5 Selenium Accumulation and Toxicity in Freshwater Fishes

David K. DeForest

William J. Adams

CONTENTS

5.1	Introduction	194
5.2	Field Examples of Se Poisoning in Fish	195
5.3	Se Bioaccumulation in Fish	195
5.3.1	Overview of Se in the Aquatic Food Chain.....	195
5.3.2	Effects of Age, Size, and Trophic Position on Se Bioaccumulation in Fish.....	196
5.3.3	Influence of Exposure Route and Form on Se Bioaccumulation.....	196
5.3.4	Se Bioaccumulation in Different Tissues	197
5.4	Se Toxicity in Fish	198
5.4.1	Maternal Transfer Se Exposures: Effects on Embryo/Larval Development and Survival.....	200
5.4.1.1	Bluegill.....	201
5.4.1.2	Brook Trout.....	204
5.4.1.3	Brown Trout.....	205
5.4.1.4	Cutthroat Trout.....	205
5.4.1.5	Dolly Varden.....	206
5.4.1.6	Fathead Minnow	206
5.4.1.7	Largemouth Bass	207
5.4.1.8	Mosquitofish.....	207
5.4.1.9	Northern Pike.....	208
5.4.1.10	Rainbow Trout	208
5.4.1.11	Razorback Sucker	208
5.4.1.12	White Sucker.....	209
5.4.2	Direct Dietary Se Exposures: Effects on Larval/Juvenile Growth and Survival.....	209
5.4.2.1	Bluegill.....	209
5.4.2.2	Chinook Salmon	211
5.4.2.3	Fathead Minnow	212
5.4.2.4	Rainbow Trout	212
5.4.2.5	Razorback Sucker	213
5.4.2.6	White Sturgeon.....	215
5.5	Recommendation for a Fish Tissue Se Threshold	215
5.5.1	Life Stage Considerations for a Se Fish Tissue Threshold	216
5.5.1.1	Relative Sensitivity of Maternal Transfer and Juvenile Toxicity Studies....	216

5.5.1.2	Consistency of Concentration-Response Relationships.....	218
5.5.1.3	Considerations for Developing and Applying Tissue-Based Toxicity Thresholds.....	219
5.5.2	Recommendation of a Fish Tissue Se Threshold.....	219
5.5.2.1	Egg Se Threshold.....	220
5.5.2.2	Ovary Se Threshold.....	220
5.5.2.3	Whole-Body Se Threshold.....	221
5.5.2.4	Summary of Fish Tissue Se Thresholds	222
5.5.3	Comparisons of Se Fish Tissue Thresholds.....	223
	Summary.....	225
	Acknowledgment	225
	References.....	225

5.1 INTRODUCTION

Selenium (Se) is a naturally occurring element that is essential to fish, but at sufficiently high concentrations can cause large-scale reproductive failure in fish populations (Lemly and Smith 1987). Many of the early studies that evaluated the chronic toxicity of Se to fish focused on aqueous exposures to inorganic selenite (e.g., Goettl and Davies 1977; Hodson et al. 1980). However, these studies were not able to duplicate the Se toxicity observed in the field (e.g., Belews Lake, NC, U.S.) at similar aqueous Se concentrations. Subsequent studies demonstrated that the critical exposure pathway for fish is dietary exposure to organic Se (Bryson et al. 1984, 1985a, 1985b, Finley 1985, Woock et al. 1987). The biogeochemistry of Se is rather complex. Human-caused releases of Se to the aquatic environment are typically in the form of inorganic selenite (e.g., coal power plant discharges) or selenate (e.g., mobilization of Se from marine shales via groundwater leaching) (Lemly and Smith 1987). Once in the aquatic environment, entry of Se into the food web depends on the Se form released (selenite is more bioaccumulative than selenate [Besser et al. 1993]) and site-specific properties that influence the reduction of Se to organic forms (such as redox conditions and biological productivity [Lemly and Smith 1987]). For example, at a given Se concentration in surface water, Se concentrations in food webs tend to be much higher in standing water (e.g., a pond or wetland) than in flowing waters (e.g., streams) (Lillebo et al. 1988, Orr et al. 2006). Accordingly, aqueous Se concentrations resulting in toxicity to fish can be highly variable between sites and, therefore, tissue Se concentrations are a better indicator of Se toxicity to fish.

The U.S. EPA has developed a draft whole-body fish tissue-based ambient water quality criterion for Se of 7.91 $\mu\text{g/g}$ dry wt., which includes a summer/fall monitoring trigger of 5.85 $\mu\text{g/g}$ dry wt. (U.S. EPA 2004). This draft criterion is based on a toxicity study in which juvenile bluegill sunfish (*Lepomis macrochirus*) were exposed to aqueous and dietary Se under simulated winter conditions (i.e., reduced temperature and photoperiod) (Lemly 1993a). This study, which will be summarized below in more detail, was expanded upon by a separate laboratory in 2007 with somewhat different results. At the time this book went to press, the final draft Se criterion for fish tissue was still pending. There has been some disagreement in the literature on appropriate Se tissue thresholds for fish (Lemly 1993b, 1996, DeForest et al. 1999, Hamilton 2002), with differences in interpretation largely due to different opinions on the environmental relevance of different Se toxicity study types. Lemly (1993b, 1996) and Hamilton (2002), for example, recommended a whole-body Se threshold of 4 $\mu\text{g/g}$ dry wt., while DeForest et al. (1999) recommended whole-body Se thresholds of 9 $\mu\text{g/g}$ dry wt. for warm water fish and 6 $\mu\text{g/g}$ dry wt. for coldwater anadromous fish. In addition, Lemly (1993b, 1996) recommended an ovary/egg Se threshold of 10 $\mu\text{g/g}$ dry wt., while DeForest et al. (1999) recommended an ovary Se threshold of 17 $\mu\text{g/g}$ dry wt. Although the differences in these values may seem small in the field of aquatic toxicology, the differences can be important due to the often narrow range between background levels of Se and toxicity.

This chapter first provides background information on Se toxicity by summarizing field examples of Se impacts on fish populations and discussing Se bioaccumulation in fish. Mechanisms of Se toxicity in fish are then summarized, followed by a detailed review of Se toxicity studies in which Se concentrations were measured in fish tissue. Finally, fish tissue-based Se thresholds are recommended.

5.2 FIELD EXAMPLES OF Se POISONING IN FISH

The most clearly documented case of Se poisoning in fish occurred in Belews Lake (NC, U.S.), a cooling plant reservoir for a coal power plant. Operation of the power plant began in 1974 and by 1976 no centrarchids in the 0–64 mm size range were found and only 8 black and flat bullheads were found in the projected young-of-the-year size range (Cumbie and Van Horn 1978). Similarly, Hyco Reservoir (NC, U.S.) served as a cooling water impoundment for a large coal-fired electric power plant (Skorupa 1998a). In four separate coves of the reservoir, 38–75% reductions in densities of adult fish were observed between 1979 and 1980, and greater than 95% reductions in larval fish densities were observed along three transects. These episodes at Belews Lake and Hyco Reservoir provided the impetus for several of the Se toxicity studies summarized later in this chapter.

Skorupa (1998a) identified 12 real-world examples of Se poisoning, five of which were associated with Se poisoning of fish populations (the other seven examples were for Se poisoning of aquatic bird populations). The five examples with fish included Belews Lake and Hyco Reservoir, and also Martin Lake, TX (U.S.), Sweitzer Lake, CO (U.S.), and the Swedish Lakes Project (Sweden). Martin Lake received unauthorized Se discharges of two fly ash settling ponds containing 2200–2700 $\mu\text{g Se/L}$. After a die-off of fish in the reservoir was detected in the late 1970s, measured Se concentrations in fish muscle ranged from 2.0 to 9.1 $\mu\text{g/g}$ wet wt. (or 8.0–37.6 $\mu\text{g/g}$ dry wt. assuming 75% moisture) (Garrett and Inman 1984). Sweitzer Lake was created near Delta, Colorado in 1954 for recreational purposes. As cited in Skorupa (1998a), Barnhart (1957) reported that mortality of seven stocked game fishes was attributed to excessive dietary intake of Se. Butler et al. (1991) state that the presence of large green sunfish and carp populations in the lake suggest they may have adapted to Se in the lake, but populations of species once present, such as suckers, are minimal. In addition, channel catfish have been stocked in the lake, but there is no evidence of catfish reproduction in the lake (although it is unclear if suitable spawning habitat was available for catfish, which is not unusual in man-made reservoirs [Skorupa 1998a]). The minimum, median, and maximum whole-body Se concentrations in fish collected in 1988–1989 were approximately 15, 30, and 50 $\mu\text{g/g}$ dry wt., respectively; levels sufficiently high to suggest Se would be toxic. In the Swedish Lakes Project, a leachable rubber matrix containing sodium selenite was suspended in a sack in 11 lakes as a means to mitigate high mercury (Hg) levels. The lakes initially had a Se concentration of about 0.1 $\mu\text{g/L}$ and the target Se concentration was 3–5 $\mu\text{g/L}$ (although up to 25–35 $\mu\text{g Se/L}$ was measured within 100 m of the leach sacks) (Skorupa 1998a). Prior to Se treatment, muscle Se concentrations in yellow perch averaged 0.8–2.8 $\mu\text{g/g}$ dry wt. After the first year of treatment the muscle Se concentrations averaged 6–36 $\mu\text{g/g}$ dry wt. and by the second year perch populations collapsed in several lakes. Ultimately, the researchers could not clearly establish the cause of the perch population collapse, but the possibility of Se poisoning was not ruled out (Skorupa 1998a).

5.3 Se BIOACCUMULATION IN FISH

5.3.1 OVERVIEW OF Se IN THE AQUATIC FOOD CHAIN

In nature, juvenile and adult fish may be simultaneously exposed to inorganic and organic Se forms in both the water and the diet; however, in general, it is exposure to organic Se forms in the diet that is most important at environmentally relevant exposure levels (Bertram and Brooks 1986, Woock et al. 1987, Besser et al. 1993, Coyle et al. 1993). Field-based Se bioaccumulation factors (BAFs)

for fish, defined as the ratio of the whole-body Se concentration in fish to the Se concentration in colocated surface water, are generally greater than 1000 (Brix et al. 2005), inversely related to exposure concentration, and can be highly variable between sites and depending on the site conditions. In terms of the BAF, most of the Se bioaccumulation in the aquatic food chain occurs at the level of primary producers and microorganisms. A number of studies have identified primary producers and microorganisms as being the critical link where Se enters the aquatic food chain (e.g., Riedel et al. 1991, Maier and Knight 1994, Sanders and Gilmour 1994, Williams et al. 1994, Malchow et al. 1995, Riedel and Sanders 1996). Although it is mostly organic Se forms that are transferred in the aquatic food chain, Se typically enters aquatic systems, either naturally or anthropogenically, in an inorganic form (e.g., selenate, selenite). Primary producers can rapidly bioconcentrate and incorporate inorganic Se into selenoamino acids, such as selenocysteine or selenomethionine (Ogle et al. 1988). Once in the aquatic food chain, Se trophic transfer factors (TTFs), defined as the Se concentration in an organism's tissue to its concentration in the organism's food item, typically range from <1 to 2 for fish in laboratory studies (DeForest et al. 2007, Luoma and Presser 2009).

5.3.2 EFFECTS OF AGE, SIZE, AND TROPHIC POSITION ON SE BIOACCUMULATION IN FISH

Unlike chemicals that truly biomagnify up successive levels of the food chain and with concentrations increasing in older organisms, such as that observed for methyl mercury, Se concentrations in fish do not appear to increase with trophic level or organism age. May et al. (2001, 2008) reported whole-body Se concentrations in various fish species of different sizes collected from the Republican River Basin (Colorado, Nebraska, Kansas—U.S.) and the Solomon River Basin (Kansas—U.S.) and did not observe a consistent pattern of whole-body Se concentrations as a function of fish size. In both basins, whole-body Se concentration in channel catfish tended to be highest in the smallest fish collected, while for other species (common carp, green sunfish, bluegill sunfish, and largemouth bass) there was no apparent trend in Se concentration as a function of size. Muscatello et al. (2008) measured Se concentrations in aquatic food chains from two Se exposure sites downstream from uranium mining and milling operations in northern Saskatchewan, Canada. They observed that Se concentrations increased by approximately 1.5 to 6-fold between plankton, invertebrates, and forage fish; however, no biomagnification was observed between forage fish and predatory fish. Overall, the data from laboratory and field studies suggest that Se concentrations in fish are not size-, age-, or trophic level-dependent. However, work by Stewart et al. (2004) has shown using stable isotope techniques that Se concentrations can increase across trophic levels in specific food webs.

5.3.3 INFLUENCE OF EXPOSURE ROUTE AND FORM ON SE BIOACCUMULATION

As summarized in Luoma and Presser (2009), the uptake of dissolved Se by aquatic animals is slow, regardless of form, and therefore makes little or no direct contribution to bioaccumulation and toxicity in animals. This observation is supported by a variety of aquatic Se studies. Lemly (1985), for example, stated that the results of waterborne Se toxicity studies do not give a true indication of the relative sensitivity of fish to Se on a long-term basis in the environment and that it is the dietary component that is responsible for the largest portion of Se present in aquatic animals in natural waters. As concluded in a recent expert Se workshop on Se in aquatic environments, the dietary route of exposure generally dominates Se bioaccumulation processes and traditional approaches for predicting bioaccumulation based on dissolved concentrations does not work for Se (Chapman et al. 2009). Further, organic Se is the critical Se form in aquatic food webs. As summarized in Palace et al. (2004), oviparous vertebrates are the most sensitive organisms to Se, as these organisms efficiently transfer selenomethionine to their eggs, and that selenomethionine is the dominant Se form in fish and bird eggs. These observations are further supported by the studies summarized below.

The Se bioaccumulation and toxicity data for bluegill reported in Cleveland et al. (1993), Coyle et al. (1993), and Hermanutz et al. (1996) support that dietary exposure to Se, and particularly

organic Se, is the critical exposure pathway. In Belews Lake, mean whole-body Se concentrations in centrarchids (e.g., bluegill) ranged from approximately 20 to >100 $\mu\text{g/g}$ dry wt. at water Se concentrations of approximately 10 $\mu\text{g/L}$ (Cumbie and Van Horn 1978). However, Cleveland et al. (1993) exposed bluegill to an aqueous 6:1 selenate:selenite mixture up to approximately 1100 $\mu\text{g/L}$ for 60 days and whole-body Se concentrations only reached approximately 10 $\mu\text{g/g}$ dry wt. In the same study, an aqueous selenite concentration of 640 $\mu\text{g/L}$ was necessary to achieve a whole-body Se concentration of approximately 5 $\mu\text{g/g}$ dry wt. in bluegill. The aqueous Se concentration of 640 $\mu\text{g/L}$ (6:1 selenate:selenite) is higher than some acute selenate and selenite LC50 values for invertebrates (U.S. EPA 2004), which questions the environmental relevance of water-only Se exposures for relating fish tissue residues to toxicity. Coyle et al. (1993) observed that Se accumulation of Se in bluegill ovaries increased with increasing dietary organic Se exposures, but not when bluegill were exposed to an aqueous selenate:selenite mixture of 8.4 $\mu\text{g/L}$. The results of Hermanutz et al. (1996) also demonstrate the importance of the dietary exposure route for Se bioaccumulation. They conducted three sets of experiments in experimental streams. In the first two experiments, bluegill were exposed to aqueous selenite and dietary Se accumulated in the food items present in the streams, while in the third experiment the aqueous selenite source was turned off and bluegill were only exposed to the residual Se present in the food items in the streams. Se levels in the bluegill from experiment III (diet-only exposure) were nearly as high as Se from the aqueous Se exposures. It is critical, therefore, that the Se exposure route in chronic Se toxicity tests include the dietary exposure pathway.

In the Cleveland et al. (1993) study discussed earlier, 5-month-old juvenile bluegill were exposed to a waterborne 6:1 selenate:selenite mixture and 3-month-old juvenile bluegill to dietary seleno-L-methionine. The relationship between whole-body Se and juvenile mortality was much different in the aqueous exposures than in the dietary exposures. For example, at day 30 mortality was 87.5% in fish exposed to an aqueous Se concentration of 2700 $\mu\text{g/L}$ (for comparison, the surface water Se concentration in Belews Lake was approximately 10 $\mu\text{g/L}$), which resulted in a whole-body Se concentration of 14.5 $\mu\text{g/g}$ dry wt. In the dietary Se study, fish exposed to the highest dietary Se concentration of 26.0 $\mu\text{g/g}$ dry wt. had 17.5% mortality after 90 days, which was not significantly different from control mortality. These fish had a whole-body Se concentration of 13.5 $\mu\text{g/g}$ dry wt. Accordingly, very similar whole-body Se concentrations were associated with almost complete mortality (when exposed to acutely toxic aqueous Se levels) or low mortality (statistically indistinguishable relative to the controls) when exposed to environmentally relevant dietary Se levels.

