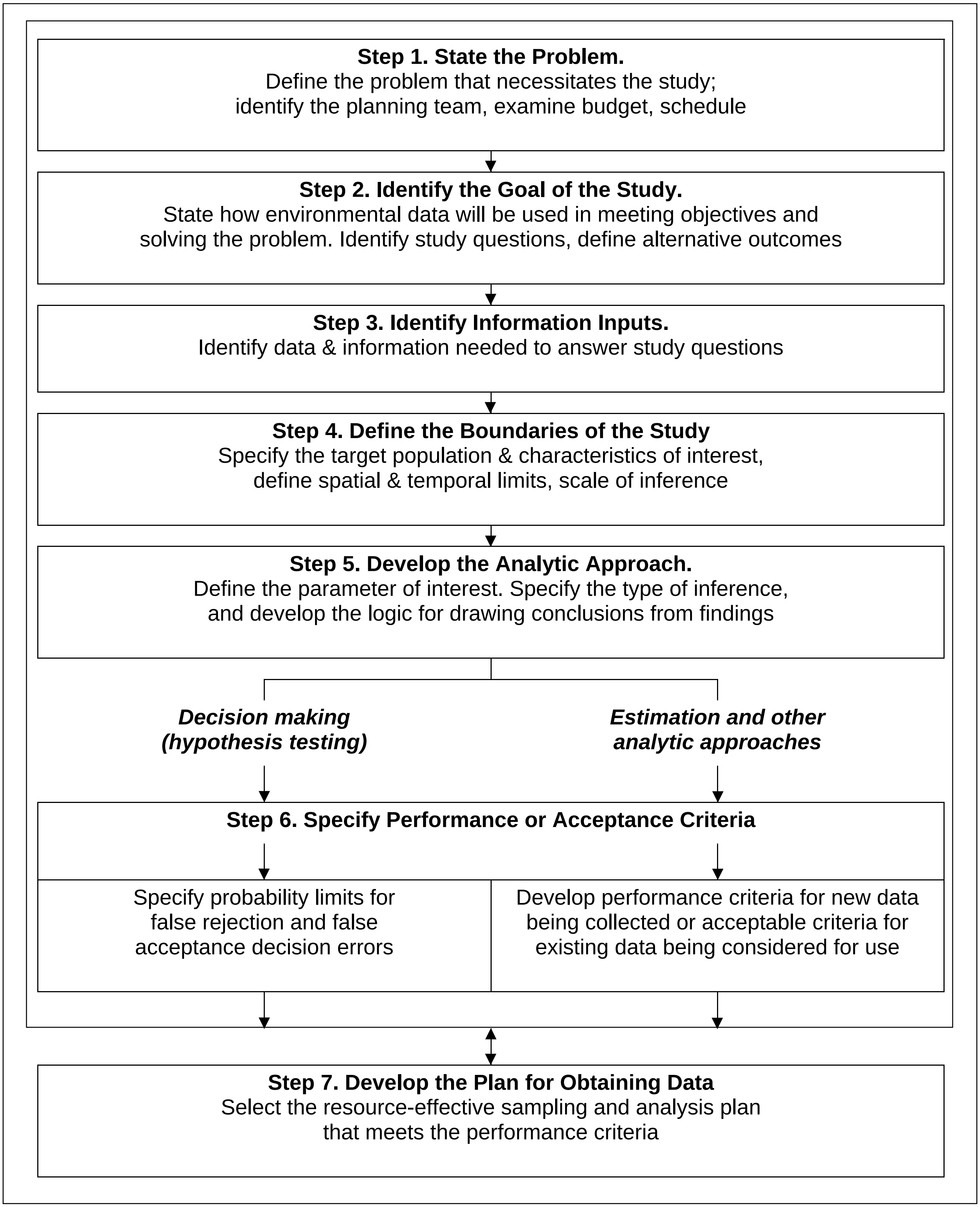

Notes:
Shaded Boxes = sources to the bay
The Conceptual Model Report (Orange County NSMP, 2006) provides details on selenium transformations between sediment and surface water (for example, bacterial processes), as well as details on loss due to volatilization.

FIGURE 3
Conceptual Model, Exposure Pathways, and Food-Web Relationships for Habitat within Newport Bay




Source: Lemly and Smith, 1987

FIGURE 4
General Selenium Processes in an Aquatic Ecosystem




Source: USEPA 2006b

FIGURE 5
The Data Quality Objective Process

# Attachment
# Case Studies

**ATTACHMENT**

# Contents

**ATTACHMENT**

# Case Studies

## Great Salt Lake

Contacts: Harry Ohlendorf (916-286-0277, hohlendo@ch2m.com) or Bill Adams (801-252-3355, william.adams@riotinto.com); see also project Web site at http://www.deq.utah.gov/Issues/GSL_WQSC/selenium.htm

### Problem Statement

In recognition of Great Salt Lake's importance to resident and migratory birds, local recreation, and the brine shrimp and mineral industries, and in response to increasing development pressures within the lake's watershed, the State of Utah (through the Department of Environmental Quality, Division of Water Quality [UDWQ]) initiated a program to support the development of a site-specific numeric water quality standard for selenium for the open waters of the lake. At the beginning of the program, those waters were protected for their beneficial uses through the application of a narrative standard in the state water quality standards (State of Utah, 2007).

The UDWQ has specified appropriate beneficial uses for waters of the State and protects those uses through the development and enforcement of water quality standards. Due to the unique geochemistry of Great Salt Lake, the application of national fresh-water selenium water quality criterion to Great Salt Lake is inappropriate (USEPA, 1987, 2004). The open waters of Great Salt Lake have instead historically been protected for their beneficial uses through the application of a narrative clause in the State water quality standards (R317-2-7). Any discharges directly to the lake are required to meet background concentrations in the lake, or the State has required the discharger to complete site-specific studies to establish a numeric standard that is protective of the lake's beneficial uses (Ostler, 2004).

Recent proposals for new discharges of wastewater to Great Salt Lake led to a recommendation that the UDWQ complete additional research to verify that the discharge of wastewaters containing selenium is not harmful to the Great Salt Lake ecosystem. The UDWQ convened the Great Salt Lake Water Quality Steering Committee, consisting of key stakeholders, and an expert Science Panel in 2004. Their role was to investigate and recommend a new, site-specific water quality standard for selenium for the open waters of Great Salt Lake.

### Approach

The UDWQ developed a public involvement, consultation, and coordination program (including the Great Salt Lake Water Quality Steering Committee, the Science Panel, and a public involvement program) and developed a technical program (including analytical methodologies, a conceptual model for selenium in Great Salt Lake, threshold values, and the research program) to address the need for a site-specific standard. Development of analytical methodologies and development of a conceptual model that characterizes selenium cycling in the open waters of Great Salt Lake were essential precursors to the research program because of the need to be able to analyze for selenium in the highly saline waters of the lake and to provide a framework for definition of information needs (that is, research) for establishment of the water quality standard.

Through development of the conceptual model (Johnson et al., 2006), the Science Panel concluded that successful reproduction and body condition of birds were the two most sensitive, or critical, endpoints to be protected in preventing impairment of the beneficial uses of the study area. These critical endpoints, as represented by the reproductive success of California gulls, American avocets and black-necked stilts (species using Great Salt Lake for nesting) and the body condition of eared grebes and common goldeneyes (species using Great Salt Lake during fall migration and over-wintering, respectively), would be the focus for the research program.

Identification of toxicity threshold values for the exposure of birds to selenium at Great Salt Lake was necessary for the development of a water quality standard that is protective for them. Based on available information, the Science Panel agreed that the most significant exposure of birds occurs through their diet (brine shrimp and/or brine flies), and that the best-documented and most readily-monitored effects are those on reproductive success (particularly egg hatchability). The Science Panel identified a range of threshold values for the birds' diet (3.6 to 5.7 µg/g, dry weight) as well as for bird eggs (6.4 to 16 µg/g) that would be evaluated as part of the research program and development of the water quality standard. Those ranges represent the $EC_{10}$ for mallard egg hatchability based on the results from six laboratory studies (Ohlendorf, 2003). The Science Panel agreed that a selenium water quality standard that prevents impairment of beneficial uses of open waters of Great Salt Lake would be defined by a waterborne or tissue concentration that is represented by those ranges.

Research projects were completed in 2006, 2007, and 2008 to:

- Determine the concentration and effect of selenium in shorebirds through the sampling of adult birds, eggs, diet, water and sediment
- Determine the concentration and effect of selenium in California gulls through the sampling of adult birds, eggs, diet, water and sediment
- Determine the concentration and effect of selenium in eared grebes and common goldeneyes through the sampling of adult birds when they arrive at Great Salt Lake and before leaving the lake
- Conduct a synoptic survey of selenium in periphyton and brine fly larvae from the benthic zone
- Conduct a synoptic survey of selenium in water, seston and brine shrimp
- Measure and model selenium loads to Great Salt Lake
- Measure selenium flux to and from sediment and atmosphere (primarily volatilization)
- Define the transfer of selenium from water and diet to brine shrimp

Data and observations from all these projects were documented in individual project reports and integrated into a quantitative model and synthesis report that is available on the Web site (http://www.deq.utah.gov/Issues/GSL_WQSC/selenium.htm).

## Results/Outcome

Results of the research focused on answering a series of questions that were identified through the Data Quality Objectives process, as follows:

1. **Are significant ecological effects occurring in aquatic wildlife? If so, to which ones and at which locations?** The Science Panel rephrased this question as follows to account for the two critical endpoints previously described:

   - Have any adverse effects been observed in the reproductive endpoints for aquatic wildlife due to selenium that were investigated as part of this program?

     No egg hatchability or teratogenic effects were observed in gulls, avocets, or stilts using the open waters of Great Salt Lake. The geometric mean selenium concentration observed for gulls was 2.89 µg/g (dry weight) and for shorebirds it was 2.72 µg/g. These values are similar to the 85th to 90th percentile of background levels and consistent with a non-contaminated site (Skorupa and Ohlendorf, 1991).

   - Have any adverse effects been observed in non-reproductive endpoints (for example, body condition) in aquatic wildlife due to selenium that were investigated as part of this program?

     A determination could not be made due to confounding variables and insufficient data; however, elevated concentrations of selenium and mercury were found in bird blood and livers. This may indicate that some of these birds were using selenium to detoxify mercury.

   - The Science Panel determined that the reproductive endpoint is considered the most sensitive endpoint for selenium on Great Salt Lake and will be the basis for the selenium water quality standard for open waters of the lake. Non-reproductive endpoints will require additional research before they can be used in assessing the water quality standard.

   - Selenium concentrations in water, sediment, food chain items, and bird liver, blood, and eggs were measured and summarized in Section 5.0 of the final report (CH2M HILL, 2008a).

2. **What is the relative importance of various food-chain exposure pathways for aquatic wildlife?**

   - Bird diets were determined and are summarized in Section 5.0 of the final report.

   - Although some birds (such as gulls and goldeneyes) are known to consume food items from offsite locations (such as fresh water sources along Great Salt Lake), the assumption in the Bioaccumulation Model is that all birds consume only items they can obtain from the open waters of Great Salt Lake. This represents a conservative scenario where birds are consuming the food item with the most likely food chain link for selenium.

- It is assumed that California gulls consume a diet of 100 percent brine shrimp and shorebirds consume a diet of 100 percent brine fly larvae. Shorebirds are also assumed to consume shore-zone sediment as 5 percent of their diet.
- Various alternatives were incorporated into the Bioaccumulation Model to allow the user to explore and evaluate effects from various combinations of bird diets.

**3. What are the transfer factors that describe relationships between selenium concentrations in water column, in bird diets, and the concentrations found in bird eggs?**

- Transfer factors, regression equations, and other methods were developed to describe these relationships (CH2M HILL, 2008b). The recommended transfer relationships are incorporated into the Bioaccumulation Model. The Model allows the user to select from various relationships and/or change transfer factors if desired.
- The Multi-step Transfer Factor (MS-TF) model should be used to model uptake of selenium by brine shrimp (CH2M HILL, 2008b). This model was developed using site-specific data that follow the uptake of selenium by brine shrimp through seston.
- Until more data are collected, the estimate of selenium in brine fly larvae and adults should be determined through a ratio relating brine fly selenium concentrations to adult brine shrimp concentrations.
- Relationships for shorebirds are site-specific and are best understood from available information. For implementation of the water quality standard, relationships for shorebirds should be used. Specifically, the Shorebird Regression Model should be used to model selenium transfer between bird diet and eggs for shorebirds and the Gull Transfer Factor (GTF) Model for gulls (CH2M HILL, 2008b). These models represent site-specific conditions.

**4. What are the most important processes that affect the partitioning, cycling, and release of selenium in the Great Salt Lake open waters?**

- Volatilization was demonstrated to be the major mechanism of selenium removal from Great Salt Lake (geometric mean of 2,108 kg/yr [could range between 820 and 5,240 kg/yr]). Permanent sedimentation follows as the second-most-important mechanism for selenium removal (geometric mean of 520 kg/yr [could range between 45 and 990 kg/yr]). Other mechanisms include shallow zone particulate sedimentation, deep brine layer dissolution and resuspension, and brine shrimp cyst removal.
- A possible loss of about 800 kg per year (geometric mean [could range from 0 to 1,600 kg/yr]) through the railroad causeway from the South Arm to the North Arm was estimated from a few, discrete sampling events. This estimate is uncertain and warrants further work to verify.

- Most selenium was present in the dissolved phase but selenium concentrations were relatively higher in the particulate fraction of the deep brine layer.
- The measured loss fluxes more than balance the measured annual load (1,480 kg per year) during the study period. The observed increase in total selenium concentration during the study period indicates that some selenium loads have not yet been measured or that some losses are overestimated and further monitoring is needed.
- Long-term cycling of selenium within Great Salt Lake was not fully addressed by this program due to the insufficient length of the study period.
- Significant variability in results was observed, but these data represent the best available information. Further work will be required to allow for accurate predictions of future waterborne selenium concentrations.

**5. What are the sources of waterborne selenium entering Great Salt Lake, and what is the relative significance of the various sources?**

- Water quality sampling and flow measurements for six tributaries to the Great Salt Lake identified total selenium loads to the lake of 1,540 kg (over the 15-month study period).
- A review of the literature identified the possibility that dry and wet atmospheric deposition could contribute a significant load of selenium to Great Salt Lake. No data from Great Salt Lake are available; however, this load could be as high as 596 kg/yr using relationships from the literature. Therefore, the selenium load attributable to atmospheric deposition could be greater than any single tributary.
- While lake water levels generally decreased during the study period, waterborne selenium concentrations were observed to increase. This indicates that potential selenium sources have not yet been measured, fate processes leading to recycling in the lake are not understood, or that some of the losses are overestimated. Possible additional sources could be (1) unmeasured surface inflows; (2) submarine groundwater discharges; (3) lake sediment pore water diffusion into the overlying water column; and (4) wind-blown dust that is deposited directly on the lake surface.
- Because of the anomalies observed in the overall mass balance of selenium in Great Salt Lake, further work is needed to better understand the mass balance of selenium in the lake.

A Bioaccumulation Model was developed from data collected from Great Salt Lake to describe the transfer of selenium from water and sediment up through the food web and into bird eggs. The Model allows the user to estimate diet and egg selenium concentrations from an assumed waterborne selenium concentration. The model also allows the user to back-calculate a waterborne selenium concentration from an assumed diet or egg selenium concentration. Resulting waterborne, diet, and egg concentrations are listed and plotted upon egg and diet toxicity curves to illustrate potential effects of selenium on egg hatchability (Ohlendorf, 2003).

The Bioaccumulation Model is composed of a series of relationships that describe the transfer of selenium from water up through the food chain. The transfer factors and regression equations that represent these relationships were developed from data collected from Great Salt Lake as part of the research program. The user has the flexibility to select from numerous options to evaluate the sensitivity and results from alternative transfer relationships and bird diet combinations

The Science Panel made the following recommendations:

1. The water quality standard should be a tissue-based standard, based upon the selenium concentration found in the eggs of birds using the open waters of Great Salt Lake. The standard should be evaluated based upon the geometric mean of eggs sampled in the course of one nesting season at locations where birds are dependent upon the open waters of Great Salt Lake.

2. A selenium water quality standard that prevents impairment for aquatic wildlife of Great Salt Lake lies within the range of 6.4 to 16 µg/g for bird eggs (See Fact Sheet, *Recommended Guidelines for a Water Quality Standard for Selenium in Great Salt Lake* on the Web site: http://www.deq.utah.gov/Issues/GSL_WQSC/selenium.htm).

3. Each Science Panel member prepared a brief position statement providing their individual recommendation for a water quality standard. This statement includes the recommended basis for the standard (all are tissue-based) selenium concentration, associated level of protection, and brief rationale for the recommendation. These position statements were forwarded to the Steering Committee and Water Quality Board for consideration. Individual recommended values were as follows:

    - 12 – 13 µg/g — Six Science Panel members (most likely value for $EC_{10}$);
    - 10.4 µg/g — One Science Panel member;
    - 5 µg/g — One Science Panel member; and
    - Abstained — One Science Panel member, agency policy did not allow member to make recommendation.

4. For implementation of a tissue-based standard, the waterborne concentration of selenium associated with the water quality standard should be derived from the Bioaccumulation Model.

5. Given the uncertainties of the current understanding of selenium cycling in Great Salt Lake, the bioaccumulative nature of selenium, the need to incorporate both waterborne and tissue-based selenium concentrations, and the desire to proactively protect and manage the water quality of Great Salt Lake, the Science Panel developed a concept for a tiered approach to implementing the selenium (tissue-based) water quality standard. The approach assumes the use of the Bioaccumulation Model developed as part of this program to relate water, diet and egg concentrations. The Science Panel recommends that the State of Utah implement a similar tiered approach for monitoring, assessment, and management options to ensure the selenium tissue-based water quality standard is not exceeded. The objectives of the approach are to perform the following:

    - Monitor Great Salt Lake to assess trends in selenium concentrations and determine whether they are approaching or exceeding the water quality standard in eggs, using water and diet (measured in brine shrimp and estimated in brine flies by a "translation factor") as indicators of whether the standard is likely to be exceeded in the egg;

- Address current uncertainty in modeled bioaccumulation relationships by validating expected bioaccumulation with new data for water or diet concentrations and, if appropriate, egg selenium and hatchability;
- Evaluate trigger selenium concentrations that initiate various monitoring, assessment and management actions identified in the assessment framework;
- Evaluate the lake with respect to the numeric tissue-based water quality standard for selenium; and
- Initiate management actions to mitigate further increases in selenium concentration if an upward trend is observed.