Due to these observations, in developing and recommending tissue-based Se thresholds we only considered Se toxicity studies that included dietary exposures to organic Se. DeForest et al. (1999) previously excluded water-only Se exposures in recommending fish tissue-based Se thresholds, as did the U.S. EPA in deriving its draft fish tissue-based Se criterion (U.S. EPA 2004). Ultimately, however, this decision had limited influence on the tissue-based thresholds we recommend since, for reasons discussed later, we considered maternal transfer studies to be the most relevant study type for developing and implementing fish tissue-based Se thresholds. In maternal transfer studies, parent fish are exposed to Se, which is transferred to the ovaries and then the eggs, where, at sufficiently high concentrations, can adversely affect larval development and survival. All of the maternal transfer studies available for deriving egg or ovary Se toxicity thresholds are based on dietary exposures to organic Se (either explicitly in the lab or implicitly in field-exposed fish).

5.3.4 SE BIOACCUMULATION IN DIFFERENT TISSUES

Se concentrations in fish, from both laboratory toxicity studies and monitoring in the field, have been measured in a wide variety of tissues, including whole-body tissue, muscle, liver, ovaries, and eggs. The disparity in tissue types analyzed in toxicity tests has made it difficult to compare the relative sensitivities of different fish species and to develop broadly applicable tissue guidelines or

criteria. In deriving its draft whole-body-based Se criterion, the U.S. EPA estimated whole-body Se concentrations from individual tissues when necessary (U.S. EPA 2004). For evaluating the reproductive endpoint of embryo/larval development and mortality, the most appropriate tissues to analyze are the ovaries or eggs, as these tissues are closely linked to the site of toxic action. However, due to practical sampling constraints (e.g., getting adequate tissue mass or seasonal issues), whole-body or muscle Se measurements can also be considered. In general, as discussed later, whole-body Se can provide a reasonable indicator of potential reproductive toxicity in fish. However, relationships between Se in tissues can vary by species, which in turn imparts uncertainty in attempting to develop, and then apply, a whole-body- or muscle-based Se threshold in the field. It is apparent that a whole-body or muscle Se guideline could be either dramatically under-protective for one species or over-protective for another species. The clearest approach for overcoming these species-specific relationships in Se bioaccumulation is to focus on an egg or ovary-based Se threshold. Such a Se threshold could still be related to a site-specific whole-body or muscle Se threshold if a species- and site-specific relationship is derived (deBruyn et al. 2008).

5.4 SE TOXICITY IN FISH

In general, adult fish do not appear to be especially sensitive to Se, and there are numerous studies that found no link between elevated egg Se concentrations and fertilization or hatching success, including Gillespie and Baumann (1986), Coyle et al. (1993), Holm et al. (2005), and Muscatello et al. (2006). The classic pathway of documented Se poisoning in fish is exposure of adult female fish to Se, maternal transfer of the Se to the ovaries and then eggs, and then, if sufficiently high egg Se concentrations are reached, larval deformities and mortality. As discussed in Lemly (2002), excess dietary Se is deposited in the developing eggs of fish, particularly in the yolk. After the eggs hatch, the larval fish rapidly use the yolk as an energy supply and a source of protein for building new body tissues. Lemly (2002) also provides a good overview of Se effects on specific fish organs, as well as Se-induced terata (e.g., spinal, craniofacial, and finfold deformities), which are permanent biomarkers of toxicity that can be lethal to the fish. Edema, or accumulation of fluids in the body cavity and head, is another effect of elevated Se levels in fish (Lemly 2002). In the field, larval terata and edema represent an endpoint that can be used as diagnostic evidence of Se poisoning in the fish. In the laboratory, it has also been shown that juvenile fish exposed to dietary organic Se can experience reduced growth and survivorship. Studies in which fish were exposed to aqueous selenite were not considered for reasons discussed in Section 5.3.3. Table 5.1 lists the toxicity studies in which Se was measured in whole-body fish tissue, but excluded from consideration in recommending a whole-body Se threshold.

Chronic dietary Se toxicity studies with fish, in which Se tissue residues were measured, fall under two general categories: (1) exposure of adults and evaluation of effects on offspring (e.g., larval mortality, teratogenesis, and edema) or (2) direct exposure of juveniles. The studies with adults can be further divided into two additional categories: (1) exposure of adults to dietary Se in the laboratory and (2) natural exposure of adults in the field, stripping of gametes, and rearing of embryos and larvae in the laboratory. This latter technique has typically been used to evaluate Se toxicity in salmonids (e.g., rainbow trout, brook trout, and cutthroat trout) and other rather large species where it is more difficult to rear adults in the laboratory. Further, this latter approach is useful for site-specific toxicity evaluations because the Se exposure history of the fish population at the site is reflected in the toxicity test results.

In DeForest et al. (1999) we provided a study-by-study review of Se toxicity studies with fish, in which Se concentrations in fish tissues were measured during and/or after the Se exposure period. That review summarized the Se toxicity studies available at the time (with the exception of Doroshov et al. [1992] and CP&L [1997], which we were not aware of) and ultimately recommended fish tissue-based Se guidelines by considering only those studies that considered dietary exposures to organic Se forms (typically selenomethionine). The following sections separately summarize

TABLE 5.1

Summary of Toxicity Studies in Which Selenium Was Measured in Whole Body or Ovary Tissue (or Specific Tests Within a Study), but Were Excluded from This Evaluation

Reference	Species	Tissue Se (µg/g dry wt.)	Summary of Test Conditions and Basis for Exclusion
Beyers and Sodergren 2002; Hamilton et al. 2005c	Razorback sucker	—	Both studies evaluated Se toxicity to larval razorback suckers, but identified widely different Se thresholds. Beyers and Sodergren (2002) identified a no effects whole-body Se threshold of >42 µg/g dry wt., while Hamilton et al. (2005c) reported that whole-body Se concentrations of 5.4–6.1 µg/g dry wt. adversely affected survival. Given the disparity in the results and because the toxicity response for each source of larvae in Hamilton et al. (2005c) was not clearly related to either the Se exposure concentrations or the internal tissue Se concentrations (see text), we did not attempt to identify a Se threshold for razorback sucker in our evaluation.
Cleveland et al. 1993	Bluegill sunfish	WB NOEC/LOEC = 3.8/5.0	Juvenile bluegill were exposed to aqueous Se (6:1 selenate:selenite) concentrations ranging from 170 to 2700 µg/L for 60 days. The NOEC and LOEC for mortality were 340 and 680 µg/L, which were associated with whole-body Se concentrations of approximately 3.8 and 5.0 µg/g dry wt., respectively. This study was excluded from our Se threshold evaluation because water-only exposures to inorganic Se were not considered environmentally relevant (see Section 5.3.3).
GEI Consultants 2008	Fathead minnow	—	Fish were collected from streams across a gradient of Se concentrations and spawned in the laboratory. However, an effect level could not be estimated due to high variation in the endpoint showing the greatest response to Se (graduated severity index of larval malformations).
Hamilton and Wiedmeyer 1990	Chinook salmon	WW WB NOEC/LOEC = 10.4/19.5 FW WB NOEC/LOEC = 8.2/14.4 BW WB NOEC = >5.3	Fish were exposed to a mixture of aqueous selenate, selenite, boron, and molybdenum in well water (WW), freshwater (FW), and brackish water (BW) for 60–90 days. This study was excluded from our Se threshold evaluation because fish were exposed to a mixture of elevated elements and because water-only exposures to inorganic Se were not considered environmentally relevant (see Section 5.3.3).
Hamilton et al. 1986	Chinook salmon	WB NOEC/LOEC = 9.6/13	Parr were fed Se-amended mosquitofish collected from areas with potential contamination from other chemicals.
Hamilton et al. 1990	Chinook salmon	WB LOEC: <3.3	In one test from this study juvenile fish were fed Se-amended mosquitofish collected from areas with potential contamination from other chemicals (this study included another test in which fish were fed a diet spiked with seleno-DL-methionine—this test was included in our evaluation [Table 5.3]).

continued

TABLE 5.1 (continued)

Summary of Toxicity Studies in Which Selenium Was Measured in Whole Body or Ovary Tissue (or Specific Tests Within a Study), but Were Excluded from This Evaluation

Reference	Species	Tissue Se (µg/g dry wt.)	Summary of Test Conditions and Basis for Exclusion
Hodson et al. 1980	Rainbow trout	WB NOEC: >1.8	Fish were exposed to five aqueous selenite concentrations ranging from 5.5 to 53 µg/L from the newly fertilized egg stage until 44 weeks posthatch, but tissue Se was only measured in the 53 µg Se/L treatment. Negligible effects were observed in the 53 µg Se/L treatment. In addition, this study was excluded from our Se threshold evaluation because water-only exposures to inorganic Se were not considered environmentally relevant (see Section 5.3.3).
Hunn et al. 1987	Rainbow trout	WB NOEC/LOEC = 2.6/4.3	Fry were exposed to five aqueous selenite concentrations ranging from 7.8 to 100 µg/L for 90 days. The aqueous NOEC and LOEC for mortality were 21.0 and 47.2 µg/L, respectively. This study was excluded from our Se threshold evaluation because water-only exposures to inorganic Se were not considered environmentally relevant (see Section 5.3.3).

WB = whole body; NOEC = no-observed-effect-concentration; LOEC = lowest-observed-effect-concentration.

the maternal transfer and juvenile toxicity studies with Se. The review is then further organized by fish species. Only studies that measured Se concentrations in whole-body tissue, ovaries, or eggs were included in this evaluation. In addition, due to species-specific differences in Se concentration relationships between tissues discussed in Section 5.3.4, this evaluation only considered measured Se concentrations in fish (no attempt was made to estimate Se concentrations in one tissue from concentrations measured in another tissue).

5.4.1 MATERNAL TRANSFER SE EXPOSURES: EFFECTS ON EMBRYO/LARVAL DEVELOPMENT AND SURVIVAL

Studies in which adult fish were exposed to dietary organic Se, and effects on offspring were evaluated, have been conducted with 13 species of fish: bluegills (*L. macrochirus*), brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), cutthroat trout (*Oncorhynchus clarki*), Dolly Varden (*Salvelinus malma*), fathead minnows (*Pimephales promelas*), largemouth bass (*Micropterus salmoides*), eastern mosquitofish (*Gambusia holbrooki*), western mosquitofish (*Gambusia affinis*), northern pike (*Esox lucius*), rainbow trout (*Oncorhynchus mykiss*), razorback suckers (*Xyrauchen texanus*), and white suckers (*Catostomus commersoni*). The toxicity results are summarized below as no-observed effect concentrations (NOECs) and lowest-observed effect concentrations (LOECs). The NOEC is the highest Se concentration in a study that did not result in statistically significant effects relative to the control, and the LOEC is the lowest Se concentration in a study that did result in statistically significant effects relative to the control. In addition, EC10 and EC20 values (10% and 20% effect concentrations) were identified, which were either reported by the study authors or which we calculated from the concentration-response data reported in the study.

5.4.1.1 Bluegill

Bryson *et al.* (1984, 1985a, 1985b)—Bryson *et al.* (1984) collected adult bluegills (*L. macrochirus*) from the Se-contaminated Hyco Reservoir (NC, U.S.) and from a reference lake. All combinations of males and females from Se-contaminated and reference lakes were artificially crossed in different ash pond concentrations (0%, 20%, and 50% ash pond water). Swim-up larvae were fed zooplankton from either Hyco Reservoir or the reference lake. Larvae were observed for 28 days after hatching. All larvae from a Hyco Reservoir female exhibited abnormal development and 100% mortality prior to reaching swim-up. The mean ovary Se concentration in the females from Hyco Reservoir was 49 µg/g dry wt. (Table 5.2).

Bryson *et al.* (1985a), based on a study similar to Bryson *et al.* (1984), reported that egg hatchability from female bluegills averaging 9.1 µg/g dry wt. Se in the ovaries was 88%, and that 93% survived through swim-up (Table 5.2). Swim-up was only approximately 5.6% in larvae from females averaging 30 µg Se/g dry wt. in the ovaries (Table 5.2). The results reported in Bryson *et al.* (1985b) are somewhat difficult to interpret because none of the control fish spawned, but hatching and swim-up success were 83.8–86.6% and 91.1–95.5%, respectively, from parents with a mean ovary Se concentration of 14.8 µg/g dry wt., and 86.0% and 83.3–97.4% from parents with a mean ovary Se concentration of 9.2 µg/g dry wt. (Table 5.2). Overall, the studies by Bryson and colleagues resulted in ovary NOECs of >9.1, >9.2, and >14.8 µg/g dry wt. and ovary LOECs of <30 and <49 µg/g dry wt. (Table 5.2).

Gillespie and Baumann (1986)—Adult bluegills (*L. macrochirus*) were collected from the Se-contaminated Hyco Reservoir and from an uncontaminated reservoir and all possible parent combinations were artificially crossed. Zygotes were reared in uncontaminated water and evaluated for percent fertilization and hatching success. In over 18 crosses during a two year period, no significant differences in percent fertilization or percent hatch were found between all parent combinations. However, all crosses involving a female from the Se-contaminated water body resulted in larvae with gross abnormal morphology (65–100%), and all larvae died before they reached the swim-up stage. The ovary Se concentration in the female producing 65% abnormal larvae was 38.6 µg/g dry wt. (assuming a moisture content of 85%, per Gillespie and Baumann [1986]). Accordingly, the ovary LOEC from this study was <38.6 µg/g dry wt. (Table 5.2).

Doroshov *et al.* (1992)—Bluegill (*L. macrochirus*) were fed Se concentrations of 5.5, 13.9, and 21.4 µg/g (as seleno-L-methionine). Natural spawning was not successful, so ovulation was hormonally induced and fertilization was conducted *in vitro*. The most sensitive endpoint was larval edema, which was observed during the first 5 days posthatch. Edema was observed in 0%, 5%, and 95.7% of the larvae in the control, 5.5, 13.9, and 21.4 µg/g Se diets, respectively. Because 5% edema was statistically significant ($p \leq .05$) in the 13.9 µg/g Se treatment, 5.5 and 13.9 µg/g were identified as the dietary NOEC and LOEC. The mean egg Se concentrations associated with the NOEC and LOEC were reported as 8.3 and 19.5 µg/g dry wt., and the mean ovary Se concentrations associated with the NOEC and LOEC were reported as 6.34 and 14.1 µg/g dry wt. In terms of identifying a Se toxicity threshold from this study, it is interesting to note that the egg Se concentrations associated with the dietary LOEC of 13.9 µg/g were variable. Edema was measured in six progenies from parents exposed to this dietary Se concentration and the associated egg Se concentrations are reported for three of these progenies in Table 21 of Doroshov *et al.* (1992). Egg Se concentrations for these three progenies from the 13.9 µg Se/g diet were 8.55, 22.06, and 30.20 µg/g dry wt. Likewise, ovary Se concentrations were 3.94, 21.82, and 20.40 µg/g dry wt. for the 13.9 µg Se/g dietary treatment. Comparison of these Se concentrations to the edema data reported in Appendix 8 of Doroshov *et al.* (1992) shows that edema was 0% at an egg Se concentration of 8.55 µg/g dry wt. (ovary Se = 3.94 µg/g dry wt.), 10% at 22.06 µg/g dry wt. (ovary Se = 21.82 µg/g dry wt.), and 10% at 30.20 µg/g dry wt. (ovary Se = 20.40 µg/g dry wt.). Thus, based on data reported for three of the six progenies, 10% edema was only observed in progeny associated with egg Se concentrations ranging from 22.06 to 30.20 µg/g dry wt. (geometric mean of 25.81 µg/g dry wt.) or ovary Se concentrations ranging from

TABLE 5.2
Summary of Toxicity Studies That Evaluated Selenium Toxicity to Embryos/Larvae Resulting from Maternal Transfer