The approach implements various trigger concentrations for water, diet, and egg selenium that increase monitoring levels and management options if and when actual selenium concentrations increase.

6. The final tissue-based water quality standard that prevents impairment of the beneficial uses of the open waters of Great Salt Lake will represent a level of protectiveness (that is, not exceeding a specified level of predicted reduction of egg hatchability) recommended by the Steering Committee and selected by the Water Quality Board.
7. Given the uncertainties of the current understanding of the Great Salt Lake ecosystem, it is prudent to identify potential actions UDWQ could take to verify and validate the current model, the new water quality standard, and future permit limits. Consequently, the Science Panel recommended that the UDWQ consider a number of research priorities and provisions incorporated into the proposed assessment framework (listed in the final report [CH2M HILL, 2008a]).

The Steering Committee received these recommendations from the Science Panel in early May 2008 and, following public meetings in June to allow further public input, subsequently made their recommendations to the State Water Quality Board in August. In October 2008, the State adopted a site-specific selenium standard of 12.5 µg/g as a tissue-based standard for eggs of aquatic-dependent birds using Gilbert Bay (i.e., most of the open waters of the lake), based on a minimum of five samples collected over the nesting season (State of Utah, 2008). Assessment procedures are incorporated as a part of this standard, as follows:

**Egg Concentration Triggers:** DWQ Responses

**Below 5.0** µg/g: Routine monitoring with sufficient intensity to determine if selenium concentrations within the Great Salt Lake ecosystem are increasing.

**5.0** µg/g: Increased monitoring to address data gaps and areas of uncertainty identified from initial Great Salt Lake selenium studies.

**6.4** µg/g: Initiation of Level II Antidegradation review for all permit renewals or new permits to Great Salt Lake; may include loading reductions.

**9.8** μg/g: Initiation of preliminary total maximum daily load (TMDL) studies to evaluate selenium loading sources.

**12.5** μg/g **and above**: Declare impairment; formalize and implement TMDL.

Additional assessment procedures associated with this standard are referenced at R317-2-7.1, Application of Standards. Antidegradation Level II Review procedures associated with this standard are referenced at R317-2-3.5.C.

## Lessons Learned

Reflecting on the approach used to achieve a selenium standard for Great Salt Lake, it is appropriate to consider the process and its strengths and weaknesses.

### What Worked?

1. The process of establishing a water quality standard for Great Salt Lake utilized a nine-member Science Panel chaired by the UDWQ. The Panel was constituted by scientists who had studied selenium toxicology extensively or were experts in environmental fate or processes on Great Salt Lake. The panel was further established to include members from the regulatory/resource community (State, USEPA, U.S. Fish and Wildlife Service, U.S. Geological Survey), academia, brine shrimp industry, private consulting, and mining industry. The Panel reflected a diversity of views and experiences but was able to reach a majority opinion. This process is viewed as a positive experience and one that has merit in terms of reflecting the best science and diversity of views.

2. The Science Panel was lead by a State Department of Environmental Quality scientist familiar with the issues related to the lake and with selenium.

3. Research projects that were recommended and supported by State, industry, and NGO funding were directed by a consultant group that facilitated the setting of data quality objectives, collection of data, review and validation of data, and preparation of reports and models. This approach ensured timely research results, data summaries, frequent conference calls, and deliverance of models and Panel recommendations.

4. Science Panel members recommended research relating to fate processes, mass loading to the lake and bioaccumulation of selenium by aquatic organisms. The outcome of this research was critical to the understanding of the fate of selenium in Great Salt Lake in terms of loading to the lake, losses to sediments, and volatilization to the atmosphere.

### What Could have Worked Better?

1. Establishing a water quality standard for such a unique water body (salinity is five times ocean) for a substance that required consideration of not only aquatic species, but also aquatic-dependent species (birds) in a 2-year time period was very time-constrained.

2. Performing research by committee design with a short time frame did not allow for repeat studies or in-depth investigation following reports on the initial question(s). Secondary questions are often as important as the initial question.

3. A 2-year time period was not a sufficient length of time to prepare a complex fate, transport and toxicity model for a large lake system.
4. Time trends of concentrations of selenium in the lake could not be adequately assessed over a 2-year period. While the data collected were helpful in understanding the risk associated with current loads to the lake system, understanding the longer-term trends in water concentrations lacked sufficient data.
5. Additional data on the use of dietary species in the lake by various birds (sensitive receptors) would have assisted in the interpretation of trophic transfer of selenium and would have helped in understanding the relationship between diet and egg concentrations.
6. Lack of time and sufficient modeling data limited the ability of the Science Panel to develop a robust relationship between water and tissue concentrations in brine shrimp in the lake.

### Recommendations

1. There is a need to better understand the relationship between water and brine shrimp and brine fly concentrations. This is critical to understanding the relationship between loading to the lake and concentration of selenium reaching bird eggs.
2. Utilization of dietary species (brine flies and brine shrimp) by birds that are nesting on the lake is needed to improve understanding of the risk that may exist to birds using lake organisms.
3. Understanding of mass loading to the lake and losses to sediments and volatilization has improved dramatically; these data sets should be enhanced by additional monitoring.
4. Water, brine shrimp and brine flies should be monitored on a regular basis in coming years, especially during the bird breeding season (April–June). The data would be collected to assess trends over time, potential to exceed the bird-egg threshold, and to improve the relationship between brine shrimp and brine flies. The data would be used to assess the need for restriction of mass loading to the lake. Further, once the relationship between brine flies and brine shrimp is improved, it would be possible to monitor water and brine shrimp and drop the brine fly monitoring.
5. A tiered water quality standard implementation approach should be established that would increase the frequency and extent of monitoring of water, avian dietary items and bird eggs as a result of increases in tissue concentrations in water, brine flies or brine shrimp.
6. The Science Panel strongly endorsed the idea of using a tissue-based approach for the protection of the Great Salt Lake ecosystem, as tissue concentrations in bird eggs best reflect the receptor endpoint that is most likely to be impacted and allows for site-specific accumulation of selenium.

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# Nitrogen and Selenium Management Program (NSMP) for Newport Bay Watershed, Orange County, California

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## Problem Statement

Total Maximum Daily Loads (TMDLs) have been established for the Sand Diego Creek/Newport Bay watershed for contaminants including selenium. The Santa Ana Regional Water Quality Control Board (Regional Board) specified waste discharge requirements for short-term (that is, 1 year or less) groundwater-related discharges and for *de minimus* discharges within the San Diego Creek/Newport Bay watershed due to the concern that groundwater-related discharges in the watershed may adversely affect surface waters and would likely not comply with established TMDLs for the watershed (Order No. R8-2003-0061). The Order established certain tasks that must be completed by a Working Group that was established to develop and implement a comprehensive Work Plan to address selenium discharges in the watershed over the 5-year permit term. As part of the Working Group recommendations a series of reports were prepared, samples were collected, analyzed, and results summarized towards establishing site-specific water quality objectives for selenium in the watershed.

## Approach

The approach to the study was to summarize existing information since the TMDL was adopted, fill information data gaps with new sampling and analysis, and structure the process of evaluating impacts from selenium with a series of conceptual models. Conceptual models were developed for the drainage canals, flood channels, creeks, and the main reaches of San Diego Creek and off-channel wetlands. In addition, a separate conceptual model was created to structure the evidence for selenium exposure and risk in the estuarine and marine environment of Newport Bay.

Data summarized as part of completing the conceptual models were then evaluated as part of ecological screening of water, sediment, and tissue chemistry results. The resulting information was used as a weight-of-evidence determination of ecological risk from selenium to different receptors for different portions of the watershed.

On a parallel track, pollutant trading programs and best management practice (BMP) options were developed as a means of reducing selenium loads to the bay. A BMP optimization model was created to link flows and loads with the placement and design of BMPs.

## Results/Outcome

The results of the project have led to a study of the use of site-specific water quality objectives (SSOs) for at least a partial solution to the problem of enhanced selenium concentrations in the Newport Bay watershed. The results of summarizing existing post-TMDL data and focused studies has been to document consistent exceedances of whole-body fish tissue concentrations of selenium as compared to the USEPA draft tissue-based criterion. In addition, bird egg selenium concentrations in the watershed exceed various low effect levels (although not as frequently as the fish tissue exceedances). There is documentation of a relative lack of bioavailability of San Diego Creek selenium, including a lack of ambient waterborne concentrations of organo-selenium compounds. Most areas of the watershed and bay surface water habitats are dominated by Se (VI), the least bioavailable of the potential selenium species and most species show reduced accumulation factors from diet to tissue or egg.

Currently, USGS research staff are modifying the Presser/Luoma model for selenium exposure and risk from San Francisco Bay (Presser and Luoma 2006) to apply a version of the model to Newport Bay. The goal is to create a model based on the empirical database from San Diego Creek/Newport Bay using the previously-created structure and linkages of the San Francisco Bay model. That work is currently in progress. The goal will be to create a model that allows the comparison of TMDL implementation alternatives for their effects on selenium exposure and risk in the watershed and from which agency regulators can select appropriately-protective values as SSO selenium levels for specific areas of the watershed and bay. Key receptors in the freshwater and marine portions of the TMDL area will be evaluated against scenarios of altered selenium loads to different portions of the watershed.