Species	Reference	Adult Exposure	Endpoint	Tissue	Se Concentration (µg/g dry wt.)				
					NOEC	LOEC	EC10	EC20	
Bluegill	Bryson et al. 1984	Field	Larval mortality	Ovary	—	<49	—	—	
	Bryson et al. 1985a	Field	Hatchability/swim-up	Ovary	>9.1	—	—	—	
		Field	Hatchability/swim-up	Ovary	—	<30	—	—	
	Bryson et al. 1985b	Field	Hatchability/swim-up	Ovary	>14.8	—	—	—	
		Field	Hatchability/swim-up	Ovary	>9.2	—	—	—	
	Gillespie and Baumann 1986	Field	Larval edema	Ovary	—	<38.6	—	—	
	Doroshov et al. 1992	Lab	Larval edema	Ovary	3.94	21.10	16 ^a	20 ^a	
		Lab	Larval edema	Egg	8.55	25.81	21 ^a	23 ^a	
	Coyle et al. 1993	Lab	Larval mortality	WB	7	16	8 ^a	8.5 ^a	
		Lab	Larval mortality	Ovary	20	35	24 ^a	27 ^a	
Brook trout		Lab	Larval mortality	Egg	22.5	41.3	22 ^a	26 ^a	
	Hermanutz et al. 1996	Mesocosm	Larval edema	WB	4.4	21.8	7.7 ^a	9.7 ^a	
		Mesocosm	Larval edema	Ovary	17.3	69	30 ^a	36 ^a	
	Holm et al. 2005	Field	Larval deformities	Egg	17	20	20 (EC06)	—	
	Formation Environmental 2009	Field	Alevin mortality	Egg	—	—	17.7	21.6	
			Larval deformities	Egg	—	—	19.3	21.7	
					—	—	—	—	
					—	—	—	—	

Cutthroat trout	Kennedy et al. 2000	Field	Larval deformities/ mortality	Egg	>21	—	—	—
	Hardy et al. 2010	Lab	Larval deformities/ mortality	WB	>11.37	—	—	—
		Lab	Larval deformities/ mortality	Egg	>16.04	—	—	—
	Rudolph et al. 2008	Field	Larval deformities	Egg	20.6	46.8	—	—
		Field	Alevin mortality	Egg	—	—	17 ^a	23 ^a
Dolly Varden	Golder 2009	Field	Larval deformities	Egg	—	—	54	60
Fathead minnow	Ogle and Knight 1989	Lab	Reproduction	WB	>7.5	—	—	—
		Lab	Reproduction	Ovary	>10.92	—	—	—
	Schultz and Hermanutz 1990	Mesocosm	Larval edema/lordosis	Ovary	—	<23.6	—	—
Largemouth bass	CP&L 1997	Lab	Larval mortality	Ovary	—	—	22 ^a	24 ^a
Northern pike	Muscatello et al. 2006	Field	Larval deformities	Egg	3.80	31.28	20.38	33.55
Rainbow trout	Holm et al. 2005	Field	Larval deformities	Egg	17	25	23 ^a	27 ^a
Eastern mosquitofish	Staub et al. 2004	Field	Brood size/offspring viability	WB	>11.85	—	—	—
Western mosquitofish	Saiki et al. 2004	Field	Fry mortality and deformities	WB	>17.5	—	—	—
Razorback sucker	Hamilton et al. 2005a, 2005b	Field	Larval deformities	Egg	37.8	46.5	—	—
White sucker	de Rosemond et al. 2005	Field	Larval deformities	Egg	—	—	25.6 (EC13)	—

^a We calculated these EC10 and EC20 values from the concentration-response data reported in the original studies.

WB = whole body; NOEC = no-observed-effect concentration; LOEC = lowest-observed-effect concentration; EC10 = 10% effect concentration; EC20 = 20% effect concentration.

20.40 to 21.82 $\mu\text{g/g}$ dry wt. (geometric mean of 21.10 $\mu\text{g/g}$ dry wt.). Accordingly, the geometric mean egg and ovary Se concentrations of 25.81 and 21.1 $\mu\text{g/g}$ dry wt., respectively, were identified as the LOECs from this study (Table 5.2). By fitting a probit model to the concentration-response data for edema, we calculated ovary Se EC10 and EC20 values of 16 and 20 $\mu\text{g/g}$ dry wt. and egg Se EC10 and EC20 values of 21 and 23 $\mu\text{g/g}$ dry wt. (Table 5.2).

Coyle *et al.* (1993)—Bluegill (*L. macrochirus*) were fed diets containing Se concentrations of 0.8, 4.6, 8.5, 16.8, and 33.3 $\mu\text{g/g}$, and simultaneously exposed to a nominal aqueous Se concentration of 10 $\mu\text{g/L}$ for 140 days. Seleno-L-methionine was used for the dietary exposures and a 6:1 ratio of selenate:selenite was used for aqueous exposures. Spawning frequency, fecundity, and hatching success were monitored during the last 80 days of the test and fry survival was monitored for 30 days after hatch (fry were exposed to the same aqueous Se concentrations as their parents over the 30-day posthatch duration). No effects on spawning frequency, fecundity, or hatching success were observed in any of the treatments. In addition, growth of bluegill fry at either 15 or 30 days posthatch was unaffected by any of the Se treatments tested. However, larvae from females fed the 33.3 $\mu\text{g Se/g}$ diet had greatly reduced survival (7%) relative to other treatments (75–90%) 5–6 days after hatching. All bluegill larvae in this study experienced increased mortality from approximately day-6 through day-30 posthatch, including control fish. Coyle *et al.* (1993) attributed this increased mortality to the likelihood of starvation resulting from the unsuccessful transition from endogenous to exogenous feeding. The dietary NOEC and LOEC, therefore, was 16.8 and 33.3 $\mu\text{g Se/g}$, respectively. The corresponding mean ovary Se concentrations at the NOEC and LOEC were 20 and 35 $\mu\text{g/g}$ dry wt., respectively, and the mean egg Se concentrations at the NOEC and LOEC were 22.5 and 41.3 $\mu\text{g/g}$ dry wt. (Table 5.2). On the basis of the whole-body Se concentrations in adults on the day spawning was initiated, the whole-body Se concentrations associated with the NOEC and LOEC were 7 and 16 $\mu\text{g/g}$ dry wt., respectively (Table 5.2). By fitting a probit model to the concentration-response data for larval mortality, we calculated EC10 and EC20 values of 24 and 27 $\mu\text{g/g}$ dry wt. for ovaries, 22 and 26 $\mu\text{g/g}$ dry wt. for eggs, and 7.8 and 9.2 $\mu\text{g/g}$ dry wt. for whole body (Table 5.2).

Hermanutz *et al.* (1992, 1996); Tao *et al.* (1999)—Adult bluegill (*L. macrochirus*) were exposed to Se in experimental streams dosed with selenite. The streams contained well-developed assemblages of fish food organisms, so an environmentally relevant dietary Se exposure pathway was simulated. Three Se exposure regimes were tested: (1) bluegills were exposed in streams dosed with 10 and 30 $\mu\text{g/L}$ selenite; (2) bluegills were exposed in streams dosed with 2.5 and 10 $\mu\text{g/L}$ selenite; and (3) bluegills were exposed in streams that had previously been dosed with either 2.5 or 10 $\mu\text{g/L}$ selenite, but the selenite source had been turned off. Of these studies, we focused on the results from the second study that evaluated streams continuously dosed to maintain aqueous Se concentrations of approximately 2.5 and 10 $\mu\text{g/L}$ (plus a control stream). At the end of the exposure period, whole-body Se concentrations in adult bluegill were approximately 1.6, 4.4, and 21.8 $\mu\text{g/g}$ dry wt. (assuming 75% moisture) and ovary Se concentrations were 5.1, 17.3, and 69.0 $\mu\text{g/g}$ dry wt. (assuming 85% moisture, per Gillespie and Baumann [1986]). The most sensitive endpoint was larval edema, which was 0% in the control stream and in the 2.5 $\mu\text{g Se/L}$ stream and 100% in the 10 $\mu\text{g Se/L}$ stream. Thus, the whole-body NOEC and LOEC were 4.4 and 21.8 $\mu\text{g/g}$ dry wt., respectively, and the ovary NOEC and LOEC were 17.3 and 69 $\mu\text{g/g}$ dry wt. (Table 5.2).

5.4.1.2 Brook Trout

Holm (2002); Holm *et al.* (2003, 2005)—Gametes from adult brook trout (*S. fontinalis*) were collected from a stream with elevated Se (Luscar Creek, downstream from active coal mining), intermediate Se (Gregg River), and a reference stream (Cold Creek) in west-central Alberta, Canada. Eggs were fertilized in the laboratory and deformities and edema in fry were evaluated. The most pronounced effect observed in the trout from Luscar Creek was a $7.9 \pm 1.8\%$ (mean $\pm$ SE) incidence of craniofacial deformities, compared with a 2.3 ± 0.8 and $2.1 \pm 0.6\%$ incidence of craniofacial deformities in fish collected from the Gregg River and the Cold Creek reference stream, respectively.

The incidence of craniofacial deformities was significantly different ($p < .05$) in Cold Creek relative to Luscar Creek, but not in the Gregg River relative Luscar Creek (despite similar levels of craniofacial deformities observed in the Gregg River and Cold Creek). The mean ($\pm$ SE) egg Se from parent fish collected from Luscar Creek was 7.8 ± 0.7 $\mu\text{g/g}$ wet wt. (20 ± 1.8 $\mu\text{g/g}$ dry wt.), compared to mean egg Se concentrations of 6.6 ± 0.4 $\mu\text{g/g}$ wet wt. (17 ± 1.0 $\mu\text{g/g}$ dry wt.) for Gregg River fish and 1.3 ± 0.1 $\mu\text{g/g}$ wet wt. (3.3 ± 0.26 $\mu\text{g/g}$ dry wt.) for Cold Creek fish (wet-to-dry weight conversions based on an assumed moisture content of 61% [Holm et al. 2005]). Accordingly, egg Se concentrations of 17 and 20 $\mu\text{g/g}$ dry wt. may be considered the NOEC and LOEC from this study, although the LOEC based on Luscar Creek fish is based on just a 6% increase in craniofacial deformities relative to the intermediate Se (Gregg River) and reference site (Cold Creek) (Table 5.2).

5.4.1.3 Brown Trout

Formation Environmental (2009)—Adult brown trout (*S. trutta*) were collected from south-east Idaho streams with varying Se levels. Gametes were stripped and eggs were fertilized in the field. The fertilized eggs were then transported to the laboratory, where they were reared through swim-up. Larvae were observed for mortality and deformities. The resulting egg Se EC10 and EC20 values were 17.7 and 21.6 $\mu\text{g/g}$ dry wt. for alevin mortality and 19.3 and 21.7 $\mu\text{g/g}$ dry wt. for larval deformities (Table 5.2).

5.4.1.4 Cutthroat Trout

Kennedy et al. (2000)—Adult cutthroat trout (*O. clarki lewisi*) were collected from a stream with elevated Se levels (Fording River) and a reference site (Connor Lake) in southeastern British Columbia, Canada. Gametes were stripped and eggs were fertilized and transported to the laboratory for rearing. Larvae and fry were then observed for deformities and mortality. Se concentrations in Fording River eggs were highly variable, ranging from 8.7 to 81.3 $\mu\text{g/g}$ dry wt., with an average of 21.2 $\mu\text{g/g}$ dry wt. No significant ($p < .05$) correlations between Se concentrations in eggs from Fording River and Connor Lake and hatching time, deformities, and mortalities were observed. However, the variability in the data may have masked some of the potential Se-related effects. For example, percent mortalities ranged from 1.5% to 43.7% after yolk absorption, with the correlation between egg Se and mortalities having a p -value of 0.15. Because no statistically significant effects could be identified from this study, the mean egg Se concentration of 21.2 $\mu\text{g/g}$ dry wt. was identified as a NOEC. Although there is added uncertainty in this value given the variability in the data and the relationship between egg Se and larval mortality postyolk absorption, the other toxicity studies with cutthroat trout, summarized later, suggest that this egg Se NOEC estimate is not unreasonable.

Hardy et al. (2010)—The sensitivity of southeast Idaho reared cutthroat trout (*O. clarki*) to dietary Se was evaluated in a multiyear laboratory study. Eyed cutthroat trout eggs were obtained from the Henry's Lake hatchery (southeast Idaho) and reared in the laboratory. Once trout reached the swim-up stage, they were fed Se diets of 1.2 (control), 3.8, 6.4, 9.0, 11.5, or 12.0 $\mu\text{g/g}$ through maturation and spawning. After 80 weeks of feeding, control mortality was 19.5%, and then generally decreased with increasing dietary Se concentration (mortality was 3.0% in fish fed the highest Se diet of 12.0 $\mu\text{g/g}$). The concentration-response relationship for larval deformities was parabolic, with the highest percentage of deformities (15.9–20.2%) being observed at the intermediate exposure levels and the percentage of deformities at the two highest treatments (6.8–7%) were comparable to in the control fish (5.6%). The percentages of total deformities are higher than the percentages of craniofacial deformities. Hardy et al. (2010) noted that for comparison, Lemly (1997) considered deformity rates of <6% for warm water fishes indistinguishable from background and that deformity rates between 6% and 12% the effect of maternal Se intake is deemed slight. Hardy et al. (2010) also noted that this categorization is based on specific deformities of the skull and jaw, not with general deformities often seen in trout and salmon, such as curled tails and cojoined twins. Accordingly, the low frequency of deformities (both total and craniofacial) in the highest dietary Se treatments,

coupled with the low incidence (<10%) of craniofacial deformities in all treatments, suggests that none of the dietary Se treatments resulted in significant effects on cutthroat trout in this study. The highest whole-body and egg Se concentrations measured across all dietary Se treatments were 11.37 and 16.04 µg/g dry wt. The study also evaluated Se depuration and found that cutthroat trout fed the highest Se diet excreted Se at the highest rate. Consequently, Hardy et al. (2010) suggested that cutthroat trout have the ability to tolerate relatively high levels of Se exposure by maintaining non-toxic body levels via enhanced excretory mechanism. The whole-body and egg Se NOECs from this study, therefore, were >11.37 and >16.04 µg/g dry wt. (Table 5.2).

Rudolph et al. (2008)—Adult westslope cutthroat trout (*O. clarki lewisi*) were collected from a reference site (O'Rourke Lake) and a site with elevated Se levels (Clode Pond) in southeastern British Columbia, Canada. Similar to Kennedy et al. (2000), gametes were stripped and eggs were fertilized in the field. The fertilized eggs were transported to the laboratory and fry were evaluated for deformities, edema, and mortality. Mortality of Clode Pond larvae was higher than in larvae from the reference site, but no significant differences ($p < .05$) for percent fertilization, percent hatch, or mortality of eggs and alevins were observed. The eggs from four of the Clode Pond fish died before they reached the laboratory—the Se concentration in these eggs ranged from 86.3 to 140 µg/g dry wt. In addition, eggs with Se concentrations >46.8 and <75.4 µg/g dry wt. were successfully fertilized, but no viable fry were produced. The remaining egg Se concentrations in Clode Pond fish ($n = 4$) ranged from 11.8 (estimated from muscle) to 20.6 (measured) µg/g dry wt. (mean egg Se concentration of 16.4 µg/g dry wt.). For comparison, Se concentrations in O'Rourke Lake eggs ranged from 12.3 to 16.7 µg/g dry wt. (mean of 14.1 µg/g dry wt.). All viable fry after yolk absorption at swim-up were assessed for the frequency and severity of skeletal, craniofacial, and finfold deformities, as well as for edema. Deformities and edema were scored from 0 (normal) to 3 (severe). The frequency and severity of skeletal, craniofacial, and finfold deformities observed in Clode Pond offspring were either statistically insignificant or significantly ($p < .05$) lower than the O'Rourke Lake fish. For edema, the frequency was significantly ($p = .007$) greater in Clode Pond offspring than in O'Rourke Lake offspring (87.7% vs. 61.2%) and the severity score was significantly ($p = .007$) greater (0.88 vs. 0.61). However, the authors noted that the edema was not severe (indicative by the total severity score of <1 for edema), as only slight fluid accumulation was observed around the eyes and no edema of the yolk sac or pericardial cavity was observed. However, there did appear to be a clear relationship between egg Se and alevin mortality, which is a Se-relevant endpoint as alevin mortality occurred during yolk sac absorption and, hence, exposure to Se. With one exception, the percentage of dead alevins was ≤15% at egg Se concentrations ranging from 11.8 to 20.6 µg/g dry wt. On the basis of the data plotted in Figure 1 of Rudolph et al. (2008), we estimated egg Se EC10 and EC20 values of 17 and 23 µg/g dry wt. for alevin mortality (Table 5.2).

5.4.1.5 Dolly Varden

Golder (2009)—Adult Dolly Varden (*S. malma*) were targeted for collection from high-Se waters (Upper Waste Rock Creek and Upper Waste Rock Creek ponds), a moderate Se water (Lower Waste Rock Creek), and a low Se water (North Kemess Creek) in northern British Columbia, Canada. However, no mature females were available from the high-Se waters. Gametes were stripped from fish collected from the moderate and low Se sites and eggs were fertilized in an on-site laboratory. The fertilized eggs were then transported to the toxicology laboratory, where they were reared through hatching and the test was terminated when at least 90% of the fry reach the swim-up stage. Fry were evaluated for deformities (skeletal, craniofacial, and finfold defects) and edema, which were scored from 0 (normal) to 3 (severe). Using a probit model, Golder (2009) derived egg Se EC10 and EC20 values of 54 and 60 µg/g dry wt., respectively (Table 5.2).

5.4.1.6 Fathead Minnow

Ogle and Knight (1989)—Approximately 60-day-old fathead minnows (*P. promelas*) were provided a diet spiked with 25% seleno-L-methionine, 25% selenate, and 50% selenite at measured

concentrations of 5.2, 10.2, 15.2, 20.3, and 29.5 $\mu\text{g Se/g}$. Every 2 weeks fish were collected and weighed, and one fish from two of four replicates was removed for Se analysis. Spawning substrates were provided on day 98. On day 105, a male and female were selected and the spawning period was extended for 30 days after the first spawning event in each replicate. Fish were collected for Se analysis at the end of the spawning period. Eggs were collected and examined for fertility and incubated for determination of hatchability. Survival of larval fish after 14 days was determined. After 98 days of exposure, growth was significantly ($p < .05$) reduced (16% relative to the control) in fish fed the diet containing 20.3 $\mu\text{g Se/g}$. The ecological significance of this level of growth reduction (16%) is questionable given the variability in the responses at which significant effects were observed (1.30 ± 0.22 g in the control and 1.09 ± 0.16 g in fish fed 20.3 $\mu\text{g Se/g}$) and, that the fish fed the highest Se diet (29.5 $\mu\text{g/g}$) had no significant ($p > .05$) effects on any reproductive parameter evaluated: number of spawns per pair, number of eggs per spawn, percent hatch, or larval survival to 14 days. For each of these endpoints, the responses were similar to the controls, suggesting that the dietary Se threshold for reproductive effects is greater than the highest dietary concentration measured (29.5 $\mu\text{g Se/g}$). Correspondingly, no reproductive effects were observed in fish with a mean ovary Se concentration of 10.9 $\mu\text{g/g dry wt.}$ or a whole-body Se concentration of 7.5 $\mu\text{g/g dry wt.}$ (Table 5.2).

Schultz and Hermanutz (1990)—Fathead minnows (*P. promelas*) were exposed to Se in experimental streams continuously dosed with 10 $\mu\text{g/L}$ selenite. The streams contained well-developed assemblages of fish food organisms, so an environmentally relevant dietary exposure pathway was simulated. Submerged spawning platforms were provided and checked daily for embryos. Samples of collected embryos were then reared in incubation cups containing stream water with the same Se concentrations as in the streams from which they were collected. Each sample was observed each posthatch day for edema and lordosis (larvae were not fed, and observations continued until all larvae died). Se concentrations in the ovaries of the adult females were also determined. The incidence of edema and lordosis in larvae from the dosed stream, 24.6% and 23.4%, respectively, was significantly ($p < .05$) greater than in the control stream (0.9% and 5.6%). The mean Se concentration in ovaries of the parental fish was 5.89 $\mu\text{g/g dry wt.}$, or 23.6 $\mu\text{g/g dry wt.}$ assuming 75% moisture in fathead minnow ovaries). Accordingly, the LOEC from this study was identified as $<23.6 \mu\text{g/g dry wt.}$ (Table 5.2).

5.4.1.7 Largemouth Bass

CP&L (1997)—Adult largemouth bass (*M. salmoides*) were provided artificial diets spiked with seleno-DL-methionine for several months prior to spawning. In 1995 bass were provided diets with measured Se concentrations of 2.9, 7.5, and 11.2 $\mu\text{g/g dry wt.}$, and in 1996 a second set of bass were provided diets with measured Se concentrations of 26.7, 53.1, and 78.4 $\mu\text{g/g dry wt.}$ The fish spawned in the laboratory and the resulting larvae were monitored until swim-up for mortality and deformities. The most sensitive endpoint was larval mortality. On the basis of a logistic concentration-response curve developed by the study authors, we calculated ovary Se EC10 and EC20 values of 22 and 24 $\mu\text{g/g dry wt.}$, respectively (Table 5.2).

5.4.1.8 Mosquitofish

Saiki et al. (2004)—Adult western mosquitofish (*G. affinis*) were collected from two Se-contaminated sites (Sand Luis Drain and North Mud Slough) and two reference sites (North Mud Slough upstream of San Luis Drain and a location on the San Joaquin River with no history of receiving seleniferous drainwater) in central California, U.S. Pregnant females were identified (they are a live-bearing fish) and placed in glass beakers. After birth, live and dead fry were counted, then females and fry were killed with an overdose of MS-222 and measured for length and weight, and fry were evaluated for gross evidence of external deformities. Fry survival averaged greater than 96% from all sites and no fry exhibited evidence of teratogenesis. The highest whole-body Se concentration measured in parent fish was 17.5 $\mu\text{g/g dry wt.}$, which we considered a “greater than” NOEC for this study (Table 5.2).

Staub et al. (2004)—Gravid female eastern mosquitofish (*G. holbrooki*) were collected from a coal ash basin system and a reference pond on the Savannah River Site in South Carolina, U.S. After acclimation in the laboratory, fish were transferred into brood chambers, which included a small opening that allowed offspring to avoid cannibalism in an attached bag. Offspring were inspected for mortality and deformities. Brood sizes and offspring viability at birth were not significantly different ($p > .05$) between ash pond and reference site fish. The mean whole-body Se concentration measured in parent females was 11.85 $\mu\text{g/g}$ dry wt., which we considered a “greater than” NOEC from this study (Table 5.2).

5.4.1.9 Northern Pike

Muscatello et al. (2006)—Gametes were obtained from northern pike (*E. lucius*) collected approximately 2 km (high Se), 10 km (intermediate Se), and 15 km (low Se) downstream of a uranium milling operation in northern Saskatchewan, Canada. The mean egg Se concentrations in the high, intermediate, and low Se exposure sites were 48.23, 31.28, and 3.80 $\mu\text{g/g}$ dry wt., respectively. Gametes were also collected from a reference site, which had a mean egg Se concentration of 3.19 $\mu\text{g/g}$ dry wt. Of 19 trace elements analyzed in the egg samples, only Se was present at elevated concentrations in the intermediate and high Se exposure sites. Eggs were fertilized in the field and transported to the laboratory. The endpoints evaluated included fertilization success, larval deformities, and larval edema. A concentration-response relationship was observed between egg Se concentrations and total embryo deformities, and the study authors calculated egg-based Se EC10 and EC20 values of 20.38 and 33.55 $\mu\text{g/g}$ dry wt., respectively (Table 5.2). The NOEC and LOEC were 3.80 and 31.28 $\mu\text{g/g}$ dry wt., respectively (Table 5.2).

5.4.1.10 Rainbow Trout

Holm (2002); Holm et al. (2003, 2005)—Gametes from adult rainbow trout (*O. mykiss*) were collected from a stream with elevated Se (Luscar Creek, downstream from active coal mining), intermediate Se (Gregg River), and reference streams (Deerlick and Wampus creeks) in west-central Alberta, Canada. Eggs were fertilized in the laboratory and embryos and larvae were reared to the swim-up stage. Fry were evaluated for deformities and other indicators of Se toxicity, such as edema. Craniofacial and skeletal deformities in larvae were more prevalent from fish collected in the elevated Se stream being, on average, 33% and 25% at Luscar Creek compared to 7.1–12% and 7.9–9.2% at the reference streams, respectively. Larval edema was also higher at Luscar Creek (34.5%) relative to Deer Creek (6.1%) and Wampus Creek (23.3%), although the difference was only significant ($p < .05$) relative to Deer Creek. The mean ($\pm$ SE) egg Se from parent fish collected from Luscar Creek (elevated Se) was 9.9 ± 1.4 $\mu\text{g/g}$ wet wt. (25 ± 3.6 $\mu\text{g/g}$ dry wt.), compared to mean egg Se concentrations of 6.5 ± 1.6 $\mu\text{g/g}$ wet wt. (17 ± 4.1 $\mu\text{g/g}$ dry wt.) for Gregg River fish, and 3.5 ± 0.4 $\mu\text{g/g}$ wet wt. (9.0 ± 1.0 $\mu\text{g/g}$ dry wt.) for both Deer Creek and Wampus Creek fish (wet-to-dry weight conversions based on an assumed moisture content of 61% [Holm et al. 2005]). Holm et al. (2005) estimated there was a 15% probability of observing craniofacial deformities, skeletal deformities, and edema at an egg Se concentration between 8.8 and 10.5 $\mu\text{g/g}$ wet wt. (or 23–27 $\mu\text{g/g}$ dry wt. assuming 61% moisture). Overall, we estimated egg-based NOEC, LOEC, EC10, and EC20 values of 17, 25, 23, and 27 $\mu\text{g/g}$ dry wt., respectively, from this study (Table 5.2).

5.4.1.11 Razorback Sucker

Hamilton et al. (2005a, 2005b)—Adult razorback suckers (*X. texanus*) were exposed for 9 months to a gradient of Se concentrations in ponds and isolated river channels of the Colorado River near Grand Junction, Colorado (U.S.). The water bodies used in the study were: (1) Horsethief ponds, reference site (2.2 $\mu\text{g Se/L}$); (2) Adobe Creek (3.8 $\mu\text{g Se/L}$); and (3) North Pond (9.5 $\mu\text{g Se/L}$). Suckers in Horsethief ponds were fed a commercial diet, while fish in Adobe Creek and North Pond were fed naturally available diets. The Se concentrations in food were 1.1 $\mu\text{g/g}$ in commercial fish food, 4–56 $\mu\text{g/g}$ in Adobe Creek, and 20–81 $\mu\text{g/g}$ in North Pond. After the 9-month-adult exposure,

fish were collected and induced to spawn. The eggs were then used in a 9-day study to evaluate egg survival, hatchability, and embryo deformities. The mean egg Se (SE) concentrations ($\mu\text{g/g}$ dry wt.) were 6.5 (0.2) at Horsethief ponds, 46.5 (4.6) at Adobe Creek, and 37.8 (1.4) at North Pond. There were no significant differences between egg sources on viability, survival, hatch, or hatchability, but the percentage of embryos with deformities was significantly ($p < .05$) increased in the Adobe Creek test (26% deformities vs. 18% in Horsethief ponds). Thus, the egg Se concentration of 46.5 $\mu\text{g/g}$ dry wt. from Adobe Creek was associated with significantly increased deformities, while an egg Se concentration of 37.8 $\mu\text{g/g}$ dry wt. was not associated with any adverse effects compared to the reference site (Table 5.2).

5.4.1.12 White Sucker

de Rosemond et al. (2005)—Gametes were obtained from white sucker (*C. commersoni*) collected from Island Lake, which is downstream of the Cluff Lake uranium mine in northern Saskatchewan, Canada. Eggs were fertilized in the field, and embryos were reared in the laboratory. The mean egg Se concentration was 25.6 $\mu\text{g/g}$ dry wt. and mean total developmental deformities were 12.8% (Table 5.2). A limitation of this study is that an insufficient number of suckers could be collected at an uncontaminated reference lake. Nevertheless, the egg Se concentration and level of deformities observed based on gametes collected from the elevated Se site is consistent with the other field-based studies for salmonids and northern pike described earlier.

5.4.2 DIRECT DIETARY SE EXPOSURES: EFFECTS ON LARVAL/JUVENILE GROWTH AND SURVIVAL

Studies in which juvenile fish were exposed to dietary organoselenium and whole-body Se concentrations were measured, and effects on growth and survival were evaluated have been conducted with six species of fish: bluegills (*L. macrochirus*), Chinook salmon (*Oncorhynchus tshawytscha*), fathead minnows (*P. promelas*), rainbow trout (*O. mykiss*), razorback suckers (*X. texanus*), and white sturgeon (*Acipenser transmontanus*). These are summarized below.

5.4.2.1 Bluegill

Lemly (1993a)—Juvenile bluegill (*L. macrochirus*) were exposed to 5.1 $\mu\text{g Se/g}$ (as seleno-L-methionine) in the diet and 4.8 $\mu\text{g Se/L}$ (as 1:1 selenate:selenite) in water for 180 days. One set of fish was exposed to Se under simulated winter conditions, whereby water temperature was gradually reduced from 20°C to 4°C over a period of 8 weeks and photoperiod was simultaneously reduced. Another set of fish was exposed to Se at a water temperature of 20°C over the entire 180-day exposure. Under simulated winter conditions, bluegills reduced activity and feeding. The increased respiratory demand combined with reduced feeding resulted in lipid depletion and subsequent mortality in a significant number of test fish. The whole-body Se concentration at the onset of winter conditions was 5.85 $\mu\text{g/g}$ dry wt., and the whole-body Se concentration after the 120-day exposure to winter conditions was 7.91 $\mu\text{g/g}$ dry wt. Bluegills exposed to the same Se level, but in 20°C water, continued to feed and did not lose sufficient lipids for mortality to occur. We considered the whole-body LOEC from this study, under simulated winter conditions, to be <5.85 $\mu\text{g/g}$ dry wt. (Table 5.3), as this was the whole-body Se concentration at the onset of the final test temperature of 4°C (the higher whole-body Se concentration of 7.91 $\mu\text{g/g}$ dry wt. at test termination simply reflected an increase in the Se concentration due to lipid depletion during the test).

This study was the basis for the U.S. EPA's draft two-part whole-body fish tissue criterion for Se (U.S. EPA 2004). The first part was a trigger concentration of 5.85 $\mu\text{g/g}$ dry wt. that would be applied to whole-body fish Se concentrations measured in summer or fall. If this concentration was exceeded, fish should be sampled in the winter to determine whether the Se concentration exceeded 7.91 $\mu\text{g/g}$ dry wt. Because Lemly (1993a) was the only study to evaluate the combined effects of Se and simulated winter stress in bluegills (or any fish), and was the basis for the U.S. EPA's draft whole-body Se criterion, the study has received an extra level of scrutiny. One limitation of the

TABLE 5.3
Summary of Toxicity Studies That Evaluated Selenium Toxicity to Juveniles from Direct Dietary Exposures (See Table 5.1 for a List of Studies Excluded from This Table)

Species	Reference	Endpoint	Tissue	Se Concentration (µg/g dry wt.)				
				NOEC	LOEC	EC10	EC20	
Bluegill	Lemly 1993a	Mortality (winter stress syndrome)	WB	—	<5.85 (4°C)	—	—	
	McIntyre et al. 2008	Mortality (winter stress syndrome)	WB	—	—	9.56 (4°C) 13.29 (9°C)	10.16 (4°C) 14.02 (9°C)	
Chinook salmon	Cleveland et al. 1993	Mortality	WB	— ^a	— ^a	—	—	
	Hamilton et al. 1990	Growth	WB	5.3	10.4	6.4 ^b	10.9 ^b	
		Fingerling mortality following seawater challenge	WB	12.6	23.2	4.3 ^b	8.6 ^b	
		Growth	WB	—	<43.0	—	—	
Fathead minnow	Bennett et al. 1986	Growth	WB	—	<43.0	—	—	
	Bertram and Brooks 1986	Growth	WB	>2.2	—	—	—	
	Dobbs et al. 1996	Growth	WB	—	<47.5 to <76.0	—	—	
Rainbow trout	Vidal et al. 2005	Larval deformities	WB	— ^a	— ^a	—	—	
	Tashjian et al. 2006	Growth	WB	14.7	22.5	15	23	

^a A NOEC and LOEC could not be identified because the concentration-response data were anomalous (see text).

^b We calculated these EC10 and EC20 values from the concentration-response data reported in the original studies.

WB = whole body; NOEC = no-observed-effect concentration; LOEC = lowest-observed-effect-concentration; EC10 = 10% effect concentration; EC20 = 20% effect concentration.

study in terms of developing a national criterion, is that there was only one Se treatment (4.8 µg Se/L in water and 5.1 µg Se/g in food) and one coldwater temperature regime (4°C). Accordingly, there was interest in evaluating additional Se exposure concentrations to ensure that a concentration-response relationship exists between Se, winter stress, and juvenile mortality. Further, because many water bodies in the country do not achieve winter water concentrations of 4°C, there was interest in whether winter stress syndrome occurred at an intermediate temperature (e.g., 9°C). To address these questions, McIntyre et al. (2008) conducted an additional series of Se toxicity tests with juvenile bluegills, which are summarized below.