## Recommendations

It is important to gain a thorough understanding of transport, fate, and effects of selenium to be able to create loading goals and effectively allocate those loads as part of TMDL implementation. The conceptual models for the Newport Bay watershed were key in structuring the varied data collection, summary, and filling of data gaps. The combination empirical and mechanistic exposure and risk model for selenium will provide the linkage between biological effects and waterborne loading.

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# Power Plant Fly Ash Receiving Streams: Little Scary Creek, Conner Run, and Stingy Run

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## Problem Statement

Little Scary Creek (WV), Conner Run (WV), and Stingy Run (OH) are effluent-dominated fly ash pond receiving streams whose abiotic and biotic components are contaminated with selenium. The selenium discharged from the ash pond outfalls (like all other inorganic constituents) originates from the leaching of fly ash particles. In the early 1980s, AEP began a comprehensive biological and chemical study of ash pond receiving streams at facilities located in four states (Ohio, Virginia, West Virginia, and Kentucky), and results of these studies were reported in several publications (e.g., Van Hassel and Wood, 1984; Specht et al., 1984; Cherry et al., 1987; Van Hassel et al., 1988; Reash et al., 1988). Starting in 1993, AEP biologists supplemented this long-term monitoring with analyses of trace metals in fish tissue and invertebrates. Elevated levels of selenium, as well as other trace metals, were documented in resident sunfish species (Lohner et al., 2001; Reash et al., 2006). Sediment and benthic invertebrate samples also contained high selenium levels.

As USEPA began the process of revising the nationally recommended aquatic life criteria for selenium, focusing on a tissue-based chronic criterion (USEPA, 1998), AEP realized that selenium-contaminated fly ash pond receiving streams presented potential compliance problems. Some of these streams had water column selenium concentrations higher than USEPA's continuous maximum criterion (CMC) of 20 µg/L, and levels of selenium in biota were regarded to be markedly higher than any anticipated laboratory-based "safe" tissue criterion. As the NPDES permits for the coal-fired power plants were renewed, AEP was vulnerable to the imposition of water quality-based effluent limits that could require either treatment installation (removal of selenium) or conversion to dry fly ash disposal. Both of these options carried significant cost implications. The challenge was to develop a defensible argument that populations of selenium-sensitive fish species were viable and persistent despite having elevated concentrations of selenium in tissue samples.

## Approach

Our general approach to addressing regulatory concerns of elevated selenium levels was to: 1) evaluate the site-specific risks associated with elevated levels of selenium in biotic compartments, using all available data ; 2) understand the economic and operational impacts of complying with applicable water quality standards using alternate fly ash treatment and disposal methods; and 3) take a proactive approach in working with state agency staff who were informed of elevated selenium tissue levels in the receiving streams. The effluent quality of Stingy Run (fly ash receiving stream for AEP's General James M. Gavin Plant, southeastern Ohio) is regulated by Ohio EPA, whereas the West Virginia Department of Environmental Protection (WVDEP) regulates effluent quality for Little Scary Creek (John Amos Power Plant) and Conner Run (Mitchell Power Plant).

At Gavin Plant, the sluicing of fly ash to the pond ceased in 1995, when the plant installed wet non-forced oxidation flue gas desulfurization (FGD) systems for both units. This change resulted in a significant decrease of selenium and other constituents to Stingy Run, as fly ash was used to fixate FGD sludge rather than being sluiced to the fly ash pond. All biotic and abiotic samples analyzed to evaluate selenium bioaccumulation, thus, were collected in 1995 or earlier years. There are currently no regulatory concerns of selenium bioaccumulation in Stingy Run, and compliance with the existing NPDES discharge is not problematic.

Both Little Scary Creek and Conner Run have significant compliance concerns. WVDEP biologists have collected fish and water samples in both effluent-dominated streams. Their results are similar to AEP’s, i.e., most fish species have whole-body selenium concentrations well above USEPA’s draft chronic water quality criterion value of 7.91 µg/g dw. The agency is conducting a statewide survey of selenium levels in various compartments at reference and water quality-impacted sites. Once USEPA finalizes the revised chronic selenium water quality criterion and WVDEP adopts this value, it is likely that the agency will develop effluent limitations for selenium using a BAF back-calculation procedure. To prepare for this, AEP is conducting further evaluations on community and population-level impacts.

In 2007, a fish tissue and community analysis was conducted in both fly ash pond receiving streams, as well as a site in Kentucky and Indiana. Surveys were conducted during two seasons (summer and late fall) to assess seasonal differences in both intraspecific and interspecific bioaccumulation patterns. Whole-body arsenic, mercury, and selenium concentrations were measured in both individual and composite samples in nine different fish species. Whole-body analyses were made for two principal reasons: 1) levels of several trace metals in other tissues (liver and gonads) were measured in fish collected during 1996; and 2) it is anticipated that USEPA will issue the final revised selenium chronic water quality criterion as a whole-body concentration.

## Results/Outcome

At four study sites having a gradient of fly ash exposure (from 100 percent fly ash effluent to Instream Waste Concentrations to less than 5 percent on a large river), a total of nine fish species were collected and analyzed for whole-body selenium, arsenic, and mercury. Of these nine species, six were centrarchids (bluegill, green sunfish, longear sunfish, largemouth bass, spotted bass, and white crappie). In order to assess possible affects of sample type on trace metal levels, both individual and composite samples were collected for several species. Though data analysis continues, the principal findings observed so far are summarized below:

- At the four sites, median water column levels of selenium varied between 4 µg/L and 103 µg/L. The principal inorganic species was selenite. Levels of both arsenic and mercury were low and did not approach acute or chronic toxicity thresholds.
- Whole-body selenium concentrations (all species at all sites) ranged between 1.81 µg/g and 65.1 µg/g (dw). In general, whole-body selenium levels tended to increase with increasing fly ash exposure.
- At high exposure sites, most of the fish analyzed for selenium tissue levels had whole-body levels that exceeded USEPA’s draft chronic criterion value (7.91 µg/g dw). Whole-body arsenic levels (range = 0.27 to 3.82 µg/g) were elevated in fish from one high

exposure site whereas whole-body mercury levels were low at all sites (range = 0.012 to 0.99 µg/g). This finding supports results of previous investigations indicating that methylation of mercury in coal ash ponds and reservoirs is very limited.

- Intraspecific seasonal variation in whole-body selenium levels was marked at high and moderate exposure sites (concentration ratios ranged from 1 to 7.5), indicating that as selenium exposure increases, the intraspecific variability of tissue selenium levels also increases. Thus, multiple seasonal collections seem to be appropriate at locations where high selenium exposure is documented or expected.
- Previous researchers have reported that, unlike mercury, the bioaccumulation of selenium is not associated with trophic level or size and age of fish. Our results corroborate this observation; among all sites, the species having the highest mean whole-body selenium concentration represented a suite of feeding guilds, and neither length nor wet weight were predictive independent variables for tissue selenium levels.
- A significant negative correlation ($r^2 = 0.67$) was observed between whole-body selenium and mercury levels (all samples combined), suggesting that high tissue selenium levels antagonistically regulate the bioaccumulation of mercury.
- Fish community indices and metrics were calculated at all four sites to assess how the structure and function of the fish community varies with fly ash pond discharge exposure. High exposure sites tended to have lowered species richness and fewer numbers of sunfish, sport, and piscivore species. However, there was no evidence of a reduced diversity-based community index (Modified Index of Well-being) at three of the four sites, suggesting that some level of community balance can be maintained at locations where many species of fish contain whole-body selenium concentrations considered "elevated".
- At a site with moderate selenium contamination (about 50 percent of fish analyzed for tissue analysis had whole body selenium levels in excess of 7.91 µg/g), several pollution-tolerant species (along with high species richness and a high diversity-based index score) were found. While many factors (biotic and abiotic) interact to influence the structure and function of fish communities, our results appear to indicate that elevated selenium tissue levels, as a single variable, do not co-vary with a less diverse, less balanced community assemblage.
- We compared AEP's tissue analysis results with those obtained from WVDEP. This comparison indicated, for some species, marked differences in whole-body selenium levels. For example, the mean whole-body selenium concentration for green sunfish collected in Little Scary Creek – collected by AEP and analyzed by Brooks Rand, Inc. – was 26.62 µg/g dw. For green sunfish collected by WVDEP and analyzed by BioChem Testing, Inc., the mean whole body concentration for this species was 6.2 µg/g. This suggests that either: 1) intraspecific variability in whole-body selenium levels is significant, at least for this species; 2) differences in field processing and/or analytical procedures accounted for the differences; or 3) both factors, in combination, affected the disparate results.

## Lessons Learned

The field and laboratory assessments conducted in 2007 were important in filling in some missing puzzle pieces at each site, in terms of the extent and magnitude of selenium contamination for several fish species. It will be necessary, however, to obtain information that more definitively answers the question: "Are selenium-sensitive populations in these streams experiencing lowered survival and/or reproductive success?" To answer this question, carefully designed laboratory studies are needed to provide more insightful diagnostic information on population-level attributes. AEP, in conjunction with EPRI, is in the process of designing such studies. It is expected that the design chosen for this study will mirror existing evaluations of population health being conducted at mining sites in western Canadian provinces, and as discussed in this guidance.