McIntyre et al. (2008)—Three Se toxicity tests were conducted with juvenile bluegill. One test was similar to the Lemly (1993a) study, in which juvenile bluegill were exposed to an aqueous Se concentration of approximately 5 µg/L (as 1:1 selenate:selenite) and a dietary Se concentration of approximately 5 µg/g dry wt. (as TetraMin® spiked with seleno-L-methionine) for 182 days. In the two Se replicates, no fish died in one and two fish died in the other. The mean whole-body Se concentration was 10.0 µg/g dry wt., which was therefore identified as a “greater than” NOEC. In the other two toxicity tests, juvenile bluegill were exposed to a series of aqueous and dietary Se concentrations at either 4°C or 9°C. In these studies, the bluegill diet was the oligochaete worm *Lumbriculus variegates*, which had been exposed to various levels of selenized yeast. From these two toxicity tests, McIntyre et al. (2008) calculated whole-body Se EC10 values, based on juvenile mortality, of 9.56 and 13.29 µg/g dry wt. in the 4 and 9°C treatments, respectively (Table 5.3).

The results from McIntyre et al. (2008) did not corroborate those from Lemly (1993a), but rather suggest that juvenile bluegill in this study were no more sensitive than offspring from adults with similar whole-body Se concentrations. At the time this book went to press, the U.S. EPA was revising the draft fish tissue Se criterion.

Cleveland et al. (1993)—Juvenile bluegill were exposed to dietary seleno-L-methionine for 90 days. An anomalous response in bluegill mortality after 90 days was observed, as fish with a whole-body concentration of 4.7 µg/g dry wt. had significantly ($p < .05$) elevated mortality relative to the control fish, while mortality in fish with whole-body concentrations of approximately 7.5–13.5 µg/g dry wt. was not significantly greater. Accordingly, neither a NOEC nor a LOEC could be estimated from this study.

5.4.2.2 Chinook Salmon

Hamilton et al. (1990)—Chinook salmon swim-up larvae (*O. tshawytscha*) were provided diets containing either high-Se mosquitofish collected from the San Luis Drain (California) or mosquitofish collected from a reference site that were subsequently fortified with seleno-DL-methionine. Hamilton et al. (1990) suggested that increased toxicity observed in the San Luis Drain diet was at least partially explained by the presence of other contaminants in the mosquitofish. Accordingly, the following focuses on the study based on the selenomethionine-spiked diet. In the first of two studies, swim-up larvae were exposed to Se-fortified food for 90 days. The larvae were reared in reconstituted water, which simulated a 1:37 dilution from the San Luis Drain (minus trace elements). Se concentrations in the water were always below the detection limits of 1.5–3.1 µg/L. Dietary Se concentrations were 3.2, 5.3, 9.6, 18.2, and 35.4 µg/g. In the second study, fingerlings were exposed to the same dietary Se concentrations for 120 days in brackish water.

Upon termination of the first study at 90 days, survival and growth in Chinook salmon fed a diet containing 18.2 µg Se/g were significantly ($p < .05$) reduced relative to control fish. No significant ($p < .05$) effects on survival or growth were observed in fish fed a dietary Se concentration of 9.6 µg/g. The whole-body Se concentrations at the 90-day NOEC and LOEC were 5.4 and 10.8 µg/g dry wt., respectively. However, the reliability of the 90-day results is questionable given that control survival decreased from 99% on day 60 to 67% on day 90. The high mortality rate in the control fish suggests that some of the mortality observed in the treatment groups may be due to factors other than Se. On the basis of the day 60 data, the whole-body Se NOEC and LOEC for growth were only slightly lower (5.3 and 10.4 µg/g dry wt.) relative to the day 90 data. Based on the

concentration-response data provided in the study, we estimated day-60 EC10 and EC20 values of 6.4 and 10.9 $\mu\text{g/g}$ dry wt., respectively (Table 5.3).

In the second study, 70 mm Chinook fingerlings were exposed to the same dietary Se concentrations as above, but in brackish water, for 120 days. After the 120-day exposure, the Chinook were exposed to a 10-day seawater (28 g/L salinity) challenge test and percent mortality was assessed. The whole-body Se NOEC and LOEC after 120 days of exposure and following the 10-day seawater challenge, for both growth and mortality, were 12.6 and 23.2 $\mu\text{g/g}$ dry wt. In terms of the level of effect, mortality from the seawater challenge study was more sensitive than the reduction in growth. We calculated whole-body EC10 and EC20 values of 4.3 and 8.6 $\mu\text{g/g}$, dry wt. (Table 5.3).

5.4.2.3 Fathead Minnow

Bennett et al. (1986)—Se was followed through a three-tiered food chain (algae-rotifer-fathead minnow larvae) in three laboratory experiments. In the first experiment, 4-day-old larvae were fed Se-contaminated rotifers for 7 days, followed by a control diet for 19 days. In the second experiment, 8-day-old larvae were fed Se-contaminated rotifers for 9 days. In experiment three, 2-day-old larvae were fed Se-contaminated rotifers for 7 days before test termination. Mean Se concentrations in rotifers fed to fathead minnow larvae were >70 and 68 $\mu\text{g/g}$ dry wt. in experiments one and two, respectively. Larval growth in both of these experiments was significantly ($p \leq .05$) reduced relative to control fish. The mean larval Se concentration at the end of the 7-day exposure period was 43.0 $\mu\text{g/g}$ dry wt. in experiment one, and was 51.7 $\mu\text{g/g}$ dry wt. in experiment two. Larval growth in experiment three was significantly reduced at $p \leq .10$. The mean larval Se concentration was 61.1 $\mu\text{g/g}$ dry wt. Accordingly, a LOEC of <43.0 $\mu\text{g/g}$ dry wt. was identified from this study (Table 5.3).

Bertram and Brooks (1986)—Fathead minnows were exposed in the laboratory to either waterborne Se, Se-contaminated food, or a combination of the two for 8 weeks. Tests of fish exposed only to dietary Se were terminated after 11 weeks. No effects on growth were observed in fathead minnows fed a diet containing 7.3 $\mu\text{g/g}$ Se (and 43.5 $\mu\text{g/L}$ Se in the water), the highest dietary concentration tested. The whole-body Se concentration in the highest dietary Se concentration tested was 2.2 $\mu\text{g/g}$ dry wt. (Table 5.3).

Dobbs et al. (1996)—A three trophic level test system consisting of algae, rotifers, and larval fathead minnows was dosed with various concentrations of selenate for 25 days. Fathead minnow larval growth was significantly ($p \leq .05$) reduced relative to the controls in fish fed rotifers with a Se concentration ranging from approximately 33–60 $\mu\text{g/g}$ dry wt. Whole-body Se concentrations in the affected fish ranged from 47.5 to 76.0 $\mu\text{g/g}$ dry wt. (Table 5.3).

5.4.2.4 Rainbow Trout

Vidal et al. (2005)—Juvenile (24 days old at test initiation) rainbow trout (*O. mykiss*) were exposed to dietary seleno-L-methionine concentrations of 4.6, 12, and 18 $\mu\text{g/g}$ dry wt. for 90 days. Fish weight, fork length, and tissue Se concentrations were measured at days 0, 30, 60, and 90. In addition, hepatic lipid peroxidation and reduced glutathione (GSH)-to-oxidized glutathione (GSSG) ratios were measured on days 60 and 90. After 90 days, no significant effects on hepatic lipid peroxidation or GSH-GSSG ratios were observed in the Se treatments; however, fish exposed to dietary Se concentrations of 4.6 and 12 $\mu\text{g/g}$ dry wt. had significantly ($p \leq .05$) reduced body weight and fork length relative to the controls. Growth (weight) of fish exposed to 4.6, 12, and 18 $\mu\text{g/g}$ dietary Se was reduced by 33%, 33%, and 26%, respectively, relative to control fish. The 26% weight reduction in the highest treatment was not statistically different ($p > .05$) from the control. Vidal et al. (2005) identified the lowest treatment (4.6 $\mu\text{g/g}$) as the dietary LOEC. The whole-body Se concentration in trout exposed to a dietary Se concentration of 4.6 $\mu\text{g/g}$ was 0.58 $\mu\text{g/g}$ wet wt., or 2.3 $\mu\text{g/g}$ dry wt. assuming 75% moisture. However, the authors did not base the whole-body Se LOEC on the concentration corresponding with the dietary treatment of 4.6 $\mu\text{g/g}$. Rather, they identified 1.20 $\mu\text{g/g}$ wet wt. as the LOEC, or 4.8 $\mu\text{g/g}$ dry wt. assuming 75% moisture, which was associated with the

dietary Se concentration of 12 $\mu\text{g/g}$ —presumably because this was the lowest exposure concentration that resulted in a Se tissue level significantly greater than the concentration in the control. This results in a circumstance where exposure to Se resulted in a whole-body Se concentration that was not significantly different than the control, but growth was still significantly reduced. Further, the whole-body-based Se LOEC of 2.3 $\mu\text{g/g}$ dry wt. is also unexpected because it appears to fall within the range of background Se concentrations. As Skorupa (1998b) reported, for example, background concentrations of Se in whole-body fish range from <1 to 4 $\mu\text{g/g}$ dry wt., and are typically <2 $\mu\text{g/g}$ dry wt.

Adding to the uncertainties in terms of identifying a whole-body Se threshold from the Vidal et al. (2005) study, whole-body Se concentrations peaked at day 60 and then decreased by day 90, even in the control fish (whole-body Se concentrations were also lower at day 90 than at day 30). The changes in whole-body Se during the course of the test were substantial enough that by day 60, whole-body Se in control fish was 1.24 $\mu\text{g/g}$ wet wt. (4.96 $\mu\text{g/g}$ dry wt.; 75% moisture), or more than two times the effects-based whole-body LOEC of 0.58 $\mu\text{g/g}$ wet wt. (2.3 $\mu\text{g/g}$ dry wt.; 75% moisture) discussed earlier (Figure 5.1a). Vidal et al. (2005) suggest that the decreases in whole-body Se from day 60 to day 90 likely occurred because of the relative increases in total body mass, which would have decreased overall concentrations. However, the observed reduction in Se concentrations does not appear to be entirely a growth dilution effect because Se burdens (μg Se per fish), calculated based on the whole-body Se concentration and the weight of the fish, also declined from day 60 to 90 (Figure 5.1b). Regardless, temporal variability in whole-body Se concentrations in this study makes it difficult to link whole-body Se concentrations to different levels of growth effects. Although statistically significant effects were not observed until day 90, these effects were a result of internal Se concentrations throughout the 90-day exposure period. Thus, the effects measured at day 90 may actually result from the higher whole-body Se concentrations earlier in the study. Given all of the above, we did not identify a whole-body Se threshold associated with adverse effects in rainbow trout based on this study.

5.4.2.5 Razorback Sucker

Beyers and Sodergren (2002)—This study simulated an algae→rotifer→larval razorback sucker (*X. texanus*) food chain. All three components of the food chain were exposed to a control water or natural waters representing a gradient of Se concentrations. The natural waters were collected from three locations along the Colorado River near Grand Junction, Colorado (U.S.). Aqueous Se concentrations ranged from <1 $\mu\text{g/L}$ in the control to 20.3 $\mu\text{g/L}$ in natural waters, from <0.183 to 3.74 $\mu\text{g/g}$ dry wt. in algae, and from <0.702 to 21.8 $\mu\text{g/g}$ dry wt. in rotifers. Larval razorback suckers (5.0 mg, 10.9 mm) were exposed to the test solutions and diets for 28 days and the toxicity endpoints were survival and growth (weight and length). At the end of the study whole-body Se was measured in the larval suckers. No effects on survival or growth were observed relative to the control fish, with whole-body Se concentrations ranging up to 42.0 $\mu\text{g/g}$ dry wt. in the highest Se treatment. Consequently, the whole-body Se NOEC from this study was identified as >42.0 $\mu\text{g/g}$ dry wt.

Hamilton et al. (2005c)—This study was a continuation of the Hamilton et al. (2005a, b) studies summarized previously. Larval razorback suckers (*X. texanus*) were reared from the Horsethief Canyon State Wildlife Area (HT) (reference area), Adobe Creek (AC), and North Pond (WW), as well as from brood stock from HT. Three spawns were used for each location, with egg Se concentrations ($\mu\text{g/g}$ dry wt.) ranging from 5.8 to 6.6 at HT, 38.0 to 54.5 at AC, 34.3 to 37.2 at WW, and 7.1 for brood stock (a single egg sample was analyzed for the latter). The study was initiated with 5-day-old larvae. Larvae, by source, were exposed to one of the following four treatments: (1) reference food and reference water; (2) reference food and site water; (3) site food and reference water; and (4) site food and site water. The reference food was brine shrimp (2.7 μg Se/g) and the reference water was from the 24-Road Fish Hatchery (<1.6 μg Se/L). For HT and brood stock larvae, the site food source was zooplankton collected from the Horsethief east wetland (HTEW) and averaged

FIGURE 5.1 (a) Whole-body selenium concentrations and (b) selenium body burdens in juvenile rainbow trout following 30, 60, and 90 days. Data from Vidal et al. (2005). Asterisks (*) denote where growth was significantly reduced relative to the control.

5.6 µg Se/g dry wt.; water Se from HT averaged 0.9 µg Se/L. For AC larvae, zooplankton collected from the site averaged 20 µg Se/g dry wt. and water Se averaged 5.5 µg Se/L. For WW larvae, zooplankton collected from the site averaged 39 µg Se/g dry wt. and water Se averaged 10.7 µg Se/L. Groups of larvae from each fish source were exposed to one of these four possible diet/water combinations for up to 30 days. For the HT, AC, and WW larvae exposed to site diets, whether simultaneously exposed to site water or reference water, 100% mortality was reached in approximately 11, 14, and 7 days, respectively. The associated whole-body Se concentrations (µg/g dry wt.) in the HT, AC, and WW fish were 8.6–8.8 (day 7), 69–70.6 (day 7), and 28.3–44.5 (day 6). For brood stock larvae exposed to site diets, mortality ranged from approximately 40–60% (depending on whether they were exposed to reference or site water) and whole-body Se concentrations were 5.4 and 6.9 µg/g dry wt.

The toxicity response for each source of larvae was not clearly related to either the Se exposure concentrations or the internal tissue Se concentrations. For example, the HT site is a reference area with water and dietary Se concentrations much lower than AC and WW, which resulted in whole-body Se concentrations in HT larvae much lower than those observed in AC and WW larvae. Yet 100% mortality was observed in HT larvae after an exposure period comparable to that required for 100% mortality to be observed in AC and WW larvae. This level of mortality would not be expected due to the low levels of Se and short period of exposure. In addition, the HT larvae and broodstock larvae, which were hatched from eggs with similar Se concentrations and were exposed to the same water and dietary Se source, had different levels of mortality with HT larvae experiencing 100% mortality after about 11 days and brood stock larvae experiencing 40–60% mortality after 30 days. Further, in AC and WW larvae exposed to reference food and reference water, whole-body Se concentrations were 7.7 and 9.7 $\mu\text{g/g}$ dry wt., respectively, which are comparable or higher to the HT larvae and brood stock larvae exposed to site food; however, mortality was much lower in the AC and WW larvae versus HT larvae and brood stock larvae despite similar whole-body Se concentrations. Hamilton et al. (2005c) suggest that this may have resulted from the protective effects of arsenic, which was elevated in the brine shrimp reference food compared to the site zooplankton diets. For these reasons, it was not possible to identify a whole-body Se threshold for larval razorback suckers from this study.

Given the inability to derive a whole-body Se threshold for razorback suckers in Hamilton et al. (2005c) and due to differences between Hamilton et al. (2005c) and a similar study by Beyers and Sodergren (2002), that is, NOEC >42 $\mu\text{g/g}$ dry wt., we determined that there was too much uncertainty in deriving a razorback sucker whole-body Se threshold based on the available data.

5.4.2.6 White Sturgeon

Tashjian et al. (2006)—Juvenile white sturgeon (*A. transmontanus*) were provided formulated diets spiked with seleno-L-methionine for 8 weeks. The dietary Se concentrations were 0.4 (control), 9.6, 20.5, 41.7, 89.8, and 191.1 $\mu\text{g/g}$. Fish were monitored for survival, growth, swimming activity, and histopathological effects. The dietary NOEC and LOEC, based on body weight increase, were 20.5 and 41.7 $\mu\text{g/g}$, respectively. The corresponding whole-body Se NOEC and LOEC were 14.7 and 22.5 $\mu\text{g/g}$ dry wt., respectively. We derived a whole-body Se EC10 of 15 $\mu\text{g/g}$ dry wt. based on percent body weight increase relative to that in the control fish.

Tashjian et al. (2007)—In a continuation of the juvenile white sturgeon study conducted by Tashjian et al. (2006), fish exposed to dietary organic Se in freshwater were transferred to water with a salinity of either 15 or 20 parts-per-thousand (ppt) in order to evaluate the possible synergistic effects of Se and salinity during seaward migration. Following the 8-week Se exposure described in Tashjian et al. (2006), eight fish from each treatment group were starved for 24 h and transferred to water with a salinity of 15 or 20 ppt for 3 days. Fish survival was defined as those that did not die or lose equilibrium. In fish transferred to 15 ppt salinity water for 3 days and in 20 ppt water for 1 day, the dietary NOEC and LOEC for survival were 89.8 and 191.1 $\mu\text{g/g}$, respectively. Survival of all fish, including control fish, was low in 20 ppt Se water after 2 days. Overall, therefore, the Se thresholds from Tashjian et al. (2006) based on 8-week Se exposures are protective of juveniles then transferred to 15 ppt salinity for 3 days and 20 ppt salinity water for 1 day.

5.5 RECOMMENDATION FOR A FISH TISSUE SE THRESHOLD

The toxicity studies summarized earlier suggest there are differences in the toxicity data and patterns for maternal transfer toxicity studies versus the juvenile toxicity studies. The following first evaluates whether Se toxicity studies based on maternal transfer or direct toxicity to juveniles is more appropriate for developing a tissue-based Se threshold and then fish tissue-based Se thresholds are recommended.

5.5.1 LIFE STAGE CONSIDERATIONS FOR A SE FISH TISSUE THRESHOLD

The Se toxicity studies reviewed here were categorized as either maternal transfer studies or juvenile direct exposure studies. We recommend that toxicity data from maternal transfer studies, in which egg or ovary Se is linked to larval mortality, deformities, or edema, be used to develop a broadly applicable fish Se tissue threshold. This recommendation is based on (1) the relative sensitivity of maternal transfer and juvenile toxicity studies to Se; (2) consistency of concentration-response relationships within either maternal transfer or juvenile toxicity studies with Se; and (3) consideration of important attributes for identifying a good biomonitor of tissue Se concentrations in the field. Each of these points are discussed further below.

5.5.1.1 Relative Sensitivity of Maternal Transfer and Juvenile Toxicity Studies

The relative sensitivity of two species exposed to a metal or metalloid in the environment is determined by their sensitivity to the external concentration, that is, the substance bioaccessibility (the organism's ability to uptake the substance and transport and sequester it or eliminate the substance). This cannot be inferred simply from relationships between internal tissue concentrations and toxicity under a given set of conditions. The amount of bioavailable metal or metalloid that reaches the internal site of action varies between species due to differences in organism strategies/abilities to detoxify or excrete excess metal and even within species depending on the exposure conditions, all of which influence the bioaccumulation rate and whether toxicity will occur (Luoma and Rainbow 2005). For example, in streams with elevated Se levels, Holm et al. (2005) observed a higher rate of teratogenesis in rainbow trout than in brook trout collected from the same streams. Holm et al. (2005) observed that rainbow trout maternally transferred a larger proportion of their body burden to the eggs than did brook trout. In other words, rainbow trout may not be intrinsically more sensitive to Se than brook trout (e.g., larval development as a function of Se concentration), but may be more sensitive at a given exposure concentration due to species differences in Se toxicokinetics (i.e., the efficiency of maternal transfer of Se to eggs from the muscle during vitellogenesis may be lower in brook trout than in rainbow trout [Holm et al. 2005]).

Following from the above, the relative sensitivity of two species, or the sensitivity of two study types for a given species, is not necessarily based on the level of effects associated with internal (i.e., tissue) Se, but rather the external (i.e., dietary) Se concentration to which they are exposed. For example, suppose a water body contains Species A and Species B. The whole-body tissue Se EC10 values for Species A and Species B are 10 and 12 $\mu\text{g/g}$, respectively. However, Species A is not necessarily more sensitive than Species B because the Se dietary TTFs (i.e., bioaccumulation potential) for the two species are not necessarily the same. If the dietary TTFs are 1 and 1.5 for Species A and B, respectively, Species B requires a lower dietary Se concentration to achieve the whole-body EC10 than Species A. Thus, the dietary EC10 would be 10 $\mu\text{g/g}$ for Species A and 8 $\mu\text{g/g}$ for Species B and, assuming a similar diet, Species B would therefore be more sensitive than Species A despite having a higher whole-body Se EC10.

The same concept applies when evaluating which fish life stages are more sensitive to Se. Accordingly, when assessing whether the larval development and survival endpoints (resulting from maternal transfer) are more sensitive than the juvenile survival and growth endpoints, one must consider the exposure concentration. For Se, as previously discussed, the most environmentally relevant exposure concentration to consider is dietary organic Se. Relevant toxicity studies for this comparison are limited to laboratory studies with known dietary Se concentrations. The fish species with the most data available for making this comparison is bluegill (*L. macrochirus*). Three laboratory studies exposed adult bluegill to dietary organic Se and evaluated effects in offspring: (1) Woock et al. (1987); (2) Doroshov et al. (1992); and (3) Coyle et al. (1993). The most sensitive endpoints in these three studies were larval mortality in the Woock et al. (1987) and Coyle et al. (1993) studies and larval edema in the Doroshov et al. (1992) study. The concentration-response curves relating dietary Se concentration to the percentage of effect are shown in Figure 5.2. The concentration-response

FIGURE 5.2 Selenium effects on bluegill larvae (from maternal transfer) and juveniles (from direct dietary exposures) as a function of dietary selenium.

relationships are similar between the studies, with low-level effects up to a dietary Se concentration of approximately 12–16 µg/g dry wt., and then a rapid increase to a 90–100% effect level.

There are two studies with juvenile bluegills (nonmaternal transfer) from which concentration-response relationships could be derived based on dietary Se: Cleveland et al. (1993) and McIntyre et al. (2008). As shown in Figure 5.2, the concentration-response curve from the Cleveland et al. (1993) study is similar to the maternal transfer studies at low dietary Se concentrations. However, the same rapid increase in adverse effects is not observed at dietary Se concentrations greater than approximately 12 µg/g dry wt. Rather, the mortality response generally reaches a plateau between dietary Se concentrations of 9–33 µg/g dry wt. The concentration-response curves from McIntyre et al. (2008) are similar to those in the maternal transfer studies (Figure 5.2). Significant juvenile mortality did not begin to occur until dietary Se concentrations reach approximately 14–15 µg/g dry wt. and then the concentration-response curves becomes relatively steep. Consequently, the juvenile bluegill in these tests appear to be similarly sensitive to, but no more sensitive than, offspring from adults exposed to similar dietary Se concentrations.

Bryson et al. (1984), as summarized earlier, collected adult female and male bluegill from Hyco Reservoir and a control lake and crossed eggs and sperm from all combinations. The mean ovary Se concentrations were 49 and 3.3 µg/g dry wt. in Hyco Reservoir and control lake bluegill, respectively. All larvae hatched from Hyco Reservoir bluegill eggs experienced 100% mortality, regardless of the source of the male sperm. The surviving larvae (28 days posthatch) from control lake females were then fed zooplankton collected from either Hyco Reservoir (mean Se ± standard deviation = 45 ± 12 µg/g dry wt.) or the control lake (mean Se ± standard deviation = 1.9 ± 2.0 µg/g dry wt.). Mortality was 97.3% in juvenile bluegills fed Hyco zooplankton and 23.7% in juveniles fed control lake zooplankton. Accordingly, Se levels in Hyco Reservoir resulted in almost complete mortality regardless of the life stage and exposure route (100% mortality prior to swim-up when exposed to Se via maternal transfer or 97.3% mortality in juveniles when only exposed to dietary Se). Given the high Se levels in Hyco zooplankton and female ovaries, however, lower

effect levels or thresholds could not be identified and used to determine if one life stage was more sensitive than the other.

Overall, the available data suggest that juvenile bluegill are not more sensitive than bluegill larvae exposed to Se via maternal transfer and, in fact, the relative sensitivities of the two life stages appear to be relatively similar based on dietary Se exposures. Although the data available for this comparison are not extensive, the results for bluegill, if translatable across species, suggest the maternal transfer endpoint is more sensitive relative to dietary exposures to juveniles. At this time, similar comparisons cannot be made for other species because data from laboratory adult exposure studies are not available.

5.5.1.2 Consistency of Concentration-Response Relationships

While egg or ovary Se concentrations are strong indicators of whether Se toxicity to fish larvae is likely to occur or not, it is less clear how Se concentration-response relationships for juvenile tissues vary in response to Se exposure concentrations, recognizing that concentrations may vary as a function of age. As discussed earlier, Vidal et al. (2005) observed highly variable whole-body Se concentrations over the 90-day exposure of juvenile rainbow trout to dietary Se, so much so that by day 60, whole-body Se in control fish was 1.24 $\mu\text{g/g}$ wet wt., or more than two times their proposed effects-based whole-body LOEC of 0.58 $\mu\text{g/g}$ wet wt. An observation from this study, which was also noted by the study authors, is that the results may demonstrate the difficulty in using whole-body tissue residues as toxicity thresholds for Se in species that are developmentally immature. However, other studies with juvenile fish, such as Chinook salmon (Hamilton et al. 1990), bluegill (Lemly 1993a, McIntyre et al. 2008), and white sturgeon (Tashjian et al. 2006), did not observe the same fluctuations in tissue Se over time. The Chinook salmon study conducted by Hamilton et al. (1990) resulted in a very different relationship between juvenile whole-body Se and growth effects relative to the Vidal et al. (2005) rainbow trout study even though the two studies have very similar test designs. This variability in basic concentration-response relationships between two closely related species and very similar study designs suggests that the sensitivity of young fish directly exposed to dietary Se is variable, or at least not well understood based on the limited data available.

Teh et al. (2004) provide another example of variability in concentration-response relationships for juvenile fish. Although this study was not included in this review because whole-body Se concentrations were not measured, muscle Se concentrations were measured. Teh et al. (2004) exposed Sacramento splittail (*Pogonichthys macrolepidotus*) to dietary organic Se for 9 months. An anomalous response was observed between dietary Se and larval deformities (the only study that has observed deformities in fish not exposed to egg Se as a developing embryo). Greater than 50% deformities were observed at a dietary Se concentration of 6.6 $\mu\text{g/g}$ dry wt. and then the level of deformities decreased with increasing dietary Se concentration. Growth and survival was not significantly reduced at dietary Se concentrations up to 12.6 $\mu\text{g/g}$ dry wt. The growth data from this study are more consistent with what was observed in Hamilton et al. (1990) for Chinook salmon—weight of fish fed a dietary Se concentration of 12.6 $\mu\text{g/g}$ dry wt. was reduced by 6% relative to the control and by 41% in fish fed 26 $\mu\text{g Se/g}$. The basis for the anomalous concentration-response data for deformities is unclear. The study is unique due to its longer duration than typical studies with juveniles so there are no results available for direct comparison.

Although Teh et al. (2004) did not measure whole-body Se concentrations during the study, muscle Se concentrations did increase with increasing dietary Se concentrations (and, by inference, whole-body Se would have also increased with increasing dietary Se concentration). As a point of comparison, Lemly (1993c) collected 22 species of fish (representing eight families) from Belews Lake (and two reference reservoirs), which were assessed for deformities and edema and whole-body Se concentrations were measured. Lemly (1993c) observed an increasing relationship between whole-body Se and the prevalence of abnormalities. As shown in Figure 5.3, the relationship between deformities and whole-body or muscle Se is much different between the field data for centrarchids and the laboratory data for the splittail.

FIGURE 5.3 Relationship between fish deformities and whole-body Se in Belews Lake centrarchids (Lemly 1993c) and muscle Se in Sacramento splittails (Teh et al. 2004).

Overall, there is sufficient variability in concentration-response relationships for Se in young fish such that dietary Se toxicity studies with juveniles may not be appropriate for developing a broadly applicable tissue-based Se threshold for fish. For reasons discussed further below, a Se toxicity threshold based on maternal transfer appears to be most reliable for developing a broadly applicable tissue Se threshold.

5.5.1.3 Considerations for Developing and Applying Tissue-Based Toxicity Thresholds

In developing tissue-based toxicity thresholds for interpreting the concentrations of metals and metalloids in field-collected tissues, an important concept to consider is that there are biomonitors of exposure and biomonitors of effects (Adams et al. 2010). The bimonitor of effects should be a sensitive metric, such as the sensitivity of a species-, population-, or community-based endpoint. However, such a bimonitor of effects may not be readily sampled at a site, particularly across an exposure gradient. As such, a bimonitor of exposure should be identified that is linked to, either directly or by correlation, the bimonitor of effects. As summarized in Adams et al. (2010), a good bimonitor of exposure has the following characteristics: (1) widely distributed; (2) not sensitive to the element of interest; and (3) a strong accumulator of the element such that the internal concentration correlates well with the external exposure concentration.

For Se, maternal transfer studies meet the above characteristics of biomonitors of exposure and biomonitors of effects. Adult fish are relatively insensitive to Se and, therefore, generally amenable to sampling over a range of Se exposure conditions. The Se concentrations measured in the ovaries or eggs collected from adults are then directly linked to the critical endpoint of larval mortality and deformities (Figure 5.4).

5.5.2 RECOMMENDATION OF A FISH TISSUE SE THRESHOLD

In this evaluation, the term “threshold” is intended to represent a Se concentration below which negligible Se toxicity is expected to occur. We selected the EC10 (i.e., 10% effect concentration) for

FIGURE 5.4 Schematic representation of selenium maternal transfer studies: Attributes of a good biomonitor.

identifying tissue-based Se thresholds in this evaluation, although it is recognized that lower (e.g., EC05) or higher (e.g., EC20) effect levels are often considered in deriving ecological thresholds, criteria, or guidelines for chemicals. The Se EC10 values identified in this review are often similar to corresponding NOECs from the same study, and always less than the corresponding LOECs. If data were available from studies with similar designs, concentration-response data were pooled for calculation of the EC10. If it was not possible to pool data from separate studies, the lowest EC10 was identified as the threshold for that tissue type. The Se tissue thresholds recommended here are based on maternal transfer studies, for the reasons concluded earlier.

5.5.2.1 Egg Se Threshold

Egg Se-based toxicity data are available for several fish species, including bluegill, brook trout, brown trout, cutthroat trout, Dolly Varden, rainbow trout, northern pike, and white suckers. The minimum EC10 estimated from an individual study was 17 $\mu\text{g/g}$ dry wt., which was based on the relationship between cutthroat trout alevin mortality and egg Se reported in Rudolph et al. (2008) (Table 5.2). This EC10 is based on a simple linear regression between egg Se and alevin mortality data for a reference site and a Se exposed site, and is less than the egg Se NOEC of 20.6 $\mu\text{g/g}$ dry wt. for larval deformities from this same study (Table 5.2). Nevertheless, 17 $\mu\text{g/g}$ dry wt. is assumed to represent a conservative egg Se criterion that should be protective of larval effects. Conveniently and perhaps not surprisingly given that Se is transferred from the ovaries to eggs and that interspecies sensitivity is not highly variable as a function of egg Se, an ovary-based EC10 of 18 $\mu\text{g/g}$ dry wt. is estimated below.

5.5.2.2 Ovary Se Threshold

All of the data relating ovary Se concentrations to adverse effects on fish larvae are for bluegills, largemouth bass, and fathead minnows. The most sensitive endpoints are larval terata, edema, or mortality. The bluegill and fathead minnow data were pooled to estimate an ovary Se EC10 of 18 $\mu\text{g/g}$ dry wt. (Figure 5.5). The raw concentration-response data for largemouth bass were not available for inclusion in Figure 5.5, but the ovary Se EC10 from the logistic regression equation reported in CP&L (1997) was similar, 22 $\mu\text{g/g}$ dry wt. Most of the ovary-based Se effects data are clustered at low effect levels and very high effect levels, which is largely driven by the steepness of the concentration-response relationship. The ovary Se EC10 of 18 $\mu\text{g/g}$ dry wt. falls within the cluster of empirical low effect data. For comparison, there were three bluegill studies with sufficient

FIGURE 5.5 Relationship between ovary selenium in parent fish versus adverse effects on offspring.

data to estimate EC10 values from the concentration-response relationships between ovary Se and effects on larvae; the EC10 values from these individual studies were 16 µg/g dry wt. (Doroshov et al. 1992), 24 µg/g dry wt. (Coyle et al. 1993), and 30 µg/g dry wt. (Hermanutz et al. 1996). For convenience due to the similarity of the egg Se and ovary Se EC10 values we derived, we recommend that the egg Se threshold of 17 µg/g dry wt. be considered a combined egg or ovary Se threshold.