As USEPA and states look ahead to finalization of the revised selenium criteria, the process by which the chronic criterion is implemented is crucial. How the variability of selenium levels in tissue and water is managed has direct implications for whether a given site attains the criterion. AEP will work with EPRI and the Utility Act Group to provide technical recommendations on how USEPA should develop implementation guidance, especially options for site-specific criterion development.

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# Selenium Investigation of the Arkansas River, Fountain Creek, Wildhorse Creek, and the St. Charles River: Pueblo, Colorado

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## Problem Statement

A significant number of water bodies throughout Colorado have documented water column selenium concentrations in exceedance of the current criterion of 4.6 µg/L[1], as well as whole-body fish selenium concentrations that exceed the draft tissue-based criterion of 7.91 µg/g (CEC/Parametrix 2005). Like many regions in the western U.S., significant deposits of selenium-rich surface materials derived from marine shales naturally elevate selenium concentrations in aquatic ecosystems. As a result, fish populations are likely exposed to naturally higher concentrations than in many other regions of the country. However, there is no empirical field evidence that selenium is adversely affecting fish populations in the West.

The objectives of this study were to conduct a comprehensive analysis through concurrent collections of aquatic biological data, background chemical data, and physical habitat characteristics of a portion of the Arkansas River and nearby tributaries in the vicinity of the City of Pueblo, Colorado, to evaluate relationships between selenium concentrations and fish populations (GEI 2007).

## Approach

Data were collected seasonally from fall 2004 to fall 2006 from multiple sites on the Arkansas River, Fountain and Wildhorse creeks, and the St. Charles River (Figure 1) to evaluate potential population-level effects due to elevated selenium (GEI 2007). Sites were specifically selected to cover a wide range of ambient selenium concentrations in the study area, based on past data collections.

Data collected to accomplish the study goals were purposely multilayered to evaluate the selenium pathways from groundwater/surface water sources through the food chain to the fish population receptors. These data included:

- Fish and macroinvertebrate populations were sampled (fish in fall 2005 and fall 2006; macroinvertebrates in fall 2004, spring 2005, and fall 2005) to determine the "health" of the aquatic biota, in terms of the species composition and relative abundance of aquatic organisms, as well as reproductive success through examination of length-frequency/age-class distribution.
- Whole-body fish tissue, composite macroinvertebrate tissue, and sediment samples were collected for the analysis of selenium bioaccumulation pathways
  - Replicate samples were collected from at least one fish species from each trophic level at each site. Trophic levels included a small forage fish (e.g., cyprinid species), a

[1] Colorado applies a "total-to-dissolved" conversion factor of 0.922 to the USEPA chronic criterion of 5 µg/L based on information in a 1995 USEPA document (Stephan 1995).

bottom feeder (e.g., catostomid species), and a top predator (e.g., centrarchid species), when present. Total selenium concentrations for whole-body fish tissue were determined.

  - Replicate samples of fine (silt/clay) and coarse (sand) sediment were collected at each study site allowing evaluation of selenium accumulation potential vs. sediment particle size.

- Physical habitat measurements, including sediment particle size, were collected to determine relationships between the biota and their environment.
  - Evaluation included both primary (main channel) and secondary (side channel, off-channel) habitat types typical of streams in the high Great Plains. Measurements within habitat types included length, wetted width, average depth, maximum depth, and substrate composition. Additional measurements for primary habitat type include bank width, length of eroded bank, and percent permanent vegetation.
  - Substrate characterization included the percent distribution of fine depositional sediments in the stream channel as a measure of relative selenium bioaccumulation potential to the aquatic biota for the sites in the study area.
- Water column samples were collected monthly as part of the larger selenium load allocation study.

Collection of this comprehensive, multilayered data set allowed detailed statistical analysis of potential relationships between fish population metrics, selenium concentrations (species-specific tissue concentrations, macroinvertebrate tissue, and sediment selenium concentrations) and habitat. Paired data could be evaluated at the site level (e.g., site mean fish tissue vs. total number of species) and at the fish family level (e.g., mean replicate tissue concentrations for species within the family Cyprinidae vs. species-specific density), and grouped to provide a regional analysis.

Based on water quality regulations in Colorado (CDPHE 2008), site-specific water quality standards can be set at "ambient" concentrations if it can be shown that elevated concentrations are "natural or human-induced/uncorrectable". For the purposes of deriving a value, Colorado defines "ambient" as the 85th percentile of representative data for a stream segment.

A companion study was conducted in the study area to evaluate the sources of selenium within the reach of the Arkansas River from upstream of the city of Pueblo to the confluence with the Huerfano River (Figure 1).

Groundwater selenium concentrations were influenced more by geology than irrigation practices, with average selenium concentrations in shale-influenced zones estimated as being 100 times higher than concentrations in the alluvial zones, regardless of irrigation practices in those areas. The Pueblo Water Reclamation Facility, a permitted source, was the seventh-largest contributor of selenium. However, selenium from this source is not originating from any of the processes occurring at the facility. Instead, selenium is entering the facility through the infiltration of groundwater into the sanitary sewer system, and was, therefore, considered a natural source of selenium as well.

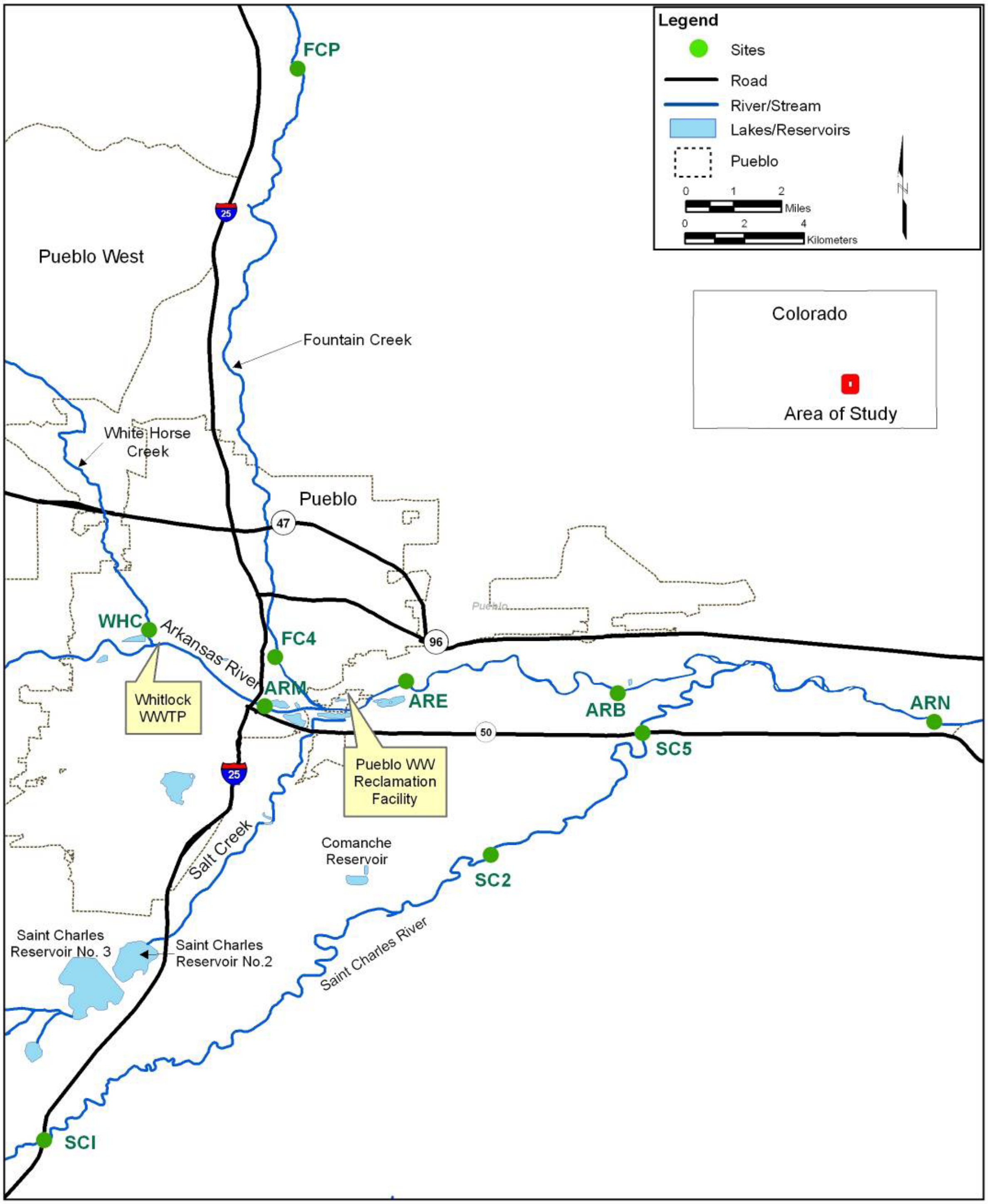


FIGURE 1
Location of Study Sites on the Arkansas River, Fountain Creek, Wildhorse Creek, and the St. Charles River, 2004 - 2006.

The results of this study indicated that selenium in this reach of the Arkansas River and tributaries occurs from natural sources, with insignificant human-induced additions to total loading. This was an important consideration in the evaluation of effects of elevated selenium to fish populations. Since elevated concentrations of selenium are naturally occurring at the study sites, this study provided a unique evaluation of selenium impacts to fish populations that have been exposed naturally to elevated selenium for potentially thousands of years.