5.5.2.3 Whole-Body Se Threshold

As previously discussed, we recommend that tissue-based thresholds for Se in fish be based on the Se concentration in the ovaries or eggs, as it is the egg Se concentration that determines whether toxicity in larvae is likely to occur. Because the efficiency with which Se is transferred to the eggs appears to vary between species (e.g., Holm et al. 2005), whole-body-based Se thresholds are expected to be more variable between species. From the earlier review of maternal transfer studies, whole-body-based concentration-response data for bluegill sunfish (Coyle et al. 1993, Hermanutz et al. 1996) and fathead minnow data (Ogle and Knight 1989) were pooled to estimate a whole-body Se EC10 of 8.1 µg/g dry wt., which was associated with adverse effects on larvae (Figure 5.6). As shown in Figure 5.6, whole-body Se concentrations up to 7.5 µg/g dry wt. are associated with ≤5% effects relative to their respective controls, while whole-body Se concentrations ≥16 µg/g dry wt. are associated with >90% effects on larvae. Accordingly, no empirical data are available for whole-body Se concentrations between 7.5 and 16 µg/g dry wt., which adds uncertainty to the concentration-response relationship between this range in whole-body Se concentrations. However, the EC10 of 8.1 µg/g dry wt. appears to be adequately conservative for a threshold, as it falls within the lower end of this range. This EC10 of 8.1 µg/g dry wt. also appears to be protective of maternal transfer toxicity in cutthroat trout, as the NOEC from Hardy et al. (2010) was >11.37 µg/g dry wt. The maternal transfer-based whole-body EC10 of 8.1 µg/g dry wt. is similar to the U.S. EPA's draft whole-body Se criterion of 7.91 µg/g dry wt. (which is being revised when this book went to press), and lower than the EC10 values for juvenile bluegill mortality from the McIntyre et al. (2008) studies and the EC10 value for reduced juvenile white sturgeon growth from Tashjian et al. (2006).

The Chinook salmon juvenile growth and seawater challenge study conducted by Hamilton et al. (1990) indicates that anadromous salmonids may be more sensitive than other fish species tested.

FIGURE 5.6 Relationship between whole-body selenium in parent fish versus adverse effects on offspring.

TABLE 5.4

Comparison of Recommended Selenium Thresholds to the Lowest LOEC and Highest NOEC below the Lowest LOEC

Tissue	Recommended Se Threshold (µg/g dry wt.)	Lowest LOEC (µg/g dry wt.)	Highest NOEC Below Lowest LOEC (µg/g dry wt.)
Egg	17	25	22.5
Ovary	17	21.1	20
Whole body	8	16	>11.85

The EC10 values we derived from Hamilton et al. (1990) were 6.4 µg/g dry wt. for reduced growth and 4.3 µg/g dry wt. for mortality in a 10-day-seawater challenge following a 120-day dietary Se exposure. Thus, a whole-body Se threshold less than 8.1 µg/g dry wt. may be warranted for monitoring Se levels in juvenile anadromous salmon that may be exposed to Se during rearing in fresh water bodies.

5.5.2.4 Summary of Fish Tissue Se Thresholds

From the above review of maternal transfer toxicity studies, we recommend an egg and ovary Se threshold of 17 µg/g dry wt. and a whole-body Se threshold of 8.1 µg/g dry wt. For comparison, and because an EC10 could not be calculated for every study, the lowest whole-body, ovary, and egg Se LOECs identified are 16, 21, and 25 µg/g dry wt., respectively, and the highest whole-body, ovary, and egg Se NOECs that fall below these LOEC values are 11.85, 20, and 22.5 µg/g dry wt., respectively (Table 5.4). Thus, the EC10s recommended as thresholds here are all not only below the lowest LOEC values, but also within the range of NOEC values for most species. Finally, as shown in Figure 5.7, the egg and ovary Se EC10s (or similar effect levels) between most species are not highly variable, with nine of the ten lowest values being within a factor of 1.5. This generally low variability in egg and ovary Se EC10s between species further supports use of egg or ovary Se in deriving a broadly applicable Se threshold.

FIGURE 5.7 Distribution of selenium thresholds for eggs or ovaries.

5.5.3 COMPARISONS OF SE FISH TISSUE THRESHOLDS

Although there is a general consensus that monitoring and regulating Se based on concentrations in fish tissue is more useful and appropriate than monitoring Se in water or sediment, there are still a variety of opinions in defining the appropriate fish tissue Se thresholds (e.g., Lemly 1996, DeForest et al. 1999, Hamilton 2002, U.S. EPA 2004, Chapman 2007). The Se toxicity thresholds recommended here are compared to those of Lemly (1996), DeForest et al. (1999), Hamilton (2002), U.S. EPA (2004), and Chapman (2007) in Table 5.5. The egg and ovary Se threshold of 17 µg/g dry wt. recommended in this review is higher than the threshold of 10 µg/g dry wt. recommended in Lemly (1996) and the whole-body Se threshold of 8.1 µg/g dry wt. recommended in this review is higher than the threshold of 4 µg/g dry wt. recommended in Lemly (1996) and Hamilton (2002). The following evaluates the differences between these Se threshold values.

The precise basis for the difference between the recommended Se threshold levels in eggs and ovaries cannot be readily determined because the lowest egg or ovary Se concentration associated with adverse effects identified in Lemly (1996) was an ovary Se concentration of 18 µg/g dry wt., which was associated with reproductive failure in bluegills (Hermanutz et al. 1992). In Hermanutz et al. (1992), a mean ovary Se concentration of 4.4 µg/g wet wt. was associated with significantly ($p \leq .05$) increased mortality, edema, and hemorrhaging in bluegills. Lemly (1996) assumed an ovary moisture content of 75%, which resulted in an estimated dry weight Se concentration of 18 µg/g, while, from Gillespie and Baumann (1986), we assumed a bluegill ovary moisture content of 85% to estimate a dry weight Se concentration of 29 µg/g (we ultimately derived an ovary-based EC10 of 30 µg/g dry wt. based on the updated Hermanutz et al. [1996] report). Therefore, the differences in egg and ovary Se thresholds of 17 µg/g dry wt. from this review and 10 µg/g dry wt. from Lemly (1996) is primarily due to a different percent moisture assumption for bluegill ovaries in the Hermanutz et al. (1992, 1996) studies and an undefined extrapolation from 18 to 10 µg/g dry wt. We believe that the body of evidence, which includes several maternal transfer studies published after Lemly (1996) and DeForest et al. (1999), continues to support that an egg or ovary Se concentration

TABLE 5.5
Comparison of Recommended Thresholds for Selenium in Fish Tissues

Tissue	Threshold ($\mu\text{g/g}$ dry wt.)	Basis	Reference
Egg	17	EC10: Se concentration in fish eggs associated with alevin mortality; data from Rudolph et al. (2008)	This review
	10	Reproductive failure	Lemly 1996
	>16–40	Range in “effects thresholds” for coldwater species	Chapman 2007
Ovary	17	EC10: Se concentration in parent fish ovaries associated with larval mortality and edema in offspring; data from multiple studies with bluegill and fathead minnows (lowered from 18 to 17 $\mu\text{g/g}$ dry wt. for consistency with egg Se threshold)	This review
	10	Reproductive failure	Lemly 1996
	17	EC10: Se concentration in parent fish ovaries associated with larval mortality and edema in offspring; data from multiple studies with bluegill and fathead minnows	DeForest et al. 1999
Whole body	8.1	EC10: Se concentration in parent fish associated with larval mortality and edema in offspring; data from Ogle and Knight (1989), Coyle et al. (1993), and Hermanutz et al. (1996)	This review
	4	Mortality of juveniles and reproductive failure	Lemly 1996
	9	EC10: Se concentration in parent fish associated with larval mortality and edema in offspring; data from Ogle and Knight (1989), Hermanutz et al. (1992), and Coyle et al. (1993)	DeForest et al. 1999
	4	Mortality of juveniles and reproductive failure	Hamilton 2002
	7.91	LOAEC for juvenile bluegill simultaneously exposed to Se and simulated winter conditions, with 5.85 $\mu\text{g/g}$ dry wt. considered a summer/fall trigger for monitoring in winter; data from Lemly (1993a)	U.S. EPA 2004

EC10 = 10% effect concentration; LOAEC = lowest-observed-adverse-effect-concentration.

of 17 $\mu\text{g/g}$ dry wt. is a broadly applicable Se threshold for these tissues (this is the lowest egg or ovary EC10 value that we identified from all available studies).

Although this review and Lemly (1996) both support that eggs and/or ovaries are the most relevant tissues for monitoring the potential for Se toxicity at a site, both studies also provide whole-body-based Se thresholds. The discrepancy in our recommended whole-body Se threshold of 8.1 $\mu\text{g/g}$ dry wt. and the whole-body Se threshold of 4 $\mu\text{g/g}$ dry wt. recommended by Lemly (1996) and Hamilton (2002) is based on the following. First, for reasons discussed earlier, we believe that the variability in relationships between whole-body Se and toxicity in direct toxicity studies with juvenile fish limit the usefulness of these studies for deriving and implementing a broadly applicable tissue-based threshold. Our recommended whole-body Se threshold is based only on maternal transfer studies. Second, we note that the lowest whole-body Se concentrations measured in adversely affected fish were observed in tests where fish were exposed to very high aqueous Se concentrations (Hunn et al. 1987, Cleveland et al. 1993) or exposed to a field-collected diet in which the authors noted that elements in addition to Se may have contributed to the toxicity observed (Hamilton et al. 1990). We identified whole-body chronic values (geometric mean of the NOEC and LOEC) from these studies of 3.3 $\mu\text{g/g}$ dry wt. (rainbow trout; Hunn et al. 1987), 4.4 $\mu\text{g/g}$ dry wt. (bluegill; Cleveland et al. 1993); and <3.3 $\mu\text{g/g}$ dry wt. (Chinook salmon; Hamilton et al. 1990). For perspective, in the study of Se toxicity to juvenile trout conducted by Vidal et al. (2005), the measured whole-body Se concentration in control fish peaked at 4.96 $\mu\text{g/g}$ dry wt. (calculated from a

wet weight concentration of 1.24 $\mu\text{g/g}$ and assuming 75% moisture). In addition, under the National Water Quality Assessment (NAWQA) program, the United States Geological Survey (USGS) measured whole-body Se concentrations in fish collected from reference sites in Alaska, Arizona, California, Oregon, Washington, and Wyoming (U.S.) (<http://water.usgs.gov/nawqa/>). Whole-body Se concentrations in approximately 40% of the reference site fish exceed 4 $\mu\text{g/g}$ dry wt., compared to approximately 5% that exceed 8.1 $\mu\text{g/g}$ dry wt. In our opinion, a whole-body Se threshold of 4 $\mu\text{g/g}$ dry wt. is impractically low for use as a broadly applicable Se threshold. Again, however, we believe that using an egg- or ovary-based Se threshold reduces much of the uncertainty in interpreting Se concentrations measured in field-collected fish.

Summary

Se is a naturally occurring element that typically enters aquatic water bodies, either from natural or anthropogenic sources, as inorganic selenite or selenate. The critical exposure route for fish is dietary exposure to organic Se, with the amount of Se that is ultimately reduced to organic Se compounds and bioavailable to fish being highly dependent on site-specific conditions and uptake by primary consumers. Bioaccumulated Se is maternally transferred to the eggs where, at sufficiently high concentrations, it can cause edema, deformities, or mortality in larval fish during yolk sac absorption. Due to the site-specific factors that influence Se bioavailability and bioaccumulation in fish, the Se concentration in fish tissue, especially in the eggs or ovaries, is a much more reliable indicator of potential toxicity than Se in the water column. The U.S. EPA developed a draft whole-body Se criterion based on the combined effects of Se and simulated winter stress on juvenile bluegill survival, which is being revised at the time this book went to press. Additional recent studies on the toxicity of Se to juvenile bluegills under simulated winter stress suggest that juvenile toxicity studies with Se are not more sensitive than maternal transfer toxicity studies. Maternal transfer studies meet the ideal attributes for deriving and implementing a tissue-based based threshold, as adults are relatively insensitive to Se and amenable for egg or ovary sample collection, with the Se concentration in the eggs or ovaries directly linked to the critical endpoints of larval mortality, deformities, and edema. Further, for development of a broadly applicable tissue-based threshold, egg and ovary Se EC10 values are not highly variable between most freshwater fish species tested to-date. We derived egg and ovary Se EC10 values of 17 and 18 $\mu\text{g/g}$ dry wt., respectively, and recommend an egg and ovary Se threshold of 17 $\mu\text{g/g}$ dry wt. (this is the lowest egg or ovary EC10 value that we identified from all available studies). Although we believe that eggs or ovaries are the more appropriate tissues for developing a broadly applicable fish tissue Se threshold, we also recommend a whole-body Se threshold of 8.1 $\mu\text{g/g}$ dry wt. when egg or ovary Se data are lacking.

ACKNOWLEDGMENT

We thank Jim Meador and an anonymous reviewer for their valuable comments on this chapter.

REFERENCES

- Adams, W., et al. 2010. Utility of tissue residues for predicting effects due to metals in aquatic organisms. *Integr. Environ. Assess. Manage.* doi 10.1002/ieam.108.
- Barnhart, R. A. 1957. Chemical factors affecting the survival of game fish in a western Colorado reservoir. M.S. Thesis, Colorado State University, Fort Collins, CO.
- Bennett, W. N., A. S. Brooks, and M. E. Boraas. 1986. Selenium uptake and transfer in an aquatic food chain and its effects on fathead minnow larvae. *Arch. Environ. Contam. Toxicol.* 15:513–517.

- Bertram, P. E., and A. S. Brooks. 1986. Kinetics of accumulation of selenium from food and water by fathead minnows. *Wat. Res.* 20:877–884.
- Besser, J. M., T. J. Canfield, and T. W. La Point. 1993. Bioaccumulation of organic and inorganic selenium in a laboratory food chain. *Environ. Toxicol. Chem.* 12:57–72.
- Beyers, D. W., and C. Sodergren. 2002. Assessment of exposure of larval razorback sucker to selenium in natural waters. *Arch. Environ. Contam. Toxicol.* 42:53–59.
- Brix, K. V., J. E. Toll, L. M. Tear D. K. DeForest, and W. J. Adams. 2005. Setting site-specific water-quality standards by using tissue residue thresholds and bioaccumulation data. Part 2. Calculating site-specific selenium water-quality standards for protecting fish and birds. *Environ. Toxicol. Chem.* 24:231–237.
- Bryson, W. T., W. R. Garrett, M. A. Mallin, K. A. MacPherson, W. E. Partin, and S. E. Woock. 1984. 1982 Environmental monitoring studies, Volume II: Hyco Reservoir bioassay studies. Carolina Power and Light Company, New Hill, North Carolina. 65 pp + appendices.
- Bryson, W. T., W. R. Garrett, K. A. MacPherson, M. A. Mallin, W. E. Partin, and S. E. Woock. 1985a. Hyco Reservoir 1983 bioassay report. Carolina Power and Light Company, New Hill, North Carolina. 55 pp.
- Bryson, W. T., K. A. MacPherson, M. A. Mallin, W. E. Partin, and S. E. Woock. 1985b. Hyco Reservoir 1984 bioassay report. Carolina Power and Light Company, New Hill, North Carolina. 51 pp.
- Butler, D. L., R. P. Krueger, B. C. Osmundson, A. L. Thompson, and S. K. McCall. 1991. Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in the Gunnison and Uncompahgre River Basins and at Sweitzer Lake, West-Central Colorado, 1988–89. U.S. Geological Survey. Water-Resources Investigation Report 91–4103. 99 pp.
- Carolina Power & Light Company (CP&L). 1997. Largemouth bass selenium bioassay. Roxboro, NC, USA. 14 pp.
- Chapman, P. M. 2007. Selenium thresholds for fish from cold freshwaters. *Hum. Ecol. Risk Assess.* 13(1):20–24.
- Chapman, P. M., et al. 2009. Ecological assessment of selenium in the aquatic environment: Summary of a SETAC Pellston Workshop. Society of Environmental Toxicology and Chemistry (SETAC). Pensacola, FL, USA.
- Cleveland, L., E. E. Little, D. R. Buckler, and R. H. Wiedmeyer. 1993. Toxicity and bioaccumulation of waterborne and dietary selenium in juvenile bluegill (*Lepomis macrochirus*). *Aquat. Toxicol.* 27:265–280.
- Coyle, J. J., D. R. Buckler, C. G. Ingersoll, J. F. Fairchild, and T. W. May. 1993. Effect of dietary selenium on the reproductive success of bluegills (*Lepomis macrochirus*). *Environ. Toxicol. Chem.* 12:551–565.
- Cumbie, P. M., and S. L. Van Horn. 1978. Selenium accumulation associated with fish mortality and reproductive failure. *Proc. Ann. Conf. S.E. Assoc. Fish Wildl. Agencies* 32:612–624.
- de Rosemond, S. C., K. Liber, and A. Rosaasen. 2005. Relationship between embryo selenium concentration and early life stage development in white sucker (*Catostomus commersoni*) from a northern Canadian lake. *Bull. Environ. Contam. Toxicol.* 74:1134–1142.
- deBruyn, A. M., A. Hodaly, and P. M. Chapman. 2008. Tissue selection criteria: Selection of tissue types for development of meaningful selenium tissue thresholds in fish. Part 1 of Selenium issue thresholds: Tissue selection criteria, threshold development endpoints, and field application of tissue thresholds. Washington (DC, USA): North America Metals Council-Selenium Working Group.
- DeForest, D. K., K. V. Brix, and W. J. Adams. 1999. Critical review of proposed residue-based selenium toxicity thresholds for freshwater fish. *Hum. Ecol. Risk Assess.* 5(6):1187–1228.
- DeForest, D. K., K. V. Brix, and W. J. Adams. 2007. Assessing metal bioaccumulation in aquatic environments: The inverse relationship between bioaccumulation factors, trophic transfer factors and exposure concentration. *Aquat. Toxicol.* 84:236–246.
- Dobbs, M. G., D. S. Cherry, and J. Cairns, Jr. 1996. Toxicity and bioaccumulation of selenium to a three-trophic level food chain. *Environ. Toxicol. Chem.* 15(3):340–347.
- Doroshov, S., et al. 1992. Development of water quality criteria for resident aquatic species of the San Joaquin River. Draft final report to the California State Water Resources Control Board for Contract No. 7–197–250–0. Department of Animal Science, University of California, Davis, CA.
- Finley, K. A. 1985. Observations of bluegills fed selenium-contaminated *Hexagenia* nymphs collected from Belews Lake, North Carolina. *Bull. Environ. Contam. Toxicol.* 35:816–825.
- Formation Environmental. 2009. Brown trout laboratory reproduction studies conducted in support of development of a site-specific selenium criterion. Unpublished draft report prepared for J.R. Simplot Company, Smoky Canyon Mine, Pocatello, ID, USA.
- Garrett, G. P., and C. R. Inman. 1984. Se-induced changes in fish populations of a heated reservoir. *Proc. Ann. Conf. S.E. Assoc. Fish and Wildl. Agencies* 38:291–301.