## Results/Outcome

Total selenium water column concentrations were generally elevated throughout the study area, with only the upper reaches on the St. Charles River and Fountain Creek with mean selenium concentrations below the Colorado chronic standard of 4.6 µg/L dissolved selenium.

Mean fine sediment selenium concentrations for all sites ranged from 1.20 µg/g to 20.9 µg/g dw. A positive relationship between total mean sediment selenium concentration (mean includes coarse and fine sediment concentrations), mean dissolved selenium water column concentration, and mean sediment TOC (mean includes coarse and fine sediment percentages) was observed when all sites are included in a regression analysis, similar to that reported by Van Derveer and Canton (1997). Fine sediment mean selenium concentration (ranging from 1.20 ± 0.92 µg/g to 20.9 ± 14.7 µg/g) was greater than coarse sediment selenium concentrations (ranging from 0.25 ± 0.21 µg/g to 8.42 ± 8.05 µg/g) at all sites, confirming Van Derveer and Canton's suggestion.

Mean invertebrate selenium tissue concentrations varied by season, year, and site and ranged from 6.0 µg/g dw to 45.5 µg/g dw among the sites. Individual tissue sample concentrations ranged from less than the minimum detection limit (<0.8 µg/g wet weight) to 90 µg/g.

Selenium tissue concentrations were measured for three cyprinids (central stoneroller [*Campostoma anomalum*], sand shiner [*Notropis stramineus*], red shiner [*Cyprinella lutrensis*]), one catostomid (white sucker [*Catostomus commersoni*]), and three centrarchids (green sunfish [*Lepomis cyanellus*], smallmouth bass [*Micropterus dolomieu*], and largemouth bass [*M. salmoides*]). Selenium tissue concentrations varied noticeably by fish family. Mean concentrations in all cyprinids were greater (21.1 µg/g dw; SE = 1.38) than either centrarchids (19.7 µg/g dw; SE = 1.32) or catostomids (17.5 µg/g dw; SE = 1.52). However, there was no statistical difference between the overall mean for the three families ($p = 0.19$). Most mean whole-body selenium concentrations were well above the draft chronic tissue criterion of 7.91 µg/g.

Media with measured selenium concentrations and sediment TOC collected simultaneously with fall fish population sampling were analyzed in an all possible regression analysis (Hintze 2000) to determine most likely parameters affecting bioaccumulation pathways for selenium throughout the study area.

Total fish density, when weighted by habitat availability, was not significantly related to tissue selenium. Rather, substrate conditions, represented by percent silt and boulder/rip rap, explained most of the variability in total fish density weighted by habitat ($R^2$=0.44, two-parameter model). Although silt by itself likely does not directly contribute to greater fish densities, a high silt percentage may be an indicator of high primary production that could influence food availability, or diverse habitat that contains slow backwater or eddy refugia.

For the fall data, site-mean fish tissue selenium concentrations (all species combined) were most influenced by macroinvertebrate tissue concentrations, followed by coarse sediment selenium concentration and sediment TOC. Out of these three variables, only macroinvertebrate selenium resulted in a significant positive relationship with whole-body fish tissue concentration ($R^2 = 0.38$, $p = 0.001$) for the fall data. Although these three variables are most influential, together they explained only 45 percent of the variability in fish tissue selenium concentrations.

Trends were also evaluated by fish family. While a slight negative relationship was observed between densities of cyprinid species and their whole-body selenium concentrations (i.e., as selenium concentrations increased, density decreased), this relationship was not statistically significant ($R^2 = 0.0239$; $p = 0.5120$). However, when habitat parameters, substrate characteristics, and selenium data were included in an all possible regressions analysis, the strongest two-parameter model suggested percent silt and whole-body selenium have the greatest influence on cyprinid densities. Together, percent silt and selenium concentration explained 49 percent of the variability in the data, with percent silt explaining most of the variability. A three-parameter model of percent silt, whole-body selenium, and percent sand explained 57 percent of the variability in the densities of cyprinid species, indicating a potential habitat-related confounding factor in the evaluation of potential selenium effects.

Grouping species-specific data by the family Catostomidae resulted in trends similar to those observed with cyprinids. Catostomid densities (equivalent to white sucker density) were not significantly correlated with whole-body selenium concentrations ($R^2 = 0.02$; $p = 0.65$). Rather, a significant positive relationship was once again observed with percent silt ($R^2 = 0.25$; $p = 0.05$).

A significant negative relationship was observed between whole-body selenium and species-specific densities for the family Centrarchidae ($R^2 = 0.53$; $p = 0.02$). However, densities of centrarchid species were also significantly correlated with primary habitat average depth ($R^2 = 0.74$; $p < 0.001$); greater population densities were associated with greater habitat depths. A three-parameter model identified primary habitat depth, percent sand, and percent cobble as having the greatest influence on centrarchid density. Together, these three parameters explained 90 percent of the variability in centrarchid density. These results and the significant negative relationship between mean primary habitat depth and centrarchid selenium suggest population differences between sites are more related to habitat parameters than whole-body selenium concentrations.

Overall, the results of the study indicate no consistent relationships between selenium concentrations in water, sediment and fish tissues, to fish taxa richness or density for the range of concentrations observed. Although some sites exhibited lower numbers of fish species (e.g., Wildhorse Creek), which may indicate an adverse effect of selenium concentrations, those concentrations are based on natural sources. In addition, the selenium tissue concentrations in fish were no higher at that site than other sites with many more species.

It is important to note that attempts to determine selenium effects were highly confounded by significant correlations between fish population metrics and primary habitat average depth. In fact, habitat parameters, in general, consistently explained more of the variability in fish parameters than whole-body fish tissue selenium, suggesting that tissue concentrations were not driving populations despite the wide range of tissue levels. Analyses indicate that while selenium may play a role in fish populations at these sites, habitat plays potentially a more important role in structuring the fish community**.**

## Recommendations

The findings summarized above highlight the importance of a comprehensive study plan, incorporating all facets of the environment that could influence fish communities. The strong interaction of habitat with selenium in describing fish population trends supports the need for future studies to include detailed information on habitat availability and quality, in addition to key components of the selenium bioaccumulation pathway such as water, sediment, and tissues.

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# Canadian Coal Mining

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## Problem Statement

Coal strata in the Elk River Valley contain selenium, whose release into the environment is accelerated by coal mining. Selenium concentrations are increasing in waters downstream of the coal mines and elevated selenium concentrations have been found in biota (e.g., fish, water birds and their prey). Increasing environmental concentrations of selenium are of concern related to possible future impacts to resident biota.

## Approach

The Elk River Valley is primarily lotic, thus initial investigations focused on determining whether adverse effects due to selenium could be occurring in fish or water birds representative of and common in the Valley's lotic environments. When these investigations indicated that there was not cause for immediate concern, the focus shifted to the less common lentic areas, representing worst-case conditions for selenium accumulation and possible toxicity to biota. The lotic studies similarly did not indicate that large-scale impacts to populations of resident biota were occurring, although there were indications of localized effects. Accordingly, the focus then shifted to management and research into treatment methods to attempt to reduce or possibly even reverse the trend of increasing selenium concentrations downstream of the mines.

## Results/Outcome

Site-specific selenium tissue thresholds have not been developed to date despite extensive effects studies. There are indications that some biota (i.e., red-winged blackbirds) can regulate selenium concentrations in their eggs below critical toxic levels. In other cases (e.g., longnose sucker, Columbia spotted frogs) results were inconclusive due to logistical and other problems with the studies. In one case, Westslope cutthroat trout, two different studies indicated that this species had a relatively high tolerance to selenium, but one indicated a threshold of effects while the other did not despite overlapping selenium tissue concentrations. Accordingly, a third study is underway to attempt to determine a selenium tissue threshold for this trout species and to resolve differences between the two studies (one lotic, the other lentic). Because effects thresholds have not been determined, site-specific standards cannot yet be developed. As noted above, the focus is now on monitoring and management.

## Lessons Learned

Initially studies were conducted independently by industry and government, and relationships between the two were more adversarial than cooperative. However, subsequently the multi-stakeholder Elk Valley Selenium Task Force (EVSTF) was formed and a range of studies were conducted jointly and relationships became cooperative. A key lesson/recommendation is the need to involve all stakeholders in work being conducted to ensure both that they approve and that the study outcomes will be generally accepted.

Emphasis has been placed on peer reviewed publication, where merited and appropriate, of study results, which provided a high level of technical acceptability; publication in the international scientific literature should be encouraged for other selenium studies/situations. However, publication was possible in many cases because new research was being done; this also meant that there was a high possibility of failure in some state-of-the-science investigations and, predictably, this occurred in some cases resulting in extensive funding and effort returning very little information. It is thus important to conduct both cutting-edge and basic studies to ensure that necessary information is obtained. A key component missing to date in the Elk River Valley is a comprehensive evaluation of the state of the fisheries throughout the Valley. Evaluations have been conducted in some but far from all aquatic environments in the Valley. Such field evaluations are a necessary complement to laboratory studies which are, by definition, simplistic and may not reflect what is occurring in the real world. Thus, future investigations need to combine appropriate laboratory and field investigations in weight-of-evidence assessments.

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# Idaho Phosphate Mining

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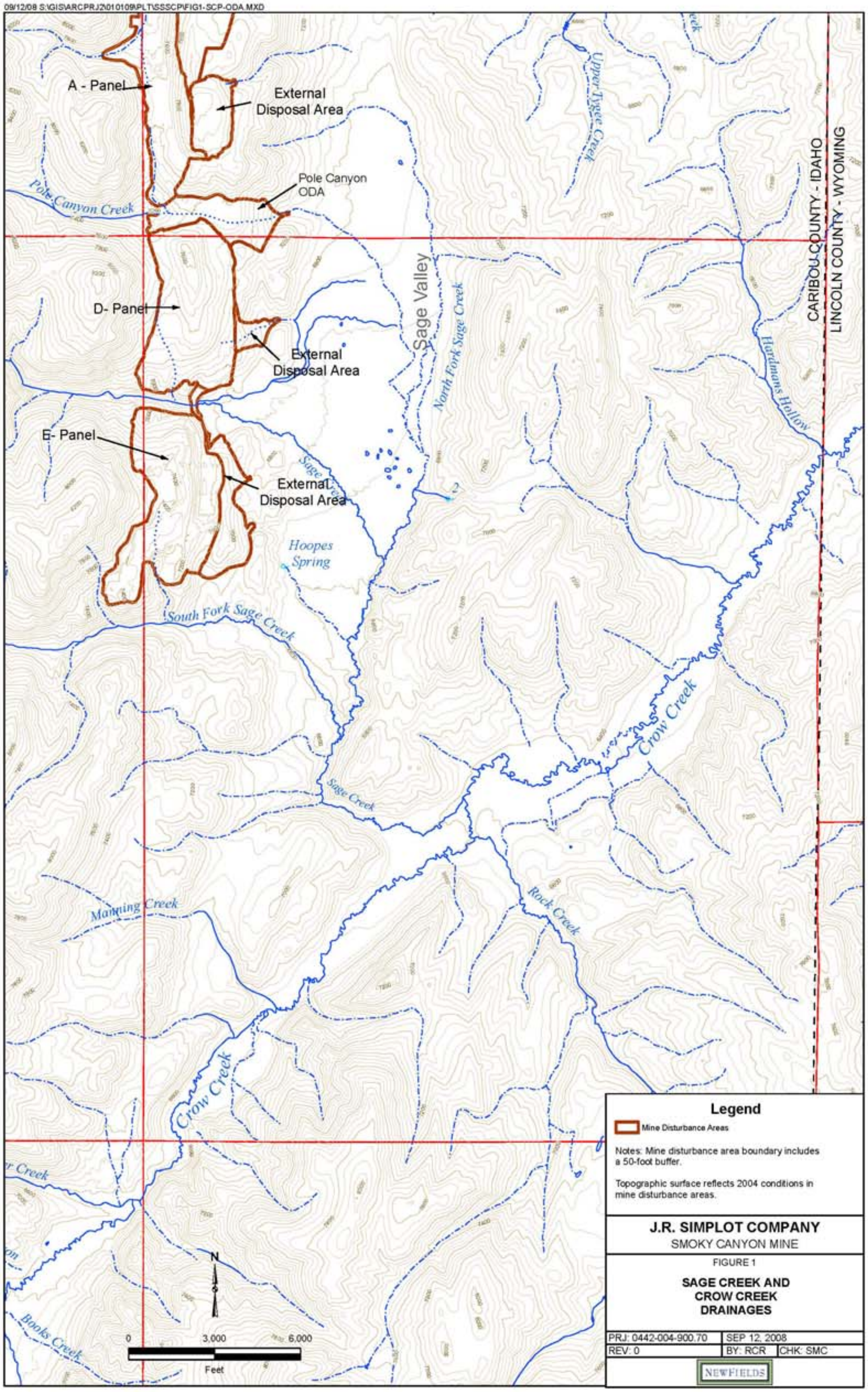
## Problem Statement

A Site Investigation (SI) (NewFields 2005) was conducted at the J.R. Simplot Company's Smoky Canyon Mine, a Southeast Idaho mining facility, as part of a 2003 Administrative Order on Consent (State of Idaho Department of Environmental Quality, United States Department of Agriculture, Forest Service Region 4, and United States Environmental Protection Agency, Region 10 [IDEQ, USFS and EPA] 2003). Investigations to date have identified elevated selenium concentrations in Hoopes Spring (located outside the mine lease boundary), which discharges to lower Sage Creek. Sage Creek ultimately discharges to Crow Creek (Figure 1). Several potential sources may contribute selenium to Hoopes Spring via groundwater flow to the spring. The largest potential source identified as a contributor of selenium to groundwater is the Pole Canyon Overburden Disposal Area (ODA) cross-valley fill. Additional potential sources of selenium to groundwater are mine panels D and E, located west-northwest of Hoopes Spring. Infiltration of water through the mine overburden transports solubilized selenium to the underlying geological formation known as the Wells Formation. Water from the Wells Formation is expressed as surface water at Hoopes Spring. A Non-Time Critical Removal Action for Pole Canyon is in progress. Two primary components of this action, including construction of a diversion pipeline and infiltration basin to isolate Pole Canyon Creek from the ODA, are completed. Conceptual groundwater modeling indicates that a significant reduction in the selenium load from the Pole Canyon ODA is expected to be observed at Hoopes Spring in about 10 years.

In the interim, modification of the selenium water quality standard is being investigated.

Rationale for pursuing such a modification includes:

- The bluegill toxicity data used to derive the current Idaho State Standard and the U.S. Environmental Protection Agency's Draft Aquatic Life Water Quality Criteria (USEPA 2004) are based on effects to bluegill in a lentic (still water) environment. Streams near the Smoky Canyon Mine are lotic (flowing water).
- Bluegill sunfish, a warm water species, is not found in Idaho mountain streams.
- Recently published literature suggests that the trout species present within the Crow Creek watershed may be less sensitive to selenium than species used to derive the current standard.
- Data collected during the SI revealed what appeared to be healthy aquatic communities at locations where selenium concentrations exceeded the current chronic surface water standard (5 µg/L).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A - Panel
External Disposal Area
Pole Canyon ODA
Pole Canyon Creek
D- Panel
External Disposal Area
E- Panel
External Disposal Area
Hoopes Spring
South Fork Sage Creek
Sage Valley
North Fork Sage Creek
Upper Tygee Creek
Hardmans Hollow
CARIBOU COUNTY - IDAHO
LINCOLN COUNTY - WYOMING
Sage Creek
Crow Creek
Rock Creek
Manning Creek
Books Creek
N
0
3,000
6,000
Feet
Legend
Mine Disturbance Areas
Notes: Mine disturbance area boundary includes a 50-foot buffer.
Topographic surface reflects 2004 conditions in mine disturbance areas.
J.R. SIMPLOT COMPANY
SMOKY CANYON MINE
FIGURE 1
SAGE CREEK AND CROW CREEK DRAINAGES
PRJ: 0442-004-900.70
SEP 12, 2008
REV: 0
BY: RCR
CHK: SMC
NEWFIELDS

- As indicated in USEPA's Draft Selenium Criteria (2004) for chronic selenium toxicity in fish, diet is the primary route of exposure. The current State of Idaho water quality standard for selenium (5 µg/L) is based on a concentration in water that is not consistent with the state of the science on aquatic life exposure.

## Approach

Simplot has been working collaboratively with the Site-Specific Selenium Criterion (SSSC) Workgroup, which includes agency personnel from IDEQ, Idaho Department of Fish and Game (IDFG), USFS, Wyoming Department of Environmental Quality (WDEQ), USEPA headquarters, and USEPA Region 10, and has moved forward with several components of the study based on review and input on various work plans. Through this collaborative process, a concerted effort has evolved that includes 1) field studies, 2) laboratory studies, and 3) literature reviews.

The overall approach is based on developing the relationships between the three primary lines of evidence outlined below:

- **Field studies** are being conducted to define exposure conditions and the condition of the aquatic community in relation to habitat. These studies will aid in understanding the relationships between aqueous, sediment, and tissue selenium concentrations across the site. Field monitoring will also provide data to evaluate the overall condition of aquatic communities, population and community structure, and habitat quality at target locations, including areas upstream of the source area (background), at the source area, downstream of the source area, and at a reference location. Results from these studies will help identify species of ecological significance, aid in the assessment of sensitive species for the site, and put the laboratory study results in perspective.
- **Laboratory studies** are being conducted to define toxic effects using site species. These studies aid in understanding mechanisms and potential magnitude of toxicity. They provide site-specific data to develop relationships between tissue concentrations and water quality. Results from these studies will identify a chronic threshold for fish, which can then be evaluated relative to fish populations and communities and to benthic communities that exist at the site.
- **Literature reviews** augment site-specific data with potentially applicable data from other sites, species, and conditions. The review of existing literature aids in the overall design of site-specific studies and toxicity of selenium to other species. Along with the field studies, the literature base contributes to the evaluation of whether the site-specific studies are representative of the more sensitive, ecologically significant species. Results of the continuing literature review augment field and laboratory studies and may reinforce site-specific interpretations to be made regarding toxicity.

To implement the approach and develop the primary lines of evidence, the following steps were identified along with key considerations for implementation.