- GEI Consultants. 2008. Maternal transfer of selenium in fathead minnows, with modeling of ovary tissue-to-whole body concentrations. Unpublished report prepared on behalf of Conoco-Phillips. Littleton, CO, USA.
- Gillespie, R. B., and P. C. Baumann. 1986. Effects of high tissue concentrations of selenium on reproduction by bluegills. *Trans. Am. Fish Soc.* 115:208–213.
- Goettl, Jr., J. P., and P. H. Davies. 1977. Water pollution studies. Job Progress Report, Federal Aid Project F-33-R-12. Department of Natural Resources, Colorado Division of Wildlife, Fort Collins, CO, USA. pp. 39–42.
- Golder. 2009. Development of a site-specific selenium toxicity threshold for Dolly Varden char. Prepared for Northgate Minerals Corporation, Smithers, BC, Canada. 17 pp. + tables, figures, and appendices.
- Hamilton, S. J. 2002. Rationale for a tissue-based selenium criterion for aquatic life. *Aquat. Toxicol.* 57: 85–100.
- Hamilton, S. J., and R. H. Wiedmeyer. 1990. Concentrations of boron, molybdenum, and selenium in chinook salmon. *Trans. Am. Fish Soc.* 119:500–510.
- Hamilton, S. J., A. N. Palmisano, G. A. Wedemeyer, and W. T. Yasutake. 1986. Impacts of selenium on early life stages and smoltification of fall chinook salmon. *Trans. 51st N. A. Wildl. Nat. Res. Conf.* 51:343–356.
- Hamilton, S. J., K. J. Buhl, N. L. Faerber, R. H. Wiedmeyer, and F. A. Bullard. 1990. Toxicity of organic selenium in the diet to chinook salmon. *Environ. Toxicol. Chem.* 9:347–358.
- Hamilton, S. J., K. M. Holley, K. J. Buhl, F. A. Bullard, L. K. Weston, and S. F. McDonald. 2005a. Selenium impacts on razorback sucker, Colorado River, Colorado. I. Adults. *Ecotoxicol. Environ. Saf.* 61:7–31.
- Hamilton, S. J., K. M. Holley, K. J. Buhl, and F. A. Bullard. 2005b. Selenium impacts on razorback sucker, Colorado River, Colorado. II. Eggs. *Ecotoxicol. Environ. Saf.* 61:32–43.
- Hamilton, S. J., K. M. Holley, K. J. Buhl, and F. A. Bullard. 2005c. Selenium impacts on razorback sucker, Colorado River, Colorado. III. Larvae. *Ecotoxicol. Environ. Saf.* 61:168–169.
- Hardy, R. W., L. Oram, and G. Möller. 2010. Effects of dietary selenomethionine on cutthroat trout (*Oncorhynchus clarki bouvieri*) growth and reproductive performance over a life cycle. *Arch. Environ. Contam. Toxicol.* 58(1):237–245.
- Hermanutz, R. O., K. N. Allen, T. H. Roush, and S. F. Hedtko. 1992. Effects of elevated selenium concentrations on bluegills (*Lepomis macrochirus*) in outdoor experimental streams. *Environ. Toxicol. Chem.* 11:217–224.
- Hermanutz, R. O., K. N. Allen, N. E. Detenbeck, and C. E. Stephan. 1996. Exposure of bluegill (*Lepomis macrochirus*) to selenium in outdoor experimental streams. USEPA Report. Mid-Continent Ecology Division, Duluth, MN.
- Hodson, P. V., D. J. Spry, and B. R. Blunt. 1980. Effects on rainbow trout (*Salmo gairdneri*) of a chronic exposure to waterborne selenium. *Can. J. Fish Aquat. Sci.* 37:233–240.
- Holm, J. 2002. Sublethal effects of selenium in rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). Masters Thesis; Department of Zoology, University of Manitoba, Winnipeg, Canada.
- Holm, J., et al. 2003. An assessment of the development and survival of wild rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) exposed to elevated selenium in an area of active coal mining. The Big Fish Bang. Proceedings of the 26th Annual Larval Fish Conference.
- Holm, J., et al. 2005. Developmental effects of bioaccumulated selenium in eggs and larvae of two salmonid species. *Environ. Toxicol. Chem.* 24:2373–2381.
- Hunn, J. B., S. J. Hamilton, and D. R. Buckler. 1987. Toxicity of sodium selenite to rainbow trout fry. *Wat. Res.* 21(2):233–238.
- Kennedy, C. J., L. E. McDonald, R. Loveridge, and M. M. Stroscher. 2000. The effect of bioaccumulated selenium on mortalities and deformities in the eggs, larvae, and fry of a wild population of cutthroat trout (*Oncorhynchus clarki lewisi*). *Arch. Environ. Contam. Toxicol.* 39:46–52.
- Lemly, A. D. 1985. Toxicology of selenium in a freshwater reservoir: Implications of environmental hazard evaluation and safety. *Ecotoxicol. Environ. Saf.* 10:314–338.
- Lemly, A. D. 1993a. Metabolic stress during winter increases the toxicity of selenium to fish. *Aquat. Toxicol.* 27:133–158.
- Lemly, A. D. 1993b. Guidelines for evaluating selenium data from aquatic monitoring and assessment studies. *Environ. Monit. Assess.* 28:83–100.
- Lemly, A. D. 1993c. Teratogenic effects of selenium in natural populations of freshwater fish. *Ecotoxicol. Environ. Saf.* 26:181–204.
- Lemly, A.D. 1996. Selenium in aquatic organisms. In: *Environmental Contaminants in Wildlife—Interpreting Tissue Concentrations*, eds. W.N. Beyer, G.H. Heinz, and A.W. Redmon-Norwood, pp. 427–445. New York, NY: CRC/Lewis Publishers.

- Lemly, A. D. 1997. A teratogenic deformity index for evaluating impacts of selenium on fish populations. *Ecotoxicol. Environ. Saf.* 37:259–66.
- Lemly, A. D. 2002. *Selenium assessment in aquatic ecosystems: A guide for hazard evaluation and water quality criteria*. New York, NY: Springer-Verlag.
- Lemly, A. D., and G. J. Smith. 1987. Aquatic cycling of selenium: implications for fish and wildlife. U.S. Dept. of the Interior, Fish and Wildlife Service. Leaflet 12. Washington, DC. 10 pp.
- Lillebo, H. P., S. Shaner, D. Carlson, N. Richard, and P. DuBow. 1988. Regulation of agricultural drainage to the San Joaquin River. State Water Resources Control Board. SWRCB Order No. W.Q. 85–1. 151 pp.
- Luoma, S. N., and P. S. Rainbow. 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. *Environ. Sci. Technol.* 39(7):1921–1931.
- Luoma, S. N., and T. S. Presser. 2009. Emerging opportunities in management of selenium contamination. *Environ. Sci. Technol.* 43:8483–8487.
- Maier, K. J., and A. W. Knight. 1994. Ecotoxicology of selenium in freshwater systems. *Rev. Environ. Contam. Toxicol.* 134:31–48.
- Malchow, D. E., A. W. Knight, and K. J. Maier. 1995. Bioaccumulation and toxicity of selenium in *Chironomus decorus* larvae fed a diet of seleniferous *Selenastrum capricornutum*. *Arch. Environ. Contam. Toxicol.* 29:104–109.
- May, T. W., et al. 2001. An evaluation of selenium concentrations in water, sediment, invertebrates, and fish from the Republican River Basin: 1997–1999. *Environ. Monit. Assess.* 72:179–206.
- May, T. W., et al. 2008. An evaluation of selenium concentrations in water, sediment, invertebrates, and fish from the Solomon River Basin. *Environ. Monit. Assess.* 137(1–3):213–232.
- McIntyre, D. O., M. A. Pacheco, M. W. Garton, D. Wallschläger, and C. G. Delos. 2008. Effect of selenium on juvenile bluegill sunfish at reduced temperature. Health and Ecological Criteria Division, Office of Water, USEPA, Washington, DC. EPA-822-R-08–020.
- Muscatello, J. R., P. M. Bennett, K. T. Himbeault, A. M. Belknap, and D. M. Janz. 2006. Larval deformities associated with selenium accumulation in northern pike (*Esox lucius*) exposed to metal mining effluent. *Environ. Sci. Technol.* 40:6506–6512.
- Muscatello, J. R., A. M. Belknap, and D. M. Janz. 2008. Accumulation of selenium in aquatic systems downstream of a uranium mining operation in northern Saskatchewan, Canada. *Environ. Pollut.* 156(2):387–393.
- Ogle, R. S., and A. W. Knight. 1989. Effects of elevated foodborne selenium on growth and reproduction of the fathead minnow (*Pimephales promelas*). *Arch. Environ. Contam. Toxicol.* 18:795–803.
- Ogle, R. S., K. J. Maier, P. Kiffney, M. J. Williams, A. Brasher, L. A. Melton, and A. W. Knight. 1988. Bioaccumulation of selenium in aquatic systems. *Lake Res. Manage.* 4(2):165–173.
- Orr, P. L., K. P. Guiguer, and C. K. Russel. 2006. Food chain transfer of selenium in lentic and lotic habitats of a western Canadian watershed. *Ecotoxicol. Environ. Saf.* 63:175–188.
- Palace, V. P., J. E. Spallholz, J. Holm, K. Wautier, R. E. Evans, and C. L. Baron. 2004. Metabolism of selenomethionine by rainbow trout (*Oncorhynchus mykiss*) embryos can generate oxidative stress. *Ecotoxicol. Environ. Saf.* 58:17–21.
- Riedel, G. F., D. P. Ferrier, and J. G. Sanders. 1991. Uptake of selenium by freshwater phytoplankton. *Water Air Soil Pollut.* 57–58:23–30.
- Riedel, G. F., and J. G. Sanders. 1996. The influence of pH and media composition on the uptake of inorganic selenium by *Chlamydomonas reinhardtii*. *Environ. Toxicol. Chem.* 15(9):1577–1583.
- Rudolph, B.-L., I. Andreller, and C. J. Kennedy. 2008. Reproductive success, early life stage development, and survival of westslope cutthroat trout (*Oncorhynchus clarki lewisi*) exposed to elevated selenium in an area of active coal mining. *Environ. Sci. Technol.* 42:3109–3114.
- Saiki, M. K., B. A. Martin, and T. M. May. 2004. Reproductive status of western mosquitofish inhabiting selenium-contaminated waters in the grassland water district, Merced County, California. *Arch. Environ. Contam. Toxicol.* 47:363–369.
- Sanders, R. W., and C. C. Gilmour. 1994. Accumulation of selenium in a model freshwater microbial food web. *Appl. Environ. Microbiol.* 60(8):2677–2683.
- Schultz, R., and R. Hermanutz. 1990. Transfer of toxic concentrations of selenium from parent to progeny in the fathead minnow (*Pimephales promelas*). *Bull. Environ. Contam. Toxicol.* 45:568–573.
- Skorupa, J. P. 1998a. Selenium poisoning of fish and wildlife in nature: lessons from twelve real-world examples. In: *Environmental chemistry of selenium*, eds. W. T. Frankenberger and R. A. Engberg, pp. 315–354. New York, NY: Marcel Dekker, Inc.
- Skorupa, J. P. 1998b. Selenium. Pages 139–184 in National Irrigation Water Quality Program Information Report No. 3: Guidelines for interpretation of the biological effects of selected constituents in biota, water, and sediment. United States Department of the Interior, November 1998. 198 pp. + appendices.

- Staub, B. P., W. A. Hopkins, J. Novak, and J. D. Congdon. 2004. Respiratory and reproductive characteristics of eastern mosquitofish (*Gambusia holbrooki*) inhabiting a coal ash settling basin. *Arch. Environ. Contam. Toxicol.* 46:96–101.
- Stewart, R. A., S. N. Luoma, C. E. Schlekot, M. A. Doblin, and K. A. Heib. 2004. Foodweb pathway determine how selenium affects aquatic systems: A San Francisco Bay case study. *Environ. Sci. Technol.* 38:4519–4526.
- Tao, J., P. Kellar, and W. Warren-Hicks. 1999. Statistical analysis of selenium toxicity data. Report submitted for US EPA, Health and Ecological Criteria Division. The Cadmus Group, Inc., Durnham, NC.
- Tashjian, D. H., S. J. Teh, A. Sogomoyan, and S. S. O. Hung. 2006. Bioaccumulation and chronic toxicity of dietary L-selenomethionine in juvenile white sturgeon (*Acipenser transmontanus*). *Aquat. Toxicol.* 79:401–409.
- Tashjian, D., J. J. Cech, Jr., and S. S. O. Hung. 2007. Influence of dietary L-selenomethionine exposure on the survival and osmoregulatory capacity of white sturgeon in fresh and brackish water. *Fish Physiol. Biochem.* 33:109–119.
- Teh, S. J., et al. 2004. Chronic effects of dietary selenium on juvenile Sacramento splittail (*Pogonichthys macrolepidotus*). *Environ. Sci. Technol.* 38:6085–6593.
- U.S. EPA (United States Environmental Protection Agency). 2004. Draft aquatic life water quality criteria for selenium–2004. Office of Water, Office of Science and Technology, Washington, DC.
- Vidal, D., S. M. Bay, and D. Schlenk. 2005. Effects of dietary selenomethionine on larval rainbow trout (*Oncorhynchus mykiss*). *Arch. Environ. Contam. Toxicol.* 49:71–75.
- Williams, M. J., R. S. Ogle, A. W. Knight, and R. G. Burau. 1994. Effects of sulfate on selenate uptake and toxicity in the green alga *Selenastrum capricornutum*. *Arch. Environ. Contam. Toxicol.* 17:449–453.
- Woock, S. E., W. R. Garrett, W. E. Partin, and W. T. Bryson. 1987. Decreased survival and teratogenesis during laboratory selenium exposures to bluegill, *Lepomis macrochirus*. *Bull. Environ. Contam. Toxicol.* 39:998–1005.

Palaemon serratus

From *The Royal Natural History*, edited by Richard Lydekker, Frederick Warne & Co.,
London, 1893–94.