**Field Studies for Receptor and Exposure Characterization** - Characterize selenium concentrations in biotic and abiotic media, along with the associated aquatic community conditions to understand selenium concentrations in the different environmental media observed during the SI, and fishery and habitat quality conditions.

Key Considerations –

- The spatial and temporal design of the study is adequate to define the range of exceedances of the current State of Idaho water quality standard.
- The appropriate and relevant media are being sampled to provide data for developing relationships between biotic and abiotic media.
- The spatial and temporal design of the study is adequate to define the range of important aquatic species.

**Define Effects and Derive Chronic Water Quality Value** – Conduct laboratory toxicity testing, including adult reproduction and early life stage (ELS) testing. Toxicity testing includes two species and two exposure scenarios. Adult reproduction studies include pre-parental exposure of eggs (i.e., parents exposed in-situ with eggs spawned and reared in laboratory). ELS studies include dietary and aqueous exposures of young fish (from the egg stage) with no pre-parental exposure. A literature review has been ongoing since the SI. New and relevant literature has been continually compiled and reviewed and has been thoroughly considered in developing this approach. In addition, a thorough selenium toxicity literature review has been conducted as part of an Environmental Impact Statement for expansion of the Smoky Canyon Mine to Panels F and G (USFS and BLM 2007). Literature is continually being compiled and reviewed throughout this process.

Key Considerations -

- Identification of the fish species that are the most sensitive receptors is supported by site-specific information and literature.
- Testing for two trout species, brown trout and Yellowstone cutthroat trout (YCT), will yield site-specific information for toxicity threshold development.
- Early life stages of fish, either through pre-parental exposure passed on to the egg or dietary intake and aqueous exposure as young developing fish are the most sensitive life stages.
- Adult wild fish collected from the exposure areas represent the range of tissue concentrations that are present.

**Modeling** – Translate tissue-based values to water quality values. Development of site-specific relationships will be the most direct method; however, models derived and presented in published literature may also provide usable translation approaches. The literature, both currently and in the future, is expected to provide different approaches to translate tissue effects values to water quality values as the base of information about selenium bioaccumulation grows.

Key Considerations -

- Appropriate and relevant relationships can be developed between effects thresholds data derived for fish tissues and selenium in surface waters.
- A water column effects threshold can be developed based on these relationships.

**Evaluate Protectiveness and Biological Relevance** – Relate chronic values for trout reproduction and ELS effects to trout populations and potential effects to other site species. An ongoing literature review is expected to provide, if available, additional toxicity data on selenium for both aquatic and wildlife species. Some of these data may provide important insights that will aid in understanding site species.

Key Considerations-

- Data from the site characterization, laboratory studies, and literature review are being used to fill any significant data gaps with respect to understanding species sensitivities, population responses, and community responses.
- Fish population, as well as community and benthic macroinvertebrate community data, provide in-situ assessment of community and population structure and abundance relative to different exposure conditions. Analysis of these data identifies the range of species exposed and aids in identifying potential tolerance and intolerance to ambient selenium concentrations.
- The water column threshold developed will be protective of the aquatic community and wildlife receptors that may use the aquatic environment.

**Propose Site-Specific Criterion** – Propose a chronic value and rationale, and work with State and Federal agencies in the process to adopt and implement this value.

Key Considerations -

- Integrated lines of evidence developed through ongoing collaboration with State and Federal agencies will provide a criterion that satisfies Agency requirements and will be appropriate for implementation at the State level.

The intent of this approach is to build upon the level of understanding developed by USEPA during the ongoing process to propose a new selenium criterion.

## Results/Outcome

Work on various components of this approach has either been completed or is underway. Agency review and input has been integrated into the process to ensure that acceptable and relevant methods are utilized. Work completed to date includes:

- Formation of the SSSC Workgroup, including development and maintenance of a secure project webpage where all literature, project reports, conference call minutes, schedules and other relevant information, are posted;
- Work Plan - Field Monitoring Studies for Developing a Site-Specific Selenium Criterion;
- Fall 2006 Field Monitoring and Fall 2006 Interim Data Report;
- Draft Summary of Approach for Developing a Site-Specific Selenium Criterion;
- Technical Memorandum – Justification and Rationale for Fish Size Selection and Water Quality Analyses for Selenium;

- Technical Memorandum - Site Boundaries and Applicability of Site-Specific Selenium Criterion;
- Technical Memorandum - Methods for Testing Adult Brown Trout Reproductive Success;
- Spring 2007 Field Monitoring and Spring 2007 Interim Data Report;
- Fall 2007 Field Monitoring and Fall 2007 Interim Data Report ;
- Field collection and laboratory testing for adult brown trout reproductive success;
- Field collection and laboratory testing for adult YCT reproductive success;
- Early Life Stage Studies for YCT exposed to aqueous and dietary selenium; and
- Spring 2008 Field Monitoring.

As of the development of this Case Study, two fall and two spring monitoring events have been completed. Data from these studies are being compiled to begin assessing and interpreting spatial and temporal trends of selenium in abiotic and biotic media as well as for the evaluation of potential relationships between selenium in water and fish tissues. Data for the benthic invertebrate community and fish population monitoring are being evaluated to examine if site differences exist and if those differences can be related to elevated concentrations of selenium in either the water or the diet. Trout adult reproduction studies are either in the data analysis phase (brown trout) or are still being conducted (YCT).

No specific conclusions can be drawn at this time, nor have the various lines of evidence been sufficiently evaluated to assess whether there is agreement or disagreement. However, some general trends or observations are emerging:

- Upstream of the source area, aqueous selenium concentrations are low, ranging from <1 µg/L to about 1.8 µg/L. Some of the upstream areas include locations with a naturally elevated selenium background.
- At the source area, aqueous selenium concentrations were less than background before 1990 and ranged from 10 to 30 µg/L from about 2000 to the present.
- There is a gradual and consistent decrease in aqueous selenium concentrations with distance away from the source area with the most downstream locations having aqueous selenium concentrations that typically range from about 1.5 to 3 µg/L.
- Biotic tissue concentrations (benthic and periphyton) of selenium generally follow the surface water selenium concentration trends. Tissue residues peak at or adjacent to the primary source area and decrease at distance away from the source area.
- Mean sculpin selenium concentrations in tissue from background and reference locations ranged from 3.75 to 8.5 mg/kg dw, while in the source area sculpin mean selenium ranged from 10.9 to 26.6 mg/kg dw. In downstream areas, mean selenium in sculpin tissues ranged from 7.78 to 20.0 mg/kg dw.

- Mean trout selenium concentrations in tissue from background and reference locations ranged from 2.25 to 8.67 mg/kg dw, while in the source area trout mean selenium ranged from 15.2 to 25.0 mg/kg dw. In downstream areas, mean selenium in trout tissues ranged from 9.2 to 11.2 mg/kg dw.
- Emerging trends between fish tissue concentrations of selenium and aqueous selenium concentrations indicate the fish tissue and aqueous selenium relationship is quite good, despite the fact that diet is cited as the primary exposure pathway.
- Generally, sculpin tissue concentrations have been higher than trout from the same location, possibly due to site fidelity.
- Trout and sculpin are the dominant species (abundance and biomass) at nearly every site. At the Crow Creek sites downstream of Sage Creek, there appears to be a transition from a more upland, higher-gradient stream system to a lower-gradient rangeland system and species compositions appear to reflect this change with sculpin being replaced by dace, higher numbers of sucker species and mountain whitefish.
- Total trout standing crop (kg/ha) data across all three seasons evaluated to date indicate the highest standing crop at Sage Creek, immediately downstream of Hoopes Spring, the primary selenium source area.
- At this time, it is not clear whether population estimates observed at the different locations are a function of selenium exposure, habitat quality and quantity, or physical characteristics (e.g., temperature).
- Wild parental brown trout and YCT have been collected, stripped of eggs and milt, and fertilized eggs were raised in a laboratory setting. Data analysis for brown trout is beginning, and evaluations of effects (i.e., deformities, growth, survival, etc) relative to egg and whole body selenium are being conducted to derive $EC_{10}$ and $EC_{20}$ value for this species. YCT data, once the tests are completed, will be similarly evaluated.

## Lessons Learned

This project is a work in progress. Based on the work conducted thus far, several lessons learned have emerged:

- Involve Agencies early on in the process to gain input from their perspective on what will be needed to satisfy the requirements for a site-specific criterion.
- Field monitoring studies are needed to characterize exposure concentrations, range of species exposed, and selenium compartmentalization in the system, and to develop the relationship between tissue-based effects values derived from the laboratory studies and the ambient tissue and aqueous selenium relationship. Without such site-specific data, generic non–site specific translation models will be needed.
- Seasonal, multi-year field characterization of the aquatic community and physical habitat will aid in refining the community characteristics and variability.

- Adult reproduction studies with pre-exposed parents are an important component in developing the effects lines of evidence. These studies can be difficult and require proper planning to complete, but are the most practical and direct approach for assessing the full range of exposure and effects.
- Early life stage studies, if conducted, should include pre-parental exposure, if practical, but if a choice has to be made between the use of wild pre-exposed parents for the adult reproduction studies or the ELS studies to examine diet and aqueous exposures, opt for the adult reproduction studies.
- While effects to fish are likely best related to the level of selenium in reproductive tissues, State regulatory agencies evaluate compliance through water quality standards; thus, translating effects based on selenium residues to selenium concentrations in water is critical for successful development of a site-specific criterion.

## References

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